A SEARCH FOR GENETIC FACTORS INFLUENCING IMMUNE RESPONSES TO A KILLED *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* VACCINE IN AUSTRALIAN FINE-WOOL MERINO SHEEP

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ABSTRACT

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A study was conducted to identify associations between genetic markers and immune responses in Australian fine-wool Merino sheep to a killed *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) vaccine (Gudair^{TM}). Blood samples and immune response data (antibody and interferon gamma, IFN-γ results) were obtained from 934 sheep from a long-term *Map* vaccination trial undertaken on three independent properties in New South Wales, Australia. Blood samples were genotyped for eight microsatellite markers that included four (DYMS1, OLADRW, OLADRB and SMHCC1) from the *Ovar-Mhc* region, two each from the SLC11A1 (OVINRA1 and OVINRA2) and IFN- γ (o(IFN)γ and OarKP6) gene regions.

Vaccination with Gudair^{TM} induced strong antibody and IFN-γ responses as early as two weeks post-vaccination. Between-property differences in magnitude and trend of immune responses, concomitant with season of vaccination and magnitude of natural infection prevalent in individual flocks, were evident. Immune responses in controls on all the three properties remained consistently low, except for slightly elevated IFN-γ levels at a few time points in controls of properties 2 and 3, concomitant with exposure to natural infection.

There were only 2 alleles and 3 genotypes for marker o(IFN)γ but other loci exhibited extensive polymorphisms, the most occurring at OLADRW which had 42 alleles and 137 genotypes. Heterozygosities varied between 33% (OVINRA2) and 87% (SMHCC1), while polymorphic information contents ranged from 0.31 (o(IFN)γ) to 0.88 (OLADRW). Genotypes at loci DYMS1, OLADRB, SMHCC1, OVINRA1 and o(IFN)γ were in Hardy-Weinberg equilibrium (HWE), while those at OarKP6 were in HWE only when rare alleles (<1.0% frequency) were pooled with the closest size class. Departure from HWE, resulting from possible preferential amplification of alleles in heterozygotes, was evident at OLADRW and OVINRA2.

Associations between immune responses and genetic polymorphisms at the marker loci were examined by analysing both genotypic and allelic affects. The study revealed several genotypes/alleles at different marker loci to be significantly associated with antibody and IFN-γ responses to vaccination with Gudair^{TM}. However, the majority of those effects were inconsistent across the three properties. Based on significance and consistency in effects across the three properties, five genotypes (two at DYMS1 and one each at OLADRB, SMHCC1 and OVINRA1) and three alleles (one each at DYMS1, OLADRB and o(IFN)γ) were considered either 'probable' or 'most likely' to be associated with low IFN-γ responses, while a genotype at o(IFN)γ was considered 'most likely' to influence high IFN-γ responses. An allele at OarKP6 was considered 'probable' to be associated with low antibody responses to vaccination. Considering the significance of IFN-γ responses in protection against *Map*, it is likely that the identified genotype/alleles influencing IFN-γ responses to vaccination would also influence immune responses to natural *Map* infections. However, further studies need to be conducted to determine the role of these marker genotypes/alleles in protection against paratuberculosis under natural infection conditions.

Key words: paratuberculosis, OJD, Johne's disease, sheep, immune response, genetic markers, gene polymorphisms, MHC, SLC11A1, IFN-γ

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LIST OF ABBREVIATIONS

1C7	MHC classIII gene
AFO	acid-fast organism
AIC	Akaikes information criterion
AR 1	first-order auto-regressive model
Bf	B factor
BLV	bovine leukemia virus
Bota	*Bos taurus*
bp	base-pairs
C2, C4A and C4B	complement factors
CARD15	caspase recruitment domain-containing protein 15
CD	Crohn's disease
CD38	cluster of differentiation 38
CD4+	T-helper cells expressing cluster of differentiation 4
CD8+	T-helper cells expressing cluster of differentiation 8
cDNA	complementary DNA
CLIP	class II associated invariant chain peptide
CMI	cell-mediated immunity
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CSRD226	microsatellite in MHC class I region
CTL	cytotoxic T lymphocytes
CYP21	MHC classIII gene
DMA and DMB	genes encoding α- and β-chains of MHC class II DM molecules
DNA	deoxyribo nucleic acid
dNTP	deoxyribo nucleotide phosphate
DP, DM, DN/DO, DQ and DR	MHC class II molecules
DQA and DQB	genes encoding α- and β-chains of MHC class II DQ molecules
DRA and DRB	genes encoding α- and β-chains of MHC class II DR molecules
DRB1	ovine functional class II DRB gene
DRB2, DRB3 and DRB3	ovine non-functional DRB genes
DTH	delayed-type skin hypersensitivity
DYA and DYB	genes encoding α- and β-chains of MHC class II DY molecules
DYMS1	microsatellite in ovine DYA gene
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EM	expectation-maximization
ER	endoplasmic reticulum
FEC	faecal egg count
FGMT	footrot gene-marker test
G15	MHC class III gene
HIV	human immuno-deficiency virus
HLA	human leukocyte antigen
HSP70	heat-shock protein 70
HWE	Hardy-Weinberg equilibrium
IFN-γ	interferon gamma
IL	interleukin
IMF	international mapping flock
Ipr1	intracellular pathogen resistance 1
kb	kilo base-pairs
kDa	kilo Daltons

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LMP	low-molecular-mass protein
LRR	leucine-rich repeat
LSM	least square mean
LST1	MHC classIII gene
LT	lymphocyte transformation
LTA and LTB	MHC class III genes
Map	*Mycobacterium avium* subspecies *paratuberculosis*
MAS	marker-assisted selection
MBL	mannan binding lectin
MHC	major histocompatibility complex
MSMD	Mendelian susceptibility to mycobacterial disease
NK	natural killer cells
NOD2	nucleotide oligomerization binding domain 2
NRAMP1	natural resistance-associated macrophage protein 1
o(IFN)-γ	microsatellite in ovine interferon gamma gene
OD	optical density
OLA	ovine leukocyte antigen
OLADRB	microsatellite in ovine DRB2 gene
OLADRW	microsatellite in ovine DRB1 gene
Ovar	*Ovis aries*
OVINRA1 and OVINRA2	microsatellites in the ovine SLC11A1 gene
PBR	peptide binding region
PCR	polymerase chain reaction
PPD	purified protein derivative
PSO	polymorphism-specific oligonucleotide
PTB	paratuberculosis
QTL	quantitative trait loci
RFLP	restriction fragment length polymorphism
RSCA	reference-strand-mediated conformation analysis
RT-PCR	reverse transcription polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate - polyacrylamide gel electrophoresis
SE	standard error
Sh-LA	sheep leukocyte antigen
SLC11A1	solute carrier family 11 member 1
SMHCC1	microsatellite in MHC class I region
SNP	single nucleotide polymorphisms
SSCP	single strand conformational polymorphism
sst1	susceptibility for tuberculosis 1
STR	simple tandem repeat
TAP	transporter-associated protein
TAPBP	transporter-associated protein binding protein
T-cells	thymus-derived lymphocytes
TCR	T-cell receptor
Th1	T-helper cells subset 1
Th2	T-helper cells subset 2
TLR	toll like receptor
TNF	tissue necrosis factor
VDR	vitamin D receptor

1. INTRODUCTION

Johne's disease or paratuberculosis (PTB) is a chronic, progressive intestinal disease of ruminants. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*), a slow-growing mycobactin-dependent acid-fast bacterium containing the insertion sequence, *IS900* (Sweeney 1996). PTB, though predominantly a disease of cattle, sheep, goat and farmed deer, has also been reported in rabbits (Greig *et al.*1997), macropods (Cleland *et al.* 2001), white-tailed deer (Chiodini and Van Kruiningen 1983), Tule elk (Manning *et al.* 2003), bison (Buergelt and Ginn 2000), foxes and stoats (Beard *et al.* 1999), mandrills (Zwick *et al.* 2002), macaques (McClure *et al.* 1987) and ferrets (de Lisle *et al.* 2002), indicating a wide host-range. Based on restriction fragment length polymorphisms (RFLP) of *IS900, Map* isolates from domestic ruminants were classified into two principal types, 'cattle (C)' and 'sheep (S)', with cattle and sheep being preferentially infected with their named types (Collins *et al.* 1990). In Australia and New Zealand, *Map* isolates from naturally occurring PTB in sheep and cattle were found exclusively conformed to 'S' and 'C' types, respectively, while those in goats and deer were predominantly of 'C' type (reviewed by Whittington *et al.* 2000). 'S' type *Map* strains were also found to cause PTB in goats (Collins *et al.* 1990) and deer (de Lisle *et al.* 1993, 2006) in New Zealand.

PTB is a primarily a disease of adult animals. Young stock usually get infected by ingestion of either faecal material, milk or colostrum containing *Map* micro-organisms. Subsequent to ingestion, the organisms get localized in the lymphoid tissue of Payer's patches in the small intestine. The majority of naturally-infected animals, by virtue of cell-mediated immune (CMI) responses, clear the infection and become resistant (Perez *et al.* 1996), while in other individuals the intracellular mycobacteria persist for long periods, usually 2 to 5 years. Depending on the range of the host's CMI responses, individuals with latent infection might either eliminate the infection without ever progressing, or remain in an incubatory state throughout their productive life without ever exhibiting clinical signs. Alternatively, they may progress to become sub-clinical shedders of mycobacteria, before finally exhibiting clinical signs of infection (Stehman 1996; Clarke 1997; Sergeant 2003).

Serological tests aimed to detect PTB are problematic and often lead to false-positives and false negatives. Animals with latent or sub-clinical infection remain sero-negative since

antibodies are produced relatively late in the progression of disease (Clarke *et al.* 1996). Despite the sub-clinical nature of the disease and suspected under-reporting, PTB has been notified in numerous countries. The Animal and Plant Health Inspection Service of the United States Department of Agriculture found 21.6±1.7% of dairy operations in United Sates to be PTB-positive (NAHMS 1997). The disease was identified as early as 1952 in New Zealand sheep flocks and has subsequently been classed as endemic (de Lisle, 2002). In Australian sheep, it was first identified in 1980 (Seaman *et al.* 1981). Since then, the disease has spread gradually in a clustered fashion, with 1,971 flocks (2.6% of total flocks) considered to be infected and a further 2,107 flocks (2.8% of total flocks) to be either suspected or under surveillance by the end of 2003 (Australian Animal Health Council 2004).

PTB does not result in high mortality rates; however, its economic implications are enormous. Economic losses mainly result from production losses (e.g. reduction in milk, body weight, wool) and condemnation of affected carcasses. The impact of PTB on reduced dairy herd productivity in the United States was estimated to be US$200-250 million annually (Ott *et al.* 1999). A recent study in Australia estimated the mean annual decrease in gross margin due to PTB over 12 infected sheep flocks to vary between 6.4% and 8.5% over a three year period, equating to an average reduction in annual income of A$13,715 per farm per year (Bush *et al.* 2006). A PTB economic evaluation study in New Zealand considered 70% of the flocks to be infected by the disease, leading to an annual national economic loss of up to $10 million (Brett 1998). This estimated loss was exclusive of the effects resulting from sub-clinical cases, which often pre-dominate the clinical cases. Further effects of the disease result from the impact of market discrimination against flocks known to be infected. Also, there is a sustained zoonotic concern with regard to the possible role of *Map* in Crohn's disease in humans (Grant 2005; Sechi *et al.* 2005). Reports on detection of viable *Map* in retail pasteurized whole milk in the U.K. (Grant *et al.* 2002) and the U.S.A. (Ellingson *et al.* 2005) further aggravate this concern.

Treatment of the disease is ineffective and economically impracticable, and there are no approved drugs to treat PTB in livestock (Harris and Barletta 2001). Vaccination against PTB can play an important role in the control of clinical disease, but cannot prevent the animals from being infected (Gwozdz *et al.* 2000). One possible way of limiting the incidence of the disease is to identify genetic markers for resistance to the disease. Such markers can be

employed to selectively breed resistant animals so as to increase the frequency of resistant alleles in the population.

A long term trial designed to evaluate the efficacy of a whole killed *Map* vaccine, Gudair ™ (CZ Veterinaria, Porrino, Spain), for the control of PTB in Merino sheep was carried out in the Central Tablelands of New South Wales, Australia, from 1999 to 2004 (Reddacliff *et al.* 2006). In total, 600 sheep from three different properties were vaccinated, while another 600 constituted the unvaccinated control group. Antibody and interferon gamma (IFN-γ) responses to vaccination, recorded at various intervals for up to 54 months post-vaccination, revealed between-individual variations in vaccine response. These variations offered the opportunity to search for possible genetic markers to immune responses to vaccination. Immune responses to vaccination determine the inherent ability of individuals to protect themselves from disease represented by the vaccine antigens and can be employed to quantify resistance in disease association studies, for example based on genes contained within the major histocompatibility complex (MHC) (Outteridge *et al.* 1988). For protection against intracellular micro-organisms like *Map*, CMI responses are of paramount importance.

MHC class I and II gene products play an important role in antigen presentation, essential for the onset of immunological responses. Several studies have investigated the polymorphisms of ovine MHC genes (reviewed by Dukkipati *et al.* 2006a) and their role in resistance and/or susceptibility to diseases in sheep (reviewed by Dukkipati *et al.* 2006b). In the studies reviewed, several genes from class I and II regions were found to be significantly associated with resistance to a number of diseases, gastrointestinal nematodiasis in particular. Polymorphisms at three microsatellites from the class II MHC region, one each at the DRB1 (OLADRW; Schwaiger and Epplen 1995), DRB2 (OLADRB; Blattman and Beh 1992) and DYA loci (DYMS1; Buitkamp *et al.* 1996), and another from the class I region (SMHCC1; Groth and Wetherall 1994) have been reported in different sheep breeds.

Genes from locations other than from the MHC considered to be immunologically important include those coding for interferon gamma (IFN-γ) and solute carrier family 11 member 1 (SLC11A1), formerly known as the natural resistance-associated macrophage protein 1 (NRAMP1). Alleles at the SLC11A1 gene in humans have been shown to be associated with susceptibility to mycobacterial infections (Awomoyi *et al.* 2002), and a diallelic microsatellite marker locus, o(IFN)-γ, within the IFN-γ gene was shown to be associated with

resistance to gastrointestinal nematodiasis in sheep (Coltman *et al.* 2001). Two microsatellites, OVINRA1/NRAMP1 (Pitel *et al.* 1996; Matthews and Crawford 1998) and OVINRA2 (Pitel *et al.* 1996), were found to be located within the 3' untranslated region of the ovine SLC11A1 region. Alleles at the OVINRA1 locus were found to be associated with probable susceptibility/resistance to clinical paratuberculosis in two naturally infected fine wool Merino flocks in Australia (Reddacliff *et al.* 2005).

Microsatellite markers located within functional genes have to date been preferentially employed in disease association studies by virtue of their tight linkage with functional gene regions, the ease and accuracy in their genotyping as well as from economic considerations. Employing eight microsatellite markers from three immunologically-significant chromosomal regions, four from the MHC and two each from IFN-γ and SLC11A1 regions, an investigation into genetic markers for immune responses to *Map* vaccination was undertaken with the following specific objectives.

1. To standardise method for effective recovery of DNA from frozen blood samples stored for prolonged periods.
2. To optimise multiplex PCR conditions so as to minimise the number of reactions required for amplification of target DNA of the employed markers.
3. To investigate genetic polymorphisms at eight microsatellite marker loci located in MHC, SLC11A1 and IFN-γ gene regions, in Australian Merino sheep.
4. To study the effects of individual marker alleles and genotypes at the investigated marker loci on antibody and IFN-γ responses to *Map* vaccination.
5. To study the chromosome-wise haplotype effects of the investigated markers on antibody and IFN-γ responses to *Map* vaccination.

2. REVIEW OF LITERATURE

In this chapter, literature pertaining to immune responses to *Map*, structure and polymorphisms of ovine MHC, SLC11A1 and IFN-γ genes, together with their role in disease resistance has been reviewed. A few other loci found to be associated with susceptibility to tuberculosis have also been briefly reviewed. More emphasis was laid on ovine MHC, considering the role of MHC molecules in antigen presentation necessary for elicit of immune responses to infectious organisms and reported associations of MHC genes with resistance/susceptibility to various diseases.

2.1 *MAP* INFECTION IN SHEEP

Ingestion of faeces from *Map*-infected sheep is the primary source of infection, although intra-uterine and mammary routes of infection are possible (Clarke 1997). Infected sheep can shed *Map* in faeces during the sub-clinical stage of infection for up to 18 months prior to the onset of clinical signs. During the clinical phase of infection shedding might be as high as 5×10^{12} mycobacteria per day (Chiodini *et al.* 1984). Experimental infection of sheep via intra-tracheal and intravenous routes was also documented (Kluge *et al.* 1968). Subsequent to ingestion, the *Map* organisms get localized in the lymphoid tissue of Payer's patch of the small intestine. The majority of the naturally infected animals clear infection by non-specific innate intracellular killing and become resistant to re-infection (Perez *et al.* 1996). In the remaining animals, mycobacteria that survive innate responses multiply within the macrophages resulting in the initiation of specific immune responses.

Depending on the range of host's specific immune responses (mainly CMI responses), individuals with latent infection might either eliminate the infection or might remain in an incubatory state throughout their productive life without ever exhibiting clinical signs or might progress to become sub-clinical light or heavy shedders of mycobacteria, before finally exhibiting clinical signs of infection (Stehman 1996; Clarke 1997; Sergeant 2003). The early cellular and molecular processes that determine whether an infection is cleared or will persist are not fully understood, although the fate of infection is likely to be influenced by the size of infective dose, route of infection, virulence of mycobacterial strain, age of the host, host resistance genes affecting antigen presentation and intra-cellular killing, and environmental factors (Clarke 1997).

CMI responses play an important role in protection against *Map* and are usually evident relatively early in infection and remain active for months or years, thereby delaying expansion of the disease to the multi-bacillary form (Clarke 1997; Perez *et al.* 1999; Whittington and Sergeant 2001). During this stage, strong Th1 responses associated with increased IFN-γ production are evident in the individuals (Burrells *et al.* 1999). However, over a protracted period of persistent infection humoral responses replace the diminishing CMI responses as the multi-bacillary form of PTB becomes evident (Clarke 1997; Perez *et al.* 1997). During this phase, a strong Th2 response associated with low IFN-γ and high concentrations of antibody is evident (Burrells *et al.* 1999). Humoral responses are evident only in the late sub-clinical and early clinical phases of infection, when increased multiplication of *Map* leads to macrophage breakdown. However, antibody effector mechanisms are of little use at these stages (Clarke *et al.* 1996).

Several studies have examined immune responses to experimental infection as well as vaccination of sheep with *Map*. The CMI and humoral responses observed are reviewed below. Also, studies that have considered genetic resistance/susceptibility to *Map* infection are reviewed.

2.1.1 Immune responses to experimental infection

Several studies have investigated immune responses to experimental infection of sheep with *Map* and revealed that CMI, rather than humoral responses, are more likely to confer resistance to progressive infection (Clarke 1997). In one of the early studies (Gilmour *et al.* 1977), the degree of delayed-type skin hypersensitivity (DTH, a manifestation of CMI) was found to be associated with viable counts of *Map* in intestine. Sheep exhibiting strong DTH reactions at three months post-infection harboured significantly lower *Map* organisms during 5-27 months post-infection, compared to those exhibiting weak reactions. Also, electron microscopy of macrophages within the lesions in sheep with strong DTH showed mainly degenerating bacilli. This was in contrast to macrophages from similar lesions in sheep with weak DTH which often contained numerous intact bacilli. In a study of experimental infection of weaner sheep with the 'S' strain of *Map*, four of the six sheep found to be infected (66.67% sensitivity) exhibited a positive DTH reaction (Reddacliff and Whittington 2003).

IFN-γ production, a good indicator of CMI, was also studied in sheep experimentally infected with *Map*. A surge in IFN-γ production, concomitant with infection status was generally evident in sheep infected with *Map* (Gwozdz and Thompson 2002; Reddacliff and Whittington 2003; Stewart *et al.* 2004; Begg *et al.* 2005). This surge was evident at 2-4 months post-infection (coinciding with the onset of faecal shedding of *Map*) and prevailed throughout the period of shredding (16-30 months), before declining in the final stage of disease progression (Stewart *et al.* 2004).

Tissue-specific differences in IFN-γ production were also observed. IFN-γ production in the pre-scapular lymph node was found to be higher than that of peripheral blood (Gwozdz and Thompson 2002). The authors attributed this to memory T-cells being potent producers of IFN-γ, compared to naïve T-cells (Sanders *et al.* 1988) and to the possible preferential migration of antigen-specific memory T-cells to peripheral lymph nodes (Premier *et al.* 1996). Contrary to this, a recent study (Begg *et al.* 2005) showed IFN-γ production in pre-scapular, anterior-jejunal and ileo-cecal lymphnodes was lower compared to that in peripheral blood. However, IFN-γ production in spleen, mid- and posterior-jejunal lymph nodes was higher compared to that in the rest of the examined tissues. It was inferred that the presence of $CD4^+$ T cells, potent producers of IFN-γ, in large numbers in the gut lymph nodes (as a result of migration from within the gut or peripheral lymphatics) might have resulted in such high IFN-γ production in those tissues.

Map strain and route of infection were found to influence IFN-γ production in peripheral blood (Begg *et al.* 2005). IFN-γ response in sheep infected with strain W was higher than in sheep infected with strain JD3. Similarly, IFN-γ responses seen for animals challenged by the intra-tonsillar route were found to be higher than those for orally challenged sheep.

Humoral responses to experimental infection with *Map* are usually not observed until the late stages of infection, when the CMI responses begin to wane. Lambs infected with a 'C' strain of *Map*, did not show a definitive increase in enzyme-linked immunosorbent assay (ELISA) values for antibodies until about six weeks post-infection (Juste *et al.* 1994). In a different study, antibody levels in sheep exposed orally to medium or high doses of tissue homogenates of *Map* remained at background levels until at least six to nine months and peaked at nine months post-challenge, just before clinical disease was evident (Begg *et al.* 2005). This shift in immune responses from CMI to humoral, during the clinical phase of

infection, was not evident in a long-term experimental infection study (Stewart *et al.* 2004). None of the clinically affected sheep developed antibody responses despite the presence of persistent faecal shedding of *Map*. However, a similar study in goats by the same authors (Stewart *et al.* 2006) noted persistently elevated IFN-γ responses two months post-challenge with a 'C' strain of *Map*, and antibody responses commenced just prior to the time of faecal shedding of bacteria.

Immune response trends in animals considered immune or susceptible to experimental infection with *Map* were investigated in two studies. Of the 14 *Map* infected lambs in a study (Gwozdz *et al.* 2000), seven animals tested positive in polymerase chain reaction (PCR) for the presence of acid-fast organisms (AFO) in necropsied ileum and ileo-caecal lymph nodes at 53 weeks post-infection. While the pattern of antibody production was similar in the AFO+ and AFO- sheep, IFN-γ responses in AFO- sheep were higher than those in AFO+ sheep between weeks 9-36 post-infection and differed significantly ($P<0.05$) at 18 weeks post-infection. In a different study (Begg and Griffin 2005) on experimental infection of *Map*, sheep considered to be immune to infection exhibited consistently higher lymphocyte transformation (LT) responses during 9-15 months post-infection, compared to diseased sheep. Antibody titres on the other hand remained at base line levels in all sheep except that the diseased sheep showed a small spike at nine months post-infection. The findings of these two studies further underlined the role of CMI responses in combating *Map.*

2.1.2 Immune responses to vaccination

Vaccination of sheep against *Map*, employing killed, live, as well as recombinant vaccines has been undertaken in different studies. In an early study investigating immune responses in New Zealand sheep to a live *Map* vaccine (Neoparasec^{TM}), humoral responses measured by complement fixation and gel diffusion tests were detected as early as three weeks post-vaccination and remained high until the end of the experiment at 36 weeks post-vaccination (Hilbink and West 1990). In line with those findings, a different trial on the same vaccine in Spanish sheep recorded an immediate and strong ELISA response that persisted for up to 272 days post-vaccination (Juste *et al.* 1994). Corroborating the fact that humoral responses have negligible role in combating *Map* infection, no change in the ELISA response trend (compared to that in un-infected vaccinates) was evident in the same study, in sheep subjected to experimental infection at 71 days post-vaccination.

A recent study considered both humoral and CMI immune responses to Neoparasec™ vaccination (Begg and Griffin 2005). Strong LT and IFN-γ (measures of CMI), as well as ELISA antibody responses were evident as early as two weeks post-vaccination. While IFN-γ responses returned to baseline levels by 10 months post-vaccination, LT and ELISA responses were persistent and even peaked higher at the end of the same period. Immune responses to Neoparasec™ vaccination of sheep experimentally infected with *Map* were also studied (Gwozdz *et al.* 2000). Infected sheep vaccinated at two weeks post-infection, compared to infected sheep that were not vaccinated, exhibited higher ELISA as well as IFN-γ responses at all time points monitored for up to 53 weeks post-infection. While ELISA responses differed significantly ($P<0.05$) at all the time points, IFN-γ responses differed significantly ($P<0.05$) between the two groups at 9 and 27 weeks post-infection.

Two recent studies tested immunogenicity of recombinant *Map* DNA vaccines in sheep. Vaccination of sheep with three DNA vaccines encoding p85A-Mav, p85A-BCG and pHsp65 elicited dominant Th1 type, rather than Th2 type immune responses by day 90 post-vaccination (Sechi *et al.* 2006). IFN-γ expression detected by PCR was remarkably higher in sheep vaccinated with p85A-Mav and pHsp65, while that in sheep vaccinated with p85A-BCG was modest. Th2 type responses measured by IL-10 responses were quite low in sheep belonging to all the three groups. In a different study, vaccination of sheep with a 22 kDa *Map* protein (p22) elicited both humoral and CMI responses as early as four weeks post-vaccination (Rigden *et al.* 2006). IFN-γ production was detected by four weeks and it increased significantly and progressively by 13 and 29 weeks post-vaccination. Three sheep that gave the highest IFN-γ response by four weeks post-vaccination also exhibited good antibody reactivity detected by Western blotting at the time.

An extensive long-term vaccination trial was undertaken on three properties in New South Wales, Australia to test the efficacy of commercially available killed *Map* vaccine, Gudair™ (Reddacliff *et al.* 2006). Two hundred lambs were vaccinated on each of the three properties and immune responses monitored for up to 54 months post-vaccination. Strong humoral and CMI responses were evident in sheep belonging to all three properties. Greater than 80% and 60% of the vaccinated sheep on each of the properties tested positive for IFN-γ and ELISA antibodies, respectively, at their peak level time points.

Significant genotypic (P<0.01) as well as genotype by management interaction (P<0.05) effects on humoral and CMI responses to *Map* vaccination have been reported (Goddard *et al.* 2000). Scottish Blackface lambs had lower antibody and LT responses than lambs from Texel crosses. Further, for both assays, Blackface lambs under extensive management showed a greater immuno-reactivity than respective lambs under semi-intensive management. The reverse was true in the case of lambs from Texel crosses.

2.1.3 Genetic resistance/susceptibility to infection

The SLC11A1was found to play an important role in resistance to mycobacterial diseases in mice and humans. A glycine→aspartate mutation at amino acid 169 of the murine SLC11A1 renders mice susceptible to intracellular pathogens including *Mycobacterium bovis* (Vidal *et al.* 1995). However, this glycine→aspartate mutation at amino acid 169 could not be detected in a study involving PTB affected and unaffected sheep (Beard *et al.* 1999). In humans, there were indications for positive (Bellamy 1999; Awomoyi *et al.* 2002) as well as negative (Shaw *et al.* 1997; Soborg *et al.* 2002) associations of NRAMP1 alleles with susceptibility to tuberculosis.

A few studies have investigated the genetic variation of susceptibility and immune responses to *Map* in cattle, goats and sheep. A Dutch study analysed complete pedigree records and infection status at slaughter of 3020 cows and found the overall heritability of susceptibility to *Map* to be 0.06 (Koets *et al.* 2000). Heritability estimates estimated separately for vaccinated and non-vaccinated subsets were found to be 0.09 and <0.01, respectively. A study in goats examining susceptibility to Johne's disease, based on faecal shedding of *Map*, estimated the heritability of susceptibility to be 0.01 and 0.15, respectively for Barbari and Jamnapari breeds (Singh *et al.* 1990). A New Zealand study looked at the genetic variation of PTB incidence in sheep by examining health records of 3645 ewes on one property spanning over eight years (Hickey *et al.* 2003). The heritability of lifetime incidence of PTB, considered as a binomial trait, was estimated to be 0.07±0.14 and 0.18±0.11 for Romneys and Merinos respectively, with an overall value of 0.14±0.09. It was concluded that the low heritability estimate for PTB in Romneys would render traditional breeding methods aimed at reducing the incidence of Johne's disease to be slow or unsuccessful. However, selection in case of Merinos was predicted to be effective initially, though likely to be hampered subsequently.

Genetic variation in the ability of cows to establish humoral responses to *Map* under natural infection was estimated in a Danish study (Mortensen *et al.* 2004). Antibody levels in milk samples from 11,535 cows from 99 herds were determined using ELISA. A bivariate model with daily milk yield and ELISA readings as dependent variables showed a significant heritability for the ability to produce *Map* antibodies of 0.102 and a non-significant genetic correlation of -0.037 between daily milk yield and ELISA readings.

A recent study focussed on genetic resistance/susceptibility to *Map* in sheep (Reddacliff *et al.* 2005). Adult fine-wool Merino sheep (N=198) from two different properties in New South Wales, Australia were classed as having severe, mild or no PTB on the basis of clinical, pathological and culture tests, and as positive or negative in tests for humoral immunity. Associations of the phenotypic classes with polymorphisms at SLC11A1, MHC, IFN-γ, lysosome and leukaemia inhibiting factor loci were analyzed. MHC class I microsatellite (CSRD226) genotypes, 163/163 in one property and 163/* in the other property, were found to be associated with different susceptibility traits to the disease. Also, a possible association of 162 and 160 bp alleles at a microsatellite (OVINRA1) locus within the SLC11A1 gene with susceptibility and resistance, respectively, to clinical PTB was revealed.

2.2 OVINE MHC: STRUCTURE AND GENE POLYMORPHISMS

The major histocompatibility complex (MHC) is an organised cluster of tightly linked genes with both immunological and non-immunological functions, and is present in all vertebrates, except the jawless fish (Tizard 2004). The MHC was discovered during tissue transplantation studies in mice (Gorer 1937) and was first known for its role in histocompatibility. Subsequently, its role in immune regulation (Benaceraff and McDevitt, 1972) and several other functions (Bonner 1986; Zavazava and Eggert 1997; Pen and Potts 1999) was discovered. The primary function of the MHC is to code for specialised antigen-presenting receptor glycoproteins, known as histocompatibility molecules or MHC molecules. These molecules bind processed peptide antigens and present them to T lymphocytes, thereby triggering immune responses.

Human and mouse MHCs have been investigated in much more detail than those of other mammals (Deverson *et al.* 1991), and among domesticated species, the sheep MHC is poorly characterised (Kostia *et al.* 1998). The MHC of humans, designated as the human leukocyte antigens (HLA), covers a region of about 3.6 megabasepairs. Its complete sequence and gene

map locus have been reported (MHC sequencing consortium 1999). It serves as a valuable reference for intra-species and inter-species comparative studies (Kulski *et al.* 2002). With over 224 genes (128 predicted to be expressed), it is the most gene-dense region of the human genome. The average gene density, including pseudogenes, over the entire region is one gene per 16 kilo base-pairs. It is believed that about 40 per cent of the expressed HLA genes are involved in immune system function.

The HLA complex is divided into three regions, the telomeric class I, the centromeric class II and the central class III (Klein 1976). Analysis of the immediate-flanking regions has revealed that the classical class I and class II regions extend much further than originally thought and are referred to as extended class I and class II regions (Stephens *et al.* 1999). A set of more than seven genes involved in inflammation, including the three members of the tumor necrosis factor (TNF) super family that is located at the telomeric end of the class II region, is sometimes specified as the class IV region (Gruen and Weissman 1997).

The general structure of the MHC is conserved among mammalian species, including three main regions with different functional roles (Amills *et al.* 1998). However, when MHCs of different mammals are compared, some regions appear to be well conserved and others vary widely (Kelly *et al.* 2005). In general, the class II and class III regions are orthologous, i.e., they are clearly derived from a single ancestor without being subjected to major rearrangements (except in ruminants) and their gene order is conserved. In ruminants, the class II region is unique in that it is split into two distinct sub-regions, ‘a’ and ‘b’, separated by a distance of at least 15 cM (Andersson *et al.* 1988; Van Eijk *et al.* 1995). The class I genes, in contrast, are paralogous, i.e., they are derived by duplication and have been reorganised several times (Kelly *et al.* 2005).

The ovine MHC was first identified about 30 years ago by serological studies on sheep lymphocyte antigens (Millot 1978). Since then, it has been generally referred to as ovine leukocyte antigen (OLA) or sheep lymphocyte antigen (Sh-LA). In accordance with a nomenclature system for the MHC of vertebrates (Klein *et al.* 1990), it has now been designated as ‘*Ovar-Mhc*’ (‘*Ovar*’ representing *Ovis aries*). However, this system of nomenclature has not been universally adopted amongst animal immunogeneticists (Rothschild *et al.* 2000). *Ovar* has been localised by in situ hybridisation to chromosome 20 between bands q15 and q23 (Mahdy *et al.* 1989; Hediger *et al.* 1991). A schematic structure

of the *Ovar-Mhc* is illustrated in Figure 1. Details of the genes harboured in the three regions and their known polymorphisms are summarised below.

2.2.1 Class I genes

The class I loci include both classical and non-classical genes. The classical class I genes are members of the immunoglobulin gene family that are involved in the presentation of peptides, predominantly derived from intracellular proteins and parasites, to CD8+ cytotoxic T cells. They have also been found to interact with natural killer (NK) cells to prevent NK-mediated cell lysis (Reyburn *et al.* 1997). The non-classical class I genes are evolutionarily related and appear to have distinct functions related to immune response and NK cell recognition in specific settings (Lee *et al.* 1998). There are three classical (HLA – A, B and C) and three non-classical (HLA – E, F and G) class I genes in the HLA complex (Rhodes and Trowsdale 1998).

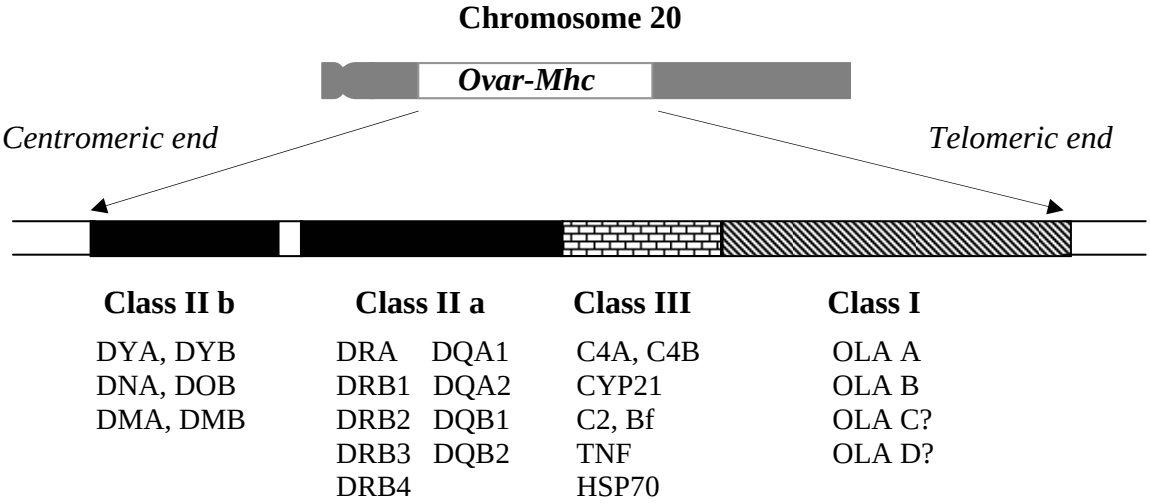

Figure 1: Schematic presentation of the structure of the *Ovar-Mhc*
(Compiled from information available in literature)

In sheep, the class I region is poorly characterised and there is a significant controversy over the number of classical class I loci. Initial studies in this regard relied mainly on the use of alloantisera in micro-lymphocytotoxicity assays. Evidence for the presence of two closely linked class I loci, OLA – A and B, was provided in 1978 (Millot 1978). Several other studies confirmed the existence of two class I loci (Stear and Spooner 1981; Cullen *et al.* 1982; Garrido *et al.* 1995; Stear *et al.* 1996; Jugo and Vicario 2001; Jugo *et al.* 2002). Three different studies, one based on the micro-lymphocytotoxicity assay (Millot 1984), one based

on immunoprecipitation followed by 2-dimensional gel analysis (Puri *et al.* 1987a) and another based on RFLP (Grossberger *et al.* 1990), have indicated the existence of a third class I locus. In a recent study aimed at haplotype characterisation of transcribed ovine MHC class I genes, at least four distinct polymorphic loci were identified (Miltiadou *et al.* 2005).

Several molecular genetic investigations have been undertaken to study polymorphisms of class I genes. A restriction fragment length polymorphism (RFLP) study conducted employing a human class I probe revealed polymorphic bands co-segregating and correlating with serologically defined lymphocyte antigens (Chardon *et al.* 1985). This was the first evidence that the serologically detected class I sheep leukocyte antigens are coded by MHC genes. In a different study, a sheep thymus cDNA library was screened with a human cDNA probe derived from HLA-B27 (Grossberger *et al.* 1990). Thirteen clones were identified and partially sequenced. Based on the sequences, the clones could be categorised into 5 distinct groups, requiring the expression of at least 3 loci. These sequences were found to be more similar to bovine than to murine class I genes.

A purine-pyrimidine repeat of the form (CA)20 was identified in an ovine class I (*Ovar-Mhc* I) positive clone from a sheep genomic library (Groth and Wetherall 1994). PCR amplification of this microsatellite region revealed the presence of 11 alleles at the locus, segregating in a Mendelian fashion. This microsatellite (SMHCC1) was found to be highly polymorphic in different breeds of sheep (Buitkamp *et al.* 1996; Paterson 1998; Paterson *et al.* 1998; Charon *et al.* 2001; Gruszczynska *et al.* 2002a). These studies revealed allele numbers ranging from 5 to 13, with high heterozygosity coefficients, indicating the usefulness of this locus as a genetic marker. This locus was found by recombination frequency to be 5.8 cM from the DRB1 locus (Buitkamp *et al.* 1996).

Recently, molecular genetic analyses in two heterozygous Scottish Blackface rams revealed 12 novel MHC class I transcripts (Miltiadou *et al.* 2005). Based on the class I sequence-specific genotypes of their progeny, these transcripts could be assigned to four individual haplotypes. Phylogenetic analyses of the more conserved exons (4 to 8) grouped the transcripts into four clusters, while a combination of phylogenetic analyses, haplotype data and transcription levels suggested the transcripts to be products of at least four loci, three of which appeared together in a number of combinations in individual haplotypes.

2.2.2 Class I molecules

Classical MHC molecules have four characteristics by which their function is defined: a high degree of polymorphism, high-level expression in particular cells, and the ability to bind small peptide molecules and present them to T cells (Kaufman *et al.* 1994). The class I MHC molecules, called class Ia molecules or class I classical molecules, are glycoproteins expressed on the surface of all nucleated somatic cells. They are found in highest concentration on lymphocytes and macrophages. The structure of the class I molecule was originally derived by X-ray crystallography (Bjorkman *et al.* 1987a,b). It is a heterodimer (Figure 2) consisting of an α or heavy chain, non-covalently linked to a light β2-microglobulin chain. The chain is composed of three extracellular domains (α1, α2 and α3), a transmembrane domain and a cytoplasmic domain. The α1 and α2 domains form the peptide-binding region (PBR), lying above the α3 domain. The groove is formed by two α helices bordering a β-pleated sheet, and residues from both α1 and α2 domains contribute to the groove (Bjorkman *et al.* 1987a). The microglobulin chain has a single extracellular domain and probably serves to stabilise the structure. The known polymorphisms of the molecule, i.e., variations in the amino acid sequence, are concentrated in three or four discrete hypervariable regions within the PBR. The rest of the molecule is highly conserved and shows little sequence variation. The α chains are encoded by polymorphic class I loci within the MHC complex, while β2 microglobulin is encoded by a non-polymorphic locus outside the MHC (Hughes and Yeager 1998).

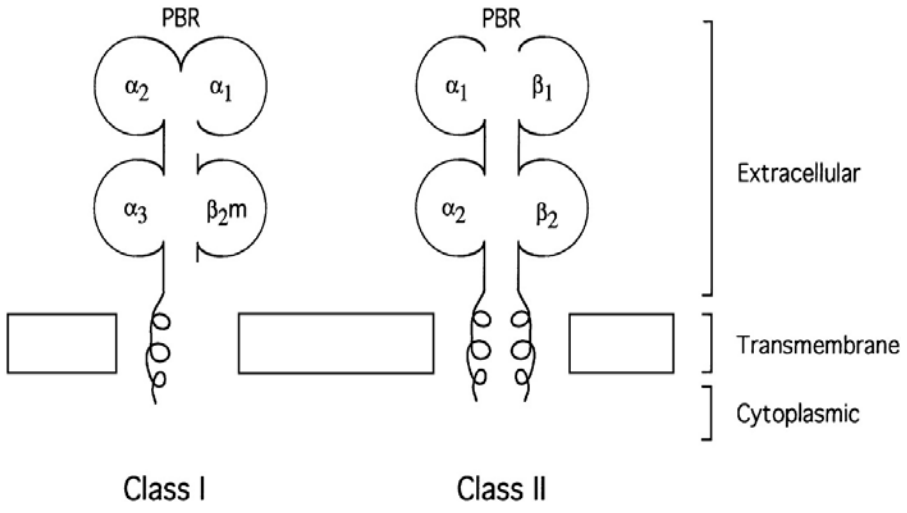

Figure 2: Schematic presentation of the structure of MHC class I and class II molecules
(Reprinted, with permission, from the Annual Review of Genetics, Volume 32 ©1998 by Annual Reviews www.annualreviews.org)

The molecular structure and tissue distribution of sheep classical class I molecules were studied using a panel of three monoclonal antibodies (Gogolin-Ewens *et al.* 1985). The class I heterodimer comprised a heavy chain of 44 kDa and a smaller β2 microglobin of 12 kDa. In similarity to the class I MHC molecules of other species, these molecules were found to be distributed on all sheep lymphocytes and many non-lymphoid tissues, with differential expression on mature and immature lymphocytes. They were found to be expressed equally on normal lymphocytes and antigen-activated lymphoblasts (Hopkins and Dutia 1990).

The class I molecules present antigenic peptides (eight or nine amino acids long) to T cell receptors (TCRs) of CD8+ cytotoxic T lymphocytes (CTLs), the principal immune function of which is considered to be the killing of virus-infected cells and tumour cells (Rammensee *et al.* 1995). In all cells, there is constant turnover of cellular proteins that are broken down into small peptides by a multimeric proteolytic complex in the cytoplasm, known as a proteasome (Rivett 1993). In mammals, there are two proteasome components encoded within the MHC class II region, called the low molecular mass polypeptide 2 (LMP2) and LMP7. The expression of class I molecules and LMPs is enhanced by the cytokine interferon gamma. The peptides derived in the proteasome are transported across the membrane of endoplasmic reticulum (ER) by a dimeric transporter-associated protein (TAP), encoded within the class II MHC region. In the ER, a complex involving the class I molecule, the peptide and β2 microglobulin is formed, and then transported to the cell surface.

The CTLs exercise a continual surveillance in the body by means of their TCRs. In the absence of any infection, the peptides bound by class I molecule are self-peptides. During infection by a virus or other intracellular parasite, some of the proteins broken down by the proteasome are of parasitic origin (non-self or foreign peptides). When CTLs encounter the complex of self-class I MHC and foreign peptide, a cytotoxic reaction is initiated that kills the infected cells. CTLs can only recognise foreign peptides in the context of self-class I MHC, a phenomenon referred to as class I MHC restriction of CTL (Zinkernagel and Doherty 1974).

2.2.3 Class II genes

Class II genes are members of the immunoglobulin superfamily of genes which are functionally specialised for presentation of antigenic peptides mainly derived from

extracellular proteins and parasites to the TCRs on CD4+ helper T cells. In the HLA complex, these include five sets of the classical genes DP, DM, DO, DQ and DR and non-classical genes such as LMP, TAP and TAPBP. Within each set of classical genes, genes for the α-chain are designated A, while genes for the β-chain are called B. The α- and β-chain genes in each set are located close together and resemble a two-gene duplication unit, with the exception of DOA and DOB genes, which are well separated from each other. Not all sets contain genes for both chains, although some contain many pseudogenes (Tizard 2004).

Among the three regions of the ovine MHC, genes of the class II region are the best characterised. They are classified into different families, as in other mammalian species, using nomenclature adapted from humans and include DQ, DR, DY, DM and DN/DO (Hein 1997). Early studies of the class II region by genomic Southern analysis employing HLA gene probes resulted in a complex pattern of cross-hybridising bands, which suggested that sheep contained homologues of DQ and DR genes but probably not DP (Chardon *et al.* 1985; Puri *et al.* 1987c; Scott *et al.* 1987). In a subsequent study on two unrelated sheep, 7 distinct class II α and 24 distinct class II β or β -related sequences were identified (Deverson *et al.* 1991). Consistent with earlier predictions, DQ and DR homologues were detected but not DP. The *Ovar*-DQ and *Ovar*-DR loci, which constitute the class IIa sub-region, have been studied in detail. A number of other *Ovar-Mhc* II genes of the class IIb type have also been identified. These include DY (Wright *et al.* 1994), DM (Schwaiger *et al.* 1996) and DN/DO (Wright *et al.* 1995a,b).

2.2.3.1 *Ovar*-DR genes

The DR genes are highly polymorphic and the classical class II molecules encoded by these genes are expressed in higher concentrations than the DQ molecules on the cell membranes of macrophages and B cells (Outteridge *et al.* 1996). Several studies have been undertaken to characterise DRA and DRB genes of sheep.

Ovar-DRA genes: An early Southern hybridisation study (Scott *et al.* 1987) employing human HLA-D probes provided evidence for the existence of a single DRA gene in sheep that was later isolated and found to be expressed (Deverson *et al.* 1991; Ballingall *et al.* 1992). Although there was an indication for the presence of a second DRA gene in sheep (Deverson *et al.* 1991) that might have been the result of gene duplication (Ballingall *et al.* 1992), it has not been confirmed in any subsequent studies. Initial sequencing of exons 1 to 4

of the expressed DRA gene indicated that it was homologous to the human DRA gene. Complete sequencing of the gene (Fabb *et al.* 1993) has revealed that it could code for a polypeptide of 253 amino acids of which 24 constitute the signal peptide and the remaining 229 form the mature polypeptide. The DRA clones in the two studies differed at only two amino acid positions, one within exon2 (H50/A50) and the other in exon3 (T109/I109). This low level of *Ovar*-DRA sequence polymorphism was similarly reflected in RFLP studies (Escayg *et al.* 1993; Fabb *et al.* 1993; Escayg *et al.* 1996). Three allelic fragments of size 6.1, 4.9 and 2.4/2.8 kb with respective frequencies of 0.05, 0.875 and 0.075 were found to be associated with the enzyme BgIII in Merino and Romney sheep.

Ovar-DRB genes: The most polymorphic among the MHC genes is the DRB locus (Andersson and Rask 1988). *Ovar*-DRB genes have been reported to exist in multiple copies, some functional and others non-functional. Early serological and biochemical work on sheep MHC class II molecules detected seven β polypeptides in association with DRA chains that provided evidence for the existence of more than one locus encoding them. Two distinct DRB-like genes were identified using RFLP studies on bacteriophage clones of a sheep genomic library (Scott *et al.* 1987), while a different study provided evidence for the expression of two distinct *Ovar*-DRB genes (Dutia *et al.* 1994). RFLP studies employing probes specific for *Ovar*-DRB exon 2 revealed 10 DRB alleles that required the presence of at least three DRB genes (Grain *et al.* 1993). Further evidence for the presence of two copies of the expressed DRB1 gene was provided in a study on single strand conformational polymorphism (SSCP) and sequence polymorphism of MHC-DRB exon 2 in Latxa and Karrantzar sheep (Jugo and Vicario 2000). Apart from red deer (Swarbrick *et al.* 1995), sheep are the only ruminants in which the existence of two expressed DRB genes has been described, although a second DRB gene (DRB2) in cattle has been found to be expressed at very low levels (Groenen *et al.* 1990).

Four *Ovar*-DRB loci have been described by Scott *et al.* (1991b). The functional DRB1 gene is located at one of them and pseudogenes, DRB2, DRB3 and DRB4, are found at the remaining three loci. The pseudogenes lack defined exons 1 and 2, and also show numerous mutations in their sequences as well as stop codons in exons 3 and 4. There are indications that additional DRB pseudogenes exist (Schwaiger *et al.* 1996).

The whole *Ovar*-DRB region numbers several thousand base pairs and its basic structure is considered similar to other mammalian species (Schwaiger *et al.* 1996). However, almost all the studies on this region have concentrated on the polymorphisms found in exon 2 and adjoining intron 2 of the expressed gene DRB1. This is because DRB1 exon 2 encodes the β1 domain, which constitutes part of the PBR of the DR molecules. The highly variable residues concentrated in this region are in close contact with the peptides presented in the PBR or the TCR (Brown *et al.* 1993), and therefore, they are likely to be related to functionality such as disease resistance / susceptibility.

Another characteristic feature of *Ovar*-DRB1 is that a simple tandem repeat (STR) of the form [(GT)n(GA)m] exists in intron 2, 30 bp downstream from the 3' splice site of exon 2 (Schwaiger and Epplen 1995). This STR with the same basic structure is present at virtually the identical positions in all the expressed DRB alleles of cattle, sheep, goat, red deer and humans, indicating that it remained unchanged at a specific location across various species for nearly 100 million years of mammalian evolution. Two sheep DRB pseudogenes, DRB3 and DRB4, also harbour this STR either in the same or degenerated forms, while another pseudogene (DRB2) lacks it (Schwaiger and Epplen 1995). In DRB3, the STR structure is highly disintegrated, and in DRB4 only three copies of each dinucleotide [(GT)3(GA)3] are detectable.

A different microsatellite of the form (AC)n is present in intron 5, adjacent to the 5'end of exon 6 of *Ovar*-DRB2 (Scott *et al.* 1991b; Blattman and Beh 1992). Typing of this microsatellite together with that found in intron 2 of DRB1 in sheep belonging to the international mapping flock (IMF), AgResearch, New Zealand indicated a distance of 2.6 cM between the two loci (Schwaiger *et al.* 1996). This distance is almost the same as that between *Ovar*-DRB2 and *Ovar-Mhc* I. Similar distances between these loci have also been reported in a subsequent study (Paterson *et al.* 1998).

Typing of *Ovar*-DRB1 genes employing different methods in various sheep breeds has revealed extensive polymorphism at these loci (Table 1). Initial studies employed RFLP techniques utilizing DRB1 exon 2-specific probes (Blattman *et al.* 1993; Grain *et al.* 1993). However, this method has been considered unsuitable to study variation at the DRB1 owing to extensive cross-hybridisation between the DRB1 probe and the DQB locus (Escayg *et al.* 1996). Sequencing of the PCR-amplified DRB1 exon 2, either alone or together with the

adjacent STR in intron 2, has revealed extensive polymorphism within the locus (Schwaiger *et al.* 1993b; Schwaiger *et al.* 1994; Paterson 1998; Konnai *et al.* 2003a; Sayers *et al.* 2005a). SSCP and sequence analysis of DRB1 exon 2 is another method for DRB typing (Kostia *et al.* 1998; Tkacikova *et al.* 2005). However, in one of the studies employing this method (Jugo and Vicario 2000), alleles from more than one DRB locus could be detected.

Another method for typing DRB1 alleles of farm animals, using PCR-RFLP analysis, has been suggested (Amills *et al.* 1996; Rasool *et al.* 2000). Using a pair of bovine specific primers, DRB1 exon 2 was amplified from cattle, buffalo, sheep and guinea pig DNA samples. The amplified fragment was the same size in all the animals from the different species. Polymorphisms in exon 2 were detected by RFLP of the amplified product. Employing this method, DRB1 exon 2 polymorphism was investigated in different sheep breeds (Konnai *et al.* 2003b; Dongxiao and Yuan 2004; Gruszczynska *et al.* 2005), while two other studies looked at polymorphisms in exon 2 of the *Ovar*-DRB3 gene (Dongxiao *et al.* 2003; Yun-Fang *et al.* 2004).

An oligonucleotide method has also been described as a means for typing DRB genes (Schwaiger *et al.* 1993a). PCR fragments including exon 2 plus adjacent intron 2 are first separated in a polyacrylamide gel based on length variations of the microsatellite repeat and then hybridised with probes for both the intron repeat and exonic sequence. This polymorphism-specific oligonucleotide (PSO) typing has been utilised for *Ovar*-DRB1 typing in various studies (Schwaiger *et al.* 1995; Stear *et al.* 1996; Buitkamp and Epplen 1996; McCririe *et al.* 1997).

PCR amplification of exon 2 together with microsatellite in intron 2 and determination of the exact length of the amplified product using an automatic capillary sequencer is another method for typing *Ovar*-DRB1 alleles (Gruszczynska 1999; Gruszczynska *et al.* 2000; Charon *et al.* 2002). Length polymorphism of the microsatellite in intron 2 of the expressed DRB gene in various artiodactyl species has been found to be strongly associated with sequence polymorphisms in exon 2 and thus could be utilised for DRB typing (Ellegren *et al.* 1993). This method was employed in several studies to detect *Ovar*-DRB1 alleles (Outteridge *et al.* 1996; Paterson 1998; Paterson *et al.* 1998; Saberivand *et al.* 1998; Griesinger *et al.* 1999).

Table 1: Polymorphism of the expressed *Ovar*-DRB1 gene in various sheep breeds

Typing method	Breed(s) analysed		Sheep screened	No. of alleles	Reference(s)
A	Soay		15	5	Paterson (1998)
A	Perendale, Coopworth, Texel, Landrace, Merino, Romney		34	34	Schwaiger *et al.* (1994)
A	-		-	13	Schwaiger *et al.* (1993b)
A	Suffolk		71	28	Konnai *et al.* (2003a)
	Cheviot		20	14	
	Corriedale		6	9	
A	Texel		155	8	Sayers *et al.* (2005a)
	Suffolk		179	7	
B	Polish Heath		675	20	Charon *et al.* (2002)
B	German Merino	*Parents*	43	36	Gruszczynska (1999)
		Progeny	37	28	
B	Polish Heath	*Parents*	52	36	Gruszczynska *et al.* (2000)
		Progeny	100	30	
C	Merinoland		105	18	Griesinger *et al.* (1999)
	Changthangi		28	16	
	Red Maasai		35	15	
C	Soay		1209	8	Paterson (1998); Paterson *et al.* (1998)
C	-		363	12	Saberivand *et al.* (1998)
C	Merino		130	8	Outteridge *et al.* (1996)
C	Merino		234	16	Bot *et al.* (2004)
D	Scottish Blackface		21	8	McCririe *et al.* (1997)
D	Scottish Blackface		299	17	Buitkamp and Epplen (1996)
D	Scottish Blackface		200	19	Stear *et al.* (1996); Schwaiger *et al.* (1995)
D	-		-	16	Schwaiger *et al.* (1993a)
E	Merino		189	29 bands	Blattman *et al.* (1993)
E	Prealpe		89	10*	Grain *et al.* (1993)
F	Latxa, Karrantzar		83, 17	12*	Jugo and Vicario (2000)
F	Finsheep, Russian Ramanov		31	19	Kostia *et al.* (1998)
G	Suffolk		52	13 haplotypes	Konnai *et al.* (2003b)
G	Mongolian, Kazakh		53, 62	7	Dongxiao and Yuan (2004)
G	Polish Heath		101	65 haplotypes	Gruszczynska *et al.* (2005)
	Polish Lowland		99	68 haplotypes	

A - PCR amplification and sequencing of exon 2 either alone or together with a part of adjacent intron 2

B - Length polymorphism of microsatellite in intron 2 together with exon 2

C - Length polymorphism of microsatellite in intron 2

D - Length polymorphism of STRs in intron 2 plus hybridization of oligonucleotides within exon 2

E - RFLP with exon 2 specific probe

F - SSCP and sequence analysis of exon 2

G – PCR-RFLP of exon 2

* - Existence of more than one loci has been indicated

Length polymorphisms of the microsatellite in intron 5 of the pseudogene *Ovar*-DRB2 has also been studied in different breeds of sheep (Table 2). High heterozygosity (>78%) at this locus, reported in these studies, suggests the potential application of this locus as a genetic marker, especially for disease resistance. It has been shown in cattle that the resolution of microsatellite- based DRB3 typing was much better when the length polymorphism of another microsatellite located in DRB1 pseudogene was included (van Haeringen *et al.* 1999). However, no such typing studies in sheep involving the microsatellites located at DRB1 and DRB2 have been reported.

Table 2: Polymorphism of pseudogenes *Ovar*-DRB2 and *Ovar*-DRB3 in different breeds

Typing method	Breed(s) analysed	Sheep screened	No. of alleles	Reference(s)
Pseudogene Ovar-DRB2				
A	German Rhonschaf	468	8	JanBen *et al.* (2002)
A	Heatherhead	190	11	Gruszczynska *et al.* (2002b)
	Polish Lowland	200	8	
A	Soay	887	6	Paterson (1998); Paterson *et al.* (1998)
A	Merino, Corriedale, Polworth, Southdown, Suffolk, Border Leicester, Romney, Dorset	58	13	Blattman and Beh (1992)
Pseudogene Ovar-DRB3				
B	Mongolian, Kazakh	-	7	Dong-Xiao *et al.* (2003)
B	Dolang	-	24 haplotypes	Yun-Fang *et al.* (2004)

A - Length polymorphism of microsatellite in intron 5

B - PCR-RFLP of exon 2

2.2.3.2 *Ovar*-DQ genes

The existence of DQ genes in sheep was first demonstrated by genomic Southern blot analysis employing probes homologous to the HLA DQ region (Chardon *et al.* 1985; Scott *et al.* 1987). In the latter study, the presence of three DQA-like and four DQB-like genes was indicated. RFLP and sequence data derived from genomic clones (Scott *et al.* 1991a) and cDNA clones (Fabb *et al.* 1993) indicated the existence of two DQA genes per haplotype in sheep. This is consistent with a detailed genomic map of the ovine DQ sub-region (Wright and Ballingall 1994), which revealed two DQ loci each containing one DQA and one DQB gene arranged in tail to tail orientation (Figure 3). The two loci are 22 kb apart and are linked on a linear tract of 130 kb of DNA. The *Ovar*-DQ sub-region is more compact than the HLA-DQ sub-region, since a distance of 70 kb separates the two HLA-DQ loci (Campbell and

Trowsdale 1993). The *Ovar*-DQA1 and DQB1 genes at the first locus are separated by 11 kb, while the DQA2 and DQB2 genes at the second locus are 25 kb apart. The HLA-DQ1 genes and *Bota* (*Bos Taurus*)-DQ1 genes are also separated by a similar distance, while the HLA-DQ2 genes lie much closer together than the *Ovar*-DQ2 genes. The equivalents of *Bota*-DQA3 (Andersson, 1988) and HLA-DQB3 pseudogene (Ando *et al.* 1989) could not be detected in sheep.

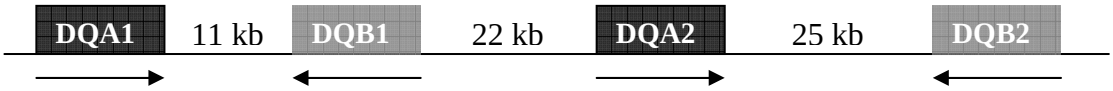

Figure 3: Schematic presentation of the structure of the *Ovar*-DQ subregion
Notes: 1. Arrows indicate the direction of transcription of the genes
2. Distances as per Wright and Ballingall (1994)

In a study on the linkage analysis between the *Ovar*-DQA1, DQA2, DQB1, DQB2 and DRA loci, no recombinants were observed between DQA1 and DQA2 loci or between DQA and DQB genes (Escayg *et al.* 1996). Also, there was no evidence of recombination between the DRA locus and any of the DQ loci. This finding, despite the lack of any available information on the distance between the DQ and DR subregion, would suggest that these loci are physically close.

Ample evidence exists for both in vitro (Wright and Ballingall 1994) and in vivo (Scott *et al.* 1991a; Fabb *et al.* 1993; Wright and Ballingall 1994) transcription of *Ovar*-DQA genes. However, cell surface expression of DQ products has been detected only for the DQ1 locus (Wright and Ballingall 1994). It is probable that despite expression of genes at the DQ2 locus, the lack of suitable monoclonal antibody (Wright and Ballingall 1994) or the possibility of the DQ2 α- and β-chains mis-pairing (Snibson *et al.* 1998) may be the reason(s) for failure in detecting their products. This view is further supported by the fact that about 10 to 18% of sheep from different breeds (Scott *et al.* 1991a; Fabb *et al.* 1993; Escayg *et al.* 1996) lack the DQA1 gene in their haplotypes, indicating that any functional DQ molecule in these sheep would be the product of expressed genes at DQ2 locus (Snibson *et al.* 1998).

Ovar-DQA genes: The nucleotide sequence of all exons and introns, excluding exon 1 of *Ovar*-DQA1 and DQA2 genes have been determined and were found to be similar to respective analogues in humans (Scott *et al.* 1991a). The second exons in these two genes

were less similar in terms of nucleotide (78%) and coding amino acid (71%) identities between them. Subsequently, full length cDNA clones coding for these two *Ovar*-DQA genes, together with that for the *Ovar*-DRA gene, have been isolated and sequenced (Fabb *et al.* 1993). All of these encode polypeptides of 255 amino acids, with 23 of them accounting for signal peptide and the other 232 encoding the mature polypeptide. DQA1 and DQA2 could be discriminated mainly based on the nucleotide sequence of exon 2. The exon 2 nucleotide dissimilarity between DQA1 and DQA2 genes (19.5%) is far more than that between the alleles within either DQA1 (8.0%) or DQA2 (10.0%). Nucleotide variation was found to be minimal in exon 4 of both genes. Similar sequence polymorphisms in exon 2 were also observed in a different study (Snibson *et al.* 1998).

Several alleles of *Ovar*-DQA1 and DQA2 have been identified based on sequence variation of the PCR amplified exon 2. Twenty-three different DQA2 sequence alleles (1-Scott *et al.* 1991a; 1-Fabb *et al.* 1993; 1-Wright and Ballingall 1994; 7-Snibson *et al.* 1998; 13-Hickford *et al.* 2004) and sixteen DQA1 sequence alleles (1-Scott *et al.* 1991a; 1-Fabb *et al.* 1993; 3-Wright and Ballingall 1994; 2-Snibson *et al.* 1998; 3-Zhou and Hickford 2001; 6-Zhou and Hickford 2003) have been identified. PCR-SSCP is an ideal method for typing DQA sequence alleles (Snibson *et al.* 1998). A single set of PCR primers could amplify all known DQA2 alleles, while a separate set of primers amplified only the DQA1 gene. Two new DQA1 and nine DQA2 alleles were identified in the study using this method.

Employing PCR-SSCP, an extensive investigation on the DQA2 gene was carried out in 2000 sheep belonging to Merino, Corriedale, Borderdale, Romney, Awassi and Finnish Landrace breeds (Hickford *et al.* 2004). As many as 23 exon 2 sequences could be identified, of which 5 were found to be more similar to bovine DQA3 or DQA4 sequences than to other sheep DQA2 and were designated as DQA2-like sequences. However, there was no evidence for the presence of the bovine DQA5-like sequences in sheep. Three or four unique DQA2 sequences could be recovered from individual sheep, suggesting the presence of two DQA2 loci.

A different study, but employing the same technique, on DQA1 in 300 sheep belonging to Merino, Corriedale, Borderdale, Romney, Awassi and Finnish Landrace breeds revealed extensive polymorphism in the exon 2 sequence, with as many as 14 alleles (Zhou and Hickford 2003). Comparison of the sheep DQA1 exon 2 sequences with those available from

cattle revealed several clusters of ovine DQA1 sequences, with some of the sheep alleles being more similar to cattle alleles than to the other sheep alleles. It was suggested that this trans-species polymorphism might be the result of balancing selection at the DQA1 locus.

Polymorphisms of DQA genes have also been reflected in RFLP studies employing exon 2 specific probes (Scott *et al.* 1991a; Fabb *et al.* 19993; Escayg *et al.* 1996; Hickford *et al.* 2000). DQA2 was found to be more polymorphic than DQA1. Up to 8 and 16 alleles have been reported for DQA1 and DQA2, respectively. Another interesting feature of these studies is that in 11 to 36 percent of the sheep screened, no DQA1 allele could be detected and the allele in such animals was considered as null. Thus, sheep do have a variable number of DQA genes in their haplotypes. In some of the sheep that possessed null DQA1 allele, two DQA2-like sequences could be detected (Hickford *et al.* 2000), retaining the pattern of two DQA loci per haplotype. Duplication of DQA2 gene was suggested in these animals. The authors' discussion with regard to the ancestry of DQA1/DQA2 and DQA1null/DQA2 (duplicated) haplotypes is interesting. Similarity between the DQA2-like sequences indicated that the two DQA2-like loci might have arisen from a recent gene duplication event. However, considering the facts that the majority (82-89%) of the studied sheep possessed the haplotype DQA1/DQA2 and that the DQA1/DQA2 haplotype was more diverse than the DQA1-null/DQA2, the authors suggested that it seemed likely that the former haplotype preceded the later.

The presence of two DQA2-like sequences in animals with DQA1 null alleles has also been reported in cattle (Ballingall *et al.* 1997). However, the two DQA2 sequences were diverse and had been categorised as DQA2 and DQA3. In sheep, it was shown that some ovine DQA2 sequences exhibited much closer similarity to the cattle DQA3 gene than to other DQA2 sequences (Snibson *et al.* 1998). This suggests that the duplicated ovine DQA2 gene in animals with DQA1 null allele may be analogous to the cattle DQA3 gene (Hickford *et al.* 2000). However, there is no evidence with regard to the expression of this gene. The presence of two additional DQA loci in cattle, *Bota*-DQA4 (Ballingall *et al.* 1997) and DQA5 (Gelhaus *et al.* 1999) has been reported, but their homologues in sheep are yet to be identified.

Ovar-DQB genes: The nucleotide sequence of *Ovar*-DQB gene, excluding exon 1 and parts of the introns, has been reported (Scott *et al.* 1991b). Comparison with human sequences

revealed similarity with both HLA-DQB1 and DQB2, suggesting the presence of a common ancestor. Subsequently, exon 2 nucleotide sequences of two separate *Ovar*-DQB genes (DQB1 and DQB2), derived from cosmid clones, have been determined (Wright and Ballingall, 1994). The two genes could not be assigned to separate loci based on the nucleotide sequences, owing to >90% similarity. However, their proximity to an *Ovar*-DQA1 or DQA2 gene could be used to discriminate between these genes.

Several new DQB sequences have been determined in subsequent studies. Difficulty still exists in assigning these sequences to separate loci because of the high similarity between the two DQB genes. Ten distinct sequences were identified from an SSCP sequence analysis of PCR-amplified DQB exon 2 in 13 Merino sheep, demonstrating considerable variation in the ovine DQB region (van Oorschot *et al.* 1994). Twenty-nine percent of the total 267 nucleotide sites in exon 2 of these alleles, translating to 46% of amino acid sites, are polymorphic. The presence of at least two separate *Ovar*-DQB genes was demonstrated in that study. Phylogenetic analyses of the exon 2 nucleotide and amino acid sequences from sheep, cattle and humans showed that the ovine and bovine sequences are more closely related to each other than either are to the human sequences. The SSCP technique was shown to be capable of discriminating between all the *Ovar*-DQB sequences identified in the study.

Sixteen distinct PCR-amplified *Ovar*-DQB exon 2 sequences have been characterised from only 18 sheep in another study (Schwaiger *et al.* 1996). While three of these sequences could be assigned to DQB1 and two to DQB2, the rest could not be assigned to either locus. Reference-strand-mediated conformation analysis (RSCA) or double-strand conformational analysis, employing two reference alleles, has been shown to be a new method for high resolution typing of the *Ovar*-DQB genes (Feichtlbauer-Huber *et al.* 2000). The use of two different reference alleles would enable high resolution of many and probably all alleles and reduce the probability of missing new alleles. Using this method, 16 new sequences (from that of van Oorschot *et al.* 1994) were obtained from 10 unrelated Scottish black-faced sheep, increasing the number of known alleles to 28. However, the alleles could not be assigned to separate loci.

2.2.3.3 *Ovar*-DNA and DOB genes

The presence of the DNA (formerly DZA) gene in sheep had been inferred from Southern analysis of genomic DNA (Scott *et al.* 1987). Cosmid clones from the sheep MHC class II

region were found to contain this gene (Deverson *et al.* 1991). Subsequently, the nucleotide sequence of the DNA gene, together with its predicted amino acid translations was reported (Wright *et al.* 1995a). It had all the salient features of a class IIA gene, including two exons coding for the two extracellular domains, and one coding for a proline rich connecting peptide, a hydrophobic transmembrane region and a cytoplasmic tail. Also, it has two conserved N-linked glycosylation sites NGT and NAT, and two conserved cysteine residues, forming a disulphide bond in the α2 domain. The ovine and human genes share 83% nucleotide identity (translating to 78% amino acid identity) at exons 2 and 3. Though transcription of the *Ovar*-DNA gene was detected by Northern hybridisation with an *Ovar*-DNA probe, there was no evidence of expression of the gene. Like that of the *Ovar*-DRA, the *Ovar*-DNA gene appears to be monomorphic (Schwaiger *et al.* 1996).

The B gene partner for HLA-DNA gene is the non-polymorphic HLA-DOB gene (Tonnelle *et al.* 1985), while the murine homologue of the *Ovar*-DNA gene expresses in combination with the H2-OB gene (Karlsson *et al.* 1991). There was an early indication in sheep for the existence of *Ovar*-DOB gene (Scott *et al.* 1987). The gene has been cloned and subsequently sequenced (Wright *et al.* 1995b). Exons 1 and 2 have been found to exhibit amino acid identities of 62% and 80% respectively, in comparison with the HLA-DOB gene. Neither transcription of the gene nor its expression in combination with *Ovar*-DNA gene could be detected in the study.

2.2.3.4 *Ovar*-DY genes

DYA and DYB (DIB) genes which are absent in HLA have been detected in cattle (Andersson *et al.* 1988). These were shown to segregate with the DOB gene in one region separated by a recombination distance of 17cM from the region that contains DQ, DR and C4 loci. The *Bota*-DYA gene has been cloned and sequenced (van der Poel *et al.* 1990), while there has been no report of cloning of its B gene partner. A unique single copy class IIB gene, *Bota*-DIB has been cloned and sequenced from a phage library (Stone and Muggli-Cockett, 1990). The homologues of *Bota*-DYA and DIB genes in sheep, designated as *Ovar*-DYA and DYB, have been identified in sheep by screening a cosmid library with *Ovar*- and HLA-DQ probes at low stringency (Wright *et al.* 1994). The presence of DY genes, together with the absence of DP genes and variability in the number of DQ genes between haplotypes, has been considered as a distinguishing feature of the ruminant class II region.

The *Ovar*-DYA gene have shown high sequence similarity to the bovine and caprine DYA genes and much less so to the *Ovar*-DRA, DNA and DQA genes (Wright *et al.* 1994). Similarly, the *Ovar*-DYB gene exhibited a higher degree of sequence similarity to the *Bota*-DIB and was different from the *Ovar*-DQB and DRB genes. It was named DYB rather than DIB because of its close proximity to DYA gene. The DYA and DYB genes lie tail to tail with a distance of 11 kb between them. While transcription of the gene could be detected, there was no evidence for its expression. The authors suggested that evolution of the DY locus may be the result of duplication of a pair of DQ genes, with subsequent rapid divergence.

A polymorphic microsatellite (DYMS1) of the form (CA)n was found to be located in the region 5' of the DYA gene, 19 cM from the DRB1 locus (Buitkamp *et al.* 1996). Nineteen alleles were identified at the locus in this study. The polymorphism at this microsatellite locus was later confirmed in a different study in German Rhonschaf sheep that revealed 6 alleles (JanBen *et al.* 2002).

Studies on the second exon of DY genes employing SSCP have revealed 3 alleles for *Ovar*-DYA and 4 in DYB, with respective heterozygosities of 0.67 and 0.61 (Maddox 1999). A recent study assessed the degree of conservation between ovine and bovine DYA gene sequences (Ballingall and Mckeever 2005). Nucleotide similarities of 97% in the immediate promoter, 94% in the coding and 91% in the intronic regions were observed between the species. The *Ovar*-DYA full length transcript revealed an open frame encoding a 288 amino acid protein compared with a 253 amino acid protein associated with the bovine DYA transcript.

2.2.3.5 *Ovar*-DM genes

The existence of DMA and DMB genes in sheep has been indicated based on PCR amplification of fragments from exons 2 and 3 of the *Ovar*-DMB gene and exon2 of the *Ovar*-DMA gene, employing primers derived from murine and human gene sequences (Schwaiger *et al.* 1996). Only two exon 2 alleles could be detected in the case of the DMB gene by SSCP (Maddox 1999).

2.2.4 Class II molecules

Class II molecules have a much more restricted expression pattern than do class I molecules, in that they are expressed primarily on cells deemed to have antigen uptake, processing and presentation functions (macrophages, dendritic cells and B cells). Their expression varies among species and is enhanced in rapidly dividing cells and in cells treated with interferon (Tizard 2004). Class II molecules are also heterodimers (Figure 2), but in contrast to class I molecules, are composed of an α- and a β-peptide chain. Each chain has two extracellular domains, a connecting peptide, a transmembrane domain and a cytoplasmic domain. A third protein chain, called γ or invariant chain (Li or CD74), is associated with intracellular class II molecules (Tizard 2004).

The class II PBR consists of two α helices bordering a β-pleated sheet (Hughes and Yeager 1998), as with the class I molecule. The difference is that in class II, one of the α helices and about half of the β-pleated sheet are contributed by the α chain, whereas the other α helix and other half of the β-pleated sheet come from the β chain. Polymorphisms in the class II molecules result from variation in the amino acid sequences of the α helices at the sides of the groove. The α- and β-chains are encoded by genes in the class II region. In mammals, the class II subregions (designated as DR, DP and DQ in humans), each contain a functional α-chain gene and one or more functional β-chain genes.

The class II molecules present peptides derived from exogenous proteins to the TCR of CD4+ helper T cells (Germain and Margulies 1993). In response to a foreign peptide, the helper T cells release cytokines that trigger the production of antibodies and cell-mediated immune responses. The class II molecules also possess the property of MHC restriction, in which the antigens bound to MHC molecules also need to be recognised by a TCR on a helper cell, in order to trigger an immune response. The peptides presented by class II molecules can vary substantially in length, between 11 and 17 residues (Rammensee *et al.* 1995).

The complex between the class II molecule and its peptide ligand is created by a mechanism quite different from that of class I. Before transport to the cell surface, the class II dimer forms a complex with the invariant chain (Li) in the ER, which acts as a chaperone to stabilise the heterodimer and prevents premature peptide loading. This complex then travels to an acidic endosome-like compartment (Peters *et al.* 1991), where the Li is degraded by a

series of proteolytic cleavage events, leaving a residual peptide (class II associated invariant chain peptide; CLIP) occupying the PBR of the MHC molecule. The release of CLIP and its replacement with antigenic peptides is catalysed by HLA-DM, which is independently targeted to endosomal compartments. The resultant MHC class II-peptide complex is then transported to the cell surface, where it awaits interaction with antigen-specific T cells. The expression of MHC class II, Li and HLA-DM genes is co-ordinately regulated at the level of transcription by a conserved set of factors and defined cis-acting elements (Boss and Jensen 2003).

Immunoprecipitation and sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) analysis of ovine class II molecules have revealed a non-covalently associated glycoprotein complex with a 30-32 kDa alpha chain and a 24-26 kDa beta chain (Puri *et al.* 1985). A similar finding on the structure of class II molecules was reported by Hopkins *et al.* (1986). Studies to categorise the sheep MHC class II molecules revealed four structurally and serologically distinct subsets of class II molecules, similar to those found in humans (Puri *et al.* 1987a,b,c; Puri and Brandon 1987). Also, these molecules exhibited structurally detectable allelic polymorphism. Three of the subsets displayed allelic polymorphism in β-polypeptides, while the fourth set showed allelic variation in both of their α- and β-polypeptides (Puri *et al.* 1987a). Approximately 10-12 different class II molecules were found to be expressed by a single sheep (Puri and Brandon 1987). Subgroup-specific monoclonal antibodies against sheep MHC class II molecules, nine specific for the β-chain and four for the α-chain, have been developed (Dutia *et al.* 1990).

2.2.5 Class III genes

Relative to other parts of the MHC, this region has the highest gene density, with the least number of pseudogenes (Kulski *et al.* 2002). However, some of the genes located in this region are not involved with the immune system. Class III genes with an obvious role in immunobiology include members of the complement cascade (C4A, C4B, C2 and Bf) and genes such as TNFα, LTA and LTB. C4, C2 and Bf are genes for complement proteins (Campbell *et al.* 1986). TNFα, LTA and LTB encode cachectin, lymphotoxin A and B molecules, respectively (Webb and Chaplin 1990). Other genes of interest located in the region include HSP70, CYP21, G15, cytochrome p450, LST1 and 1C7. Of these, HSP70 is important as it encodes heat shock protein 70, which presents intracellular contents of cancer cells to the immune system and thus has a role in tumor rejection (Srivastava *et al.* 1998).

The gene coding for HSP70 is duplicated and it has been shown recently that the loss of one of the duplicated genes in Holstein cattle is responsible for hereditary myopathy of diaphragmatic muscles (Sugimoto *et al.* 2003).

The class III region is poorly characterised in sheep. The existence of this region is based on circumstantial evidence derived from comparisons with related species, namely goats and cattle, and synteny between several loci (Schwaiger *et al.* 1996). The authors described a preliminary map of the *Ovar*-class III region. Cosmid clones containing C4 genes were isolated from a sheep genomic library by hybridisation with a bovine C4 cDNA probe. Additional cosmid clones containing the genes for 21-hydroxylase (CYP21), complement factor 2 (C2) and factor B (Bf) could also be obtained by a cosmid walking procedure employing respective human DNA probes. Relative positions of these loci were mapped within an approximate 150 kb DNA segment. Evidence could be obtained for duplication of C4 and CYP21 loci. Also, the order of CYP and C4 loci in sheep (CYP21B…C4…C4…CYP21) is quite different from that in humans, mouse and cattle (CYP21B…C4B…CYP21A…C4A). Furthermore, the two *Ovar*-C4 loci lie in tail-to-tail orientation. This evidence suggests the occurrence of a chromosomal inversion in this region of the sheep chromosome.

2.2.5.1 Complement cascade genes

The presence of the C4 gene in sheep was first indicated in RFLP studies employing human C4 cDNA probe (Chardon *et al.* 1985). Neither any polymorphism nor linkage to MHC could be demonstrated. Subsequently, linkage between the C4 gene and OLA-SY1b antigen was established (Groth *et al.* 1987a). The presence of two polymorphic C4 loci has been indicated in a study on C3 and C4 concentrations in Merino and Suffolk sheep (Groth *et al.* 1987b). A rapid procedure for the isolation of complement factor, C4, from ovine plasma has been described, and two isotypes of C4 molecules, C4A and C4B, have been detected (Groth *et al.* 1988). The isotypes differed in the molecular weight of the alpha chain (108 and 95 kDal. respectively). An RFLP of the C4 gene, employing Taq1 enzyme and the HLA-C4 probe, revealed linkage disequilibrium between C4 and DQB genes in unrelated sheep. Similar linkage of the C4 and DRB genes has also been reported (Wetherall *et al.* 1991). A C4*A2 phenotypic allele was found to be associated with a 19-kb DRB RFLP fragment in 18 of the 27 sheep studied.

In another study based on cloning and sequencing of DNA fragments obtained by PCR amplification of thioester and isotype determining sites of the sheep C4 genes, up to five distinct C4 loci were detected (Ren *et al.* 1993). The number of C4 genes per haplotype is thus similar to that in both humans and mice (Schwaiger *et al.* 1996). However, the sheep and cattle genes are believed to have evolved independently of those in primates and mice (Ren *et al.* 1993). Close to another complement factor gene, Bf, a polymorphic microsatellite locus, BfMS, has been detected (Groth and Wetherall 1995). Eight alleles, differing in base-pair length, were detected at the locus in an Australian fine-wool Merino flock (Bot *et al.* 2004).

2.2.5.2 TNFα gene

Tumour necrosis factor alpha (TNFα) is a cytokine with a wide range of effects on both lymphoid and non-lymphoid cell types. The existence of a single copy of the TNFα gene in sheep has been demonstrated (Nash *et al.* 1991). Ovine TNFα cDNAs were cloned and sequenced by three independent groups (Young *et al.* 1990; Green and Sargan 1991; Nash *et al.* 1991). The sequences obtained in the first two studies were exactly the same, encoding for a 76-amino acid leader sequence and a 157-amino acid mature protein. The amino acid sequence was up to 88% homologous to the human TNFα protein. The cDNA sequence obtained in the third study was similar to that obtained in the first two studies, except that it lacked one amino acid in the leader sequence.

A recent study investigated allelic variation at the *Ovar*-TNFα locus (Alvaez-Busto *et al.* 2004). SSCP and sequence analysis of a 273-base-pair fragment, comprising part of the fourth exon and the 3’ un-translated region of the gene, revealed three different alleles. These alleles differed in one deletion and one single nucleotide polymorphism. However, no difference was found in their frequencies in Latxa and Rasa breeds. An earlier attempt to detect polymorphism at this locus, employing RFLP with the use of human cDNA probes, was unsuccessful (Engwerda *et al.* 1996).

2.2.5.3 Other class III genes

There has been little research in the characterisation of genes other than the complement cascade and TNF genes of the *Ovar*-class III region. A dinucleotide microsatellite of the form (CA)n has been found to occur in at least one of the two cattle CYP21 genes (Moore *et al.* 1991). However, no such microsatellite could be detected in any of the *Ovar*-CYP21 genes

either by PCR using an oligonucleotide primer (Moore *et al.* 1991) or by Southern hybridisation (Schwaiger *et al.* 1996).

2.2.6 Inheritance and polymorphism of MHC genes

A characteristic feature of the MHC antigens is their co-dominant expression, i.e., both the alleles at a given locus are expressed in a heterozygote individual. Also, the MHC is inherited en bloc as a haplotype with the exception of rare recombination (1-3 % frequency). Hence, in the case of MHC genes, an association based on haplotypes is usually stronger and more meaningful than an allelic association (Dorak 2005). Despite the enormous number of alleles at each expressed locus, the number of haplotypes observed in a population is much smaller than the theoretical expectations. This is because of certain alleles tending to occur together on the same haplotype rather than randomly segregating, a phenomenon referred to as linkage disequilibrium (Begovich *et al.* 1992).

Among the expressed loci in the human genome, the MHC shows the greatest degree of polymorphism (Dorak 2005). The level of polymorphism is at such a degree that it is theoretically possible for each human to possess a different set of MHC alleles. Certain of the class I and class II loci that are involved in antigen presentation show extraordinarily high levels of polymorphism with several hundreds of allelic variants of the genes within the population (Klein 1986). Polymorphism is more predominant in the protein domains constituting the PBR of the MHC molecules, enabling the molecules to bind a diverse array of peptides. The allelic diversity in the PBR is considered to have formed primarily by accumulation of point mutations over many generations. The alleles have accumulated and form lineages or families with similar sequences (Gyllensten *et al.* 1990). Some of the presumably ancient allelic lineages are conserved across related species, a phenomenon referred to as trans-species polymorphism (Klein *et al.* 1993).

Genes at these polymorphic loci are usually present as multiple copies, some of them being pseudogenes. The pseudogenes lack either one or more exons in them and even in the exons that exist, numerous mutations occur, rendering them non-functional. The presence of multiple copies is of evolutionary significance. Since it involves a birth and death process, new genes are created and some of them are maintained in the genome for a long time, while others are deleted or become non-functional through deleterious mutations (Klein *et al.* 1998). Class I loci undergo a faster rate of birth and death evolution than class II loci, and

hence, it is difficult to establish the orthologous relationships of different class I genes among different orders of mammals (Hughes and Nei 1989). On the other hand, the high longevity of class II genes enables such orthologous class II loci to be shared by different orders of mammals (Takahashi *et al.* 2000).

The mechanisms responsible for polymorphism in the MHC genes have been intensely debated and reviewed (Hughes and Yeager 1998; Meyer and Thompson 2001; Bernatchez and Landry 2003). Parasite-mediated balancing selection and reproductive mechanisms constitute the two main types of mechanisms that operate to maintain the unusually high level of MHC polymorphism. Three different non-exclusive forms of balancing selection, symmetrical overdominance, negative frequency dependent selection and fluctuation in selection pressure, are known to exist (Charbonnel and Pemberton 2005).

According to the hypothesis of heterozygote advantage or symmetrical overdominance (Doherty and Zinkernagel 1975), an individual that is heterozygous, rather than homozygous, at the MHC loci has better immune surveillance against infectious organisms. The domains α1/α2 and α1/β1 of class I and class II molecules, respectively, that form the peptide-binding groove in each case, constitute the driving force for heterozygote selection, in the presence of challenge from infectious agents. Several studies (Thursz *et al.* 1997; Carrington *et al.* 1999; Penn *et al.* 2002; Stear *et al.* 2005) have confirmed this selective advantage of MHC heterozygosity against infectious agents.

Under negative frequency-dependent selection or rare allele advantage (Clarke and Kirby 1996), MHC genotypes with a rare allele are supposed to have a strong selective advantage as few pathogens have been exposed and adapted to it. Conversely, the relative fitness of the common genotypes would be decreased. A study on the association between class II DRB alleles and resistance to gastro-intestinal parasitism in Soay sheep (Paterson *et al.* 1998) has provided evidence for rare allele advantage.

The third form of balancing selection results from fluctuation in the selection pressure. Spatial and (or) temporal variation in the presence or density of pathogens could result in constant changes in the intensity of pathogen-mediated selection, thus maintaining polymorphism at the level of metapopulation (Hedrick 2002). A recent study pertaining to a

long-term genetic survey of Soay sheep supported this hypothesis (Charbonnel and Pemberton 2005).

One early hypothesis explaining the high level of polymorphism within the MHC was the neutral theory of molecular evolution (Kimura 1968). This theory suggested that the molecular mechanisms that result in polymorphism include point mutations, reciprocal recombination and gene conversion. Though point mutation rate in MHC is by no means higher than elsewhere in the genome (Parham *et al.* 1995), accumulation of point mutations over millions of years as a result of the sharing of allelic lineages by related species might result in extensive allelic polymorphisms (Klein *et al.* 1993). Other mechanisms that might bring about MHC gene diversity include MHC-based non-assortative mating preferences (Penn and Potts 1999) and maternal-foetal incompatibility (Ober *et al.* 1998). However, these mechanisms together with the neutral theory have been discarded as a main cause of MHC polymorphism, as these should affect gene regions at random, rather than being concentrated in the PBRs (Jeffery and Bangham 2000).

2.2.7 Conclusion

Several studies have been undertaken to characterize *Ovar-Mhc* genes. However, majority of those were focused on the class II region in general and on DR and DQ genes, in particular. The length polymorphisms of microsatellites within the DRB1 and DRB2 genes, and the exon 2 sequence variations at DRB1, DQA and DQB genes have been extensively studied in different breeds of sheep. In contrast, the class I region is poorly characterised. Controversy still exists with regard to the number of classical class I loci and there is no information on the non-classical class I genes. Studies pertaining to this region have focused mainly on the length polymorphism of a microsatellite located at one of the loci. Several genes, across the three regions of the *Ovar-Mhc*, are yet to be characterised.

Recent advances in large-scale cloning and sequencing have helped generate long genomic sequences, even complete MHC sequences, in several species (Kumanovics *et al.* 2003). The genomic sequences, in contrast to the cDNA sequences, provide the complete and ordered set of the MHC genes, including pseudogenes. The complete sequence of the HLA complex was available in late 1990s (MHC sequencing consortium 1999) and it was evident that the class I and II regions extend well beyond the original boundaries (Stephens *et al.* 1999). Among the domesticated species, such large MHC genomic sequences have been reported for the B locus

of the chicken (Kaufman *et al.* 1999), the class I region of the quail (Shiina *et al.* 1999), the class II region of the cat (Beck *et al.* 2001) and the class I region of the pig (Renard *et al.* 2001; Chardon *et al.* 2001). However, there are no reports of such sequences with regard to the *Ovar-Mhc*. Availability of the complete sequence of the *Ovar-Mhc* will enable the design of multiple markers that are more dense, equidistant and expansive throughout the region. This will facilitate the characterisation of individual animals in terms of haplotypes rather than individual genes. MHC haplotypes are more meaningful, considering the existence of linkage disequilibrium among the MHC genes.

2.3 OVINE MHC: ROLE IN DISEASE RESISTANCE

Owing to the immunological importance of MHC genes, several studies have investigated the relationship between polymorphisms of *Ovar-Mhc* genes and resistance and/or susceptibility to various diseases in sheep, which are summarised in Table 3. The association of MHC genes with other traits such as bodyweight, growth and fertility has also been documented.

2.3.1 Association with gastrointestinal nematodiasis

Gastrointestinal nematodes are perhaps the most important parasites of domestic sheep world-wide. These include strongyles, nematodes of the order Strongylida (*Oesophagostomum, Chabertia, Bunostomum, Trichostrongylus, Cooperia, Ostertagia, Teladorsagia* and *Haemonchus* spp.*)* and nematodes of the *Nematodirus* spp. In temperate areas, *Teladorsagia circumcinta* (formerly *Ostertagia circumcinta*) is predominant, causing significant morbidity and loss of production (Coltman *et al.* 2001). Anthelmintic chemotherapy aimed at curtailing nematode infections has become increasingly expensive and has led to drug resistance (Roos 1997). Increasing concerns regarding drug residues in food have resulted in a growing number of producers adopting husbandry programmes that are less dependent on drugs. For these reasons, selection for parasite resistance in domestic sheep is being promoted in many countries.

Although resistance/susceptibility to nematode infection, as measured by faecal egg counts (FEC), is a moderately heritable trait, traditional selection based on FEC values can be problematic due to the cost and difficulty of measuring phenotypes in a commercial production setting. An alternative approach involves selection of animals using DNA marker haplotypes corresponding to genes associated with reduced FEC (Sonstegard and Gasbarre 2001). It is with this background that MHC loci have become attractive candidates for several

studies aimed at a search for DNA markers for gastrointestinal nematodes. Different genes from classes I to III of *Ovar-Mhc* have been implicated in resistance/susceptibility to various sheep nematodes.

Table 3: Association of *Ovar*-Mhc genes with disease resistance

Class	Gene/marker	Association	Reference
Gastrointestinal nematodes			
I	Class I antigens	Yes	Windon and Dineen (1984); Luffau *et al* (1990); Stear *et al* (1996)
I	Class I antigens	No	Riffkin and Yong (1984); Cooper *et al* (1989)
I	OMHC I	Yes	Charon *et al* (2001)
II	DRB1	No	Hulme *et al* (1991); Blattman *et al* (1993); McCririe *et al* (1997)
II	DRB1	Yes	Buitkamp *et al* (1994); Schwaiger *et al* (1995); Stear *et al* (1996, 2005); Charon *et al* (2002); Sayers *et al* (2005a)
II	DYA	Yes	Buitkamp *et al* (1996)
III	C4	Yes	Whetherall *et al* (1991)
Bacterial Footrot			
I	Class I antigens	Yes	Outteridge *et al* (1989)
II	DQA, DRB	Yes	Litchfield *et al* (1993)
II	DQA1, DQA2, DQB, DRA	Yes	Escayg *et al* (1997)
Johne's disease			
I	CSRD226	Yes	Reddacliff *et al* (2005)
BLV induced ovine lymphoma			
II	DRB1	Yes	Yoshiko *et al* (1999); Aida (2001); Konnai *et al* (2003c)
III	TNFα	Yes	Kabeya *et al* (2001)

2.3.1.1 Role of class I genes

The earliest studies (reviewed by Hohenhaus and Outteridge 1995) on associations between OLA-type and resistance to gastrointestinal nematodes were conducted at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), McMaster Laboratory, Australia (Windon and Dineen 1984; Outteridge *et al.* 1985). In those studies, an association was found between the serologically detectable Class I antigen SY1 and resistance to *T. colubriformis* in lines of sheep selected for high and low responsiveness to that parasite. In the later study, SY1 was split into two distinct entities, SY1a and SY1b. The proportion of SY1a or SY1b or SY1a+SY1b was significantly higher in high responders in comparison to low responders. Subsequently, weak evidence was found for an association between SY1a+SY1b and low

FEC in high/low responder lines as well as in random-bred sheep (Outteridge *et al.* 1986). In the between-line comparison, SY1a+SY1b was found to be associated with low FEC. However, analysis within the high responder line showed that the effect of SY1a+SY1b on low FEC was statistically non-significant. Furthermore, in random-bred vaccinated sheep the effect of this genotype on FEC was significant in ewes, but not in rams. Those same authors, in a subsequent study (Outteridge *et al.* 1988), demonstrated that selection of out-bred sheep for the OLA-SY1 type alone would confer responsiveness to vaccination against *T. colubriformis*.

Further observation in New Zealand Romney sheep also suggested the association of SY1a and SY1b alleles with low FEC under natural challenge with *T. colubriformis*, while another allele, SY6, was found to be associated with significantly higher FEC (Douch and Outteridge 1989). Re-examination of the effects of SY1a+SY1b on FEC in 11 Merino sire groups in Australia (Outteridge 1991) revealed that while SY1 had an effect in lowering the FEC in high responder groups, it had very little effect in low responder groups. It was suggested that other genes involved in the expulsion of parasites had been selected against in the low responder groups, and consequently the resistant SY1 marker had no effect in these groups.

Studies on the association of Class I antigens with resistance to *H. contortus* have also been undertaken (Riffkin and Yong 1984; Cooper *et al.* 1989). None of the OLA types were strongly associated with resistance/susceptibility to the parasite. Contrary to those results, Luffau *et al.* (1990) reported significant effects of various OLA Class I haplotypes on the humoral response as well as on FEC in experimental infections with *H. contortus* in Romney sheep. In a study on Scottish Blackface sheep following natural infection, predominantly with *T. circumcinta*, a Class I antigen G13br was found to be significantly associated with low FEC in 4-month-old lambs (Stear *et al.* 1996). Animals with G13br had 10-fold lower FEC compared to animals that lacked the G13br antigen. In the same study, this antigen was also found to be in linkage disequilibrium with the g2 allele at the DRB1 locus that had earlier been found to be associated with reduced FEC in a different lamb crop from the same farm (Schwaiger *et al.* 1995).

A microsatellite in the Class I region, SMHCC1, was also found to be associated with resistance to gastrointestinal nematodes (Buitkamp *et al.* 1996). It was noted that in Scottish Blackface sheep following natural infection with *T. circumcinta*, resistant alleles at this locus

would result in an 8-fold reduction in FEC in comparison with the most common alleles. Similar association of the alleles at this locus with nematode resistance was also detected in Polish Heatherhead (Charon *et al.* 2001).

2.3.1.2 Role of class II genes

The lack of appropriate serological reagents to type ovine Class II molecules promoted the use of molecular methods to investigate the influence of Class II genes on gastrointestinal nematode resistance. Employing restriction fragment length polymorphism (RFLP) with human DQB and DRB probes, Blattman *et al.* (1993) searched for an association between the RFLP bands and resistance to *H. contortus.* Using mixed model best linear unbiased prediction (BLUP) procedures to obtain unbiased estimates of band effects by dissociating background gene effects from MHC effects, no significant associations were found between any band or haplotype and FEC. Using similar methods, no association could be detected between Class II RFLP bands and FEC, as a measure of immune responsiveness to *T. colubriformis* (Hulme *et al.* 1991). However, in another RFLP study employing an HLA-DRB probe a significant association between a 19-kb DRB fragment and susceptibility to gastrointestinal nematodes was detected in ewes, but not in rams (Wetherall *et al.* 1991). The failure of RFLP studies to identify associations between MHC Class II genes and nematode resistance in those studies was attributed to the inability of the RFLP method to discriminate a sufficiently large number of alleles (Buitkamp and Epplen 1996).

DRB1 alleles, based on length polymorphisms of a microsatellite in intron 2 plus hybridization of oligonucleotides within exon 2, were found to be associated with FEC in Scottish Blackface lambs following natural infection with *T. circumcinta* (Buitkamp *et al.* 1994; Schwaiger *et al.* 1995; Stear *et al.* 1996; Buitkamp and Epplen 1996). The data were analysed by least squares after fitting sex, sire and dam nested within sires as main effects. A significant MHC effect was detected with substitution of the most common allele, I, by a resistant allele, G2 (equivalent of allele *Ovar*-DRB1*0203, in terms of exon 2 sequence), resulting in a drastic reduction in FEC. It was implied that the success in identification of the nematode-resistant DRB1 allele was due to the advanced typing method employed in the studies (Buitkamp and Epplen 1996). The association of the G2 allele with faecal nematode egg counts was confirmed in a recent study in Scottish Blackface sheep (Stear *et al.* 2005).

A different study in Scottish Blackface sheep using serum antibody to detect *T. circumcinta* antigens could not reveal any obvious relationship between recognition of any parasitic antigen and polymorphism at the DRB1 locus (McCririe *et al.* 1997). It was found that the G2 allele at the locus, implicated in nematode resistance in some of the previous studies (Buitkamp *et al.* 1994; Schwaiger *et al.* 1995; Buitkamp and Epplen 1996; Stear *et al.* 1996), could not recognise the same set of parasitic antigens in all the sheep that possessed the allele. This suggested that the strong association of DRB1 alleles with resistance previously observed was not simply due to the production of an antibody to a single protective molecule by all sheep with the G2 allele, but might be due to mere linkage of G2 allele with a resistant allele at another locus (McCririe *et al.* 1997).

Sequence-based typing of DRB1 exon 2 in Suffolk and Texel sheep revealed an allele (*Ovar*-DRB1*0203) at the locus to be associated with a decrease in FEC (Sayers *et al.* 2005a). This association was observed only in Suffolk sheep, and two other alleles were associated with an increase in FEC. The locus accounted for 14% of the phenotypic variation in FEC in the breed. However, there was no evidence for such association in the Texel sheep, and the DRB1 locus accounted for only 3% of the phenotypic variation in FEC. The difference in FEC between the breeds was attributed in part to the different allele profile at the locus.

The influence of length polymorphism of the microsatellite present in intron 2 of DRB1 on nematode resistance has also been documented. A significant effect of genotype at the microsatellite locus was detected on resistance to *H. contortus* and *T. colubriformis* (Outteridge *et al.* 1996; Charon *et al.* 2002), and to *T. circumcinta* (Paterson *et al.* 1998). Another microsatellite, DYMS1, at the *Ovar*-DYA locus was also found to be significantly associated with resistance to *T. circumcinta* in a flock of Scottish Blackface sheep (Buitkamp *et al.* 1996). Substitution of the most common alleles by resistant alleles identified at the locus resulted in a 218-fold reduction in FEC.

2.3.1.3 Role of class III genes

There has been one report on the association of the complement factor (C4) gene and resistance to gastrointestinal nematodes in sheep (Wetherall *et al.* 1991). No significant associations were detected between C4 RFLP alleles defined by restriction enzyme *Taq*I and FEC. However, a C4*A2 phenotypic allele was found to be in linkage disequilibrium with a 19-kb DRB-RFLP fragment that had significant association with high FEC. This finding

provided circumstantial evidence that an allele of the ovine C4 gene might be involved in determining the resistance/susceptibility to internal parasites.

2.3.1.4 Genome-wide scans

Investigators at AgResearch (New Zealand), CSIRO (Australia) and the University of Melbourne (Australia) have developed three sets of parasitic quantitative trait loci (QTL) resource families (Beh and Maddox 1996). Those have been derived from crosses of divergent lines selected over several generations for high or low resistance to nematodes, predominantly *H. contortus* and *T. colubriformis*. Independent full genome scans carried out on flocks at CSIRO and AgResearch (Beh *et al.* 1998; Crawford 1998) revealed three to six putative QTL related to FEC. However, none of those QTLs were located in the MHC region, while a large effect was detected on the 'q' arm of Chromosome 3, near the positional candidate gene, IFN-γ. In a recent genome-wide scan performed on the CSIRO resource flock, one region on Chromosome 6 attained chromosome-wide significance, while five other regions on Chromosomes 1, 3, 6, 11 and 12 attained point-wise significances in relation to resistance to *T. colubriformis* (Beh *et al.* 2002). No significant association could be detected for two markers in the MHC region with resistance to the nematodes.

2.3.2 Association with bacterial diseases

There are only a few reports on the involvement of MHC genes on resistance to bacterial diseases in sheep. One of the earliest studies in this respect tested the involvement of OLA Class I antigens in resistance to *Corynebacterium pseudotuberculosis* in the 'Prealpes du Sud' sheep flock (Millot 1989). No association between the OLA antigens and occurrence of abscesses could be detected, although some OLA antigens were either positively or negatively associated with delayed occurrence of abscesses.

Footrot, a common and highly contagious disease of sheep, is the result of a mixed bacterial infection, principally involving *Dichelobacter nodosus*. Variation in susceptibility between breeds has been well documented, and heritability values of 0.28 for New Zealand Romney sheep under natural challenge (Skerman *et al.* 1988), and 0.31 for Merino sheep under artificial challenge (Raadsma *et al.* 1990) have been reported. Three studies revealed associations between *Ovar-Mhc* and resistance to footrot. Outteridge *et al.* (1989), employing antisera for ovine Class I antigens, showed two antigens, SY6 and SY1b, significantly

influenced resistance to footrot. The second study (Litchfield *et al.* 1993) was based on RFLP, employing human cDNA-DQA and DRB probes. One DRB band was significantly associated with susceptibility to footrot and two other DRB bands were associated with residual footrot infection after vaccination. Several bands belonging to DRB as well as DQA loci were found to be associated with antibody titres during infection.

The association of Class II genes with resistance/susceptibility to footrot was also suggested in a subsequent study involving RFLP using ovine DQA1, DQA2, DQB and DRA probes (Escayg *et al.* 1997). In the first of two trials in the study, a significant association was detected between the MHC haplotype and footrot status in progeny of one of the four sires that were classified as resistant, susceptible and self-curing. This association turned to be more significant either when the self-curing and resistant animals were combined or when the self-curing animals were excluded from the analysis. However, this association was non-significant in the second trial when the resistant and self-curing animals were combined or separated or when the latter were excluded from the analysis. The difference was attributed to dry weather conditions prevailing during the second trial, which led to poor transmission and unreliable disease classification.

A preliminary study of genetic influences on the susceptibility of Merino sheep in Australia to Johne's disease indicated a possible role of MHC in susceptibility to the disease (Reddacliff *et al.* 2005). A 163 base-pair allele at a dinucleotide microsatellite locus, CSRD226, in the MHC Class I region was associated with susceptibility to the disease in two independent flocks.

2.3.3 Association with viral diseases

The association of MHC genes with resistance to viral diseases has also been shown in a small number of studies. An investigation of the distribution and density of ovine MHC Class I and Class II antigens in normal, acanthotic and malignantly-transformed ovine skin was carried out using monoclonal antibodies labelled with immunoperoxidase (Townsend *et al.* 1995). An association between tumour invasiveness and low level expression of MHC Class I was apparent in the study.

Two studies investigated the association between the *Ovar*-DRB1 gene and development of tumours in sheep after experimental infection with bovine leukemia virus (BLV; Yoshiko *et*

al. 1999; Aida 2001). The results of both studies indicated that the differences in immune response were due to differences in DRB1 alleles and reflected the risk of BLV-induced leukaemogenesis. DRB alleles encoding the serine-arginine (SR) motif at positions $\beta^{70/71}$ were positively related to susceptibility to development of tumours, while the alleles encoding the arginine-lysine (RK) motif at the same position were related to resistance. In a recent study in sheep carrying these alleles, different immune responses were investigated for 30 weeks subsequent to artificial infection with BLV (Konnai *et al.* 2003c). It was found that sheep with the resistant genotype, RK/RK, strongly expressed IFN-γ, which is important for cellular immunity against viral infections, while the animals with the susceptible genotype, SR/SR, strongly expressed interleukin-2, a major proliferative factor for B-cell malignant transformations in BLV infections.

The involvement of an *Ovar-Mhc* Class III gene, TNFα, in the pathogenesis of BLV infection in sheep has also been investigated (Kabeya *et al.* 2001). It was found that the expression of the TNFα Type 1 receptor, R1, was down-regulated in peripheral blood monocytes from BLV-positive compared to BLV-negative sheep. Also, proliferative responses of peripheral blood monocytes in the presence of TNFα were observed from the BLV-positive but not BLV-negative sheep. It was concluded that the expression of TNFα and its receptors was closely associated with lymphocytosis induced by BLV.

2.3.4 Association with other traits

MHC genes have also been found to be associated with traits other than disease resistance. Alleles at *Ovar*-DRB, a microsatellite in the Class II region, were significantly associated with the production of antibodies against equine chorionic gonadotropin in dairy ewes (Roy *et al.* 1999). In high responders, the residual antibodies against the hormone, usually administered for induction of ovulation, would deplete the hormone during subsequent administration and thus might result in infertility. In a different study (Gruszczynska *et al.* 2000), alleles at another Class II locus, DRB1, were found to be significantly associated in Polish Heath sheep with bodyweight at birth and weight gain during the first month of the lamb's life.

In a candidate gene approach to detect QTL, evidence was found for a locus affecting marbling in Texel and Suffolk sheep (Walling *et al.* 2002). However, it was concluded that

further studies, aimed at techniques to improve the resolution of its position and effects, would be needed. A recent study in Merinos in Australia examined the association of five markers within the MHC region with production traits such as bodyweight, wool weight, faecal scouring, fibre diameter and fibre strength (Bot *et al.* 2004). A 178 base-pair allele at the Class III B-factor locus and a 226 base-pair allele at the Class II DRB1 microsatellite locus were found to be significantly associated with increased wool, resulting in 0.4 kg and 0.36 kg more wool weight than the most frequent alleles at the respective loci.

2.3.5 Conclusion

Several studies, over the past two decades, have focussed on the associations of the *Ovar-Mhc* genes with disease resistance. Most of them are confined to gastrointestinal nematodes. The involvement of Class II DRB genes was predominant in these studies. A few studies have revealed an association of Class I antigens with resistance/susceptibility to gastrointestinal nematodes, however those associations could not be utilised for screening sheep flocks for increased genetic resistance, owing to the complexity and labour-intensiveness of MHC antigen serotyping methods.

Association of *Ovar*-DRB1 allele, G2 (*Ovar*-DRB1*0203) with FEC was documented in several studies (Buitkamp *et al.* 1994; Schwaiger *et al.* 1995; Stear *et al.* 1996; Buitkamp and Epplen 1996; Sayers *et al.* 2005a; Stear *et al.* 2005). However, in a separate study this allele could not recognise the same set of parasitic antigens in all the sheep that possessed the allele, suggesting that the strong association of the allele with reduced FEC might be due to linkage of the G2 allele with a resistant allele at a different locus (McCririe *et al.* 1997). Also, care should be taken with regard to the phenotypes chosen for such association studies. Associations based on total FEC should not be interpreted as a particular marker to be resistant/susceptible to all the nematode species, as it has been shown in a recent study (Stear *et al.* 2006) that lambs with high total FEC had high numbers of *Cooperia* and *Trichostronglylus* spp., rather than *T. circumcincta.* Also, differences do exist in terms of host's immune responses to *Nematodirus* and strongyles (Winter 2002). Immunity to strongyle infection develops slowly and the animals, during the first season of parasite exposure, are comparatively susceptible. In contrast, immunity to *Nematodirus* is extremely rapid, with sheep over the age of six months having solid age-immunity. Further, a recent study (Davies *et al.* 2006) reported differences in localization of QTL for stronglye and *Nematodirus* FEC. These studies stress the need for identification of genetic markers specific

to individual nematode species based on larval culture or species-specific FEC, rather than total FEC.

Availability of the complete genome sequence of the *Ovar-Mhc* region would enable development of accurate and simplified typing methods for various MHC loci involving multiple markers that are more dense, evenly spaced and span the entire region. This would facilitate the unambiguous characterization of individuals in terms of haplotypes, rather than individual genes. Once such methods are available, studies on important diseases in sheep can be carried out, aiming to unveil MHC marker alleles that causally influence resistance or susceptibility to disease. Genetic markers could then be employed to screen animals at an early stage of their life, aiming to decrease the incidence of the disease concerned. The enormous potential of such MHC markers in curtailing a disease is exemplified by a 5-year selection programme, employing disease susceptibility markers in the MHC region, that brought down the prevalence of dermatophilosis in Zebu Brahman cattle in Martinique from 0.76 to 0.02 (Maillard *et al.* 2003). However, care should be exercised in selection leading to either fixation or loss of such an MHC allele in a population as it might lead to fixation or loss of the associated haplotype. This might be undesirable, considering the importance of MHC heterozygosity in immune response traits. Equally important, prior to initiation of selection, is to look at the possible association of the alleles of the haplotype in question with other disease and production traits.

Currently in New Zealand, Lincoln University offers a commercial MHC-based diagnostic test (footrot gene-marker test, FGMT) that enables commercial ram-breeders to selectively breed rams that are more tolerant to footrot. This test, based on scoring polymorphism at *Ovar*-DQA2 locus, was developed from results of an earlier study on the association between alleles of *Ovar-Mhc* and footrot (Escayg *et al.* 1997). A recent New Zealand-wide survey into the costs of footrot and the impact of FGMT predicted a potential saving of three to six million dollars per annum to the New Zealand fine-wool and mid-micron industries by the year 2013-2014 (Greer 2004). It can be hoped that QTLs identified in the MHC region will be fine mapped and more diagnostic tests become available in the coming years. MHC genes, with their immunological significance, still continue to be fascinating subjects for geneticists aiming at increased genetic resistance to diseases.

2.4 OVINE SLC11A1 GENE

The natural-resistance-associated macrophage protein 1 (NRAMP1) gene plays an important role in protection against several intracellular pathogens and was first identified in mice by positional cloning (Vidal *et al.* 1993). It was earlier denoted as the *Bcg/Lsh/Ity* locus because of its role in resistance/susceptibility to *Mycobacterium bovis* strain *bacilli Callmette-Guerin*, *Leishmania donovani* and *Salmonella typhimurium* infections (Skamene 1994). NRAMP1, based on its function as a divalent cation-proton antiporter, has been renamed as solute carrier family 11a member 1 (SLC11A1, Goswami *et al.* 2001). SLC11A1, by virtue of pleiotropic effects on macrophage activation, has potential for antimicrobial defences (including anti-viral), anti-tumour defences and autoimmunity (Blackwell *et al* 2000). Its effects include generation of antimicrobial hydroxyl radicals and modulation of TNFα, interleukin-1β, MHC class II molecules, chemokine KC, nitric oxide and nitric oxide synthetase.

A natural glycine→aspartate mutation at amino acid 169 of the murine SLC11A1 renders mice susceptible to *Leishmania donovani*, *Salmonella typhimurium* and *Mycobacterium bovis* (Vidal *et al.* 1995). The SLC11A1 gene is evolutionarily conserved in many phylogenetically distant organisms like mammals, birds, insects, worms, plants, fungi and bacteria (Cellier *et al.* 1996). A fragment of the ovine SLC11A1 was first isolated by screening of a sheep cosmid library (Pitel *et al.* 1994) and was later assigned to chromosome 2q41→q42 by *in situ* hybridization (Pitel *et al.* 1995).

2.4.1 Structure and polymorphism

Sequencing of ovine SLC11A1 cDNA cloned by reverse transcription polymerase chain reaction (RT-PCR) of RNA from macrophages yielded a sequence of 2083 nucleotides (GenBank accession # AF005380, Matthews and Crawford 1998). The open reading frame (exclusive of stop codon TGA) was 1644 nucleotides long, with the predicted protein being 548 amino acids long. The SLC11A1 amino acid sequence in sheep was found to be ≈98% similar to that in cattle and deer. A 2172-bp full length cDNA sequence of the sheep SLC11A1 gene was also reported by a different research group (GenBank accession # U70255, Bussmann *et al.* 1998). The lengths of open reading frame and predicted protein and were similar to those reported by Matthews and Crawford (1998). While the in-frame initiator codon followed a 134-nucleotide 5’ untranslated region, the TGA termination codon was located downstream from glycine 548 (nucleotide 1778).

The exon-intron boundaries of the sheep SLC11A1 gene were determined by PCR amplification, cloning and complete or partial sequencing of introns (Bussmann *et al.* 1998). The ovine gene possessed 15 exons. The exon-intron organization was found to be perfectly conserved among human, mouse and sheep genes, although intron sizes varied from species to species. The complete sheep SLC11A1 gene was predicted to span up to 10.5 kb of genomic DNA.

The sheep SLC11A1 protein was predicted to be 59.4 kDa in molecular weight and its structure to be in agreement with the putative murine, human, bovine and chicken SLC11A1 protein (Bussmann *et al.* 1998). The sheep protein is highly hydrophobic with 12 putative transmembrane domains, two potential amino-linked glycosylation sites and four protein kinase C phosphorylation sites. Sequence alignment of the bovine, human, mouse and chicken SLC11A1 predicted proteins, in comparison with the sheep sequence, exhibited identities of 98%, 89%, 86% and 68%, respectively.

Cell-specific expression of sheep SLC11A1 mRNA in various organs and cell types was studied employing RT-PCR (Bussmann *et al.* 1998). SLC11A1 mRNA could be detected in liver, spleen and lung, but not in the lymph node and heart. Also, an increase in SLC11A1 expression was observed in alveolar macrophages subsequent to activation with live *Salmonella abortusovis*. It was concluded that the SLC11A1 gene in sheep, as in other mammals, expressed specifically in the reticulo-endothelial system, macrophages in particular.

Two polymorphic microsatellite markers were identified close to the sheep SLC11A1 gene (Pitel *et al.* 1996). A $(TG)_n$ repeat (OVINRA1, EMBL accession # X89268) was found to be located in the 3' untranslated region, while a second $(GT)_n$ repeat (OVINRA2, EMBL accession # X89269), associated with a short interspersed repetitive element (SINE), was in the same 40 kilo bp cosmid as the SLC11A1 gene. The two microsatellites were found to be genetically linked. Eight and four alleles could be detected for the two microsatellites respectively in 129 two-generation families sired by 15 rams. While the $(TG)_n$ repeat is absent in the 3' region of human and mouse SLC11A1 genes, there are two such repeats in the bovine SLC11A1 gene (Matthews and Crawford 1998). While eight OVINRA1 alleles were detected in 15 unrelated New Zealand Romney sheep (Matthews and Crawford 1998), seven and four alleles were found at the same locus in two Australian Merino flocks

(Reddacliff *et al.* 2005). In highly structured populations of wild sheep, nine and three alleles were found at OVINRA1 and OVINRA2 loci, respectively (Worley *et al.* 2006).

2.4.2 Association with disease resistance

Studies in mice indicated SLC11A1 to be associated with natural resistance/susceptibility to intracellular infections (Vidal *et al.* 1993, 1995; Puliti *et al.* 1995; Leclercq *et al.* 1996). Controversy exists with regard to the role of SLC11A1 in susceptibility to clinical tuberculosis in humans. There were indications for positive (Bellamy 1999; Awomoyi *et al.* 2002) as well as negative (Shaw *et al.* 1997; Soborg *et al.* 2002) associations of alleles at the locus with susceptibility to tuberculosis. A recent review of literature in this area, by meta-analysis, concluded that polymorphisms at SLC11A1 loci were not associated with susceptibility to tuberculosis in subjects of European descent, while SLC11A1 gene polymorphisms significantly influenced susceptibility to the disease in Asian and African subjects (Li *et al.* 2006). Further, it has been reported that SLC11A1 gene polymorphisms would influence progression to severe forms of pulmonary tuberculosis, rather than influencing susceptibility to the disease (Zhang *et al.* 2005).

A few studies investigated the association of polymorphisms in SLC11A1 gene with disease resistance/susceptibility in cattle and sheep. A preliminary study aimed at association between SLC11A1 alleles and susceptibility to ovine PTB (Beard *et al.* 1999). No evidence of the glycine→aspartate mutation at amino acid 169 of SLC11A1 (that was implicated in susceptibility of mice to intracellular parasites including mycobacteria, Vidal *et al.* 1995) was found in the investigated PTB-effected as well as unaffected sheep . A recent study on Australian Merinos revealed possible associations of 162 and 160 bp alleles at OVINRA1 microsatellite locus within the SLC11A1 gene with susceptibility and resistance, respectively, to clinical PTB (Reddacliff *et al.* 2005).

SLC11A1 protein was found to play an important role in T-cell reactions combating bovine tuberculosis. High-level expression of SLC11A1 protein was detected in peripheral blood cells and granulomas of *Mycobacterium bovis*-infected cattle (Estrada-Chavez *et al.* 2001; Pereira-Suarez *et al.* 2006). However, no associations could be detected between resistance/susceptibility to *Mycobacterium bovis* infection and polymorphism in the SLC11A1 gene in cattle (Barthel *et al.* 2000). Studies have also been undertaken to investigate the association of polymorphism at a $(GT)_n$ microsatellite locus within the bovine

SLC11A1 gene and resistance to infection with *Brucella abortus*, a facultative intracellular pathogen. Alleles $(GT)_{13}$ and $(GT)_{14\text{-}16}$ at the microsatellite locus were implicated with resistance and susceptibility, respectively, to *Brucella abortus* infection in Holstein Friesian cattle (Adams and Templeton 1998). However, the $(GT)_{13}$ allele, even in homozygous condition, was found to be incapable of protecting Indian Zebu and their crosses with *Bos taurus* cattle against brucellosis (Kumar *et al.* 2005). Also, a recent study in Caucasian Spaniards found no difference in SLC11A1 allelic frequencies between brucellosis-positive and healthy individuals (Bravo *et al.* 2006).

2.5 OVINE IFN-γ GENE

Interferons (IFN) constitute a class of cytokines having an important role in immune responses. Apart from having direct antiviral effects (Lane *et al.* 1988), they are known to induce expression of cell-surface class II MHC molecules and increase the activity of natural killer cells and the anti-microbicidal activity of macrophages and neutrophils (Wallach *et al.* 1982; Metcalf 1987). IFN-γ differs from IFN-α and IFN-β in its biochemical and biological properties (Hovanessian 1985) and plays a central role in induction and modulation of immune responses (Young and Hardy 1995).

IFN-γ is produced by T cells in response to stimulation with an antigen or a mitogen (Havell *et al.* 1982). More specifically, it is produced by TH1 subset of T cells, rather than by the TH2 subset (Mosmann and Coffman 1987). IFN-γ secretion by antigen-sensitized lymphocytes could be considered as a good measure of T-cell activity (Rothel *et al.* 1990).

Ovine IFN-γ was detected for the first time by bioassay and found to possess properties similar to those of murine and human IFN- γ (Entrican *et al.* 1989). Ovine IFN-γ was found to be functionally cross-reactive with the bovine IFN- γ, indicating structural similarity of the ovine and bovine molecules (Rothel *et al.* 1990). The gene encoding the ovine IFN-γ was cloned by RT-PCR of mRNA from stimulated lymphocytes (McInnes *et al.* 1990; Radford *et al.* 1991). It was later mapped to 3q23 by *in situ* hybridization (Goldammer *et al.* 1996).

2.5.1 Structure and polymorphism

The ovine IFN-γ gene was cloned and sequenced by two independent groups (McInnes *et al.* 1990; Radford *et al.* 1991). cDNA was obtained by RT-PCR of lymphocyte mRNA, employing primers based on the bovine IFN-γ sequence. The ovine sequence was found to be

93% identical to the bovine one. The cDNA sequence had a putative signal sequence coding for 20 amino acids, followed by sequence for a mature IFN-γ molecules spanning 145 amino acids. Complete sequence of the gene was also reported (Crawford and McEwan 1998). It was 4842 nucleotides long, with four exons spanning 114, 69, 183 and 135 nucleotides, respectively. A total of 36 (34 in the non-coding and two in the coding regions) single nucleotide polymorphisms (SNP) and two insertions/deletions were identified in the gene.

A diallelic tetranucleotide microsatellite [o(IFN)-γ] of the form (GTTT)5/6 was identified in the ovine IFN-γ gene (Schmidt *et al.* 1996). PCR amplification of the larger and shorter alleles resulted in 128 and 124 bp long fragments, respectively. Several studies investigated polymorphism at this locus. While 128 and 124 bp length alleles were identified in the majority of studies (Crawford and McEwan 1998; Dukkipati *et al.* 2005; Reddacliff *et al.* 2005; Sayers *et al.* 2005b), alleles of sizes 126 and 130 bp were found in Soay sheep (Coltman *et al.* 2001). Also, a 122 bp allele was found fixed in a large structured population of wild sheep (Worley *et al.* 2006). It was also revealed that in New Zealand Romney-Coopworth crosses, the larger allele had 'G' 49 bp downstream of the microsatellite (referred to as haplotype A) and the smaller allele had 'A' at the corresponding position (referred to as haplotype B; Crawford and McEwan 1998). Similar haplotypes were reported to exist in Texel sheep, while two additional haplotypes, C (with 'A' at the corresponding position in the larger allele) and D (with 'G' at the corresponding position in the smaller allele), were identified in Suffolks (Sayers *et al.* 2005b).

A different microsatellite (OarKP6) of the form (AC)n was identified in a BAC containing exon 1 sequence of the ovine IFN-γ gene (Paterson and Crawford 2000). Seven alleles were observed in 170 unrelated sheep belonging to five breeds. No recombination was observed between the OarKP6 and o(IFN)-γ microsatellites. However, there were no subsequent reports on the polymorphism of OarKP6 microsatellite in sheep. Ovine IFN-γ exon 3 sequence polymorphism in 310 sheep (Merino, Corriedale, Romney, Poll Dorset and cross-bred) was studied employing SSCP analysis (Zhou *et al.* 2007). Five unique SSCP patterns, corresponding to five different alleles, *01 to *05 (GenBank accession # DQ311095-DQ311099), were identified. Alleles *01 and *02 were most common, together accounting for 86% of the allelic population.

2.5.2 Association with disease resistance

The ovine IFN-γ gene received increasing attention because of its association with nematode resistance in both domestic and feral sheep breeds. Independent full genome scans carried out on flocks at the CSIRO and AgResearch (Beh *et al.* 1998; Crawford 1998) revealed three to six putative QTL related to FEC. A large effect was detected on the 'q' arm of Chromosome 3, near the positional candidate gene IFN-γ. Subsequently, alleles at the o(IFN)-γ microsatellite locus were found to be significantly ($P<0.05$) associated with resistance/susceptibility to gastrointestinal nematodiasis in sheep belonging to two divergent nematode selection lines at AgResearch, New Zealand (Crawford and McEwen 1998). The larger (128 bp) and smaller (124 bp) alleles at the locus were more frequent in the nematode-resistant and susceptible lines, respectively. Association of alleles at this locus with FEC was also reported in feral Soay sheep (Coltman *et al.* 2001). However, the smaller 126 bp allele (rather than the larger 128 bp allele linked to nematode resistance in domestic sheep) was found to be associated with reduced FEC in lambs as well as yearlings. Also, the 126 bp allele was associated with increased *T. circumcincta*-specific antibodies in lambs.

Two other studies examined the association of this microsatellite locus with nematode resistance in domestic sheep. While no significant influence of the alleles at the locus on FEC could be detected in New Zealand Romneys (Dukkipati *et al.* 2005) and Irish Suffolks (Sayers *et al.* 2005b), the 124 bp allele was found to be associated with resistance to nematode infection in Irish Texel sheep (Sayers *et al.* 2005b).

Association of genotypes at the o(IFN)-γ microsatellite locus on susceptibility to natural ovine PTB in two Australian Merino sheep flocks was also investigated (Reddacliff *et al.* 2005). There were no consistent findings for the effect of IFN-γ genotype on resistance/susceptibility to PTB. Genotype 128/128 was found to be significantly ($P<0.01$) associated with clinical signs of the disease in flock B. However, no such association of genotype with phenotype was evident in flock A. In flock A, allele 124 was ranked high for association with severe disease, but in flock B, allele 128 was ranked higher.

2.6 OTHER LOCI FOUND TO BE ASSOCIATED WITH SUSCEPTIBILITY TO TUBERCULOSIS

Several studies have investigated the role of genetic variations at many loci other than MHC, SLC11A1 and IFN-γ, in susceptibility to tuberculosis in humans and mice. Such loci include

NOD2/CARD15, CD38, mannan-binding lectin, vitamin D receptor, sst1/IPR1, TLR2, and genes involved in type 1 cytokine cascade (IL12B/IL12RB1, IFN-γR1/IFN-γR2 and STAT1).

2.6.1 NOD2/CARD15

Nucleotide oligomerization binding domain 2 (NOD2), expressed in monocytes, is an intracellular pattern recognition receptor for bacterial components (Stockton *et al.* 2004). It has been renamed by the Human Genome Organization as the caspase recruitment domain-containing protein 15 (CARD15, Vermeire 2004). CARD15 has been shown to interact with microbial components like muramyl dipeptides, which are breakdown products of peptidoglycans from Gram-negative as well as Gram-positive bacteria, to induce early innate immune responses that subsequently orchestrate the adaptive response (Kufer *et al.* 2006). It was demonstrated that CARD15 is an essential recognition system of *M. tuberculosis* and hence could be considered as a candidate gene in tuberculosis (Ferwerda *et al.* 2005).

Two independent research groups (Hugot *et al.* 2001; Ogura *et al.* 2001) identified that genetic variants within or adjacent to the leucine-rich repeat (LRR) region of the CARD15 gene are associated with Crohn's disease (CD) in humans, for which *Map* is a possible etiological agent. One particular variant of CARD15, a C insertion at nucleotide position 2936 (2936insC), results in a premature stop codon and a truncated protein lacking the terminal 33 amino acids of the LRR (Hampe *et al.* 2001). Two individual studies, one on Gambians (Stockton *et al.* 2004) and the other on South African coloureds (Moller *et al.* 2007) reported no associations between the CD-associated CARD15 variants and tuberculosis. While no CD-associated variants were detected in the Gambians, they occurred at very low frequencies in the South African coloureds.

2.6.2. CD38

Cluster of differentiation 38 (CD38) is a glycoprotein found on the surface of many immune cells, including $CD4^+$, $CD8^+$, B and natural killer cells (Deaglio *et al.* 2001). It is a multifunctional ectoenzyme possessing signalling and adhesion properties, as well as extra-cellular/intracellular enzymatic activity (Deaglio and Malavasi 2000). Apart from its role in T-cell dependent humoral responses against extracellular pathogens, CD38 was shown to be functionally important in macrophages (Viegas *et al.* 2007).

A recent study (Viegas *et al.* 2007) investigated the role of CD38 in immune response against mycobacterial infection in mice. Subsequent to intraperitoneal infection with *M. avium*, the immune response in CD38 knockout mice (CD38KO) was compared with that in parental strain mice, C57B1.6. The absence of CD38 rendered mice more susceptible to mycobacterial infection and this susceptibility was attributed to ineffective Th1 differentiation and polarization.

2.6.3 Mannan binding lectin

Mannan binding lectin (MBL) is a pattern recognition receptor protein found in plasma; it is produced by hapatocytes and plays an important role in innate immunity (Alagarasu *et al.* 2007). Upon binding to specific carbohydrate structures present on various pathogens including *M. tuberculosis* and HIV, MBL can initiate complement activation and phagocytosis as well as induction of inflammatory cytokine responses (Petersen *et al*. 2001). Serum MBL concentration is widely variable mainly due to the presence of three common point mutations in exon 1 of the MBL2 gene at codons 52, 54 and 57, which encode for the variant alleles D, B and C, respectively. The mutant alleles are collectively known as 'O', while the wild type allele is known as 'A'. Heterozygotes for O alleles are very common in most populations and these individuals possess about 10% of wild-type serum concentrations of MBL, while MBL levels are quite low or absent in individuals that are homozygous for O alleles (Petersen *et al*. 2001).

A number of nucleotide substitutions in the MBL2 promoter region are also known to influence serum MBL concentrations. Particularly important among those is a base-pair substitution (G→C) at position -221, leading to the presence of allele X (instead of Y allele) that has a marked down-regulating effect on serum MBL levels (Garred *et al*. 2003). Strong correlation was observed between MBL genotypes and serum MBL levels in different populations and hence, genotyping could serve as a good indicator of serum MBL levels (Petersen *et al*. 2001; Garred *et al*. 2003).

There has been no clear evidence regarding MBL deficiency and susceptibility to specific pathogens. However, in humans, MBL deficiency might confer an increased risk of susceptibility to a wide range of pathogens during infancy as well as during adulthood (Bellamy 2005). Also, based on high prevalence of heterozygote carriers of O alleles in different populations, it was hypothesised that low MBL levels in such carriers might confer

selective advantage against infection by intracellular pathogens like mycobacteria (Garred *et al*. 1994). Several studies relating to tuberculosis (Garred *et al*. 1997; Bellamy *et al*. 1998; Hoal-van *et al*. 1999), leprosy (Garred *et al*. 1994) and *M. avium* (Polotsky *et al*. 1997) provided support for this hypothesis. Two recent studies on susceptibility to tuberculosis in human immunodeficiency virus-infected Western-Europeans (Garcia-Laorden *et al.* 2006) and South Indians (Alagarasu *et al*. 2007) have also corroborated the selective advantage of heterozygygous mutant MBL alleles in protection against tuberculosis.

2.6.4 Vitamin D receptor

The active form of vitamin D (1,25 dihydroxyvitamin D3) is an important immulomodulatory hormone has been found to activate macrophages and restrict the intracellular growth of *M. tuberculosis* (Rockett *et al*. 1998). This effect might be influenced by polymorphisms at three sites in the vitamin D receptor (VDR) gene (Wilkinson *et al*. 2000). VDR and 1,25 dihydroxyvitamin D3 mediate the antimicrobial response triggered by toll-like receptors (TLRs), leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular *M. tuberculosis* (Liu *et al*. 2006).

A study on VDR gene polymorphisms in Gambians revealed that a VDR genotype that produces higher circulating levels of 1,25 dihydroxyvitamin D3 was significantly under-represented amongst tuberculosis affected patients compared to ethnically matched controls (Bellamy *et al*. 1999). VDR gene polymorphisms were also found to be associated with tuberculosis among Gujarati Indians in West London (Wilkinson *et al*. 2000) and with leprosy type in patients from India (Roy *et al*. 1999). A recent study in South Africa found no association between genotypes at four SNP loci within the VDR gene and tuberculosis in the Venda population (Lombard *et al*. 2006). However, a four-locus haplotype within the VDR gene was identified in the study that significantly protected participants from the disease.

2.6.5 sstI/Ipr1

Kramnik and colleagues at the Harvard School of Public Health, Boston, U.S.A., investigated the susceptibility of inbred strains of mice to tuberculosis (Kramnik *et al*. 2000). A new locus named susceptibility for tuberculosis 1 (sst1) was found to be linked to tuberculosis susceptibility in the study. The sst1 locus mapped to murine chromosome 1, 10-19 cM distal to the NRAMP1. The candidate gene for sst1, intracellular pathogen resistance 1 (Ipr1), was subsequently identified by positional cloning (Pan *et al*. 2005). The study showed that Ipr1

mediates innate immunity to tuberculosis. The Ipr1 gene is upregulated in the sst1 resistant macrophages after activation and infection, but is not expressed in the sst1 susceptible macrophages. It was also shown that expression of Ipr1 transgene sst1 susceptible macrophages limited the multiplication of *M. tuberculosis* as well as *Listeria monocytogenes*.

2.6.6 TLR2

Toll like receptors (TLR) play a crucial role in initiating innate immune responses. TLR2 and TLR4 in humans can activate NF-κB and induce expression of inflammatory cytokines and co-stimulatory molecules (Kang *et al*. 2002). TLR2 is important in lipopolysaccharide-mediated signalling, while TLR4 can respond to a variety of bacterial cell wall components (Takeuchi *et al*. 1999). TLR2 has been found to be specifically important in mediating mycobacteria-induced proinflammatory signalling in macrophages (Underhill *et al*. 1999; Wang *et al*. 2000). A single base-pair mutation (Arg677Trp) in one of the conserved intracellular domain of TLR2 has been identified in 10 out of 45 lepromatous leprosy patients from South Korea (Kang and Chae 2001). It was absent in all of the 41 tuberculoid leprosy patients and 45 controls that were investigated, suggesting a role in susceptibility to lepromatous leprosy.

The Arg677Trp mutation was also detected in tuberculosis patients in Tunisia (Ben-Ali *et al*. 2004). Its frequency in tuberculosis patients was significantly ($P < 0.0001$) higher than in healthy controls, suggesting that this polymorphism could be a risk factor for tuberculosis. TLR2 triggered responses in monocytes to *M. leprae* have also been investigated (Kang *et al*. 2002). It was found that *M. leprae* stimulation induced the production of TNFα and interleukin 12. Anti-TLR2 monoclonal antibody blocked greater than 50% of interleukin 12 responses. Also, monocytes from patients with the Arg677Trp mutation were significantly less responsive to *M. leprae*, compared to those from normal individuals.

2.6.7 Genes involved in type 1 cytokine cascade

During the past decade, human patients with Mendelian susceptibility to mycobacterial disease (MSMD) were found to possess defects in the type-1 cytokine pathway leading to severe infections with mainly environmental mycobacteria and salmonellae species (Sahiratmadja *et al*. 2007). MSMD patients are unable to produce or respond to IFN-γ in response to intracellular pathogens, resulting in an inability to mount CMI responses. These defects are mainly due to mutations in five genes encoding proteins in the IL-12/IFN-γ axis

(reviewed by van de Vosse *et al*. 2004). They include IL12B (encoding IL12p40), IL12RB1 (encoding the β1 chain of the IL-12 receptor), IFN-γR1 and IFN-γR2 (encoding the two chains of the IFN-γ receptor) and STAT1 (encoding the signal transducer and activator of transcription 1). Several studies have demonstrated the associations of polymorphisms at these loci with susceptibility to tuberculosis in humans (Akahoshi *et al*. 2003; Fraser *et al*. 2003; Remus *et al*. 2004; Tso *et al*. 2004; Sahiratmadja *et al*. 2007).

2.7 CONCLUSION

A review of the literature pertaining to ovine PTB indicates CMI responses to be of paramount importance in protection against natural as well as experimental infections of *Map*. Humoral responses are usually not evident until the onset of clinical signs of the disease. However, both humoral and CMI responses are evident as early as 2-4 weeks post-vaccination. The current study is aimed at identification of genetic markers for immune responses to a killed *Map* vaccine. MHC class I and class II molecules play a predominant role in antigen presentation required for immune responses. The literature suggests that polymorphisms at several loci within the MHC region may be associated with resistance/susceptibility to gastro-intestinal nematodiasis, foot-rot and BLV infection in sheep.

SLC11A1 protein plays an important role in protection against intracellular pathogens including mycobacteria and a point mutation at amino acid 169 has been shown to render mice susceptible to intracellular pathogens. Though no such association was reported in sheep, a recent study indicated possible associations of alleles at a microsatellite locus within the gene with resistance/susceptibility to clinical PTB. Also, cytokine IFN-γ plays an important role in immune responses, especially in CMI responses. A small number of studies have shown association of genotypes at a diallelic microsatellite locus within the gene with nematode resistance.

After consideration of the literature, eight microsatellite markers, four (DYMS1, OLADRB, OLADRW and SMHCC1) from the *Ovar-Mhc* region and two each from SLC11A1 (OVINRA1 and OVINRA2) and IFN-γ [o(IFN)-γ and OarKP6] gene regions, were selected for the current study. Microsatellite markers were specifically chosen with consideration of the ease and low costs of genotyping methods. Moreover, the majority of the selected markers (excepting OLADRB, OVINRA2 and OarKP6) have been implicated in

resistance/susceptibility to diseases in earlier studies. Markers from the NOD2/CARD15, CD38, MBL, vitamin D receptor, sst1/IPR1, TLR2, type-1 cytokine cascade gene regions were not included in this study, since the majority of these associations with tuberculosis in humans have only been reported recently (after the start of this study). Also, most of these genes are yet to be fully characterised in cattle and sheep.

3. MATERIALS AND METHODS

A five-year field trial was undertaken in Australia to determine the efficacy of a killed *Map* vaccine, Gudair^{TM}, for the control of PTB in sheep (Reddacliff *et al.* 2005). Blood samples from the sheep involved in the trial, together with phenotype data pertaining to antibody and IFN-γ responses recorded for up to 54 months post-vaccination were obtained to form the basis of the current study. A detailed description of the flocks employed for the vaccination study and the measurement of immune responses were provided in the project final report (Reddacliff 2005). A brief description is provided below. This chapter also includes a detailed description of methods for obtaining genotypes from blood samples and statistical procedures to summarise genotypes and to explore genotype-phenotype associations.

3.1 EXPERIMENTAL FLOCKS AND ANIMALS

The vaccination trial was undertaken on three independent properties (P1, P2 and P3) in the Central Tablelands of New South Wales, Australia. Each property produced at least 800 fine-wool Merino lambs and the three flocks had been run on a self ewe replacement basis for several decades. All three properties had >5% losses per annum due to PTB in susceptible age groups (2-4 year olds), with an estimated sero-prevalence (ELISA) of >7% on P1 and P2. On each property, 400 (200 vaccinates and 200 unvaccinated controls) predominantly female lambs were selected for the trial. While all selected lambs on P1 and P2 were females, selected lambs from P3 included 142 wethers. Vaccinates and controls on each property were grazed together throughout the trial period. To increase the likelihood of *Map* challenge, the trial sheep were depastured in paddocks previously grazed by older sheep, some which were clinically affected. Also, suspected clinical cases from the remainder of the flock were regularly put in with the trial sheep.

3.2 VACCINATION

Randomly selected lambs (200 on each property) were vaccinated with Gudair^{TM} (CZ Veterinaria, Porrino, Spain) when they were 1-3 months old, while control lambs (200 on each property) were sham vaccinated with physiological saline. One ml of either vaccine or saline was administered to the lambs by sub-cutaneous injection high on the neck behind the ear using a 6 mm needle. On P1, spring 1999 born lambs were vaccinated in December 1999,

while on P2 and P3, autumn 2000 born lambs were vaccinated in June 2000. Following vaccination, all lambs were given fly strike preventive treatment over the head and neck.

3.3 IMMUNE RESPONSES

Antibody and IFN-γ responses in vaccinates and controls were assessed at different time points for up to 54 months post-vaccination. The time-points varied between the properties. On P1, immunological responses were first assessed at 12 months post-vaccination and subsequently at 18, 24, 30, 42, 54 months post-vaccination. Immunological testing in P2 and P3 commenced at the time of vaccination. Subsequent assessments in P2 were made at 2, 8, 12, 18, 24, 36, 48 months, while in P3 at 2, 8, 12, 18, 24, 36, 42 months post-vaccination. At each time point, 10 ml blood was obtained from each sheep into lithium-heparin tubes and stored at 4˚C until immune responses were assayed.

3.3.1 Antibody responses

Antibody responses in individual sheep at different time points post-vaccination were measured using a commercial kit, Parachek™ (Commonwealth Serum Laboratories, Australia). Plasma harvested from whole blood was transported overnight at 4˚C to the Commonwealth Serum Laboratories (Geelong, Vicroia, Australia) for enzyme immunoassay (EIA) testing. Antibody EIA optical density (OD) values read at 450 nm, resulting from the test were used as a phenotype to describe humoral immunity.

3.3.2 IFN-γ responses

CMI responses in individual sheep at different time points post-vaccination were assessed by measuring IFN-γ production in blood in response to stimulation with *Map* antigens. Stimulation of whole blood with Johnin purified protein derivative (PPD), avian PPD, phosphate buffered saline (nil, negative control) and pokeweed mitogen (positive control), was carried out in separate reactions, within eight hours of blood collection. Stimulated plasma was then consigned to Commonwealth Serum Laboratories at 4˚C overnight for EIA of IFN-γ using the Bovigam™ kit (Commonwealth Serum Laboratories, Australia). For each individual, four different IFN-γ EIA OD values (Johnin, avian, nil and pokeweed) read at 450 nm were obtained. While pokeweed IFN-γ OD values determined the suitability of samples for IFN-γ testing, Johnin-nil (difference between Johnin and nil OD values) and Johnin-avian (difference between Johnin and avian) OD values were employed as two phenotypes for CMI in the current study.

3.4 GENOTYPING

3.4.1 Blood samples

Heparinised whole blood samples (0.5 to 1.0 ml from each animal) were obtained from 934 of the original 1200 sheep that began the trial. These were remnants of samples utilized for immune assays and were under storage at -20˚C for prolonged periods before they were obtained. The current study was not a component of the original vaccine trial. However, a large between-individual variation in the immune responses to vaccination provided an opportunity for this investigation. By the time blood samples were received in New Zealand (late 2003), some of the sheep were either deceased or culled due to various reasons. Hence, of the original 1200 sheep (600 vaccinates and 600 controls) from the three properties, blood samples from only 934 sheep (detailed in table 4) were available.

Table 4: Source of blood samples from control and Johne's vaccinated animals

Property	Controls	Vaccinates	Total
P1	161	171	332
P2	138	165	303
P3	131	168	299
Overall	430	504	934

3.4.2 DNA extraction

Before being received from Australia to New Zealand, the heparinised whole blood samples were in storage at -20°C for prolonged periods. This, together with retention of samples for extended periods for bio-security clearance during transit resulted in low DNA yields. Initial attempts to isolate DNA using DNAzol® (Invitrogen Corporation, Carlsbad, California, USA) were unsuccessful. A column-based isolation kit, QIAMP® DNA blood mini kit (Qiagen Private Limited, Clifton Hill, Victoria, Australia), was found to yield more consistent and higher DNA yields. DNA was isolated as per the kit protocol. In brief, leucocytes in whole blood were lysed by incubation in lysis buffer and protease at 56°C for 10 minutes. DNA in lysate was adsorbed onto silica-gel membrane of spin-column and washed twice with supplied wash buffers. Purified DNA was finally eluted using either the supplied elution buffer or plain autoclaved double-distilled water. The kit protocol advocates 200µl of blood as starting material. However, since leucocyte counts were quite low in most of the blood samples, leucocytes separated from around one ml of blood by centrifugation at 1500 rpm for

30 minutes at 4°C were re-suspended in 200µl of phosphate buffered saline and employed as starting material. Quality and quantity of DNA was assessed following electrophoresis through 0.75% agarose gels.

3.4.3 Genetic markers employed

Genotyping of sheep at eight microsatellite loci was undertaken employing nine markers. Four microsatellites were from *Ovar-Mhc* and two each from SLC11A1 and IFN-γ gene regions. These loci were selected based on reports in the literature regarding their significance in disease resistance. The primer sequences along with the forward primer 5' end fluorescent label for the employed markers, together with the location of the microsatellites they amplify are detailed in table 5. Markers OVINRA1 and NRAMP1 amplify the same microsatellite repeat; however, NRAMP1 alleles are 56 base pairs (bp) larger than the respective OVINRA1 alleles. Due to this known difference in allele length, inclusion of both markers provided an internal control for the genotyping method (detailed in section 3.4.5).

3.4.4 Microsatellite DNA amplification

Annealing temperatures were optimised for amplification of target DNA for different markers. Firstly, the target DNA for each marker was amplified in individual reactions and based on proximities in annealing temperatures and primer compatibility, mutiplex amplifications were tested. It was found that target DNA for the employed nine primer-pairs could be amplified in five PCR reactions. Markers OVINRA1, OVINRA2 and OarKP6 were amplified in one reaction (annealing temperature: 59°C), while markers OLADRB and NRAMP1 were amplified in a different reaction (63°C). Markers DYMS1 and o(IFN)-γ were amplified together (54°C), while markers SMHCC1 (58°C) and OLADRW (54°C) were amplified individually. Each 20µl-PCR reaction consisted of 5 nmol of each dNTP, 37.5-45.0 nmol of $MgCl_2$, 10 pmol each of forward and reverse primers for each marker, 1.25 units of Platinum® Taq DNA polymerase (Invitrogen Corporation, Carlsbad, California, USA), 2 µl of 10X PCR buffer and 50 to 100 ng of template DNA. PCR was performed in a GeneAmp® PCR system 9600 (Applied Biosystems, Foster City, California, USA) with an initial hold at 95°C for 5 min, followed by 30 cycles (94°C for 30 sec; annealing temperature, see table 5, for 30 sec; 72°C for 1 min) and a final extension at 72°C for 7 min. Presence of amplification products and approximate amount of DNA in the PCR products were assessed by running 3.0 µl of each reaction on a 2.5% UltraPure™ agarose 1000 (Invitrogen Corporation, Carlsbad, California, USA) gel.

Table 5: Details of markers employed in the study

Marker*	Gene locus*	Chromo -some*	Position (cM)*†	Repeat	Primers		Annealing temperature	PCR reaction‡	Forward primer 5' label
					Sequences	Reference			
DYMS1	DYA	20	17.9	(CA)n	F: 5'AACAACATCAAACAGTAAGAG3' R: 5'CATAGTAACAGATCTTCCTACA3'	Buitkamp *et al.* (1996)	54ºC	Three	PET™ (Red)
OLADRW	DRB1	20	56.8	(GT)n(GA)m	F: 5'TCTCTGCAGCACATTTCCTGG3' R: 5'CGTACCCAGAGTGAGTGAAGTATC3'	Gruszczynska (1999)	54ºC	Five	VIC® (Green)
OLADRB	DRB2	20	57.7	(AC)n	F: 5'CTGCCAATGCAGAGACACAAGA3' R: 5'GTCTGTCTCCTGTCTTGTCATC3'	Gruszczynska *et al.* (2002b)	63ºC	Two	NED™ (Yellow)
SMHCC1	MHC1	20	60.6	(CA)n	F: 5'ATCTGGTGGGCTACAGTCCATG3' R: 5'GCAATGCTTTCTAAATTCTGAGGAA3'	Groth and Wetherall (1994)	58ºC	Four	FAM™ (Blue)
NRAMP1	SLC11A1	2	248.5	(TG)n	F: 5'GATGAGTGGGCACAGTGGCCT3' R: 5'TTCAAGTGTCTTATTTACACCCATTG3'	Matthews and Crawford (1998)	63ºC	Two	FAM™ (Blue)
OVINRA1	SLC11A1	2	248.5	(TG)n	F: 5'GCCACGGGTGGGATGAGT3' R: 5'TGAGCTAGGAGATAGCAGG3'	Pitel *et al.* (1996)	59ºC	One	VIC® (Green)
OVINRA2	SLC11A1	2	248.5	(GT)n	F: 5'GGGACACTGAGCAGGACA3' R: 5'CCATAGGGAGAGTCTTAGGT3'	Pitel *et al.* (1996)	59ºC	One	FAM™ (Blue)
o(IFN)-γ	IFNG	3	192.1	TGT(GT3)5 or 6	F: 5'TTGTGACTGTTAGCTAGATGTGTT3' R: 5'ATACACATATTATGCCCATCTTTT3'	Schmidt *et al.* (1996)	54ºC	Three	NED™ (Yellow)
OarKP6	IFNG	3	192.1	(AC)n	F: 5'GCCCTGTGTCTCGTGTAACTCAC3' R: 5'CCACAGGGTTGCAAAGAATCA3'	Paterson and Crawford (2000)	59ºC	One	NED™ (Yellow)

* Marker, locus and chromosome details as per the Australian sheep gene mapping website, available online at http://rubens.its.unimelb.edu.au/~jillm/jill.htm (last accessed 15/01/2007)

† Positions of marker loci on respective chromosomes are sex-averaged values; ‡ Nine markers were amplified in five different PCR reactions

F = forward primer; R = reverse primer

3.4.5 Determining PCR product lengths and scoring genotypes

Fluorescent labeling at the 5' end of forward primers for the nine markers with four different dyes facilitated automated length determination of the PCR products employing an ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). For each individual, PCR products from the five different PCR reactions were diluted and pooled based on their DNA concentrations and 1.0 µl of the pooled product used for the capillary run. Resulting chromatogram data from the genetic analyzer were analyzed using ABI Prism® GeneMapper™ software version 3.7 (Applied Biosystems, Foster City, California, USA), to obtain the individual genotypes for all the markers, based on the amplified product lengths. Genotypes from 771 and 865 sheep out of the 934 DNA samples were recovered for the NRAMP1 and OVINRA1 marker loci, respectively. Genotypes were available for both the loci in 741 individuals and in each of those individuals, NRAMP1 alleles were longer exactly by 56 bp than corresponding OVINRA1 alleles. Hence, in 30 individuals having exclusive amplification for NRAMP1, genotypes were assigned to OVINRA1 locus based on corresponding NRAMP1 genotypes, increasing the number of available OVINRA1 genotypes to 895. Since these two markers amplify the same microsatellite region, only OVINRA1 genotypes were employed for further analyses.

Prior to the actual genotyping study, exactness of genotype scoring in the pooled PCR products was validated as follows. Individual amplifications were carried out for the nine markers, employing ten randomly chosen DNA samples and the lengths of resulting products determined individually in the genetic analyzer. From the same 10 DNA templates, amplification of the nine marker regions was also carried out in five PCR reactions as detailed in table 5 and the resulting products pooled and analyzed as a single run in the analyser for length determination. Chromatogram data from the two methods resulted in identical genotypes at all the nine marker loci for each of the ten individuals.

3.5 STATISTICAL ANALYSES

3.5.1 Immune responses

Immune response data pertaining to 934 vaccinated and control animals (detailed in table 4) were kindly provided by the Department of Primary Industries, NSW, Australia. They included antibody and IFN-γ responses recorded at different time-points post-vaccination. Two measures of IFN-γ responses were available - IFN-γ (Johnin-nil) and IFN-γ (Johnin-

avian). The antibody and IFN-γ responses that were in the form of EIA OD values were analysed as detailed below.

3.5.1.1 Effect of group and time

The effect of group and time on each of the immune response variables was tested separately for each property employing PROC MIXED procedure in SAS® 9.1 (SAS Institute Inc. 2004). The linear mixed model (Littell *et al.* 1998) included the fixed effects of treatments (vaccinates and controls), time (different time-points between 0 and 54 months post-vaccination) and their interaction, and random intercepts for animals within group. The covariance error structure for repeated measures over time-points within animals within group was determined using Akaikes information criterion (AIC). A first-order auto-regressive model (AR 1) was found to be the most appropriate error structure. Least square means (LSM) along with standard errors (SE) were obtained for group, time-points and their interactions and were used in multiple comparisons among treatment effects. Graphs were plotted in Excel™ so as to denote the trends of mean antibody and IFN-γ responses over time in vaccinates and controls at each of the three properties. SAS code employed to execute the model is given below.

```
Proc Mixed Data=one;
Class Animal Group Time;
Model Phenotype=Group Time Group*Time;
Random Intercept / subject=Animal Group=Group;
Repeated / type=AR(1)subject=Animal Group=Group;
Lsmeans Group Time Group*Time/Pdiff;
Quit;
```

3.5.1.2 Correlations among immune responses

Correlations among antibody, IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) levels recorded at different time-points post-vaccination in vaccinates of three properties were fitted in SPSS® 13.0 (SPSS Inc., Chicago, IL, USA). The output correlation matrix for each phenotype measure contained Pearson's correlations between different time points, along with their respective 2-tailed *P* values. Correlations between antibody and IFN-γ responses at each time-point in vaccinates were also calculated separately for each property, using SPSS® 13.0.

3.5.2 Genetic analyses

3.5.2.1 Estimating allelic and genotypic frequencies

Individual genotype data obtained from analysis of chromatograms using GeneMapper™ software was subjected to analysis employing the Proc Allele procedure in SAS/Genetics™ (SAS Institute Inc. 2002) in order to obtain allelic and genotypic frequencies for the markers in different properties. Also, measures of marker informativeness - polymorphic information content (PIC), heterozygosity (observed) and allelic diversity (expected heterozygosity) were similarly obtained.

3.5.2.2 Tests for Hardy-Weinberg equilibrium and linkage disequilibrium

Genotypic frequencies were tested for evidence of departure from Hardy-Weinberg equilibrium (HWE) for each marker on each property as well as for the three properties together, employing a Markov chain method providing an exact test (Guo and Thompson 1992) in GENEPOP 3.4 software (available online at http://wbiomed.curtin.edu.au/genepop/index.html), which is an updated version of GENEPOP 1.2 (Raymond and Rousset 1995a). The test results were found to be identical to those of a permutation version of the same test in the Proc Allele procedure of SAS/Genetics™. Since exact *P*-values associated with a null hypothesis of equilibrium were significant for some of the markers on certain properties, two alternative hypotheses, one for an excess of heterozygotes and the other for heterozygote deficiency were tested employing the score (U) test in GENEPOP 3.4. Within-individual correlations between two uniting gametes at each locus, F_{IS} (Weir and Cockerham 1984), were also estimated for each property and over all properties, using GENEPOP 3.4. Linkage disequilibrium (LD) among chromosome-wise marker loci (MHC, SLC11A1 and IFNG chromosome regions) was tested employing Fisher's exact test in GENEPOP 3.4.

3.5.2.3 Genetic differentiation of properties

Similarities in allelic as well as genotypic frequencies at each marker locus, among the three properties and between controls and vaccinates, within each property, were tested in GENEPOP 3.4. While allelic similarity was assessed based on Fisher's exact test (Raymond and Rousset 1995b), a log-likelihood (G) based exact test (Goudet *et al.* 1996) was used to compare genotypic frequencies.

3.5.2.4 Chromosome-wise haplotype analyses

Chromosome-wise marker haplotypes were worked out by employing PROC HAPLOTYPE procedure in SAS/Genetics™. Markers DYMS1, OLADRB, OLADRW and SMHCC1 are located within the *Ovar-Mhc* region on chromosome 20. Markers OVINRA1 and OVINRA2 are located within the SLC11A1 gene on chromosome 2, while markers o(IFN)-γ and OarKP6 are located within the IFN-γ gene on chromosome 3. Allelic details pertaining to the chromosome-wise marker loci for individuals within each property were inputted, along with group of the individual (controls or vaccinates). The PROC HAPLOTYPE procedure uses the expectation-maximization (EM) algorithm to generate maximum likelihood estimates of haplotype frequencies. Apart from haplotype frequencies, the programme also outputs individual's genotype with each of the possible haplotype pairs that can comprise the genotype, and the probability the genotype can be resolved into each of the possible haplotype pairs.

3.5.3 Genetic effects on immune responses

3.5.3.1 Effect of marker genotypes on immune responses

Distributions of antibody as well as IFN-γ responses at each time-point were positively-skewed. Hence, antibody, IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) OD values were log (OD+0.00001) transformed so as to normalize the data. The effects of genotypes were tested separately for each marker locus within each property, employing the PROC MIXED procedure in SAS® 9.1. For each marker, only genotypes occurring in at least five individuals each of vaccinated and control groups were evaluated for their effects. Animals with less frequent genotypes were excluded from the analysis. The employed model was as follows.

$$Y_{ijkl} = Group_i + Genotype_j + Time_k + (Group*Genotype)_{ij} + (Group*Time)_{ik} + \sum_{m=0}^{3} \alpha_m \theta_m(Animal_{ijkl}) + E_{ijkl}, \text{ where,}$$

Y_{ijkl} = phenotype (antibody or IFN-γ response) in logarithmic scale

Group, i = 1, 2 (1 = controls, 2 = vaccinates)

Genotype, j = 1, 2, 3, …. n (number of genotypes varied for each marker within a property)

Time, k = 0, 2, …. 54 months post-vaccination (time points varied between properties)

α_m = random regression coefficient

Θ_m = corresponding transformed time (Legendre) for $animal_l$

E_{ijkl} = residual error assumed to have an auto-regression structure for $animal_l$, within a $group_i$

An auto-regressive model of first order (AR 1) was found to be the most appropriate error structure for repeated measures, based on AIC values. Least square means (LSM) along with standard errors (SE) were obtained for group, genotype, time, group*genotype and group*time and were used in multiple comparisons among different treatment effects. For graphical presentations, LSM in log-scale were back-transformed to the observed scale. Generalized SAS code employed to execute the model is given below.

```
Proc Mixed Data=one Method=ML;
Class Animal Group Genotype Time;
Model logPhenotype=Group Genotype Time Group*Genotype Group*Time;
Random lt0 lt1 lt2 lt3 / subject=Animal Group=Group;
Repeated / type=AR(1)subject=Animal Group=Group;
Lsmeans Group Genotype Time Group*Genotype Group*Time/Pdiff;
Quit;
```

Time Legendres lt0, lt1, lt2 and lt3 were computed as below.

```
lt0=1/SQRT(2)
lt1=SQRT(3/2)*xt
lt2=(SQRT(45/8)*(xt*xt))-(SQRT(5/8))
lt3=0.5*((5*xt*xt*xt)-(3*xt)), where
xt=(2*time-(HT+LT))/(HT-LT), HT and LT being highest and lowest time-points
```

3.5.3.2 Effect of marker alleles on immune responses

The effects of presence versus absence of marker alleles were determined separately for each allele at each locus within each property, employing PROC MIXED procedure in SAS® 9.1. For each marker, only alleles occurring in at least six individuals each of vaccinated and control groups were evaluated for their effects. For determining the effect of a particular allele, individuals possessing at least a single copy of that allele were classified as 'allele 1' (allelic presence), while the individuals that lacked at least a single copy of the allele as 'allele 2' (allelic absence). The employed model was as follows.

$$Y_{ijkl} = Group_i + Allele_j + Time_k + (Group*Allele)_{ij} + (Group*Time)_{ik} + \sum_{m=0}^{3} \alpha_m\theta_m(Animal_{ijkl}) + E_{ijkl}, \text{ where,}$$

Y_{ijkl} = phenotype (antibody or IFN-γ response) in logarithmic scale

Group, i = 1, 2 (1 = controls, 2 = vaccinates)

Allele, j = 1, 2 (1 = allelic presence, 2 = allelic absence)

Time, k = 0, 2, …. 54 months post-vaccination (time points varied between properties)

α_m = random regression coefficient

Θ_m = corresponding transformed time (Legendre) for $animal_l$

E_{ijkl} = residual error assumed to have an auto-regression structure for $animal_l$, within a $group_i$

An auto-regressive model of first order (AR 1) was found to be the most appropriate error structure for repeated measures, based on AIC values. Least square means (LSM) along with standard errors (SE) were obtained for group, allele, time, group*allele and group*time and were used in multiple comparisons amongst different treatment effects. For graphical presentations, LSM in log-scale were transformed back to normal scale. The generalized SAS code employed to determine the effect of an allele at a marker locus is given below.

```
Proc Mixed Data=one Method=ML;
Class Animal Group Allele Time;
Model logPhenotype=Group Allele Time Group*Genotype Group*Time;
Random lt0 lt1 lt2 lt3 / subject=Animal Group=Group;
Repeated / type=AR(1)subject=Animal Group=Group;
Lsmeans Group Allele Time Group*Allele Group*Time/Pdiff;
Quit;
```

Time Legendres lt0, lt1, lt2 and lt3 were computed as explained in section 3.5.3.1.

3.5.3.3 Effect of chromosome-wise haplotypes on immune responses

Because of high numbers of probable MHC haplotypes on all three properties, the effects of MHC haplotypes were not tested. Effects of SLC11A1 and IFN- γ haplotypes on antibody and IFN- γ responses were tested employing the PROC MIXED procedure in SAS® 9.1. For each individual, possible haplotype pairs that could comprise the genotype, along with the probabilities that the genotype could be resolved into each of the possible haplotype pairs, were obtained from haplotype analysis detailed in section 3.5.2.4. Probabilities of haplotypes featuring in different possible haplotype pairs for each individual were pooled separately for each haplotype. For an individual having only one possible haplotype pair that included a particular haplotype in homozygous condition, the pooled probablility of that haplotype in the individual was 2. The haplotype probabilities pooled separately for each haplotype in each individual were arranged in the form of a matrix that had rows corresponding to the number

of individuals in the group (vaccinates or controls) and columns corresponding to the number of possible haplotypes. The sum of haplotype probabilities in each row (each individual) was 2. For illustration, possible IFN- γ haplotype pairs (along with their probabilities) for three individuals and their arrangement in the form of matrix are shown below.

Possible haplotype pairs and their probabilities:

Individual	o(IFN)-γ genotype	OarKP6 genotype	Possible haplotype pairs		Probability
1	124/124	200/204	124-200	124-204	1.00
2	--*	200/204	124-200	124-204	0.55
			124-200	128-204	0.01
			124-204	128-200	0.43
			128-200	128-204	0.01
3	124/128	200/202	124-200	128-202	0.65
			124-202	128-200	0.35

* genotype unavailable

Pooled haplotype probabilities arranged in the form of a matrix:

Individual	Pooled haplotype probabilities					
	124-200	124-202	124-204	128-200	128-202	128-204
1	1.00	0.00	1.00	0.00	0.00	0.00
2	0.56	0.00	0.98	0.44	0.00	0.02
3	0.65	0.35	0.00	0.35	0.65	0.00

Using this haplotype probability matrix, the effects of haplotypes on antibody and IFN- γ responses were analyzed separately for controls and vaccinates of each property. The employed mixed model included the fixed effects of haplotype probabilities and random effects of animals. Covariance error structure for repeated measures over time-points within animals within group was determined based on Akaikes information criterion (AIC). Auto-regressive model of first order (AR 1) was found to be the most appropriate error structure. Differences between different haplotype combinations were tested using estimate and contrast statements. Since the phenotype data (OD readings) were log-transformed to normalize data, haplotype effects were in log-scale and were back-transformed to normal scale for graphical presentations.

4. RESULTS AND DISCUSSION

Data pertaining to humoral and CMI responses to vaccination against *Map*, recorded in a long-term study undertaken on three independent properties in New South Wales, Australia were obtained and analysed. Blood samples from 934 trial sheep were obtained and DNA recovered. Genetic analyses were carried out employing eight microsatellite markers and the effects of marker genotypes/alleles on immune responses determined using suitable statistical procedures. Results from genetic and immune response data analyses, together with the finding of genotype-phenotype association studies, are presented and discussed in this chapter.

4.1 IMMUNE RESPONSES

Individual antibody and IFN-γ responses recorded at different time-points for control as well as vaccinated sheep of the three properties are presented in appendix (tables A7-A12).

4.1.1 Effect of vaccination treatment (group) and time on antibody responses

The effects of group and time were significant ($P<0.01$) on antibody responses in sheep belonging to the three properties. The trends of antibody responses in vaccinates as well as controls of the three properties are depicted in figure 4. Antibody responses in control sheep of all the three properties were fairly constant and remained persistently low throughout the experimental period. In property 1, immunological responses were monitored only between 12 and 54 months post-vaccination. The antibody responses in vaccinates of this property, which were at a maximum at 12 months post-vaccination, decreased with time until 24 months. This was followed by an upsurge between 36 and 42 months and the responses eventually decreased by 54 months. The vaccinates had significantly ($P<0.01$) higher responses than that of controls between 12 to 42 months post-vaccination.

Antibody responses in vaccinates from property 2 peaked by around eight months post-vaccination and decreased progressively until the end of experimental period, except for a small rise at 24 months. Vaccinates on this property had significantly higher ($P<0.01$ between 2 to 36 months and $P<0.05$ at 48 months) antibody levels during 2-48 months post-vaccination, compared to that of controls. Property 3 vaccinates exhibited maximum antibody

levels as early as two months post-vaccination and their levels decreased progressively until 18 months, except for a slight upsurge at 12 months. Their antibody levels remained fairly constant and higher from 18 months till the end of the experimental period (42 months). Antibody levels in vaccinates of property 3 were significantly (P<0.01) higher than those in controls at all time points between 2-42 months.

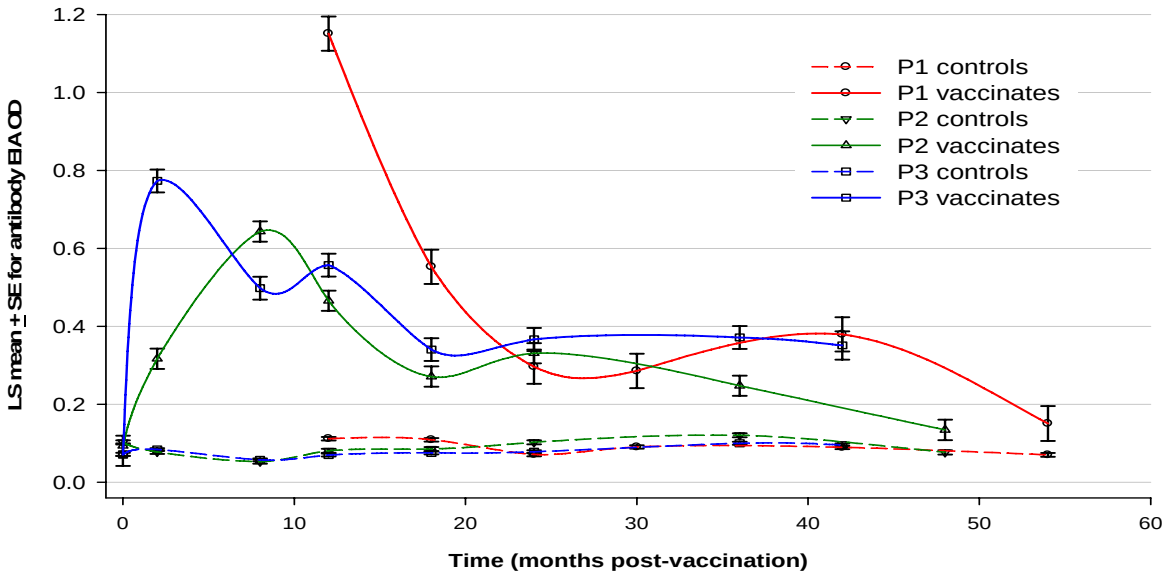

Figure 4: Trends of antibody production in response to Johne's vaccination in sheep belonging to three properties

A critical look at the antibody response trends in vaccinates on the three properties (figure 4) revealed property-specific differences. Antibody responses to vaccination in property 1 were high during the first two years post-vaccination, compared to those seen in properties 2 and 3. Immune responses were monitored from the day of vaccination in properties 2 and 3. Antibody responses in vaccinates on property 3 peaked earlier and higher compared to those in vaccinates on property 2. Also, the responses in property 3 vaccinates were consistently higher than those of property 2 vaccinates, during 12-42 months.

4.1.2 Effect of vaccination treatment (group) and time on IFN-γ responses

Vaccination induced significant Johnin-specific IFN-γ responses in peripheral leukocyte stimulation assays carried out in sheep belonging to the three properties at different time-points post-vaccination. The trends of IFN-γ responses measured as (Johnin-nil) and (Johnin-avian) OD readings in vaccinates as well as controls of the three properties are shown in figures 5 and 6, respectively. It can be seen from the graphs that the trends for IFN-γ (Johnin-

nil) and IFN-γ (Johnin-avian) were quite similar except for minor differences in magnitude, with (Johnin-avian) levels being slightly lower than (Johnin-nil) levels.

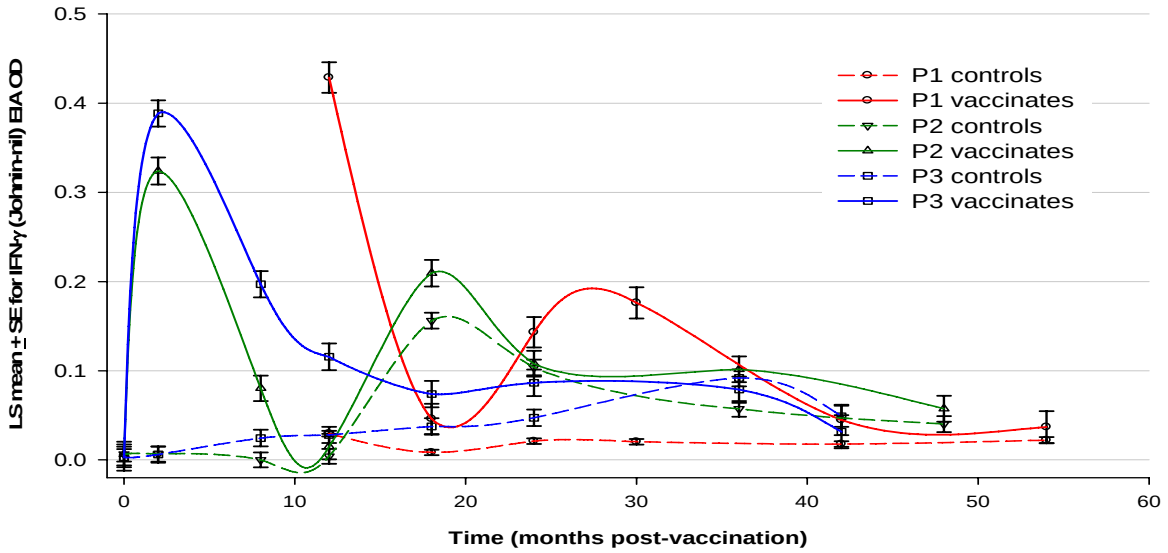

Figure 5: Trends of IFN-γ (Johnin-nil) production in response to Johne's vaccination in sheep belonging to three properties

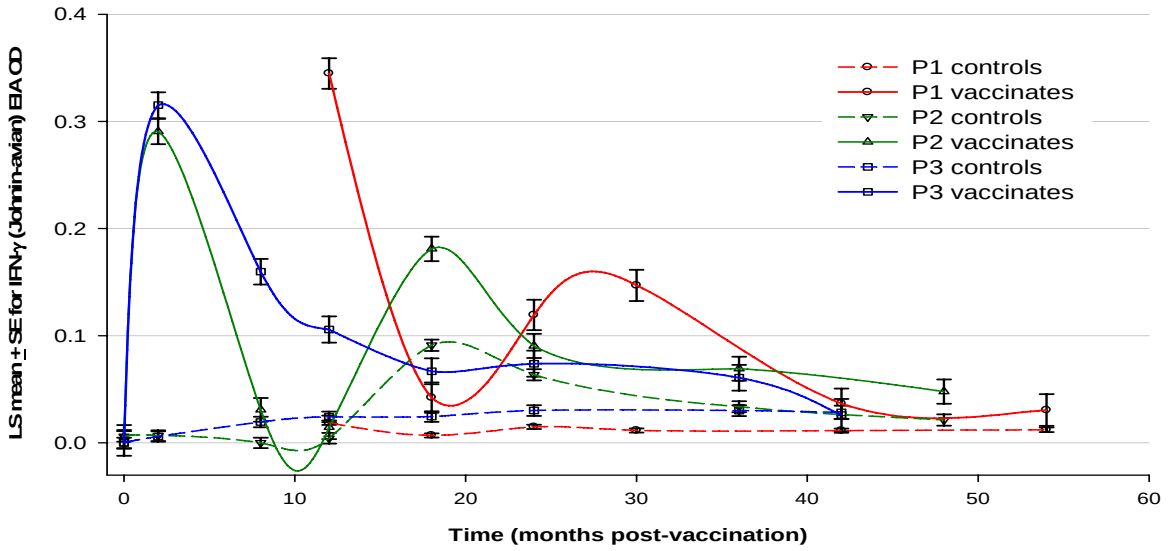

Figure 6: Trends of IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep belonging to three properties

IFN-γ responses in property 1 vaccinates (figures 5 and 6) were higher than those in properties 2 and 3. Their levels at 12 months post-vaccination in property 1 were higher when compared to those observed at any time point in properties 2 and 3. Since no readings were obtained in property 1 within 12 months and considering the likelihood for occurrence of

maximum peak at around 2-8 months (based on trends for properties 2 and 3), the IFN-γ levels in this property at the point of maximum peak were likely to be even higher. A second peak was evident in this property at 30 months following which the IFN-γ levels receded to control group levels by 42 months post-vaccination. IFN-γ levels in vaccinates were found to be significantly ($P<0.01$ at 12, 24 and 30 months, and $P<0.05$ at 18 months) higher at 12-30 months post-vaccination, compared to the control group levels. IFN-γ levels in property 1 controls were consistently low throughout the experimental period.

IFN-γ levels in vaccinates of properties 2 and 3 peaked by two months post-vaccination, with levels in property 3 being slightly higher. However, their trends differed thereafter. In property 2, IFN-γ production returned to baseline level by 12 months and peaked again to a significant level at 18 months, before remaining consistently high for rest of the experimental period. In property 3, the IFN-γ production decreased by 12 months to a lower but still significantly higher level (compared to control group level) that persisted for up to 24 months, before returning to baseline level by 42 months post-vaccination.

Unvaccinated controls of property 1 had consistently low IFN-γ (both Johnin-nil and Johnin-avian) responses throughout the experimental period. In contrast to this, controls of properties 2 and 3 had significantly peaked (Johnin-nil) levels at 18 and 36 months, respectively. It is likely that natural infection in the flocks might have caused these spikes. This can be substantiated by the fact that control sheep on properties 2 and 3 had high prevalences of shedding *Map* in faceces during the said periods (Reddacliff *et al*. 2006). However, it is interesting to note the absence of such peak for Johnin-avian responses in controls on property 3, while the Johnin-avian responses in property 2 controls peaked to a much lower level (compared to Johnin-nil) at 18 months. This suggests that the sheep on those two properties may have also been exposed to environmental *Mycobacterium avium* species.

4.1.3 Correlations among immune responses

4.1.3.1 Correlations among antibody levels

Correlations among antibody levels in vaccinates at different time-points post-vaccination in the three properties are presented in table 6. Within each property, the correlations of antibody levels in vaccinates at a particular time-point post-vaccination with those observed at subsequent time-points were high and significant ($P<0.01$). Correlations were particularly high in property 1, while all correlations with 12 months in property 3 vaccinates were

moderate but significant (P<0.01). In general, the correlations between antibody levels at successive time-points increased with progression of time in all the three properties. High correlations between time-points suggested consistency of antibody production in sheep in response to vaccination. It implies that high and low responder sheep consistently produced either more or less antibodies, respectively, throughout the experimental period. The antibody levels in vaccinates in properties 2 and 3, just prior to vaccination, were quite low (almost zero) and no different from that in controls. Hence, it is obvious that the correlations of pre-vaccination antibody levels in these properties with those at subsequent time-points post-vaccination were quite low and non-significant (P>0.05).

Table 6: Correlations among antibody levels recorded at different time-points post-vaccination in vaccinates belonging to three properties

Property 1							
	Ab18	Ab24	Ab30	Ab42	Ab54		
Ab12	.910**	.632**	.839**	.822**	.806**		
Ab18		.599**	.887**	.829**	.801**		
Ab24			.772**	.726**	.766**		
Ab30				.905**	.915**		
Ab42					.947**		
Property 2							
	Ab2	Ab8	Ab12	Ab18	Ab24	Ab36	Ab48
Ab0	-.162*	-.119	-.106	-.118	-.128	-.111	-.115
Ab2		.402**	.363**	.351**	.412**	.374**	.306**
Ab8			.689**	.684**	.648**	.605**	.557**
Ab12				.900**	.896**	.818**	.753**
Ab18					.933**	.856**	.741**
Ab24						.868**	.808**
Ab36							.811**
Property 3							
	Ab2	Ab8	Ab12	Ab18	Ab24	Ab36	Ab48
Ab0	-.076	.012	-.027	-.026	.024	-.027	-.052
Ab2		.728**	.315**	.649**	.542**	.677**	.607**
Ab8			.356**	.851**	.709**	.746**	.799**
Ab12				.377**	.338**	.292**	.396**
Ab18					.777**	.888**	.911**
Ab24						.757**	.775**
Ab36							.918**

Ab = Antibody OD at different months post-vaccination

** = correlation highly significant (P<0.01); * = correlation significant (P<0.05)

4.1.3.2 Correlations among IFN-γ responses

Correlations among IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) levels recorded at different time-points in vaccinates of three properties are presented in tables 7 and 8, respectively. As seen in antibody levels, post-vaccination IFN-γ (Johnin-nil) levels at a given point within each property are in general significantly correlated with those at subsequent time-points (table 7). It implies that high and low responder sheep consistently had either more or less

Table 7: Correlations among IFN-γ (Johnin-nil) levels recorded at different time-points post-vaccination in vaccinates of three properties

Property 1							
	(J-n)18	(J-n)24	(J-n)30	(J-n)42	(J-n)54		
(J-n)12	.403**	.458**	.373**	.377**	.313**		
(J-n)18		.316**	.397**	.187**	.194**		
(J-n)24			.627**	.378**	.255**		
(J-n)30				.223**	.466**		
(J-n)42					0.125		
Property 2							
	(J-n)2	(J-n)8	(J-n)12	(J-n)18	(J-n)24	(J-n)36	(J-n)48
(J-n)0	-.138	-.114	-.161*	.009	.001	.204*	.139
(J-n)2		.508**	.136	.469**	.340**	.181*	.275**
(J-n)8			.497**	.505**	.349**	.226**	.232**
(J-n)12				.300**	.158*	0.087	0.054
(J-n)18					.479**	.232**	.349**
(J-n)24						.482**	.269**
(J-n)36							.589**
Property 3							
	(J-n)2	(J-n)8	(J-n)12	(J-n)18	(J-n)24	(J-n)36	(J-n)42
(J-n)0	.009	-.06	-.061	-.064	-.006	-.074	-.114
(J-n)2		.616**	.379**	.335**	.237**	.197**	.231*
(J-n)8			.551**	.559**	.429**	.577**	.461**
(J-n)12				.466**	.376**	.400**	.399**
(J-n)18					.654**	.601**	.391**
(J-n)24						.332**	.339**
(J-n)36							.534**

(J-n) = IFN-γ (Johnin-nil) OD at different months post-vaccination

** = correlation highly significant (P<0.01); * = correlation significant (P<0.05)

Table 8: Correlations among IFN-γ (Johnin-avian) levels recorded at different time-points post-vaccination in vaccinates of three properties

(J-a) and (J-a)							
	(J-a)18	(J-a)24	(J-a)30	(J-a)42	(J-a)54		
(J-a)12	.434**	.463**	.416**	.389**	.299**		
(J-a)18		.305**	.405**	.211**	.172*		
(J-a)24			.627**	.368**	.256**		
(J-a)30				.222**	.401**		
(J-a)42					.131		
(J-a) and (J-a)							
	(J-a)2	(J-a)8	(J-a)12	(J-a)18	(J-a)24	(J-a)36	(J-a)48
(J-a)0	-.153	-.097	-.176*	.01	-.053	.021	.065
(J-a)2		.486**	0.124	.456**	.384**	.370**	.290**
(J-a)8			.496**	.479**	.397**	.417**	.268**
(J-a)12				.282**	.133	.15	.055
(J-a)18					.510**	.417**	.369**
(J-a)24						.568**	.262**
(J-a)36							.529**
(J-a) and (J-a)							
	(J-a)2	(J-a)8	(J-a)12	(J-a)18	(J-a)24	(J-a)36	(J-a)42
(J-a)0	-.062	-.073	-.048	-.049	-.036	-.053	-.102
(J-a)2		.605**	.380**	.334**	.232**	.207**	.215*
(J-a)8			.568**	.546**	.427**	.616**	.473**
(J-a)12				.470**	.387**	.440**	.404**
(J-a)18					.699**	.627**	.412**
(J-a)24						.389**	.372**
(J-a)36							.612**

(J-a) = IFN-γ (Johnin-avian) OD at different months post-vaccination

** = correlation highly significant (P<0.01); * = correlation significant (P<0.05)

IFN-γ responses, respectively, throughout the experimental period. However, the magnitude of correlations was comparatively less when compared to those seen for antibody responses. There was no correlation between IFN-γ (Johnin-nil) levels recorded prior to vaccination and those at subsequent time-points post-vaccination. Similar trends in correlations at different time points were also observed for IFN-γ (Johnin-avian) responses (table 8).

Correlations between IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) at each of time-points in the three properties were also estimated (table 9). On all three properties, there was quite a high and significant (P<0.01) correlation between the two IFN-γ phenotypes measured at each of the post-vaccination time-points. Thus animals with high and low IFN-γ (Johnin-nil) responses at a given time-point also exhibited high and low IFN-γ (Johnin-avian) levels, respectively at the same time-point. The observed high correlation between the two IFN-γ assays as a measure of CMI responses to *Map* vaccination indicates that IFN-γ (Johnin-nil) assay alone may be adequate to assess CMI to *Map* infections, if there is no exposure to environmental *Mycobacterium avium* species. However, a detailed study on the effect of environmental mycobacteria on CMI responses would need to be undertaken to confirm this.

Table 9: Correlations between IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) levels recorded at different time-points post-vaccination in vaccinates of three properties

Property 1		*Property 2*		*Property 3*	
IFN-γ responses	r	IFN-γ responses	r	IFN-γ responses	r
(J-n)12 and (J-a)12	.989**	(J-n)0 and (J-a)0	.812**	(J-n)0 and (J-a)0	.442**
(J-n)18 and (J-a)18	.997**	(J-n)2 and (J-a)2	.996**	(J-n)2 and (J-a)2	.993**
(J-n)24 and (J-a)24	.986**	(J-n)8 and (J-a)8	.981**	(J-n)8 and (J-a)8	.989**
(J-n)30 and (J-a)30	.985**	(J-n)12 and (J-a)12	.953**	(J-n)12 and (J-a)12	.996**
(J-n)42 and (J-a)42	.945**	(J-n)18 and (J-a)18	.992**	(J-n)18 and (J-a)18	.993**
(J-n)54 and (J-a)54	.957**	(J-n)24 and (J-a)24	.963**	(J-n)24 and (J-a)24	.981**
		(J-n)36 and (J-a)36	.665**	(J-n)36 and (J-a)36	.982**
		(J-n)48 and (J-a)48	.958**	(J-n)42 and (J-a)42	.963**

r = Pearson's coefficient of correlation

(J-n) = IFN-γ (Johnin-nil) OD at different months post-vaccination

(J-a) = IFN-γ (Johnin-avian) OD at different months post-vaccination

** = correlation highly significant (P<0.01)

4.1.3.3 Correlations between antibody and IFN-γ responses

Correlations of antibody responses with IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) responses at different time-points in vaccinated sheep of three properties are presented in tables 10 and 11, respectively. The correlations in properties 1 (at all time-points) and 3 (at all

time-points except at 12 months) were almost zero (P>0.05). This implies that animals eliciting strong and weak antibody responses at a given time point need not necessarily elicit strong and weak IFN-γ response, respectively at the same time-point. Alternatively, it can be interpreted that antibody and IFN-γ responses observed at each time-point in vaccinated sheep are independent of each other. In contrast to that seen in properties 1 and 3, moderate and significant (P<0.05) correlations were detected in property 2 between antibody and IFN-γ (Johnin-nil) levels at 2, 8, 18, 24, 36 and 48 months, and between antibody and IFN-γ (Johnin-avian) levels at 2, 18 and 24 months post-vaccination.

Table 10: Correlations between antibody and IFN-γ (Johnin-nil) levels recorded at different time-points post-vaccination in vaccinates of three properties

Property 1		*Property 2*		*Property 3*	
Immune responses	r	Immune responses	r	Immune responses	r
Ab12 and (J-n)12	-.035	Ab0 and (J-n)0	.007	Ab0 and (J-n)0	-.063
Ab18 and (J-n)18	.011	Ab2 and (J-n)2	.313**	Ab2 and (J-n)2	-.052
Ab24 and (J-n)24	.004	Ab8 and (J-n)8	.217**	Ab8 and (J-n)8	-.004
Ab30 and (J-n)30	-.035	Ab12 and (J-n)12	.079	Ab12 and (J-n)12	.204*
Ab42 and (J-n)42	.008	Ab18 and (J-n)18	.286**	Ab18 and (J-n)18	.106
Ab54 and (J-n)54	.094	Ab24 and (J-n)24	.213**	Ab24 and (J-n)24	.087
		Ab36 and (J-n)36	.301**	Ab36 and (J-n)36	.119
		Ab48 and (J-n)48	.184*	Ab42 and (J-n)42	.189

r = Pearson's coefficient of correlation; Ab = Antibody OD at different months post-vaccination

(J-n) = IFN-γ (Johnin-nil) OD at different months post-vaccination

** = correlation highly significant (P<0.01); * = correlation significant (P<0.05)

Table 11: Correlations between antibody and IFN-γ (Johnin-avian) levels recorded at different time-points post-vaccination in vaccinates of three properties

Property 1		*Property 2*		*Property 3*	
Immune responses	r	Immune responses	r	Immune responses	r
Ab12 and (J-a)12	-.022	Ab0 and (J-a)0	.041	Ab0 and (J-a)0	-.044
Ab18 and (J-a)18	.018	Ab2 and (J-a)2	.301**	Ab2 and (J-a)2	-.068
Ab24 and (J-a)24	-.008	Ab8 and (J-a)8	.191*	Ab8 and (J-a)8	-.031
Ab30 and (J-a)30	-.009	Ab12 and (J-a)12	.056	Ab12 and (J-a)12	.205**
Ab42 and (J-a)42	.009	Ab18 and (J-a)18	.282**	Ab18 and (J-a)18	.096
Ab54 and (J-a)54	.128	Ab24 and (J-a)24	.235**	Ab24 and (J-a)24	.075
		Ab36 and (J-a)36	.156	Ab36 and (J-a)36	.075
		Ab48 and (J-a)48	.154	Ab42 and (J-a)42	.18

r = Pearson's coefficient of correlation; Ab = Antibody OD at different months post-vaccination

(J-a) = IFN-γ (Johnin-avian) OD at different months post-vaccination

** = correlation highly significant (P<0.01); * = correlation significant (P<0.05)

4.2 MARKER ALLELES, GENOTYPES AND HAPLOTYPES

Genotypic results are summarized in tables 12-15. Individual genotypes recorded at eight microsatellite marker loci for control as well as vaccinated sheep of the three properties are presented in the appendix (tables A1-A6). Details of frequencies of marker alleles (tables A13-A15) and genotypes (tables A16-A21) are also listed in the appendix. A description of allelic and genotypic polymorphisms, evidence for departure from HWE, or chromosome-wise LD among the markers is presented below.

4.2.1 Allelic and genotypic frequencies

4.2.1.1 Markers located in the *Ovar-Mhc* region

The four markers from the MHC region were highly polymorphic in all three properties (table 12). Twenty one alleles were observed for marker DYMS1 (tables 12 and A13). This is consistent with the only available report (Buitkamp *et al.* 1996) of 19 alleles for the marker in Scottish Blackface sheep. In the current study, the highest number of alleles (42) was recorded at the marker locus OLADRW (tables 12 and A14). The primers employed (Gruszczynska 1999) for this marker amplify the microsatellite in intron 2, together with exon 2 of the functional DRB1 gene. Using these primers, 36 alleles were reported in Polish Heath flock (Gruszczynska *et al.* 2000) and German Merino flock (Gruszczynska 1999), although only 20 alleles were identified in a different Polish Heath flock (Charon *et al.* 2002). With primers amplifying just the microsatellite region in intron 2, fewer alleles were detected in Soay (8; Paterson 1998), Merino (16; Bot *et al.* 2004) and wild (14; Worley *et al.* 2006) sheep.

The OLADRB marker locus, within the pseudogene DRB2, was the least polymorphic among the four MHC marker loci investigated in the current study, with 12 alleles over the three properties (tables 12 and A13). A similar number of alleles (11) was reported for Merino (Blattman and Beh 1992) and Polish Heatherhead (Gruszczynska *et al.* 2002b) breeds. While 23 OLADRB alleles were identified in 900 wild sheep (Worley *et al.* 2006), a mere 6 alleles were scored in 887 Soay sheep (Paterson 1998). Marker SMHCC1, located within a class I gene, showed 16 alleles over the three properties, in the current study (tables 12 and A13). This number is the highest for the marker locus when compared to the reported 12 alleles in Scottish Blackface sheep (Buitkamp *et al.* 1996), 13 in Polish Heath (Gruszczynska *et al.* 2002a), 11 in wild sheep (Worley *et al.* 2006) and 5 in Soay sheep (Paterson 1998).

A notable feature with regard to the four MHC marker loci in the current study is the presence of rare alleles (<1.0% frequency; table 12). Over the three properties, as many as 30 out of the 42 OLADRW, 9 out of the 21 DYMS1, 5 out of the 16 SMHCC1 and 3 out of the 12 OLADRB alleles were rare. These figures indicate that microsatellite mutation rate varied between loci within the MHC, with the mutation rate at OLADRW and DYMS1 considerably higher in comparison to that at SMHCC1 and OLADRB. Such rare alleles also featured in some of the earlier studies (Buitkamp *et al.* 1996; Gruszczynska 1999; Gruszczynska *et al.* 2000; Charon *et al.* 2002). It had been suggested that individuals with rare antigen-binding abilities should be more capable of combating diseases than individuals with common antigen-binding capabilities and thus, positive selection for rare alleles would lead to the occurrence rare MHC alleles (Potts 2002; Borghans *et al*. 2004; Knapp 2007). Although the microsatellite regions investigated in the current study are themselves non-coding, it is likely that positive selection at linked adjacent coding regions might have resulted in rare alleles.

The high level of allelic polymorphism at the four MHC marker loci was also reflected in their genotype diversity (table 12), with markers OLADRW and OLADRB having the maximum (137; table A19) and minimum (47; table A17) genotypes, respectively. Markers SMHCC1 (table A18) and DYMS1 (table A16) had 76 and 92 genotypes respectively. One hundred and eleven genotypes at OLADRW, 58 at DYMS1, 50 at SMHCC1 and 23 at OLADRB loci were rare (<1.0% frequency; table 12).

In general, MHC loci are typically highly (80-90%) heterozygous in humans as well as in other mammals (Hughes and Nei 1988). Heterozygosity, both observed and expected (allelic diversity), and PIC values observed at the MHC marker loci, in the current study, were higher than those at the marker loci located in the SLC11A1 and IFNG regions. Observed overall heterozygosity ranged from 53.58% (OLADRW) to 86.59% (SMHCC1; table 12). The observed heterozygosity for OLADRW is much less than the expected heterozygosity (89%). Heterozygosity greater than 79.6% was reported at this locus in three different studies (Paterson 1998; Gruszczynska 1999; Gruszczynska *et al.* 2000). It is probable that the observed heterozygosity at OLADRW in the present study is underestimated due to preferential amplification of alleles in heterozygote individuals. Heterozygosity, as low as 31.1%, as a result of preferential amplification of alleles in heterozygotes, was recently reported in wild sheep (Worley *et al.* 2006).

Table 12: Allelic and genotypic information pertaining to markers located in the MHC region on chromosome 20

Particulars		DYMS1				OLADRW				OLADRB				SMHCC1			
		P1	P2	P3	O	P1	P2	P3	O	P1	P2	P3	O	P1	P2	P3	O
Number of sheep		301	300	292	893	273	263	261	797	308	289	284	881	313	302	280	895
Number of alleles		20 (7)	15 (5)	11 (2)	21 (9)	32 (15)	22 (9)	20 (11)	42 (30)	11 (2)	9 (2)	9 (2)	12 (3)	15 (5)	14 (4)	13 (5)	16 (5)
Allelic range (bp)		160-210	158-202	164-206	158-210	450-590	442-560	450-560	442-590	264-296	270-296	264-296	264-296	180-210	176-210	180-210	176-210
Most frequent allele[†]		192 (21.76)	190 (29.50)	202 (24.14)	190 (15.62)	480 (26.19)	480 (24.90)	478 (27.97)	480 (18.44)	276 (29.55)	276 (36.68)	296 (25.00)	276 (29.97)	194 (24.76)	194 (26.16)	194 (23.75)	194 (24.92)
Number of genotypes		75 (40)	51 (24)	43 (13)	92 (58)	91 (64)	64 (41)	46 (23)	137 (111)	43 (17)	30 (8)	27 (9)	47 (23)	54 (29)	52 (22)	39 (17)	76 (50)
Most frequent genotype[†]		186/192 (6.98)	190/196 (8.33)	184/202 (12.67)	184/202 (4.59)	480/480 (17.95)	480/480 (18.25)	478/478 (11.11)	480/480 (12.80)	276/276 (11.36)	276/296 (19.72)	270/296 (14.44)	276/296 (11.46)	192/194 (14.38)	194/202 (8.94)	194/198 (14.29)	192/194 (10.17)
Allelic diversity (%)		87.43	83.60	85.25	88.55	88.17	86.24	83.64	88.97	82.77	77.51	79.76	81.97	83.56	85.91	82.64	85.68
Heterozygosity (%)		83.39	82.33	90.07	85.22	50.18	47.53	63.22	53.58	76.30	79.93	85.92	80.59	86.90	87.75	85.00	86.59
PIC		0.862	0.818	0.836	0.874	0.873	0.849	0.818	0.880	0.808	0.746	0.768	0.798	0.817	0.845	0.804	0.842
F_{is}		0.048	0.017	-0.055	0.004	0.432	0.450	0.246	0.379	0.080	-0.030	-0.075	-0.005	-0.038	-0.020	-0.027	-0.028
HWE test Exact *P*	Ho[A]	0.156[NS]	0.039*	0.026*	0.000**	0.000**	0.000**	0.000**	0.000**	0.041*	0.332[NS]	0.327[NS]	0.000**	0.079[NS]	0.547[NS]	0.069[NS]	0.001**
	H1, Excess[B]	1.000[NS]	0.999[NS]	0.021*	1.000[NS]	1.000[NS]	1.000[NS]	1.000[NS]	1.000[NS]	0.994[NS]	0.170[NS]	0.002**	0.977[NS]	0.045*	0.138[NS]	0.294[NS]	0.279[NS]
	H1, Less[C]	0.000**	0.001**	0.980[NS]	0.000**	0.000**	0.000**	0.000**	0.000**	0.006**	0.820[NS]	0.998[NS]	0.022*	0.947[NS]	0.860[NS]	0.720[NS]	0.764[NS]
LD test Exact *P*	DYMS1	–	–	–	–												
	OLADRW	0.231[NS]	0.192[NS]	0.003**	0.013*	–	–	–	–								
	OLADRB	0.000**	0.395[NS]	0.217[NS]	0.001**	0.000**	0.000**	0.000**	0.000**	–	–	–	–				
	SMHCC1	0.057[NS]	0.005**	0.034*	0.001**	0.000**	0.001**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	–	–	–	–

P1 = property 1; P2 = property 2; P3 = property 3; O = overall for the three properties; bp = base pairs; HWE = Hardy-Weinberg equilibrium; LD = linkage disequilibrium

PIC = polymorphic information content; F_{IS} = F statistic, denoting within-individual correlation between two uniting gametes at each locus

[†] Values in the parentheses denote number of alleles/genotypes with less than one percent frequency

[‡] Values in the parentheses denote percent frequencies of respective alleles/genotypes

[A] Exact probability of being wrong in rejecting HWE hypothesis

[B, C] Exact probabilities of being wrong in rejecting HWE hypothesis because of heterozygote excess or deficiency, respectively

[NS] Non-significant (P>0.05); * significant (P<0.05); ** highly significant (P<0.01)

Observed heterozygosities for DYMS1 and OLADRB were slightly less than those expected, while for SMHCC1, the observed heterozygosity was slightly more than that expected. There are no earlier reports on the heterozygosity at DYMS1 locus in sheep. However, in line with the current findings, heterozygosities of >78% were reported for OLADRB in different breeds (Paterson 1998; Gruszczynska *et al.* 2002b). In case of SMHCC1, heterozygosity >77% was reported in two Polish sheep breeds (Gruszczynska *et al.* 2002a), while in two other studies heterozygosity of less than 58% was observed (Groth and Wetherall 1994; Paterson 1998).

High PIC values (>0.798; table 12) observed in the current study for the four MHC markers indicate their usefulness in genotype-phenotype association studies. Similar PIC values for different MHC markers featured also in different studies (Blattman and Beh 1992; Groth and Wetherall 1994; Gruszczynska *et al.* 2002a; Gruszczynska *et al.* 2002b). Excessive homozygosity at OLADRW locus resulted in a high F-statistic (F_{is}) value (0.379), while the F_{is} values at the other three loci were almost zero (table 12).

4.2.1.2 Markers located in the SLC11A1 gene

The OVINRA1 and OVINRA2 marker loci located in the SLC11A1 gene region, were also highly polymorphic, with 10 and 9 alleles respectively (tables 13 and A15). Their respective genotype numbers were 30 and 24 (tables 13 and A20). Alleles ranging from 7 to 9 were observed at OVINRA1 locus in four different studies (Pitel *et al.* 1996; Matthews and Crawford *et al.* 1998; Reddacliff *et al.* 2005; Worley *et al.* 2006), while only a maximum of 4 alleles could be detected at OVINRA2 locus in two earlier studies (Pitel *et al.* 1996; Worley *et al.* 2006). As seen above for MHC marker loci, rare alleles/genotypes (table 13) also featured at the two marker loci in the SLC11A1 region. One notable feature concerning the OVINRA2 locus is that allele 312 and genotype 312/312 had frequencies of >50% and >40%, respectively, in all the three properties studied (table 13).

While the OVINRA1 locus was highly heterozygous (79%, over the three properties), only 33% of OVINRA2 genotypes were heterozygous (table 13). There was little difference between the observed and expected heterozygosites at OVINRA1. However, only 56% of genotypes at this locus were found to be heterozygous in wild sheep (Worley *et al.* 2006). Though the observed heterozygosity at OVINRA2 is similar to that found in wild sheep (Worley *et al.* 2006), in the current study, the expected heterozygosity is almost double that

of the observed value (table 13). This finding, together with very high F_{is} value (0.457) at the locus strongly suggest preferential amplification of alleles in heterozygotes, as in case of OLADRW discussed earlier. The F_{is} value at the OVINRA1 locus was almost zero (table 13). The OVINRA1 and OVINRA2 microsatellites had PIC values of 0.761 and 0.574 respectively, over the three properties. While a similar PIC value was reported for OVINRA1 in Romney sheep (Matthews and Crawford *et al.* 1998), there are no reports on PIC for OVINRA2 in sheep.

Table 13: Allelic and genotypic information pertaining to markers located in the SLC11A1 gene region on chromosome 2

Particulars		OVINRA1				OVINRA2			
		P1	**P2**	**P3**	**O**	**P1**	**P2**	**P3**	**O**
Number of sheep		316	293	286	895	233	247	241	721
Number of alleles[†]		9 (2)	8 (2)	8 (1)	10 (4)	8 (2)	7 (1)	6 (1)	9 (2)
Allelic range (bp)		150-170	154-168	156-170	150-170	312-328	312-326	310-326	310-328
Most frequent allele[‡]		162 (36.08)	162 (24.57)	162 (36.71)	162 (32.51)	312 (58.15)	312 (50.00)	312 (56.43)	312 (54.79)
Number of genotypes[†]		27 (9)	21 (4)	22 (4)	30 (11)	21 (8)	15 (3)	13 (3)	24 (15)
Most frequent genotype[‡]		162/164 (14.87)	162/164 (14.68)	160/162 (17.48)	162/164 (14.97)	312/312 (41.20)	312/312 (40.89)	312/312 (46.47)	312/312 (42.86)
Allelic diversity (%)		78.16	79.80	76.29	79.04	61.79	62.10	59.57	62.23
Heterozygosity (%)		79.75	77.13	79.37	78.77	41.20	28.34	30.71	33.29
PIC		0.753	0.767	0.729	0.761	0.587	0.555	0.539	0.574
*F*is		-0.019	0.035	-0.039	-0.007	0.335	0.545	0.486	0.4573
HWE test Exact *P*	Ho[A]	0.506 [NS]	0.053 [NS]	0.150 [NS]	0.002**	0.000**	0.000**	0.000**	0.000**
	H1, Excess[B]	0.236 [NS]	0.822 [NS]	0.052 [NS]	0.786 [NS]	1.000 [NS]	1.000 [NS]	1.000 [NS]	1.000 [NS]
	H1, Less[C]	0.747 [NS]	0.168 [NS]	0.948 [NS]	0.261 [NS]	0.000**	0.000**	0.000**	0.000**
LD test Exact *P*	OVINRA1	–	–	–	–				
	OVINRA2	0.000**	0.001**	0.000**	0.000**	–	–	–	–

P1 = property 1; P2 = property 2; P3 = property 3; O = overall for the three properties; bp = base pairs

HWE = Hardy-Weinberg equilibrium; LD = linkage disequilibrium

PIC = polymorphic information content

F_{IS} = F statistic, denoting within-individual correlation between two uniting gametes at each locus

[†] Values in the parentheses denote number of alleles/genotypes with less than one percent frequency

[‡] Values in the parentheses denote percent frequencies of respective alleles/genotypes

[A] Exact probability of being wrong in rejecting HWE hypothesis

[B, C] Exact probabilities of being wrong in rejecting HWE hypothesis because of heterozygote excess or deficiency, respectively

[NS] Non-significant (P>0.05); ** highly significant (P<0.01)

4.2.1.3 Markers located in the IFN-γ gene

Table 14: Allelic and genotypic information pertaining to markers located in the IFNG gene region on chromosome 3

Particulars		o(IFN)γ				OarKP6			
		P1	P2	P3	O	P1	P2	P3	O
Number of sheep		304	294	292	890	311	286	278	875
Number of alleles		2 (0)	2 (0)	2 (0)	2 (0)	11 (7)	12 (8)	9 (4)	14 (9)
Allelic range (bp)		124-128	124-128	124-128	124-128	186-206	180-208	186-206	180-208
Most frequent allele[†]		124 (67.11)	124 (73.30)	124 (84.59)	124 (74.89)	200 (63.83)	200 (45.28)	200 (53.78)	200 (54.57)
Number of genotypes		3 (0)	3 (0)	3 (0)	3 (0)	21 (14)	16 (10)	17 (10)	34 (27)
Most frequent genotype[†]		124/124 (45.72)	124/124 (52.72)	124/124 (71.23)	124/124 (56.40)	200/200 (42.44)	200/204 (36.36)	200/204 (32.01)	200/204 (34.06)
Allelic diversity (%)		44.15	39.14	26.07	37.61	52.51	64.45	60.26	59.89
Heterozygosity (%)		42.76	41.16	26.71	36.97	50.16	62.94	57.55	56.69
PIC		0.344	0.315	0.227	0.305	0.470	0.577	0.538	0.536
Fis		0.033	-0.050	-0.023	-0.009	0.046	0.025	0.047	0.039
HWE test Exact *P*	Ho[A]	0.603 [NS]	0.456 [NS]	0.824 [NS]	0.595 [NS]	0.000**	0.000**	0.000**	0.000**
	H1, Excess[B]	0.759 [NS]	0.244 [NS]	0.447 [NS]	0.738 [NS]	0.983 [NS]	0.995 [NS]	0.995 [NS]	0.998 [NS]
	H1, Less[C]	0.328 [NS]	0.840 [NS]	0.723 [NS]	0.328 [NS]	0.018*	0.007**	0.005**	0.003**
LD test Exact *P*	o(IFN)γ	–	–	–	–				
	OarKP6	0.000**	0.000**	0.001**	0.000**	–	–	–	–

P1 = property 1; P2 = property 2; P3 = property 3; O = overall for the three properties; bp = base pairs

HWE = Hardy-Weinberg equilibrium; LD = linkage disequilibrium

PIC = polymorphic information content

F_{IS} = F statistic, denoting within-individual correlation between two uniting gametes at each locus

[†] Values in the parentheses denote number of alleles/genotypes with less than one percent frequency

[‡] Values in the parentheses denote percent frequencies of respective alleles/genotypes

[A] Exact probability of being wrong in rejecting HWE hypothesis

[B, C] Exact probabilities of being wrong in rejecting HWE hypothesis because of heterozygote excess or deficiency, respectively

[NS] Non-significant (P>0.05); * significant (P<0.05); ** highly significant (P<0.01)

Polymorphism at the o(IFN)γ microsatellite marker has been well studied (Schmidt *et al.* 1996; Crawford and McEwan 1998; Coltman *et al.* 2001; Dukkipati *et al.* 2005; Reddacliff *et al.* 2005; Worley *et al.* 2006). In the current study, two alleles (124 bp and 128 bp) were identified at the locus. While alleles of similar sizes featured in 4 of the cited works (Schmidt *et al.* 1996; Crawford and McEwan 1998; Dukkipati *et al.* 2005; Reddacliff *et al.* 2005), alleles of sizes 126 and 130 bp were found in Soay sheep (Coltman *et al.* 2001). Interestingly, a single 122 bp allele was found fixed in a large structured population (n=922) of wild sheep

(Worley *et al.* 2006). In the current study, the 124 bp allele was abundant in all the three properties, with an overall frequency of 75% (table 14). Allelic frequencies varied greatly in the cited works, with both alleles being abundant in different flocks. For marker OarKP6, 14 alleles translating to 34 genotypes were found in the current study, over the three properties (tables 14, A15 and A21). However, 9 alleles and 27 genotypes were rare (<1.0% frequency). It is interesting to note such a high instability (reflected by high allelic and genotypic variability) at this microsatellite locus, considering its close proximity to the stable IFNG microsatellite. While as many as 16 OarKP6 alleles were identified in 922 wild sheep (Worley *et al.* 2006), only between 1 and 4 alleles were found in 5 different sheep breeds (Paterson and Crawford 2000).

Heterozygote frequency at o(IFN)γ and OarKP6 markers was comparatively low (table 14). While the observed (37%) and expected (38%) heterozygosities at the o(IFN)γ locus were similar, the observed (57%) heterozygosity at OarKP6 locus was slightly less than that expected (60%). For the o(IFN)γ marker, heterozygosities of 40% and 47% were observed respectively, in Romney (Crawford and McEwan 1998) and Soay (Coltman *et al.* 2001) sheep. In a different study involving Romney sheep (Dukkipati *et al.* 2005), heterozygosities of 42.5% and 6.5% were recorded for two genetically independent flocks. For OarKP6, a much higher heterozygosity (75%) than in the current study was reported for wild sheep (Worley *et al.* 2006). In the current study, overall PIC values of 0.305 and 0.536 were observed for o(IFN)γ and OarKP6 respectively, while F_{is} values were -0.009 and 0.039 for the respective markers (table 14).

4.2.2 Fit for Hardy-Weinberg equilibrium

4.2.2.1 Markers located in the *Ovar-Mhc* region

Results from an exact test for HWE, together with those from tests for alternate hypotheses (heterozygote excess and deficiency) are shown in tables 12, 13 and 14 for the markers located in MHC, SLC11A1 and IFNG regions, respectively. Genotypes at MHC marker loci, except those at OLADRW, were in HWE (P>0.05) with a few exceptions. DYMS1 genotypes in properties 2 and 3 and OLADRB genotypes in property 1 deviated significantly (P<0.05) from HWE. While excess heterozygosity was the cause for deviation at DYMS1 in property 3 (P<0.05), heterozygote deficiency resulted in departure from HWE at DYMS1 in property 2 (P<0.01) and at OLADRB in property 1 (P<0.01). For marker locus OLADRW, significant

(P<0.01) departure from HWE was evident on all the three properties, owing to a huge heterozygote deficit and high F_{is} values (table 12).

Results from testing for HWE over all three properties together indicated significant (P<0.01) departure from HWE at all the four MHC marker loci. This is not surprising considering the significant allelic and genotypic differences observed among the three properties (table 15, see below).

For the four MHC markers investigated in the current study, there are only a few available reports in the literature regarding tests for HWE. While genotypes at OLADRW, OLADRB and SMHCC1 were found to be in HWE in Soay sheep (Paterson 1998), departure from HWE (caused by heterozygote deficiency) was noticed at the same loci in a population of wild sheep (Worley *et al.* 2006). Deviation from HWE also featured in studies pertaining to exon 2 polymorphisms within the functional DRB gene in Chinese sheep (Dong-Xiao *et al.* 2003) and South American cattle (Miretti *et al.* 2001), and for exon 2 polymorphisms within the DQB gene in Chinese pigs (Meiying *et al.* 2005).

4.2.2.2 Markers located in the SLC11A1 gene

Genotypes at OVINRA1 locus did not significantly depart from HWE (P>0.05) on all three properties (table 13). However, when all three properties were considered together, a significant (P<0.01) departure from HWE was evident. A huge deficit in heretozygotes at OVINRA2 locus resulted in significant (P<0.01) departures from HWE on all three properties. While there are no available reports in the literature on HWE at these marker loci in domestic sheep, a study on wild sheep (Worley *et al.* 2006) revealed HWE at both OVINRA1 and OVINRA2.

4.2.2.3 Markers located in the IFN-γ gene

Genotypes at marker o(IFN)γ within the IFNG gene were found to be in HWE (P>0.05) on all three properties (table 14). In fact, this is the only one out of the eight marker loci in the current study to be in HWE when individuals belonging to the three properties were considered together. Genotypes at the other marker locus within the IFNG gene, OarKP6, significantly (P<0.01) departed from HWE on all three properties, considered either individually or together (table 14). However, this departure turned out to be non-significant (P>0.05, data not shown) when rare alleles at the locus were pooled with the closest size

class. In a study on wild sheep, a single allele was found fixed at the o(IFN)γ locus, and genotypes at OarKP6 locus departed significantly from HWE (Worley *et al.* 2006).

4.2.3 Chromosome-wise linkage disequilibrium

Results from chromosome-wise LD tests between MHC markers located on chromosome 20 are presented in table 12. With a few exceptions, significant ($P<0.05$) LD was observed among the four marker loci located within the MHC. The exceptions include no significant ($P>0.05$) LD between markers OLADRW and DYMS1 in properties 1 and 2, between markers OLADRB and DYMS1 in property 2, and between markers SMHCC1 and DYMS1 in property 1. However, when rare alleles for each of these markers were pooled with the closest size class, the LD between these markers became significant ($P<0.05$). When individuals from all three properties were considered together, LD between different MHC marker pairs was highly significant ($P<0.01$).

In general, MHC genes are tightly linked and LD among MHC loci is a common feature. Significant LD was reported in Soay sheep (Paterson 1998) between markers within the MHC, with genetic distances of 2.6, 2.9 and 5.5 cM between OLADRW and OLADRB, OLADRB and SMHCC1, and OLADRW and SMHCC1, respectively. In a different study in Scottish Blackface sheep (Buitkamp *et al.* 1996), recombination distances of 5.8 and 19 cM were observed between OLADRW and SMHCC1 and OLADRW and DYMS1, respectively. While results from the current study confirm the significant LD between different MHC marker pairs, no map distances could be estimated as no pedigree information was available.

Significant ($P<0.01$) LD was observed between markers OVINRA1 and OVINRA2 located on chromosome 2 and between markers o(IFN)γ and OarKP6 located on chromosome 3 in the three properties, considered either alone or together (tables 13 and 14). This corroborates the genetic linkage between these respective marker loci reported in earlier studies (Pitel *et al.* 1996; Paterson and Crawford 2000).

4.2.4 Genetic differentiation of properties

Results from Fisher's exact test (Raymond and Rousset 1995b) and a log-likelihood based exact test (Goudet *et al*.1996) for allelic and genotypic differentiation of properties, respectively, are summarized in table 15. Using the same tests, allelic and genotypic similarities between vaccinates and controls in each property were also examined (table 15).

Consideration of results over all 8 marker loci indicated strong genetic (both allelic and genotypic) differences among the three properties and genetic similarity between vaccinates and controls within each property. The genetic differences between properties were the result of variation in both number and frequency of alleles/genotypes within each marker locus. Since the flocks at the three properties were independent with self-replacement for several decades at least, the observed genetic differences among the properties are unsurprising.

There were no significant (P>0.05) allelic or genotypic differences between vaccinates and controls within all three properties at marker loci OLADRB, SMHCC1, OVINRA1 and o(IFN)γ. However, some between-group differences (P<0.05) were evident with regard to markers DYMS1, OLADRW and OarKP6 (table 15), that appear to be due to uneven sampling sizes between groups resulting from loss of sheep in the unvaccinated control group due to PTB. Between group comparisons, on the whole, corroborate random allotment of animals to treatments within each property.

Table 15: Allelic and genotypic differentiation of properties and groups within properties

Particulars		DYMS1	OLADRW	OLADRB	SMHCC1	OVINRA1	OVINRA2	o(IFN)γ	OarKP6
Allelic differentiation[†]									
Group-wise	P1C Vs P1V	*	*	NS	NS	NS	**	NS	**
	P2C Vs P2V	*	**	NS	NS	NS	NS	NS	**
	P3C Vs P3V	NS	NS	NS	NS	NS	**	NS	NS
Property-wise	P1 Vs P2	**	**	**	**	**	**	*	**
	P1 Vs P3	**	**	**	**	**	**	**	**
	P2 Vs P3	**	**	**	**	**	**	**	**
Genotypic differentiation[‡]									
Group-wise	P1C Vs P1V	NS	NS	NS	NS	NS	**	NS	NS
	P2C Vs P2V	**	*	NS	NS	NS	NS	NS	NS
	P3C Vs P3V	NS	NS	NS	NS	NS	*	NS	NS
Property-wise	P1 Vs P2	**	**	**	**	**	**	*	**
	P1 Vs P3	**	**	**	**	**	**	**	**
	P2 Vs P3	**	**	**	**	**	**	**	**

P1, P2, P3 = properties 1, 2 and 3 respectively; C, V = controls and vaccinates, respectively, on each property

[†] Fisher exact test (Raymond and Rousset 1995); [‡] log-likelihood (G) based exact test (Goudet *et al.* 1996)

[NS] Non-significant (P>0.05); * significant (P<0.05); ** highly significant (P<0.01)

4.2.5 Chromosome-wise haplotype frequencies

4.2.5.1 *Ovar-Mhc* haplotypes

A haplotype analysis with the PROC HAPLOTYPE procedure in SAS/Genetics™ revealed several probable haplotypes for the four investigated microstaellite markers from the *Ovar-Mhc* region. As many as 317, 287 and 181 haplotypes were identified for properties 1, 2 and 3, respectively. Haplotypes (DYMS1-OLADRW-OLADRB-SMHCC1) 192-490-270-180 (2.21%), 192-496-276-192 (2.64%) and 202-478-296-198 (7.10%) were the most frequently observed for properties 1, 2 and 3 respectively. There were only 10, 19 and 24 haplotypes on these properties, respectively, with frequencies greater than one per cent. Frequencies of the remaining haplotypes were quite low.

The predicted haplotypes for MHC markers in the current study might be due to several reasons. All four MHC markers were highly polymorphic with as many as 42 alleles and 137 genotypes at the OLADRW locus, over the three properties. Such a high degree of polymorphism would invariably result in high haplotype numbers, despite linkage between the marker loci. Missing genotypes for some individuals at one or more of the four loci might be another factor. In the current study, of the total 934 individuals, genotypes could be recovered from between 797 and 895 animals for the four markers and as a result, several probable haplotypes were listed for animals with one or more missing genotypes. No pedigree information was available for any of the sheep. Availability of at least sire details would have resulted in a substantial reduction in probable haplotype numbers, making the haplotype analysis more meaningful. Because of an insufficient number of individuals in the most numerous MHC haplotype categories, the effects of MHC haplotypes on immune responses could not be investigated in this study.

4.2.5.2 SLC11A1 haplotypes

There were 38, 28 and 23 possible OVINRA1-OVINRA2 haplotypes in properties 1, 2 and 3, respectively; their frequencies are presented in table 16. Nineteen, 11 and 9 haplotypes in respective properties occurred at frequencies less than one per cent. Haplotype 162-312 was the most frequent haplotype in all the three properties with respective frequencies of 24.1%, 12.6% and 22.8%. As for the case of MHC markers, there were 39 and 215 missing genotypes for OVINRA1 and OVINRA2 markers respectively over the three properties and these would have resulted in greater than actual haplotype numbers. This was evident from the fact that 82, 45 and 42 individuals in properties 1, 2 and 3, respectively had missing

genotypes at OVINRA2 locus, but had genotype details for OVINRA1. Consequently, haplotype numbers were highest and lowest in properties 1 and 3, respectively.

Table 16: Frequencies of OVINRA1-OVINRA2 haplotypes in three properties

Property 1		*Property 2*		*Property 3*	
OINRA1-OVINRA2 haplotype (bp)	Frequency±SE	OINRA1-OVINRA2 haplotype (bp)	Frequency±SE	OINRA1-OVINRA2 haplotype (bp)	Frequency±SE
162-312	0.241±0.017	162-312	0.126±0.014	162-312	0.228±0.018
158-312	0.126±0.013	164-312	0.118±0.013	164-312	0.132±0.014
164-312	0.112±0.013	156-312	0.105±0.013	162-314	0.127±0.014
162-314	0.055±0.009	156-314	0.093±0.012	160-318	0.100±0.013
160-316	0.054±0.009	160-312	0.09±0.012	158-312	0.089±0.012
164-326	0.039±0.008	162-314	0.084±0.011	160-312	0.057±0.01
162-326	0.039±0.008	164-314	0.082±0.011	164-314	0.045±0.009
168-312	0.037±0.008	158-312	0.052±0.009	158-314	0.041±0.008
160-318	0.036±0.007	160-314	0.043±0.008	160-316	0.037±0.008
156-314	0.034±0.007	158-314	0.043±0.008	168-312	0.036±0.008
156-312	0.032±0.007	160-316	0.028±0.007	160-314	0.023±0.006
164-314	0.027±0.006	162-326	0.027±0.007	156-314	0.02±0.006
162-320	0.018±0.005	160-318	0.024±0.006	156-312	0.016±0.005
160-326	0.017±0.005	158-316	0.019±0.006	156-316	0.012±0.005
160-312	0.016±0.005	164-326	0.011±0.004	166-312	0.007±0.004
158-314	0.015±0.005	168-312	0.010±0.004	162-326	0.007±0.003
166-312	0.013±0.004	162-322	0.008±0.004	168-314	0.006±0.003
160-314	0.011±0.004	164-322	0.008±0.004	162-310	0.004±0.003
168-314	0.011±0.004	160-326	0.007±0.003	160-326	0.004±0.003
168-326	0.009±0.004	156-326	0.005±0.003	166-314	0.004±0.003
156-326	0.007±0.003	158-326	0.004±0.003	164-326	0.003±0.002
156-316	0.006±0.003	160-322	0.004±0.003	170-314	0.002±0.002
168-320	0.006±0.003	168-314	0.002±0.002	158-326	0.001±0.001
158-326	0.005±0.003	168-320	0.002±0.002		
162-322	0.004±0.003	154-314	0.002±0.002		
158-322	0.004±0.003	166-316	0.002±0.002		
164-320	0.004±0.002	168-326	0.002±0.002		
156-320	0.002±0.002				
158-320	0.002±0.002				
160-322	0.002±0.002				
162-318	0.002±0.002				
164-316	0.002±0.002				
162-328	0.002±0.002				
158-328	0.002±0.002				
158-316	0.002±0.002				
170-312	0.002±0.002				
150-316	0.002±0.002				
162-316	0.001±0.001				

4.2.5.3 IFN-γ haplotypes

Haplotype numbers pertaining to o(IFN)γ and OarKP6 markers located in the IFN-γ gene region were lowest when compared to those for either MHC markers or OVINRA1-OVINRA2 markers. There were only 17, 10 and 11 o(IFN)γ-OarKP6 haplotypes in properties 1, 2 and 3, respectively. Their frequencies are listed in table 17. Haplotype 124-200 had

highest frequency in properties 1 (35.9%) and 3 (39.6%), while haplotype 124-204 was the most frequent (35.8%) one in property 2. Six haplotypes in each property had frequencies higher than one. The observed low numbers for o(IFN)γ-OarKP6 haplotypes were most probably due two main reasons. Firstly, comparatively fewer missing genotypes for o(IFN)γ and OarKP6 markers (44 and 59, respectively) and secondly, low polymorphism at o(IFN)γ marker locus (just two alleles in all the three properties).

Table 17: Frequencies of o(IFN)γ-OarKP6 haplotypes in three properties

Property 1		*Property 2*		*Property 3*	
o(IFN)γ-OarKP6 haplotype (bp)	Frequency±SE	o(IFN)γ-OarKP6 haplotype (bp)	Frequency±SE	o(IFN)γ-OarKP6 haplotype (bp)	Frequency±SE
124-200	0.359±0.019	124-204	0.358±0.020	124-200	0.396±0.020
128-200	0.278±0.018	124-200	0.255±0.018	124-204	0.311±0.019
124-204	0.247±0.017	128-200	0.204±0.017	128-200	0.142±0.015
128-202	0.032±0.007	124-202	0.097±0.012	124-202	0.074±0.011
124-202	0.023±0.006	128-202	0.058±0.010	124-206	0.044±0.008
124-186	0.018±0.005	124-186	0.018±0.005	124-186	0.013±0.005
128-186	0.008±0.003	124-180	0.004±0.002	128-204	0.008±0.004
128-204	0.006±0.003	128-194	0.004±0.002	124-198	0.005±0.003
124-192	0.005±0.003	124-182	0.002±0.002	124-188	0.002±0.002
124-194	0.004±0.003	124-208	0.002±0.002	124-192	0.002±0.002
128-194	0.004±0.002			124-196	0.002±0.002
124-190	0.003±0.002				
124-196	0.003±0.002				
124-198	0.003±0.002				
124-206	0.003±0.002				
124-188	0.002±0.002				
128-196	0.002±0.002				

4.3 EFFECT OF MARKER GENOTYPES ON IMMUNE RESPONSES

The influence of genetic polymorphisms at each of the eight genetic marker loci on immune responses was tested in two ways – genotype and allele effects. Also, the effects of SLC11A1 and IFN-γ haplotypes on immune responses were tested, while those of MHC haplotypes could not be determined due to insufficient numbers. This section details the effects of individual marker genotypes on immune responses. Perusal of literature revealed the current study to be first of its kind aiming at genetic markers for variations in immune responses to Johne's vaccination in sheep as well as in other domesticated species. Hence, there was little scope for comparison of the current findings with previously reported findings.

Effects of marker genotypes on immune responses were tested employing a mixed model analysis in SAS. Immune responses recorded at different time points post-vaccination were

log-transformed so as to normalize data. The model tested the effects of genotypes within vaccinated and control individuals on each property on immune responses to vaccination. In general, the effects of marker genotypes in controls on antibody as well as IFN-γ responses on all the three properties were non-significant (P>0.05). This is not surprising considering that the immune responses in control individuals remained consistently low (except for a small surge in IFN-γ responses on properties 2 and 3; detailed in section 4.1.2) throughout the experimental period. Hence, only the effects of marker genotypes on immune responses in vaccinates are presented here.

4.3.1 Effect on antibody responses

Mean antibody responses (LSM in logarithmic scale) for different genotypes at the eight marker loci in vaccinated sheep belonging to three properties are presented in the appendix (tables A22-A27). LSM (transformed to normal scale) for marker genotypes are depicted in figures 7-14 for each marker. Also, genotypes at individual marker loci found to have significant effects on antibody responses, along with their levels of significance are presented in tables 18-23.

4.3.1.1 DYMS1

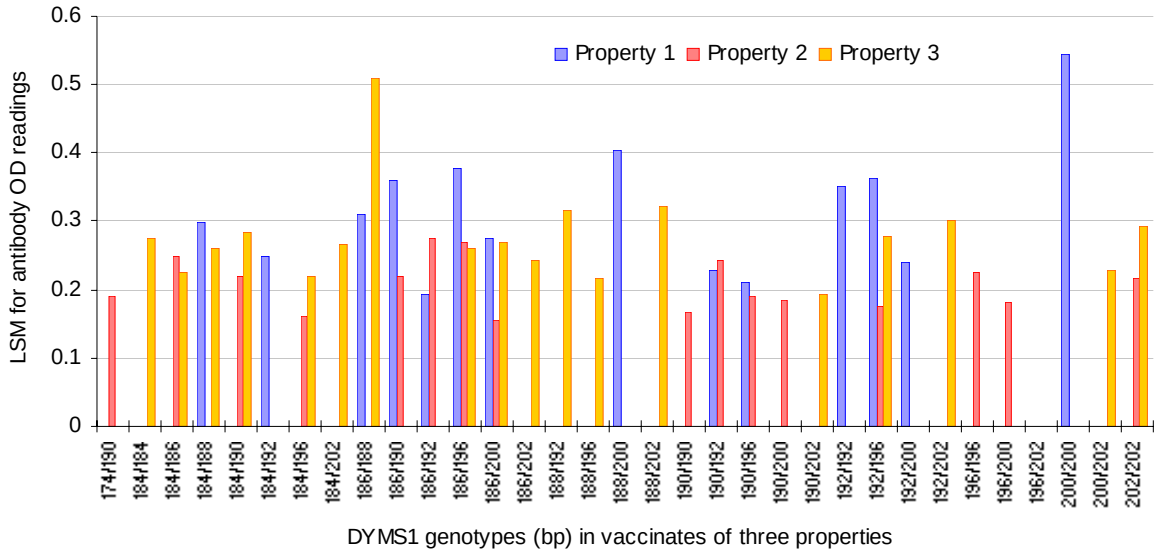

Figure 7: Effect of DYMS1 genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Vaccinated individuals in property 1 possessing DYMS1 genotype 200/200 had significantly higher antibody responses compared to those possessing 186/192, 190/192, 190/196 and 192/200 (table 18). Genotype 200/200 was only in low frequency for properties 2 and 3 and hence its effect on immune responses was not evaluated in those properties. On property 2, animals with genotype 186/192 had the highest antibody responses (figure 7). This genotype had insufficient numbers in property 3 and was associated with mediocre responses in property 1. Genotype 186/188 in property 3 was associated with increased antibody responses compared to nine other genotypes (table 18). Genotype 186/188 was absent in property 2 and in property 1, it resulted in mediocre responses.

Table 18: DYMS1 genotypes found to have significant effect on antibody responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with								
	Genotype (bp)	Effect									
Property 1	200/200	▲	**186/192**	190/192	190/196	192/200					
Property 2	186/192	▲	190/190								
Property 3	186/188	▲	184/186	**184/196**	184/202	186/196	186/202	188/196	**190/202**	196/202	200/202

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

4.3.1.2 OLADRB

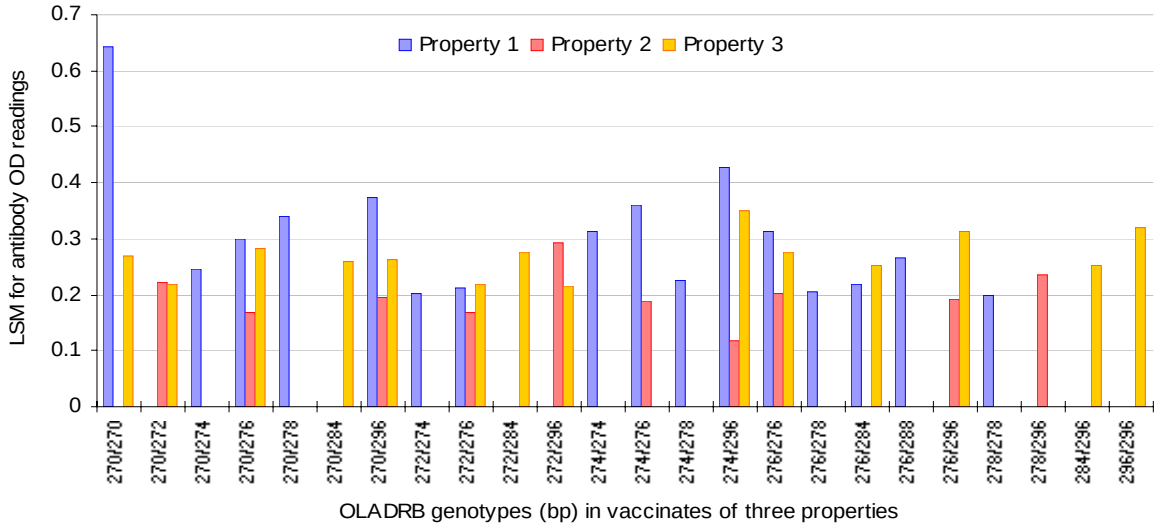

Figure 8: Effect of OLADRB genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

In property 1, OLADRB genotypes 270/270 and 276/278 resulted in significantly increased and decreased antibody responses, respectively (table 19). Genotype 270/270 had insufficient numbers in property 2 and individuals in property 3 possessing 270/270 had mediocre responses. In property 2, genotypes 272/296 and 274/296 were found to be significantly associated with increased and decreased antibody responses respectively (table 19). Contrary to its effect in property 2, genotype 274/296 resulted higher responses in individuals on properties 1 and 3 (figure 8). Animals with Genotype 272/296 had mediocre responses on property 2. None of the OLADRB genotypes were found to possess significant effects on antibody responses in property 3 vaccinates (table 19).

Table 19: OLADRB genotypes found to have significant effect on antibody responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with
	Genotype (bp)	Effect	
Property 1	270/270	▲	270/274 270/276 **272/274** 272/276 274/278 276/276 **276/278** **276/284** 276/288 **278/278**
	276/278	▼	**270/270** 274/276 274/296
Property 2	272/296	▲	270/276 **272/276** **276/276** **276/276** 276/296
	274/296	▼	270/272 **272/296** 276/276 278/296
Property 3	-	-	-

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

4.3.1.3 OLADRW

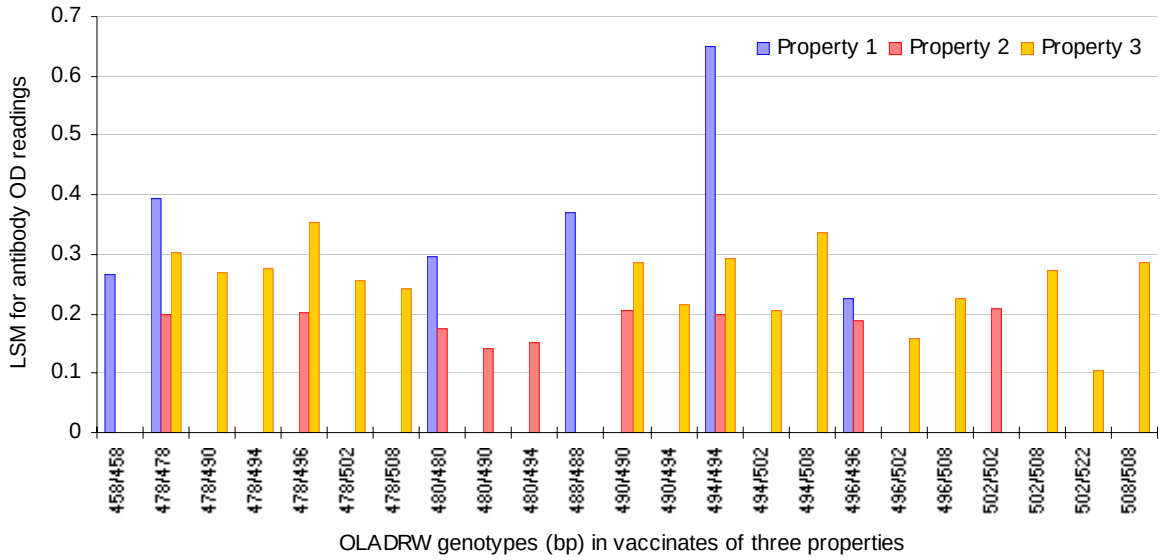

Figure 9: Effect of OLADRW genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

OLADRW genotype 494/494 was found to associate with significantly increased antibody responses in individuals on property 1 (table 20). However, no such effect of this genotype was evident in properties 2 and 3. There was no significant effect of OLADRW genotypes on antibody responses in property 2 vaccinates. In property 3 vaccinates, genotypes 478/496, 494/508 and 478/478 were found to be significantly associated with increased antibody responses, while two other genotypes (496/502 and 502/522) resulted in decreased responses (table 20 and figure 9).

Table 20: OLADRW genotypes found to have significant effect on antibody responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with						
	Genotype (bp)	Effect							
Property 1	494/494	▲	458/458	480/480	496/496				
Property 2	-	-	-						
Property 3	478/496	▲	494/502	**496/508**	502/522				
	494/508	▲	496/502	502/522					
	478/478	▲	496/502	502/522					
	496/502	▼	478/478	478/494	**478/496**	490/490	494/494	494/508	508/508
	502/522	▼	478/478	**478/496**	494/508				

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

4.3.1.4 SMHCC1

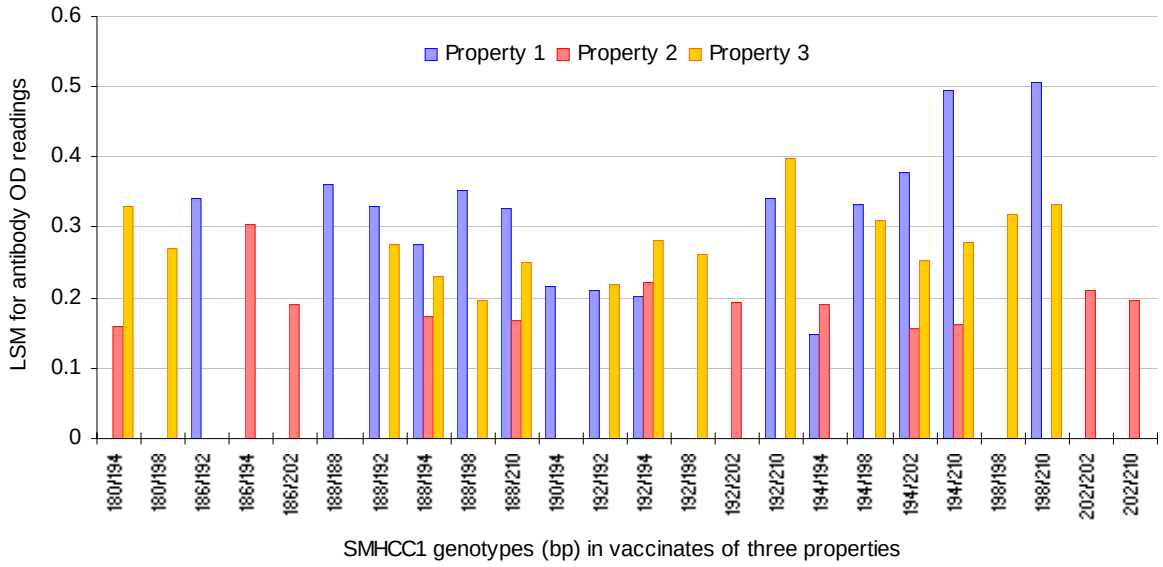

Figure 10: Effect of SMHCC1 genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Table 21: SMHCC1 genotypes found to have significant effect on antibody responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with							
	Genotype (bp)	Effect								
Property 1	194/210	▲	190/194	192/192	**192/194**	**194/194**				
	198/210	▲	192/192	192/194	**194/194**					
	194/194	▼	186/192	188/188	188/192	192/210	194/198	194/202	**194/210**	**198/210**
Property 2	186/194	▲	188/194	188/210	194/194	**194/202**	194/210			
	194/202	▼	**186/194**	192/194						
Property 3	192/210	▲	188/198	192/192						
	188/198	▼	192/210	194/198	198/210					

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

SMHCC1 genotypes 194/210 and 198/210 in property 1 were significantly associated with high antibody responses in vaccinates (table 21). While genotype 194/210 resulted in moderate responses in the other two properties, property 3 sheep with genotype 198/210 had high responses (figure 10). The effect of genotype 198/210 was not evaluated in property 2. Property 1 animals with genotype 194/194 had significantly lower antibody levels compared to animals possessing eight other genotypes (table 21). Genotypes 186/194 and 192/210 were found to be associated with increased antibody levels in properties 2 and 3 respectively, while animals with genotypes 194/202 and 188/198 had low responses in the respective properties.

4.3.1.5 OVINRA1

Several significant differences were found with regard to the effects of OVINRA1 genotypes on antibody responses (table 22). However, as seen in case of MHC markers, there was no consistency in their effects across the three properties. Vaccinates with genotypes 158/162, 156/162 and 160/164 in property 1 had significantly higher antibody responses than animals with some other genotypes (table 22). Genotypes 160/162 and 162/162 were found to be associated with lower antibody levels in vaccinates on this property.

Genotypes 156/158 and 164/164 resulted in significantly higher and lower antibody responses, respectively, in property 2 vaccinates. OVINRA1 genotypes in property 3 did not differ much in their effects on antibody responses (figure 11), except for genotype 158/160 having lower responses compared to genotype 160/162.

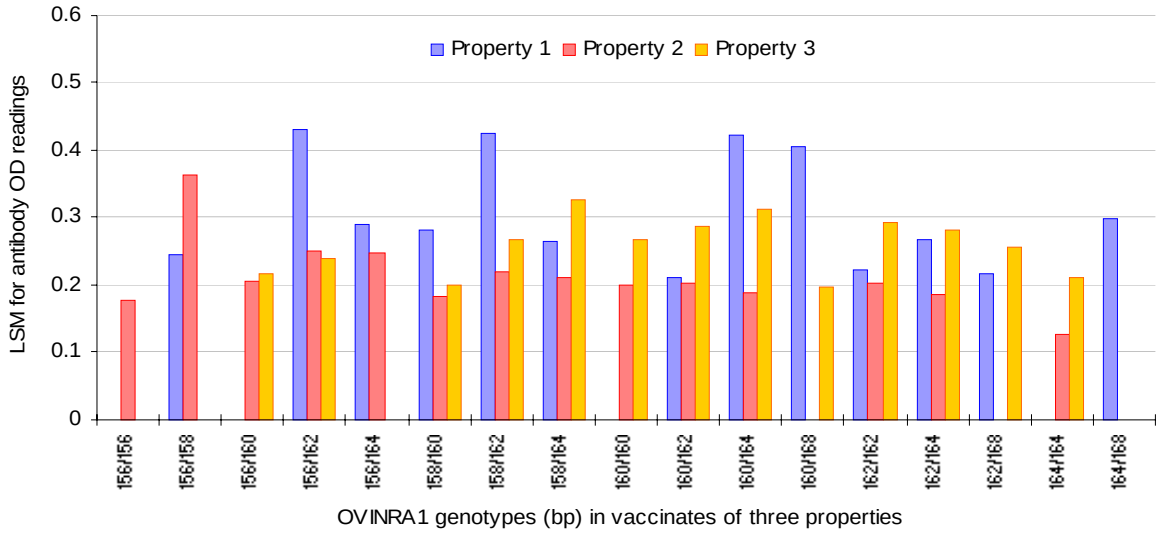

Figure 11: Effect of OVINRA1 genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Table 22: OVINRA1 genotypes found to have significant effect on antibody responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with							
	Genotype (bp)	Effect								
Property 1	158/162	▲	**160/162**	**162/162**	162/164	162/168				
	156/162	▲	160/162	162/162						
	160/164	▲	160/162	162/162						
	160/162	▼	156/162	**158/162**	160/164	160/168				
	162/162	▼	156/162	**158/162**	160/164					
Property 2	156/158	▲	156/160	158/160	160/162	160/164	162/162	**162/164**	**164/164**	
	164/164	▼	**156/158**	156/160	**156/162**	**156/164**	158/162	158/164	160/162	162/162
Property 3	158/160	▼	160/162							

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

4.3.1.6 OVINRA2

There was only a slight variation in the effects of OVINRA2 genotypes on antibody responses (figure 12) and none of the differences reached statistical significance ($P>0.05$) on any of the three properties.

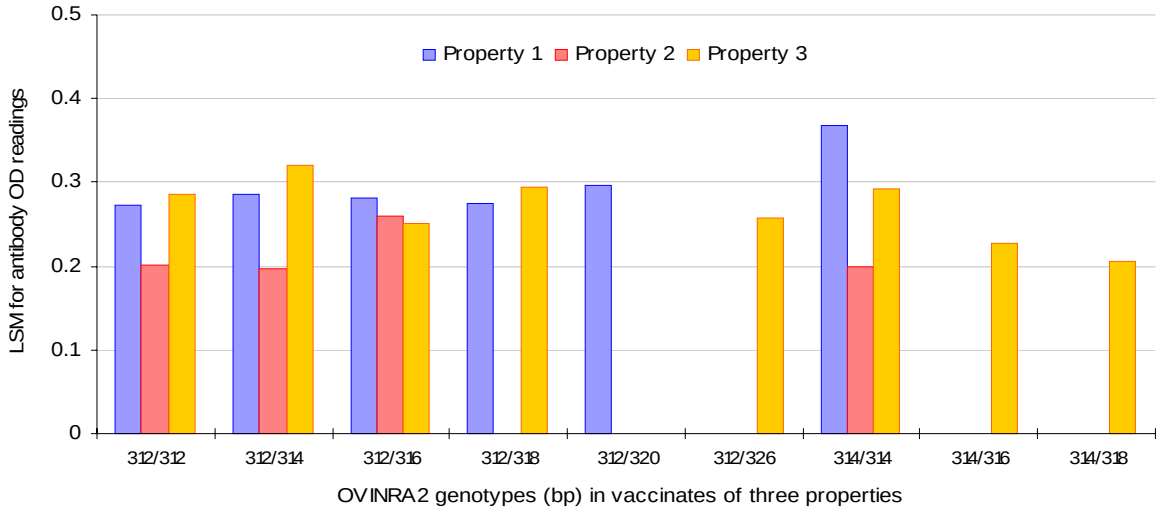

Figure 12: Effect of OVINRA2 genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.3.1.7 o(IFN)-γ

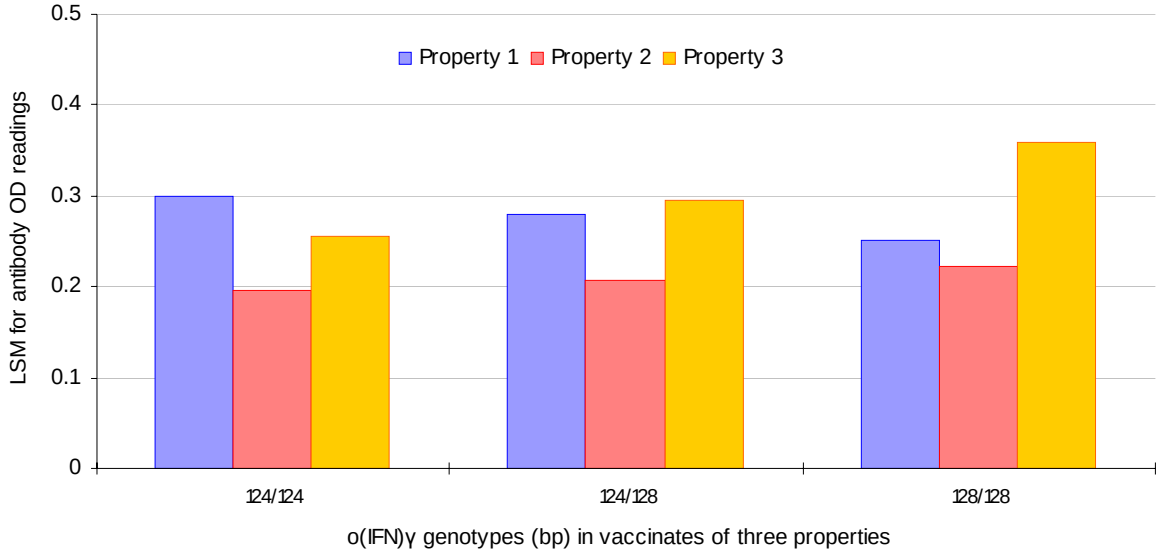

Figure 13: Effect of o(IFN)γ genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Differences between the effects of the three genotypes at the o(IFN)-γ marker locus on antibody responses were non-significant on all three properties. All the three genotypes at the

marker locus had a similar effect on antibody responses in property 2 vaccinates, while genotypes 124/124 and 128/128 in properties 1 and 3 respectively, resulted in non-significantly higher antibody responses compared to the other two genotypes on respective properties (figure 13).

4.3.1.8 OarKP6

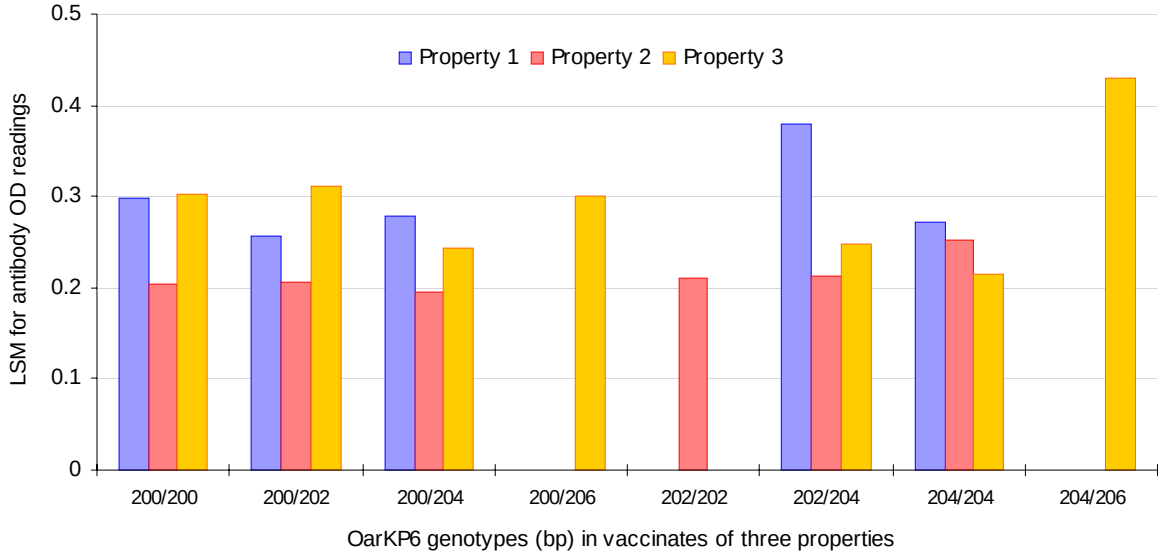

Figure 14: Effect of OarKP6 genotypes on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

OarKP6 genotypes had almost similar and non-significant effects on antibody responses in vaccinates on properties 1 and 2 (figure 14). Property 3 vaccinates with genotype 204/206 had significantly higher immune responses to those possessing two other genotypes (table 23). This genotype had insufficient numbers for evaluation in the other two properties.

Table 23: OarKP6 genotypes found to have significant effect on antibody responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with	
	Genotype (bp)	Effect		
Property 1	-	-	-	
Property 2	-	-	-	
Property 3	204/206	▲	200/204	204/204

* Genotypes in normal font differ significantly (P<0.05)

4.3.1.9 Power analysis

Post-hoc power analysis was carried out to determine an approximate power of the employed statistical model in detecting a significant difference in antibody responses between vaccinated sheep possessing two different genotypes at a particular marker locus. *A priori* analysis of the required sample size could not be undertaken as there were no prior studies in the literature on the genetic effects on immune responses. Power was estimated for two marker loci (o(IFN)-γ, having 3 genotypes and OVINRA1, having 14 genotypes) on property 2, employing proc power in SAS® 9.1 (SAS Institute Inc. 2004) as well as a specialised power analysis software, G*power (Faul *et al.* 2007). A simple fixed effects one-way analysis of variance model was assumed. For each marker locus, overall standard deviation for antibody response at the maximum response time-point (8 months post-vaccination), mean antibody response values at the maximum response time-point for each genotype, together with respective number of individuals for each genotype were used as input values. Level of significance was set at 5%.

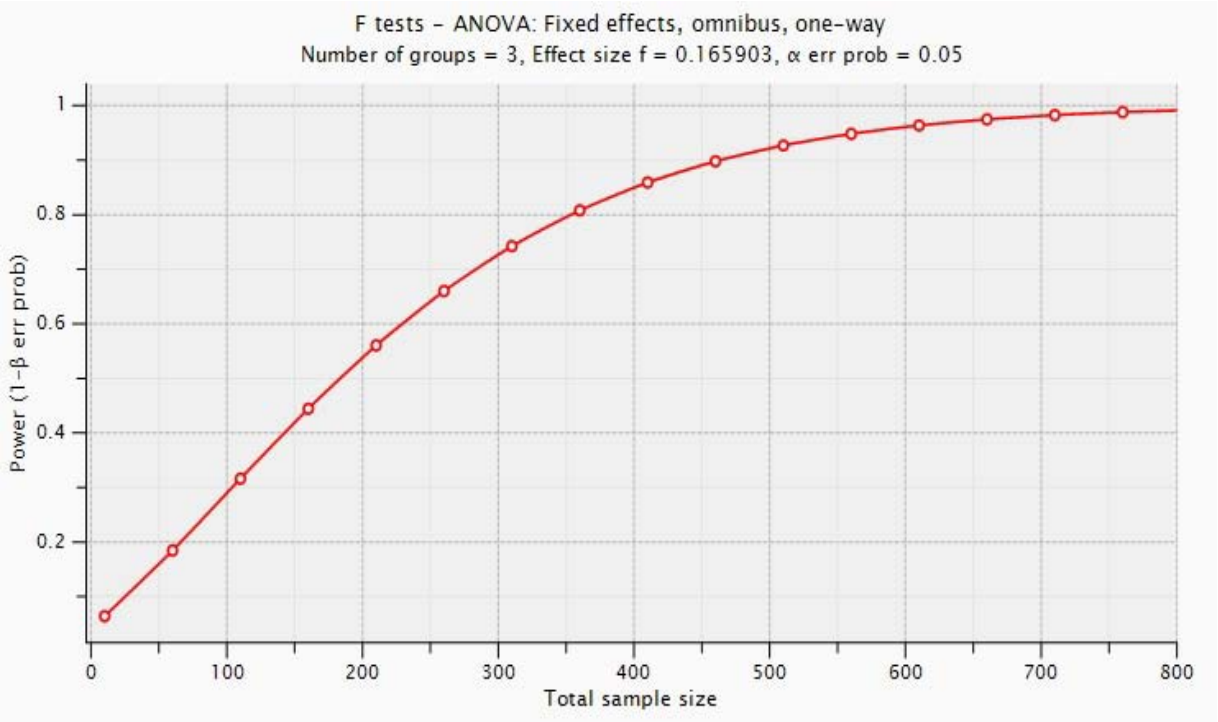

Figure 15: Sample size versus power of detecting a significant effect of genotypes at o(IFN)-γ locus on antibody responses to Johne's vaccination

Results from proc power and G*power analyses were the same. The estimated power values based on the observed differences in means were 0.47 and 0.78 for genotypes at o(IFN)-γ and OVINRA1 loci, respectively. Based on the observed standard deviation and differences in

means, the total number of individuals required to achieve power values up to 1.00 were extrapolated for genotypes at both the marker loci (figures 15 and 16). For genotypes at markers o(IFN)-γ and OVINRA1, total sample size of around 350 and 150, respectively, would be required to achieve a power of 0.80.

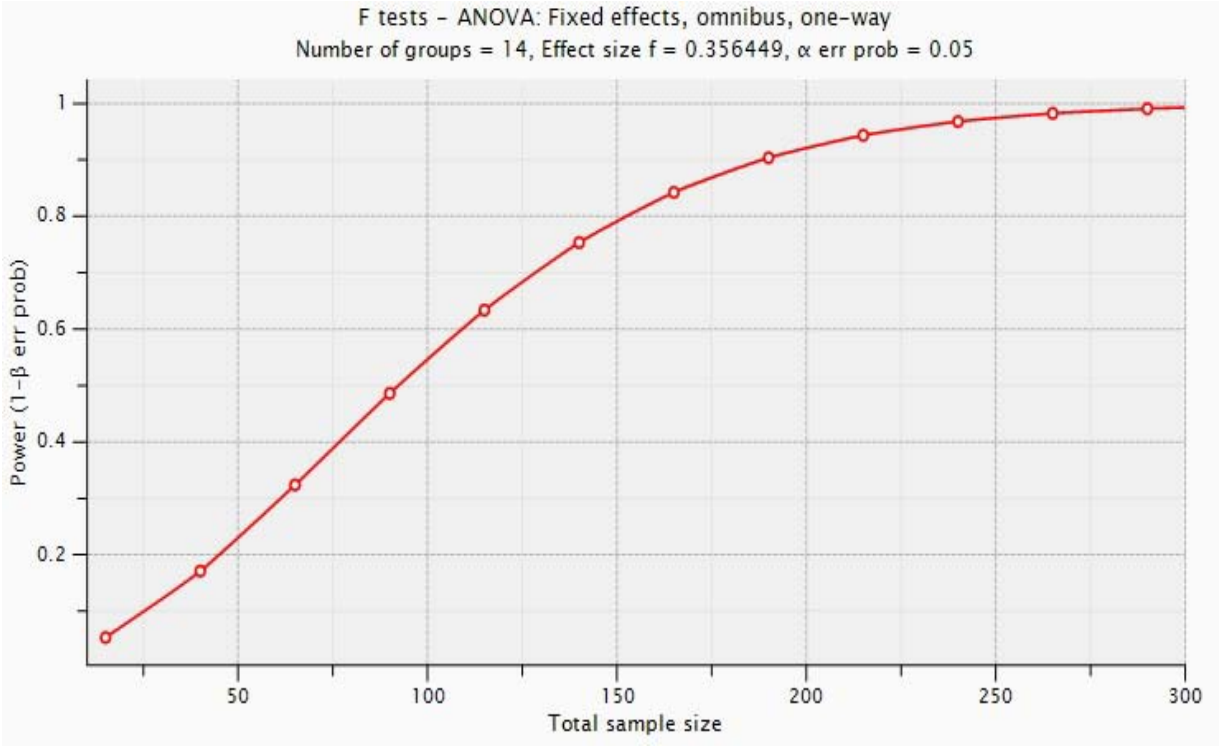

Figure 16: Sample size versus power of detecting a significant effect of genotypes at OVINRA1 locus on antibody responses to Johne's vaccination

4.3.1.10 Summary

Several genotypes at DYMS1, OLADRB, OLADRW, SMHCC1 and OVINRA1 marker loci were found to be associated with high as well as low antibody responses to Johne's vaccination. However, none of the effects were consistent across the three properties. Genotypes found to be significantly associated with either high or low responses in one or two properties either had opposite effects or were absent in the other property/properties. None of the genotypes at OVINRA2 and o(IFN)-γ loci had significant effects on antibody responses. While the effect of a genotype (204/206) at OarKP6 locus was found to be significant in property 3, the genotype was not evaluated in the other two properties, due to insufficient numbers.

4.3.2 Effect on IFN-γ responses

Mean IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) responses (LSM in logarithmic scale) for different genotypes at the eight marker loci in vaccinated sheep belonging to three properties are presented in appendix (tables A22-A27). The IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) response-trends for each of the marker genotypes were almost similar. Hence, to avoid repetition, only LSM (transformed to normal scale) for IFN-γ (Johnin-avian) responses for each of the marker genotypes are depicted in figures 17-24. However, within each marker, the level of significance for between-genotype differences varied slightly between IFN-γ phenotypes. Therefore, between-genotype differences for both the IFN-γ phenotypes were listed in tables 24-31. For simplicity, the discussion pertaining to the effects of genotypes at each marker locus was generalised based on the effects on either of the IFN-γ phenotypes.

4.3.2.1 DYMS1

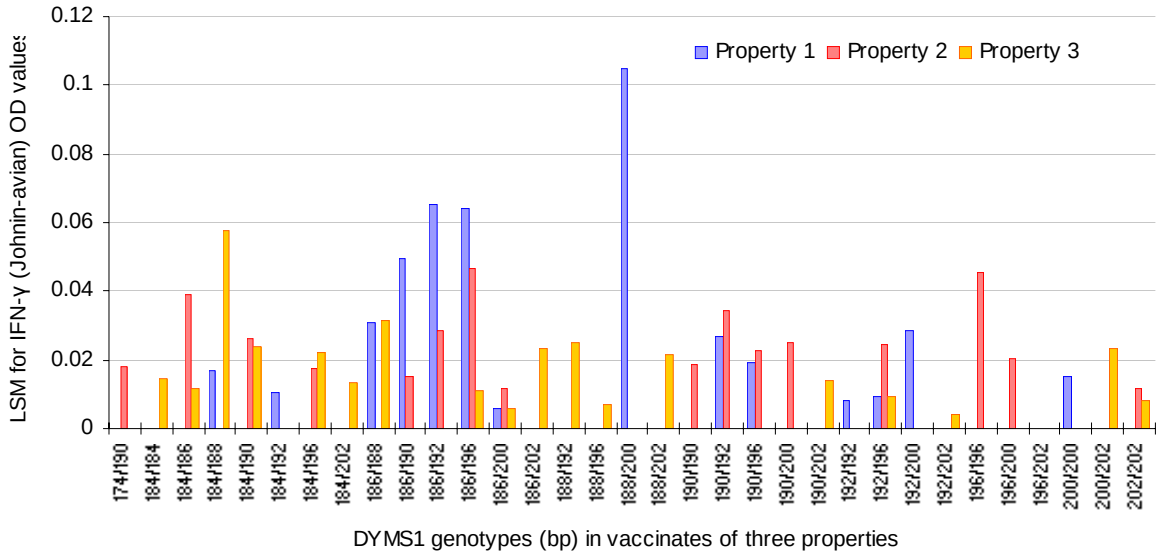

Figure 17: Effect of DYMS1 genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Several significant effects of DYMS1 genotypes were evident in the three properties (table 24). Genotype 186/200 was found to be significantly associated with very low IFN-γ responses in all the three properties. Individuals with genotype 192/196 exhibited significantly low responses in properties 1 and 3, and moderate responses in property 2. Another genotype (202/202) was also associated with remarkably low IFN-γ responses in vaccinates of properties 2 and 3, while it had insufficient numbers for testing in property one.

Table 24: DYMS1 genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes significant: Genotype (bp)	Effect	Genotypes* (bp) in comparison with									
IFN-γ (Johnin-nil) production												
Property 1	186/192	▲	184/188	**184/192**	186/188	**186/200**	190/192	190/196	**192/192**	**192/196**	192/200	
	188/200	▲	**184/192**	**186/200**	192/192	**192/196**						
	186/196	▲	184/192	192/196	186/200							
	192/200	▲	184/192	192/196	186/200							
	184/192	▼	**186/192**	186/196	**188/200**	192/200						
	192/196	▼	**186/192**	186/196	**188/200**	190/192	192/200					
	186/200	▼	**186/192**	186/196	**188/200**	192/200						
Property 2	196/196	▲	184/196	**186/200**								
	186/196	▲	184/196	186/200								
	190/192	▲	184/196	186/200								
	184/190	▲	184/196	186/200								
	186/200	▼	184/190	186/196	190/192	**196/196**						
	184/196	▼	184/190	186/196	190/192	196/196						
Property 3	184/188	▲	184/184	**184/202**	186/200	**188/196**	**192/196**	**192/202**	196/202	**202/202**		
	192/202	▼	184/196	186/188	186/196	186/202	188/192	188/202	200/202			
	202/202	▼	**184/188**	188/192	188/202							
	192/196	▼	**184/188**									
	186/200	▼	184/188									
IFN-γ (Johnin-avian) production												
Property 1	188/200	▲	184/192	**186/200**	**192/192**	**192/196**	**200/200**					
	186/192	▲	184/192	**186/200**	192/192	**192/196**						
	186/196	▲	**186/200**	192/192	192/196							
	186/200	▼	186/190	186/192	186/196	**188/200**	190/192	192/200				
	192/192	▼	186/192	186/196	**188/200**							
	192/196	▼	**186/192**	186/196	**188/200**							
	184/192	▼	186/192	188/200								
Property 2	186/196	▲	184/196	186/190	**186/200**	190/190	202/202					
	196/196	▲	184/196	186/190	**186/200**	190/190	**202/202**					
	184/186	▲	186/200	202/202								
	190/192	▲	186/200	202/202								
	202/202	▼	184/186	186/196	190/192	**196/196**						
	186/200	▼	184/186	**186/196**	190/192	**196/196**						
	186/190	▼	186/196	196/196								
	184/196	▼	186/196	196/196								
Property 3	184/188	▲	184/186	184/202	186/196	**186/200**	**188/196**	190/202	192/196	**192/202**	196/202	**202/202**
	192/202	▼	**184/188**	184/190	184/196	**186/188**	186/202	188/192	188/202	196/202	200/202	
	186/200	▼	**184/188**	184/196	186/188	186/202	188/192	188/202	200/202			
	188/196	▼	**184/188**									
	192/196	▼	184/188									
	202/202	▼	184/188									

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

Genotype 186/196 resulted in significantly high responses in individuals of properties 1 and 2, while it had opposite effect in property 3 (figure 17). Several other genotypes exhibited

significant effects. However, such genotypes were either confined to a particular property or were inconsistent across the three properties in their effects (table 24). They included 188/200 in property 1, 184/188 in property 3 and 196/196, having increased effects on IFN-γ responses, and genotypes 192/202 and 188/198 in property 3 that were associated with very low responses.

4.3.2.2 OLADRB

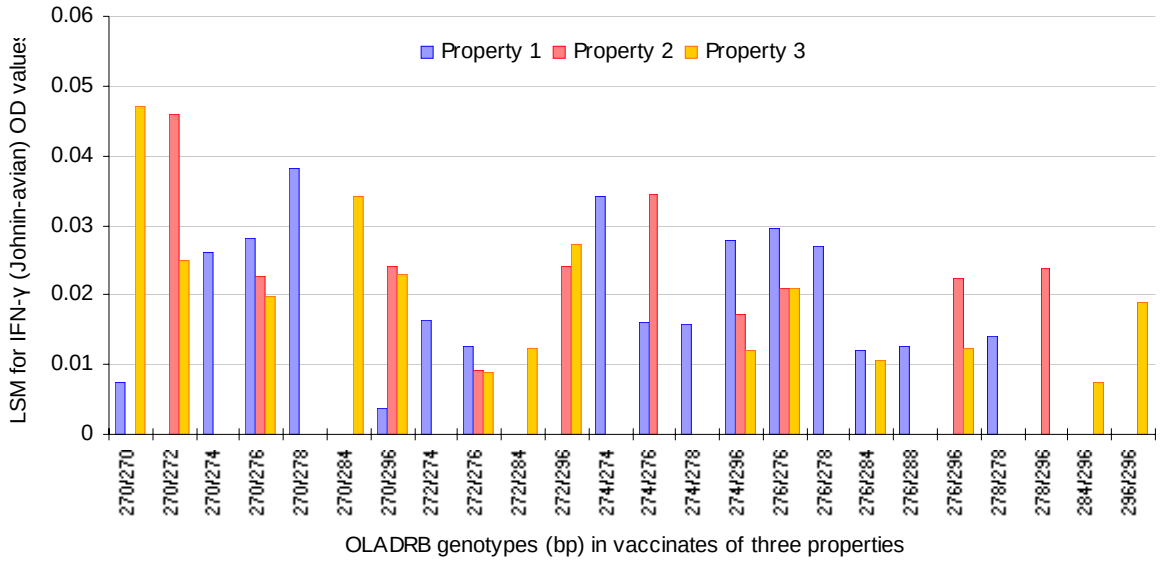

Figure 18: Effect of OLADRB genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

OLADRB genotype 270/296 in property 1 resulted in significantly lower IFN-γ levels in property 1, compared to four other genotypes (table 25). However, animals with this genotype on the other two properties had significantly high responses (figure 18). Two genotypes (270/278 and 274/274) that had sufficient numbers for evaluation only in property 1 vaccinates resulted in high antibody responses. However, their effects differed significantly only from that of genotype 270/296. Genotype 272/276 in properties 2 and 3 was found to be significantly associated with low IFN-γ levels (table 25). Animals in property 1 with this genotype possessed low but non-significant IFN-γ responses. Genotypes 270/270 and 270/284 resulted significantly high IFN-γ responses in property 3 vaccinates. While the former genotype had an opposite effect in property 1 vaccinates, the later had insufficient numbers in properties 1 and 3. Two other genotypes (284/296 and 276/284) in property 3

were found to be associated with significantly low IFN-γ responses. While effects of the genotype 284/296 were not evaluated in the other two properties, genotype 276/284 was found to possess a similar but non-significant effect in property 1.

Table 25: OLADRB genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with							
	Genotype (bp)	Effect								
IFN-γ (Johnin-nil) production										
Property 1	-	-	-							
Property 2	272/276	▼	270/272	270/276	**270/296**	272/296	276/276	**276/296**		
Property 3	270/270	▲	272/276	**276/284**	**276/296**	**284/296**				
	270/284	▲	276/284	276/296	**284/296**					
	270/272	▲	276/284	276/296	**284/296**					
	270/296	▲	276/284	276/296	**284/296**					
	284/296	▼	**270/270**	**270/272**	**270/276**	**270/284**	**270/296**	**272/296**	276/276	296/296
	276/284	▼	**270/270**	270/272	270/284	270/296				
	276/296	▼	**270/270**	270/272	270/284	270/296				
IFN-γ (Johnin-avian) production										
Property 1	270/296	▼	270/276	270/278	274/274	276/276				
Property 2	272/276	▼	270/272	270/276	270/296	272/296	**274/276**	276/276	**276/296**	
Property 3	270/270	▲	270/276	**272/276**	272/284	274/296	**276/284**	**276/296**	**284/296**	
	270/284	▲	272/276	276/284	276/296	284/296				
	270/296	▲	272/276	276/284	284/296					
	284/296	▼	**270/270**	270/272	270/284	270/296	272/296			
	272/276	▼	**270/270**	270/272	270/284	270/296				
	276/284	▼	**270/270**	270/284	270/296					

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

4.3.2.3 OLADRW

Vaccinated sheep in property 1 that possessed OLADRW genotype 494/494 exhibited significantly very low IFN-γ responses. However, this genotype resulted in significantly high responses in vaccinates of properties 2 and 3 (table 26 and figure 19). Genotypes 458/458 and 496/496 were associated with high IFN-γ levels in vaccinates belonging to property 1. Animals possessing genotype 478/478 showed significantly low responses on property 2 and moderate responses on properties 1 and 3. Genotypes 494/508, 478/494, 508/508, 478/508, 494/502 and 478/490 had sufficient numbers to be evaluated only in property 3 vaccinates. While the former two genotypes resulted in significantly high responses, the later four were associated with low IFN-γ responses.

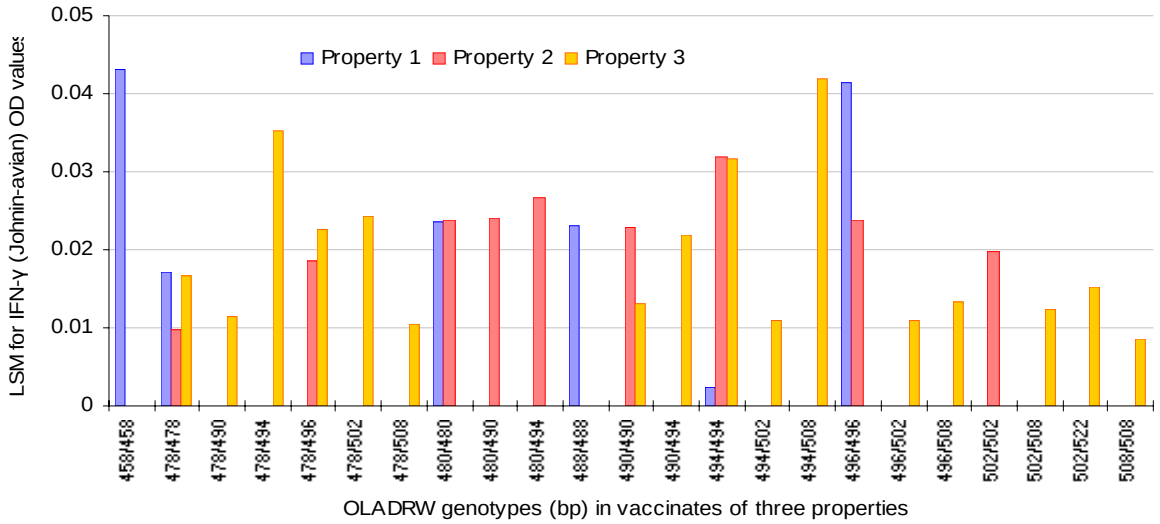

Figure 19: Effect of OLADRW genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Table 26: OLADRW genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with					
	Genotype (bp)	Effect						
IFN-γ (Johnin-nil) production								
Property 1	496/496	▲	494/494					
	494/494	▼	496/496					
Property 2	-	-	-					
Property 3	494/508	▲	478/490	**478/508**	490/490	**494/502**	**508/508**	
	478/494	▲	478/490	**478/508**	490/490	**508/508**		
	494/494	▲	478/490	**478/508**	490/490	**508/508**		
	478/508	▼	**478/494**	478/496	478/502	**494/494**	**494/508**	
	508/508	▼	**478/494**	478/502	**494/494**	**494/508**		
	490/490	▼	478/494	494/494	494/508			
	478/490	▼	478/494	494/494	494/508			
IFN-γ (Johnin-avian) production								
Property 1	494/494	▼	**458/458**	478/478	**480/480**	488/488	**496/496**	
Property 2	478/478	▼	480/480	494/494	496/496			
Property 3	494/508	▲	478/490	478/508	490/490	494/502	496/502	508/508
	478/494	▲	478/490	478/508	490/490	494/502	496/502	**508/508**
	494/494	▲	478/490	478/508	490/490	494/502	**508/508**	
	508/508	▼	**478/494**	**494/494**	494/508			
	478/508	▼	478/494	494/494	494/508			
	494/502	▼	478/494	494/494	494/508			
	478/490	▼	478/494	494/494	494/508			
	490/490	▼	478/494	494/494	494/508			

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

4.3.2.4 SMHCC1

Table 27: SMHCC1 genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes		Genotypes* (bp) in comparison with								
	Genotype	Effect									
IFN-γ (Johnin-nil) production											
Property 1	188/210	▲	**186/192**	188/188	188/198	**192/194**	192/210	**194/198**	**194/202**	**198/210**	
	194/210	▲	186/192	188/188	**192/194**	194/198	**194/202**	198/210			
	188/194	▲	186/192	**192/194**	194/198	194/202	198/210				
	192/194	▼	**188/194**	**188/210**	192/192	**194/210**					
	194/202	▼	188/194	**188/210**	**194/210**						
	198/210	▼	188/194	**188/210**	194/210						
	194/198	▼	188/194	**188/210**	194/210						
	186/192	▼	188/194	**188/210**	194/210						
	188/188	▼	188/210	194/210							
Property 2	186/202	▲	**188/194**	188/210	192/202	194/202	**202/202**				
	202/202	▼	**186/202**	192/194							
	188/194	▼	**186/202**	192/194							
Property 3	194/202	▼	180/194	**180/198**	188/194	**188/198**	188/210	**192/194**	**192/210**	**194/198**	**198/210**
	192/192	▼	188/198	192/194	192/210	**194/198**					
IFN-γ (Johnin-avian) production											
Property 1	188/210	▲	**186/192**	**188/188**	192/192	**192/194**	194/198	**194/202**	**198/210**		
	188/194	▲	**186/192**	**188/188**	**192/194**	**194/202**	**198/210**				
	194/210	▲	188/188	192/194	**194/202**	198/210					
	188/192	▲	186/192	188/188	192/194	**194/202**					
	192/210	▲	186/192	188/188	192/194	**194/202**					
	194/202	▼	**188/192**	**188/194**	**188/210**	190/194	**192/210**	194/194	**194/210**		
	188/188	▼	188/192	**188/194**	**188/210**	192/210	194/194	194/210			
	192/194	▼	188/192	**188/194**	**188/210**	192/210	194/210				
	186/192	▼	188/192	**188/194**	**188/210**	192/210					
	198/210	▼	**188/194**	**188/210**	194/210						
Property 2	186/202	▲	**188/194**	188/210	192/202	194/202	**202/202**				
	202/202	▼	180/194	**186/202**	194/194						
	188/194	▼	180/194	**186/202**	194/194						
Property 3	194/198	▲	192/192	**194/202**							
	194/202	▼	180/194	188/194	188/198	192/210	**194/198**	194/210	198/210		

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

SMHCC1 genotype 188/210 was significantly associated with high IFN-γ levels in property1 sheep (table 27). However, it had an exactly opposite effect in properties 2 and 3, with animals of this genotype having low responses (figure 20). Property 1 animals with genotypes 188/194, 194/210, 188/192 and 192/210 also had significantly higher IFN-γ responses compared to those possessing at least four other genotypes. The effects of these genotypes were inconsistent across the other two properties. Animals in properties 1 and 3 with genotype 194/202 had the lowest IFN-γ responses in those properties, while those in property

2 also had significantly low levels, but compared only to animals with genotype 186/202. Four other genotypes (188/188, 192/194, 186/192 and 198/210) resulted in significantly low IFN-γ responses in property 1 vaccinates. These genotypes were either confined to property 1 or had inconsistent effects in the other two properties. Genotypes 186/202 and 202/202, which had sufficient numbers only in vaccinates of property 2 resulted in highest and lowest IFN-γ responses, respectively, in that property. Animals with genotype 194/198 had higher responses in property 3 than animals with two other genotypes.

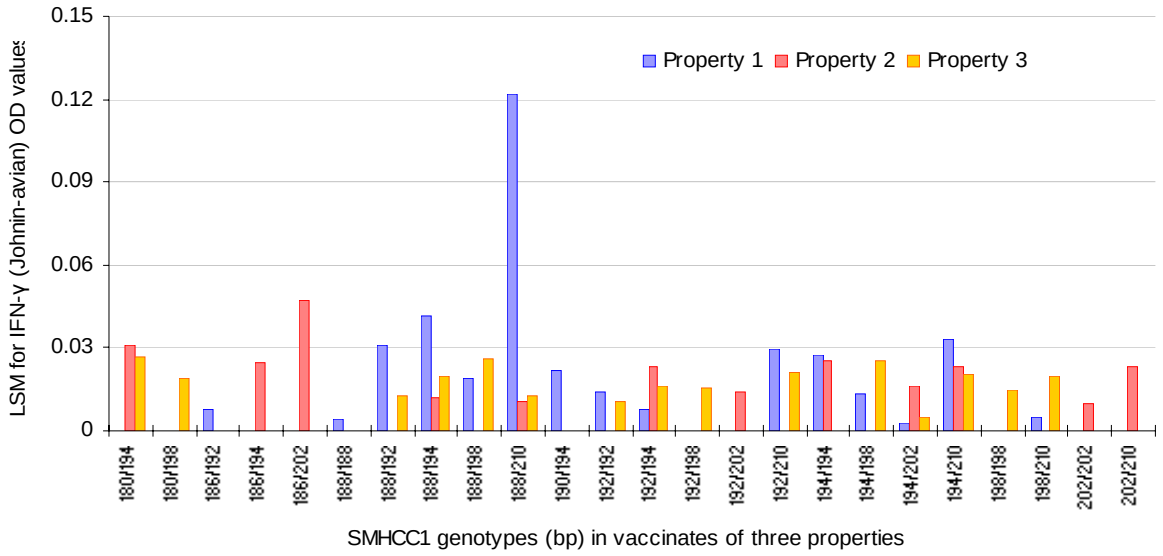

Figure 20: Effect of SMHCC1 genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.3.2.5 OVINRA1

OVINRA1 genotypes 158/160 and 164/168 were found to be associated with significantly high IFN-γ levels in property 1 vaccinates (table 28). While genotype 164/168 had insufficient numbers in properties 2 and 3, animals with genotype 158/160 showed remarkably low IFN-γ responses in those two properties (figure 21). Animals with genotypes 160/168 and 162/168 exhibited lowest responses on property 1; however, both the genotypes resulted contrastingly high responses in property 3. The effect of genotype 156/158 was consistent across properties 1 and 2, resulting significantly low IFN-γ responses in those properties. It had insufficient numbers on property 3. In vaccinates of property 2, genotype 158/164 resulted in highest IFN-γ responses. Animals with this genotype exhibited higher responses also in property 1, while those on property 3 had contrastingly low IFN-γ levels.

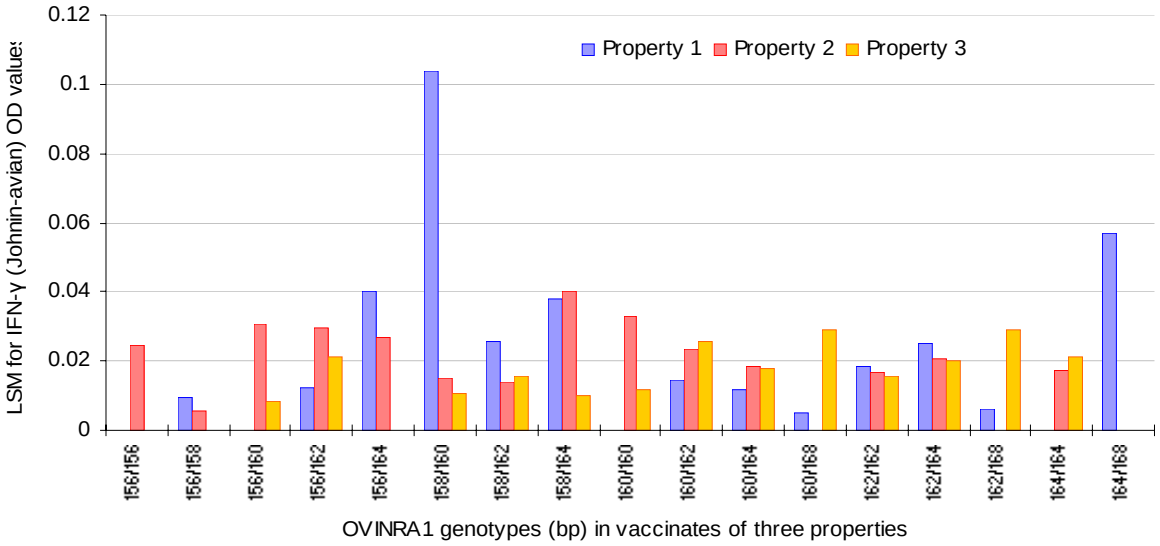

Figure 21: Effect of OVINRA1 genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Table 28: OVINRA1 genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with
	Genotype (bp)	Effect	
IFN-γ (Johnin-nil) production			
Property 1	158/160	▲	**156/158 160/164 160/168** 162/162 **162/168**
	164/168	▲	156/158 160/164 160/168 162/162 **162/168**
	156/164	▲	160/164 **160/168** 162/168
	160/168	▼	156/162 **156/164 158/160 158/162 158/164** 160/162 162/162 **162/164** 164/168
	162/168	▼	156/164 **158/160** 158/162 158/164 162/164 **164/168**
	160/164	▼	156/164 **158/160** 164/168
	162/162	▼	158/160 160/168 164/168
	156/158	▼	**158/160** 164/168
Property 2	156/162	▲	**156/158 158/160 158/162** 162/164 164/164
	156/160	▲	**156/158** 158/160 158/162
	156/158	▼	**156/160 156/162** 156/164 158/164 160/160 160/162 160/164 162/162
	158/160	▼	156/160 **156/162**
Property 3	162/168	▲	158/160 158/164
	158/160	▼	**160/162** 160/168 162/164 162/168 164/164
IFN-γ (Johnin-avian) production			
Property 1	158/160	▲	**156/158 156/162** 158/162 **160/162** 160/164 **160/168** 162/162 162/164 **162/168**
	164/168	▲	156/158 156/162 160/162 160/168 **162/168**
	160/168	▼	156/164 **158/160** 158/162 158/164 162/164 164/168
	162/168	▼	156/164 **158/160** 158/162 158/164 **164/168**
Property 2	158/164	▲	**156/158** 158/160 158/162 162/162
	156/158	▼	**156/156 156/160 156/162 156/164** 158/160 158/162 **158/164 160/160 160/162 160/164** 162/162 **162/164** 164/164
	158/162	▼	156/158 156/160 156/162
Property 3	158/160	▼	160/162

* Genotypes in normal font differ significantly ($P<0.05$), while those in bold font differ highly significantly ($P<0.01$)

4.3.2.6 OVINRA2

OVINRA2 genotypes did not differ significantly in their effects on IFN-γ production in *Map* vaccinated sheep on properties 1 and 2. Even on property 3, there was only one significant difference, animals with genotype 312/326 showing a greater response than those possessing 314/314 (table 29 and figure 22).

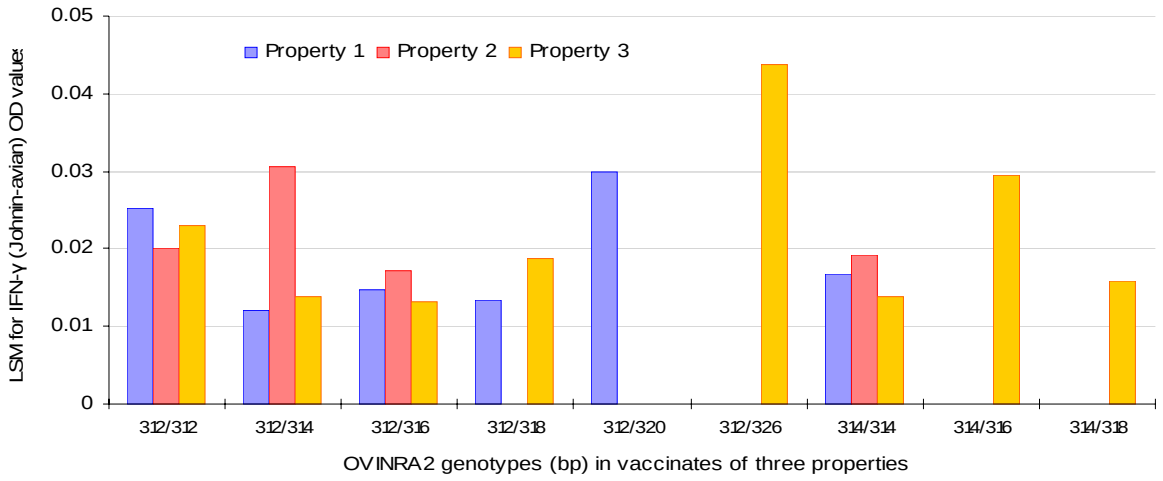

Figure 22: Effect of OVINRA2 genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Table 29: OVINRA2 genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with
	Genotype (bp)	Effect	
IFN-γ (Johnin-nil) production			
Property 1	-	-	-
Property 2	-	-	-
Property 3	312/326	▲	314/314
IFN-γ (Johnin-avian) production			
Property 1	-	-	-
Property 2	-	-	-
Property 3	312/326	▲	314/314

* Genotypes in normal font differ significantly ($P<0.05$)

4.3.2.7 o(IFN)-γ

The effects of genotypes at this marker locus on IFN-γ responses were consistent across all the three properties (figure 23 and table 30). In vaccinates of all the three properties,

genotypes 124/124 and 124/128 had a lowering effect on IFN-γ production, while genotype 128/128 was associated with high responses. Genotype 128/128 resulted in significantly higher responses than either 124//128 alone (in property 1) or both 124/128 and 124/124 (property 3). In property 2, animals with genotype 128/128 had significantly higher IFN-γ (Johnin-nil) levels than those possessing either 124/128 or 124/124. Genotype 128/128 in this property also resulted in higher, but statistically non-significant, IFN-γ (Johnin-avian) responses, compared to genotypes 124/128 and 124/124.

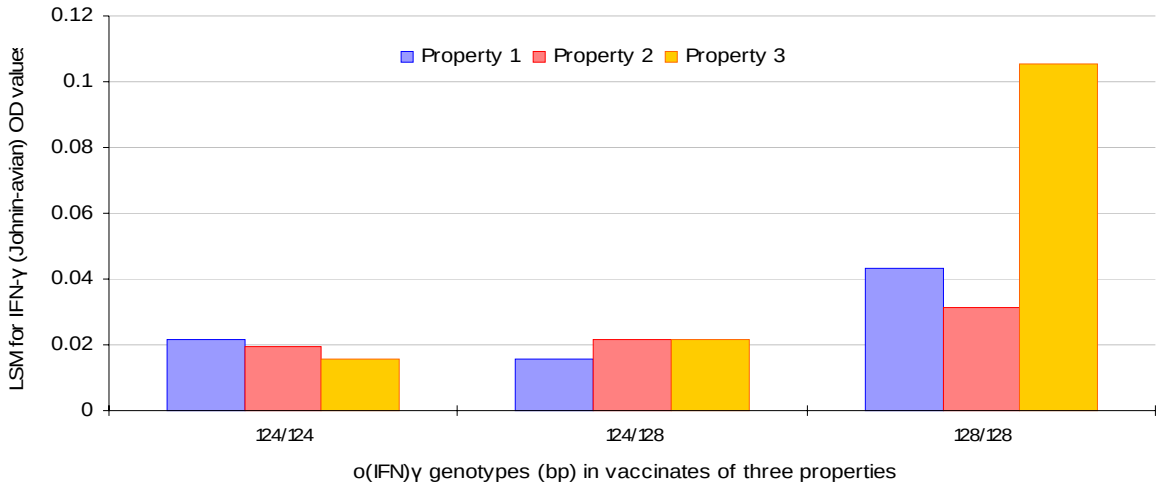

Figure 23: Effect of o(IFN)γ genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Table 30: o(IFN)γ genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with	
	Genotype (bp)	Effect		
IFN-γ (Johnin-nil) production				
Property 1	128/128	▲	124/128	
Property 2	128/128	▲	124/124	124/128
Property 3	128/128	▲	**124/124**	**124/128**
IFN-γ (Johnin-avian) production				
Property 1	128/128	▲	**124/128**	
Property 2	-	-	-	
Property 3	128/128	▲	**124/124**	**124/128**

* Genotypes in normal font differ significantly (P<0.05), while those in bold font differ highly significantly (P<0.01)

4.3.2.8 OarKP6

OarKP6 genotype 200/202 resulted in significantly low IFN-γ responses in vaccinates of property 1 (table 31 and figure 24). This genotype resulted in moderate to high responses in vaccinates belonging to properties 2 and 3. In property 2, individuals with genotype 200/200 showed significantly higher IFN-γ responses than individuals possessing genotype 200/204. None of the genotypes exhibited significant effects on IFN-γ production in property 3 vaccinates.

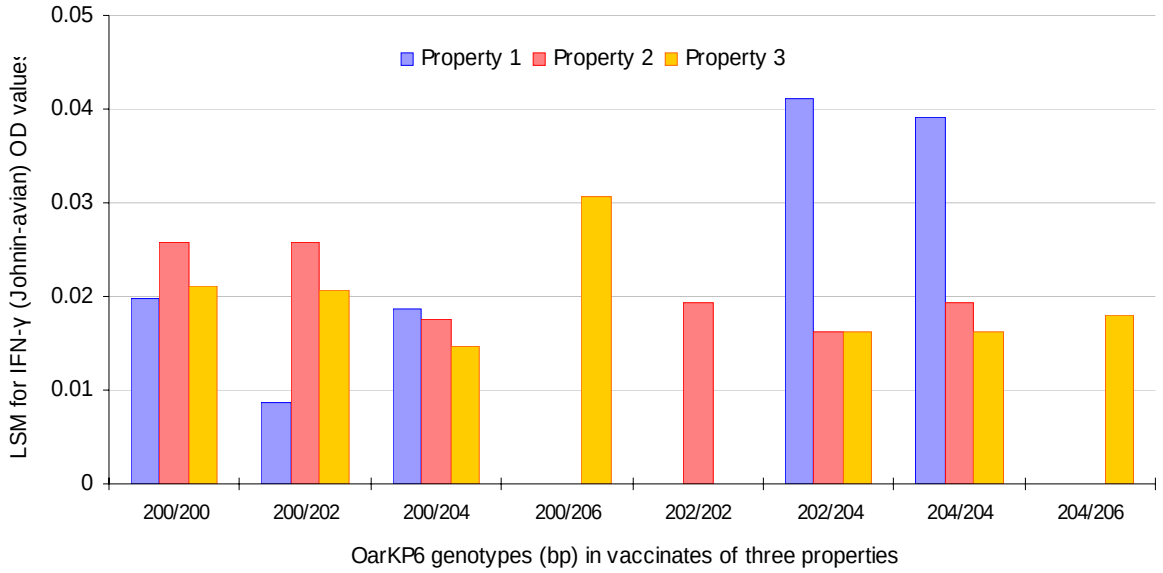

Figure 24: Effect of OarKP6 genotypes on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Table 31: OarKP6 genotypes found to have significant effect on IFN-γ responses to Johne's vaccination in sheep

Property	Genotypes significant		Genotypes* (bp) in comparison with		
	Genotype (bp)	Effect			
IFN-γ (Johnin-nil) production					
Property 1	200/202	▼	200/204	202/204	**204/204**
Property 2	200/200	▲	**200/204**		
Property 3	-	-	-		
IFN-γ (Johnin-avian) production					
Property 1	200/202	▼	202/204	204/204	
Property 2	-	-	-		
Property 3	-	-	-		

* Genotypes in normal font differ significantly (P<0.05), while those in bold font differ highly significantly (P<0.01)

4.3.2.9 Power analysis

Post-hoc power analysis was carried out to determine an approximate power of the employed statistical model in detecting a significant difference in IFN-γ responses between vaccinated sheep possessing two different genotypes at a particular marker locus. Power was estimated for markers o(IFN)-γ and OVINRA1 in a similar way as done for antibody responses (detailed in section 4.3.1.9). For each marker locus, overall standard deviation for IFN-γ (Johnin-avian) response at maximum response time-point (2 months post-vaccination), mean IFN-γ (Johnin-avian) values at the maximum response time-point for each genotype, together with respective number of individuals for each genotype were input. The estimated power values based on the observed differences in means were 0.68 and 0.45 for genotypes at o(IFN)-γ and OVINRA1 loci, respectively. Based on the observed standard deviation and differences in means, the total number of individuals required to achieve power values up to 1.00 were extrapolated for genotypes at both the marker loci (figures 25 and 26). For genotypes at markers o(IFN)-γ and OVINRA1, total sample size of around 225 and 275, respectively, would be required to achieve a power of 0.80.

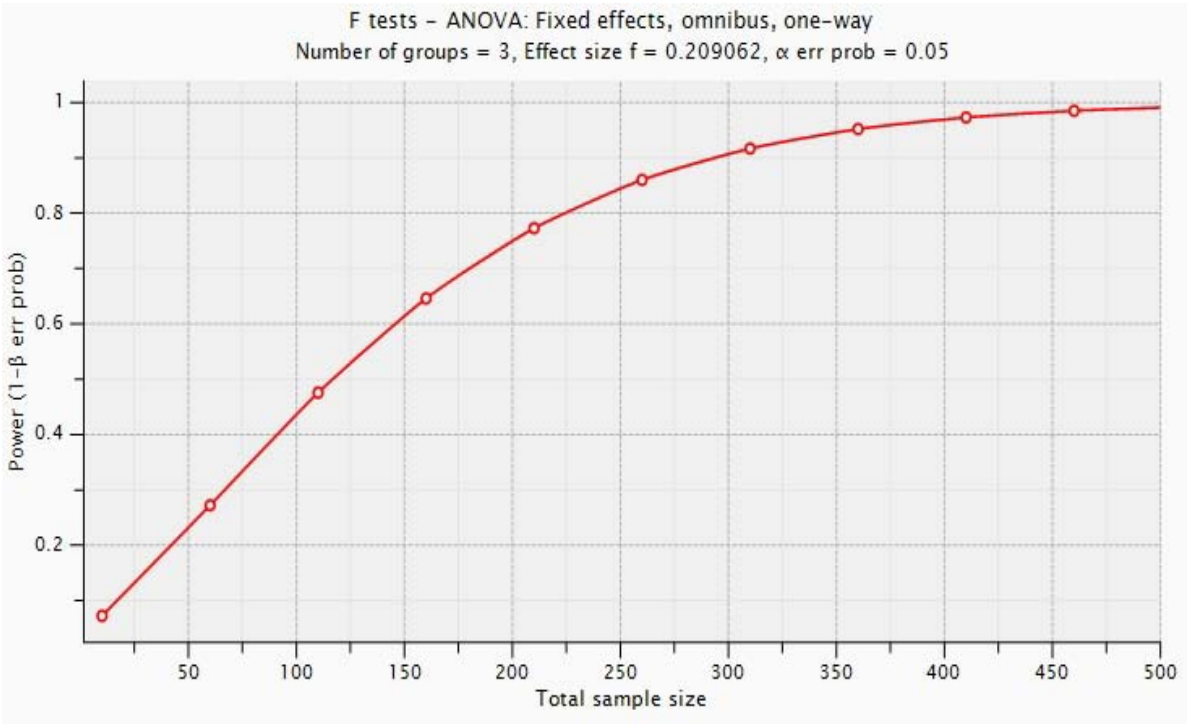

Figure 25: Sample size versus power of detecting a significant effect of genotypes at o(IFN)-γ locus on IFN-γ responses to Johne's vaccination

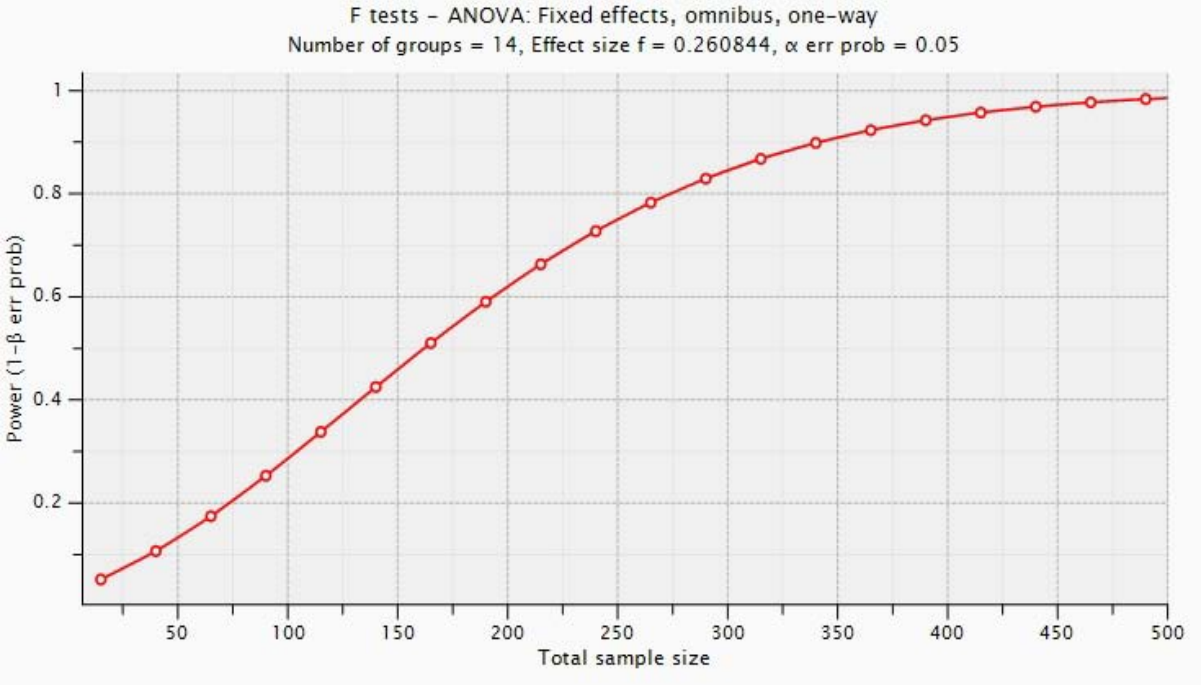

Figure 26: Sample size versus power of detecting a significant effect of genotypes at OVINRA1 locus on IFN-γ responses to Johne's vaccination

4.3.2.10 Summary

Several genotypes at DYMS1, OLADRB, OLADRW, SMHCC1, OVINRA1 and o(IFN)-γ marker loci were found to possess significant effects on IFN-γ responses to Johne's vaccination of sheep belonging to three properties. The majority of these effects were inconsistent across the three properties. Genotypes found to be significantly associated with either high or low responses in one or two properties either had opposite effects or were absent in the other property/properties. Only, the effects of two genotypes (186/200 and 202/202) at DYMS1 and one genotype at each of the OLADRB (272/276), SMHCC1 (194/202), OVINRA1 (156/158) and o(IFN)-γ (128/128) were consistent across the three properties. While the o(IFN)-γ genotype 128/128 was associated with high IFN-γ responses, the other five genotypes with consistent effects were associated with low responses. For marker loci OVINRA2 and OarKP6, only a few genotypes were found to influence IFN-γ responses and those effects were confined to a particular property.

4.4 EFFECT OF MARKER ALLELES ON IMMUNE RESPONSES

As mentioned in section 4.3, the effects of marker alleles on immune responses (for possible dominant effects) were also assessed in addition to the effects of marker genotypes

(indicative of additive effects). Effects of marker alleles on immune responses were tested employing a mixed model analysis in SAS. Immune responses recorded at different time points post-vaccination were log-transformed so as to normalize data. The model tested the effects of presence versus absence of different alleles at each of the eight marker loci on immune responses in vaccinated and control individuals on each property. As seen earlier when testing for genotype effects, the effects of marker alleles on antibody as well as IFN-γ responses in controls on all the three properties were insignificant ($P>0.05$). Hence, only the allelic effects in vaccinates are presented.

4.4.1 Effect on antibody responses

Mean antibody responses (LSM in logarithmic scale) for presence versus absence of different alleles at the eight marker loci in vaccinated sheep belonging to three properties are presented in the appendix (tables A28-A33). LSM (transformed to normal scale) for marker alleles are depicted separately for each marker in figures 27-34. Alleles at different marker loci found to have significant effects on antibody responses in *Map* vaccinates belonging to three properties are listed in table 32 and allelic effects are detailed separately for each marker.

Table 32: Significant effects of presence of different marker alleles on antibody responses to vaccination in sheep

Marker	Property 1		Property 2		Property 3	
	Allele[1] (bp)	Effect[2]	Allele[1] (bp)	Effect[2]	Allele[1] (bp)	Effect[2]
DYMS1	-	-	186	▲	188	▲
OLADRB	-	-	274	▼	272	▼
OLADRW	-	-	490	▲	502	▼
SMHCC1	210	▲	194	▼	-	-
OVINRA1	-	-	156	▲	-	-
OVINRA2	312	▼	-	-	-	-
o(IFN)γ	-	-	-	-	-	-
KP6	186	▼	186	▼	204	▼

[1] Listed alleles had significant ($P<0.05$) effect, in comparison with absence of respective alleles.

[2] Effect of presence of an allele in comparison to its absence

4.4.1.1 DYMS1

The presence of DYMS1 alleles 198 and 204 resulted in higher antibody responses in property 1 vaccinates, compared to their absence (figure 27). However, their effects were statistically non-significant. Allele 186 had a significant effect on increased antibody responses in property 2 vaccinates (table 32). It had no significant effect in the other two properties. The presence of allele 188 had a significantly effect on increased antibody

responses in sheep belonging to property 3. While this allele had insufficient numbers for evaluation in property 2, its presence resulted in non-significantly low antibody responses in property 1.

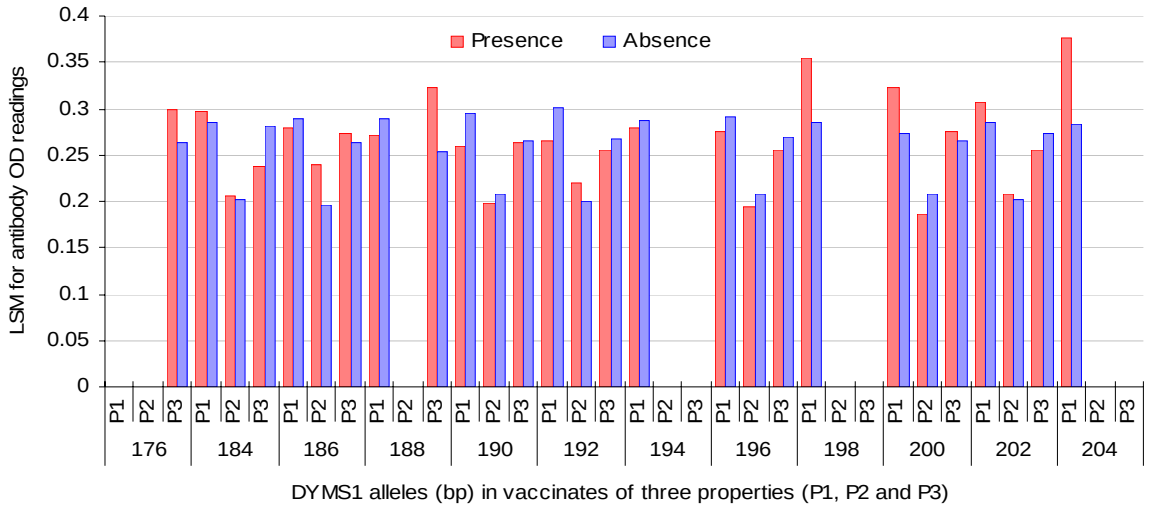

Figure 27: Effect of DYMS1 alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.4.1.2 OLADRB

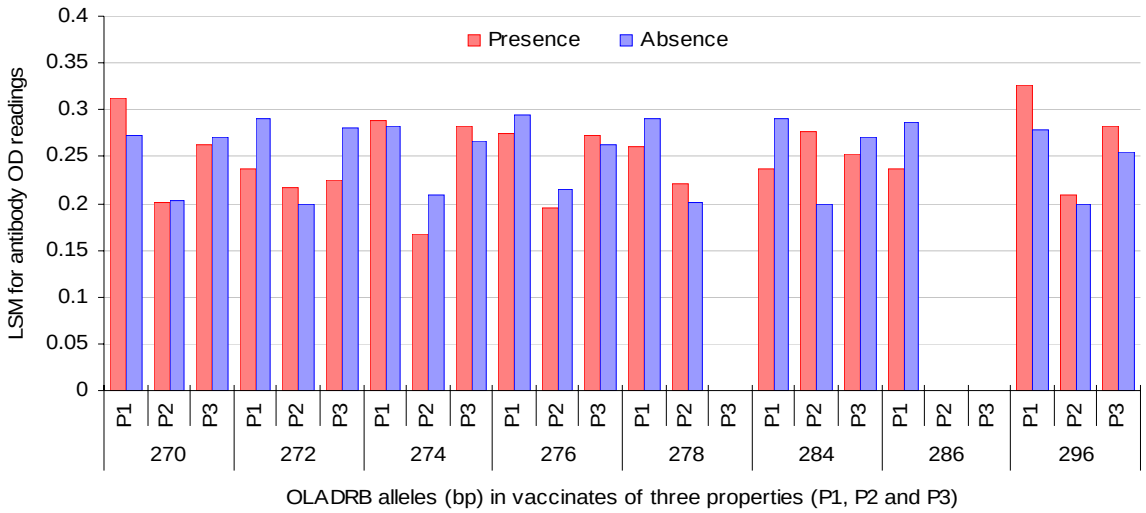

Figure 28: Effect of OLADRB alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Alleles at OLADRB locus had variable but non-significant effects on antibody production in property 1 vaccinates (figure 28 and table 32). Presence of allele 274 resulted in significant reduction in antibody levels in property 2 vaccinates, compared to its absence. The presence of this allele had no significant effect on properties 1 and 3. A different allele, 272, was found to be significantly associated with decreased responses in property 3. This allele had a similar but non-significant effect in property 1, but the effects of its presence and absence were almost similar in property 2 vaccinates.

4.4.1.3 OLADRW

The presence of OLADRW alleles 490 and 494, compared to their absence resulted in high antibody responses in property 1 vaccinates (figure 29), while presence of allele 500 is associated with decreased levels. However, none of these effects were statistically significant (table 32). In property 2, allele 490 was significantly associated with increased antibody levels. This allele had a slight reducing, but non-significant effect in property 3. Property 3 animals with allele 502 had significantly low antibody levels compared to those lacking the allele. This allele had insufficient numbers for testing in property 1 and in property 2 it had a mild reducing effect.

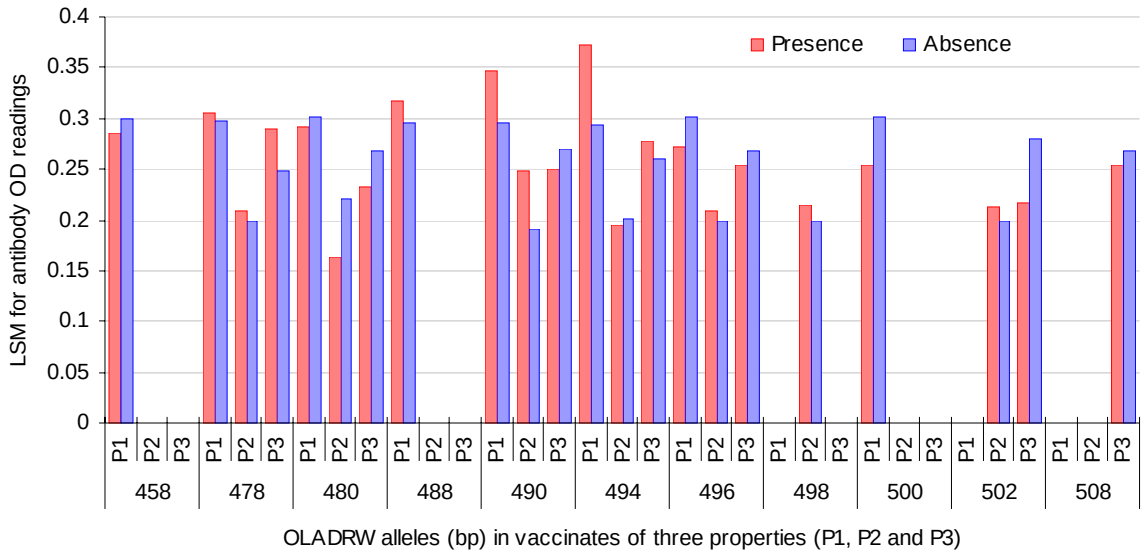

Figure 29: Effect of OLADRW alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.4.1.4 SMHCC1

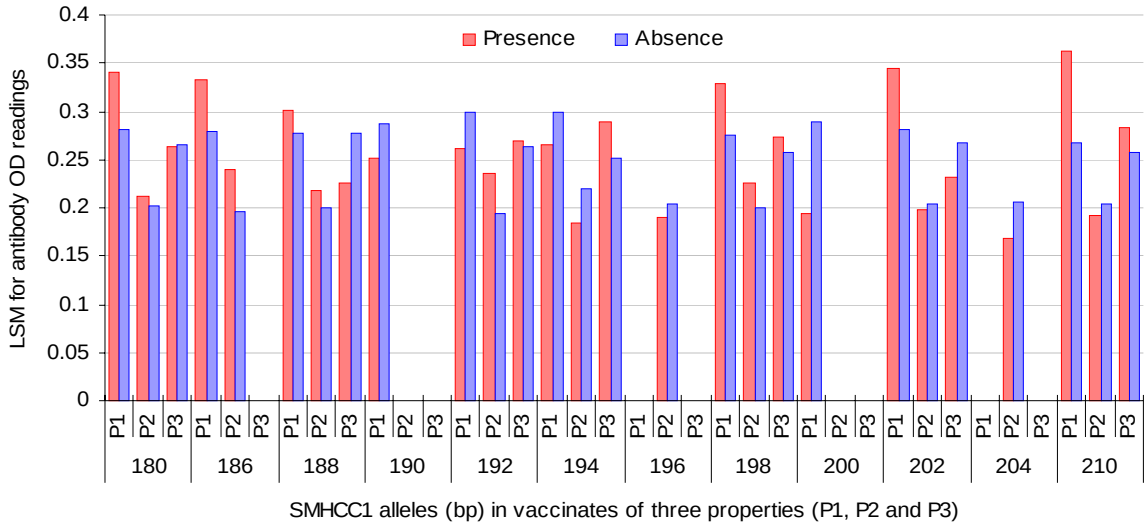

Figure 30: Effect of SMHCC1 alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

SMHCC1 allele 210 significantly increased antibody production in property 1 (table 32 and figure 30). It had non-significant and inconsistent effects in the other two properties. The presence of several other alleles also resulted in increased antibody responses in property 1. However, none of those effects were statistically significant, mostly because of low allelic frequencies. Allele 194 significantly reduced antibody responses in property 2 vaccinates. Its effects in the other two properties were conflicting and non-significant – reduced and increased levels, respectively, in properties 1 and 3. None of the SMHCC1 alleles had significant effects on antibody production in property 3 vaccinates.

4.4.1.5 OVINRA1

None of the OVINRA1 alleles significantly influenced antibody responses in sheep on properties 1and 3. The presence of allele 156 in property 2 significantly increased antibody responses (table 32 and figure 31). This allele resulted in non-significantly high or low responses in properties 1 and 2 respectively.

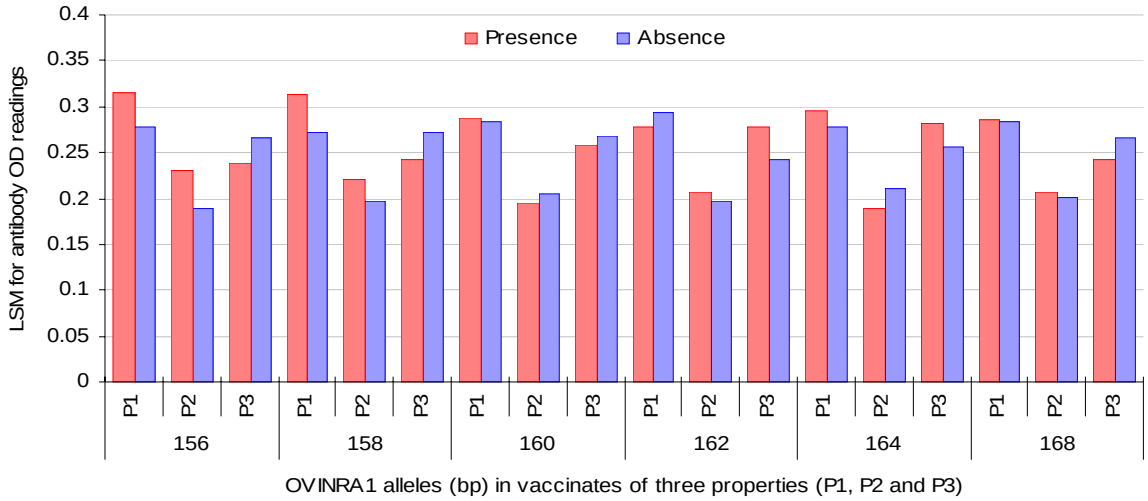

Figure 31: Effect of OVINRA1 alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.4.1.6 OVINRA2

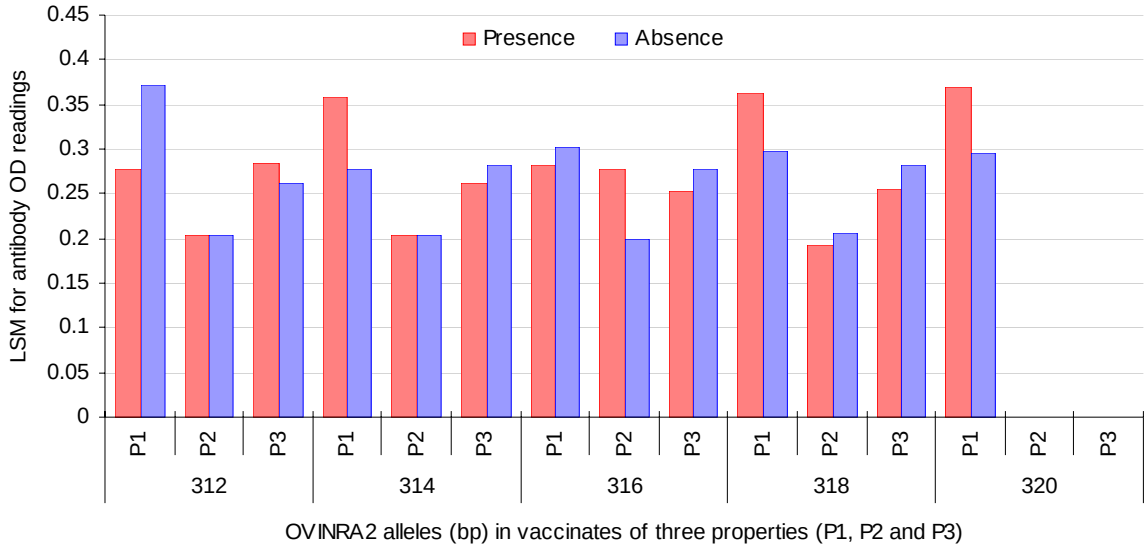

Figure 32: Effect of OVINRA2 alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Property 1 vaccinates with OVINRA2 allele 312 had significantly low antibody levels compared to those lacking the allele (table 32 and figure 32). However this allele had no

significant effect in the other two properties. The remaining alleles at this locus had variable but non-significant effects in the three properties.

4.4.1.7 o(IFN)-γ

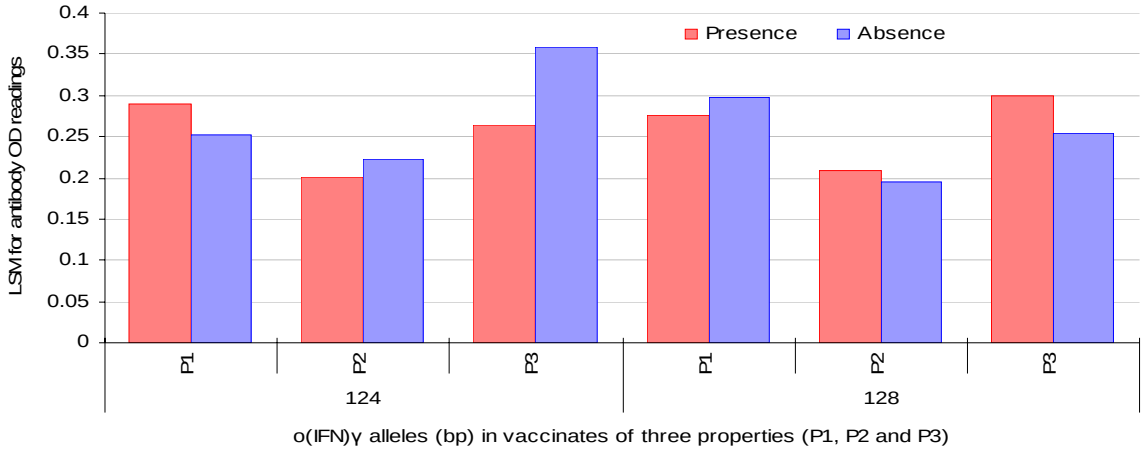

Figure 33: Effect of o(IFN)-γ alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

The two alleles at this locus had varied and inconsistent effects on antibody responses in vaccinates across the three properties (figure 33). None of those effects were significant.

4.4.1.8 OarKP6

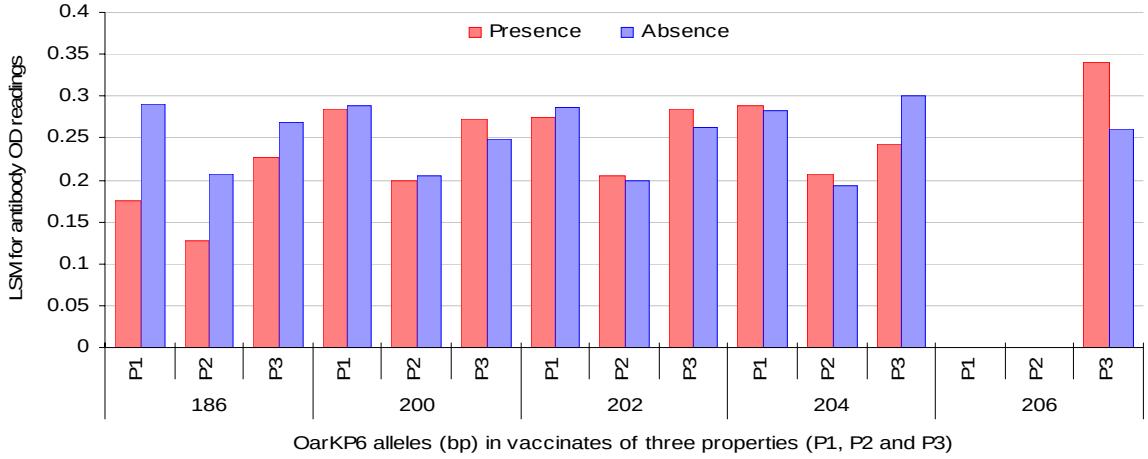

Figure 34: Effect of OarKP6 alleles on antibody production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

A 186 bp allele at this locus significantly decreased antibody responses in properties 1 and 2 (table 32 and figure 34). It had a similar but statistically non-significant effect in property 3. The presence of a different allele, 204, resulted in significantly low antibody levels in property 3. This allele had insufficient numbers in vaccinates belonging to properties 1 and 2.

4.4.1.9 Power analysis

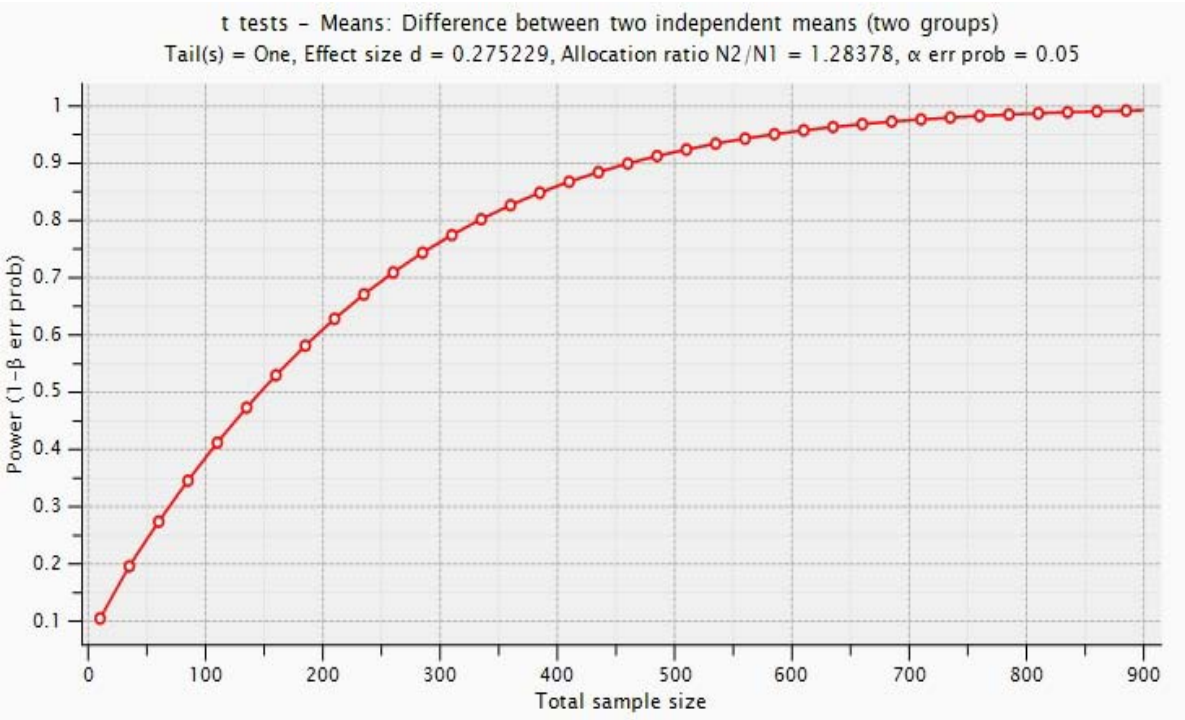

Figure 35: Sample size versus power of detecting a significant effect of allele 202 at DYMS1 locus on antibody responses to Johne's vaccination

Post-hoc power analysis was carried out to determine an approximate power of the employed statistical model in detecting a significant difference in antibody responses between vaccinated sheep possessing a particular allele and sheep lacking that allele, at a particular marker locus. *A priori* analysis of required sample size could not be done as there were no prior studies in the literature on the genetic effects on immune responses. As an illustration, power of testing the effect of allele 202 on antibody responses in property 3 vaccinates was estimated employing G*power (Faul *et al.* 2007). A two independent means model (t-test) was assumed for simplicity. Mean and standard deviation for antibody response at the maximum response time-point (2 months post-vaccination) for allelic presence and absence, together with the respective number of individuals in each category were input; the level of

significance was set at 5%. The estimated power value based on the observed differences in means was 0.55. Based on the observed standard deviation and differences in means, the total number of individuals required to achieve power values up to 1.00 were extrapolated (figure 35). A total sample size of around 340 would be required to achieve a power of 0.80.

4.4.1.10 Summary

Several alleles belonging to the investigated marker loci, excepting o(IFN)-γ, were found to significantly influence antibody responses to Johnes's vaccination, some with increasing and others with decreasing effects. However, the effects of all such alleles, excepting OarKP6 allele 186, were inconsistent across the three properties. Alleles found to be significantly associated with either high or low responses in one or two properties either had opposite effects or were absent in the other property/properties. The effect of OarKP6 allele 186 was consistent across the properties, with significantly low antibody levels in vaccinates of properties 1 and 2 and non-significantly low responses in property 3 vaccinates. The effects of the two alleles at the o(IFN)-γ locus were non-significant in the three properties.

4.4.2 Effect on IFN-γ responses

The effects of marker alleles on IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) responses are presented in this section. Mean IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) responses (LSM in logarithmic scale) for presence versus absence of different alleles at the eight marker loci in vaccinated sheep belonging to three properties are presented in the appendix (tables A28-A33). As seen with the genotypic effects, the IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) response-trends for each of the marker alleles were almost similar. Hence, to avoid repetition, only LSM (transformed to normal scale) for IFN-γ (Johnin-avian) responses for marker alleles are depicted in figures 36-43 for each marker. However, for some of the alleles within each marker locus, the level of significance for allelic presence versus absence comparisons varied between the two IFN-γ phenotypes. Thus, alleles at different marker loci found to have significant effects on IFN-γ (Johnin-nil) as well as IFN-γ (Johnin-avian) responses in *Map* vaccinates belonging to three properties are listed in table 33 and the allelic effects detailed separately for each marker. For simplicity, the discussion pertaining to the effects of alleles at each marker locus was generalised based on the effects on either of the IFN-γ phenotypes.

4.4.2.1 DYMS1

Table 33: Significant effects of presence of different marker alleles on IFN-γ responses to vaccination in sheep

	Property 1		Property 2		Property 3	
	Allele[1] (bp)	Effect[2]	Allele[1] (bp)	Effect[2]	Allele[1] (bp)	Effect[2]
IFN-γ (Johnin-nil) production						
DYMS1	-	-	202	▼	202	▼
	-	-	-	-	188 (0.05)	▲
OLADRB	-	-	270	▲	**270**	▲
	-	-	284 (0.06)	▼	284	▼
	-	-	-	-	276	▼
OLADRW	**458**	▲	496	▲	**494**	▲
	-	-	-	-	508	▼
SMHCC1	188	▲	-	-	-	-
	202	▼	-	-	-	-
OVINRA1	-	-	**158**	▼	158	▼
OVINRA2	-	-	-	-	-	-
o(IFN)γ	124	▼	124	▼	**124**	▼
	-	-	-	-	128	▲
KP6	204 (0.06)	▲	204	▼	204 (0.05)	▼
IFN-γ (Johnin-avian) production						
DYMS1	-	-	-	-	188	▲
OLADRB	-	-	284	▼	270	▲
	-	-	270 (0.05)	▲	276 (0.05)	▼
OLADRW	458	▲	490	▲	480	▼
	494	▼	-	-	494	▲
SMHCC1	188	▲	-	-	-	-
	210	▲	-	-	-	-
OVINRA1	158	▲	**158**	▼	158 (0.06)	▼
OVINRA2	-	-	-	-	-	-
o(IFN)γ	124	▼	-	-	**124**	▼
	-	-	-	-	128	▲
KP6	-	-	204	▼	204 (0.06)	▼

[1] Alleles in normal font had significant ($P<0.05$) effect, in comparison with absence of respective alleles. Alleles in bold font had highly significant ($P<0.01$) effect, in comparison with absence of respective alleles. Values in parenthesis indicate the level of significance for those alleles whose effect was nearing significance

[2] Effect of presence of an allele in comparison to its absence

Vaccinates with DYMS1 allele 202 showed significantly lower IFN-γ (Johnin-nil) responses in properties 2 and 3, compared with those lacking the allele (table 33 and figure 36). This allele also resulted in decreased, but non-significant (Johnin-avian) responses in those properties. Property 2 animals with allele 202 exhibited similar low, but non-significant IFN-γ responses. The presence of allele 188 was associated with significantly high responses in

property 3 vaccinates. This allele had insufficient numbers in property 2 and in property 1 it had a similar, but non-significant effect on IFN-γ responses. Though a few alleles in property 1 had varied effects on IFN-γ responses (figure 31), none of those effects turned out to be significant, mainly because of low frequencies of such alleles.

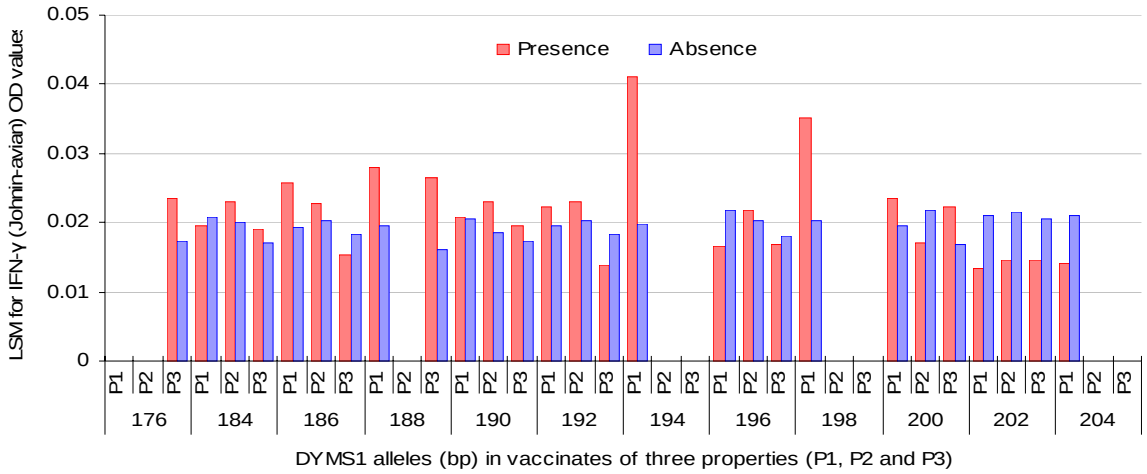

Figure 36: Effect of DYMS1 alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.4.2.2 OLADRB

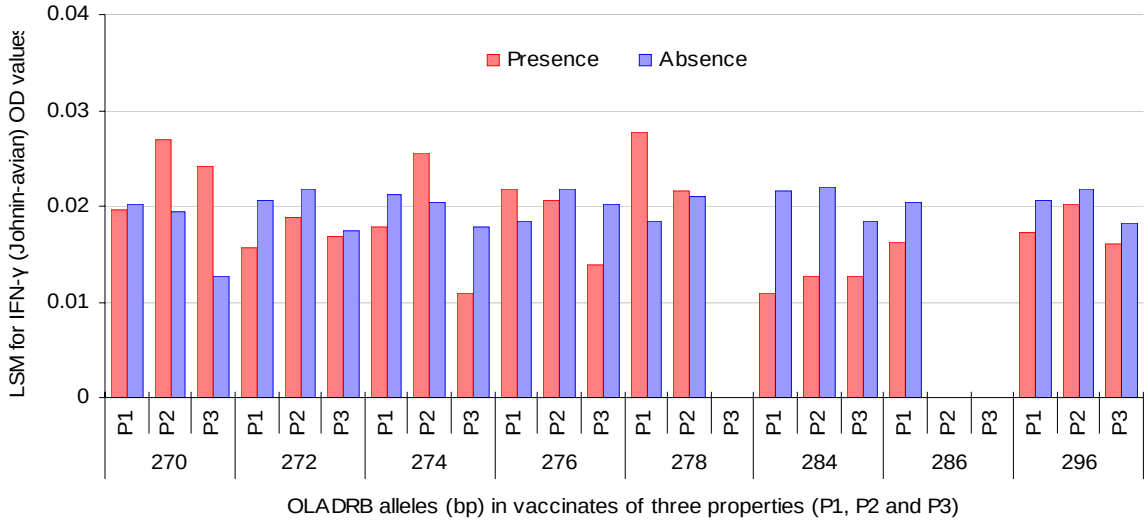

Figure 37: Effect of OLADRB alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Presence of OLADRB alleles 270 and 284 significantly increased or decreased IFN-γ production, respectively, in vaccinates on properties 2 and 3 (figure 37 and table 33). In property 1, allele 284 had a similar decreasing, but non-significant effect, while the effects of presence and absence of allele 270 were quite similar. Allele 276 resulted in significantly low IFN-γ levels in vaccinates belonging to property 3. This allele exhibited mixed and non-significant effects in the other two properties. None of the OLADRB alleles had significant effects on IFN-γ responses in property 1.

4.4.2.3 OLADRW

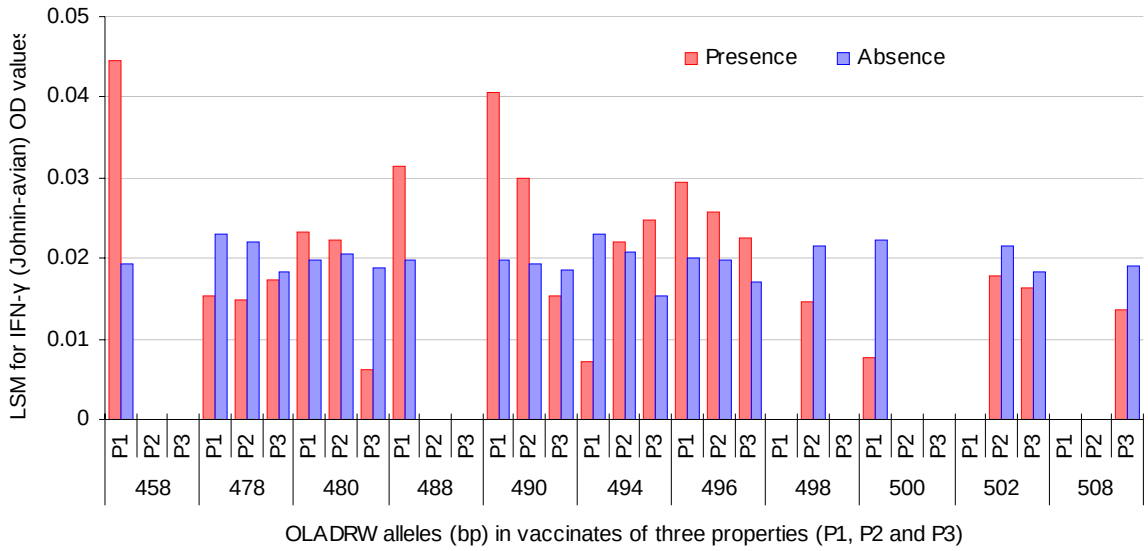

Figure 38: Effect of OLADRW alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

The presence of allele 458 resulted in significantly higher IFN-γ responses in property 1, compared to its absence (figure 38 and table 33). The effect of this allele could not be evaluated in properties 2 and 3 due to insufficient numbers. Allele 494 significantly lowered IFN-γ responses in property 1, while having an exactly opposite effect in property 3 and no significant effect in property 2. Two alleles, 490 and 496, were found to be significantly associated with increased responses in property 2. Presence of allele 490 had a similar increasing, but non-significant effect in property 1 and a slight decreasing effect in property

3. Allele 496 resulted in increased but non-significant IFN-γ levels in properties 1 and 3. Property 3 individuals with alleles 508 and 480 had significantly lower IFN-γ levels, compared to those lacking them. While allele 508 had insufficient numbers in properties 1 and 2, allele 480 had an opposite but non-significant effect in properties 1 and 2.

4.4.2.4 SMHCC1

Property 1 vaccinates with SMHCC1 alleles 188, 210, 180 and 200 showed high IFN-γ responses (figure 39). However, only the effects of alleles 188 and 210 attained statistical significance (table 33). Property 1 animals with allele 202 possessed significantly low IFN-γ levels than those lacking the allele. SMHCC1 alleles exhibited varied effects in vaccinates of properties 2 and 3 (figure 34). However, none of those effects were statistically significant (table 33).

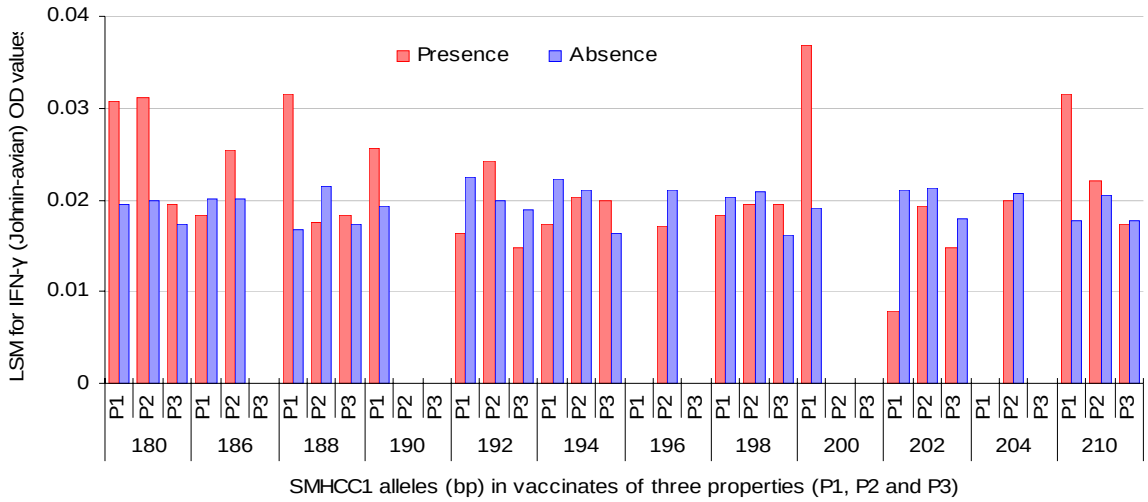

Figure 39: Effect of SMHCC1 alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.4.2.5 OVINRA1

Of the six OVINRA1 alleles tested, only allele 158 had a significant effect on IFN-γ responses (figure 40 and table 33). However, its effect was inconsistent across the three properties, with a significant increasing effect in property 1 and a significant lowering effect in properties 2 and 3.

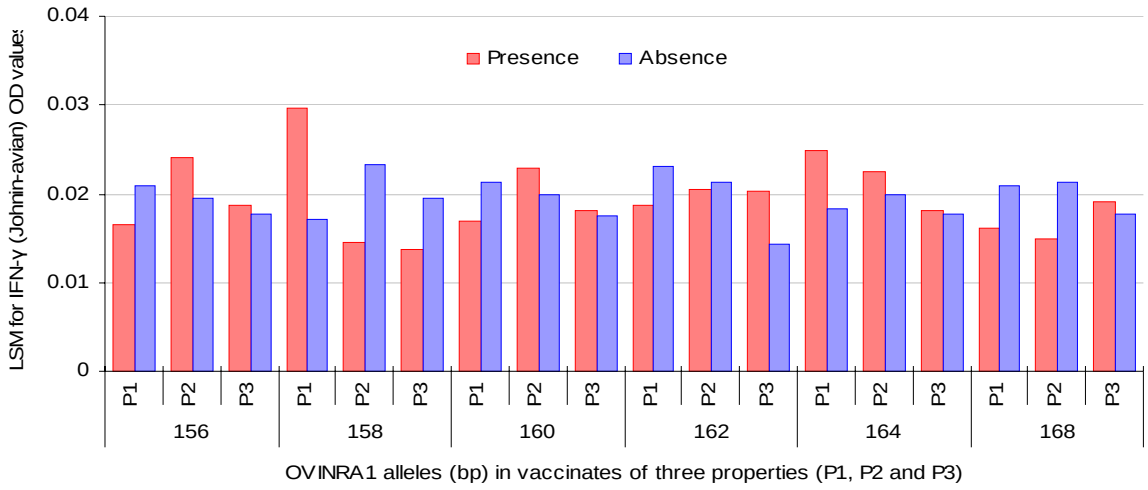

Figure 40: Effect of OVINRA1 alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

4.4.2.6 OVINRA2

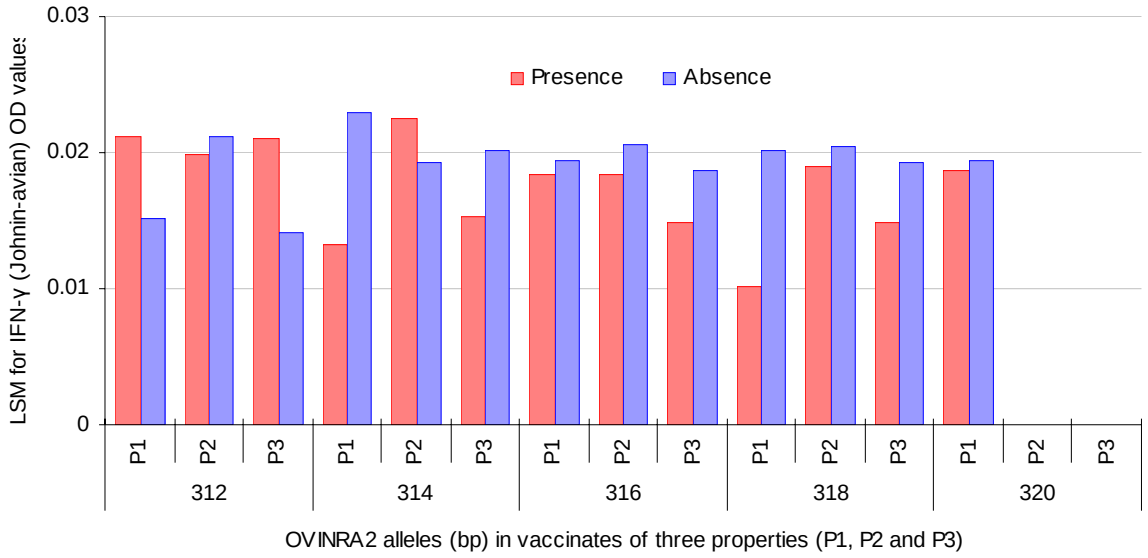

Figure 41: Effect of OVINRA2 alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep

LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Alleles at the OVINRA2 locus showed varied effects on IFN-γ responses in vaccinates of the three properties (figure 41). However, none of those effects were statistically significant in any of the three properties (table 33).

4.4.2.7 o(IFN)-γ

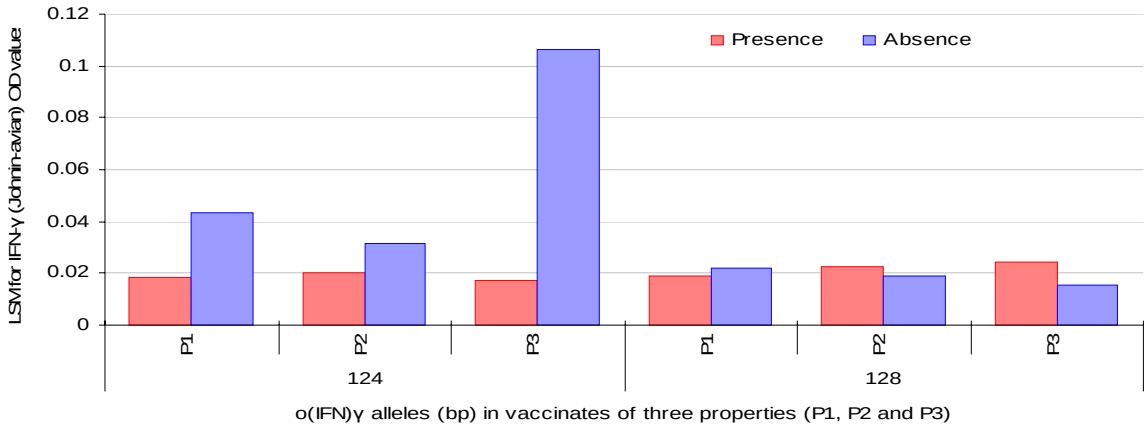

Figure 42: Effect of o(IFN)-γ alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep
LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

A 124 bp allele at this marker locus was found to exhibit a significant and consistent effect on IFN-γ responses in vaccinates of the three properties (table 33 and figure 42). Individuals with at least a single copy of the allele had significantly lower responses in all the three properties, compared to those lacking the allele. This effect was more marked in property 3. Also, allele 128 resulted in significantly higher IFN-γ responses in vaccinates belonging to property 3. However, it had no similar effect in the other two properties.

4.4.2.8 OarKP6

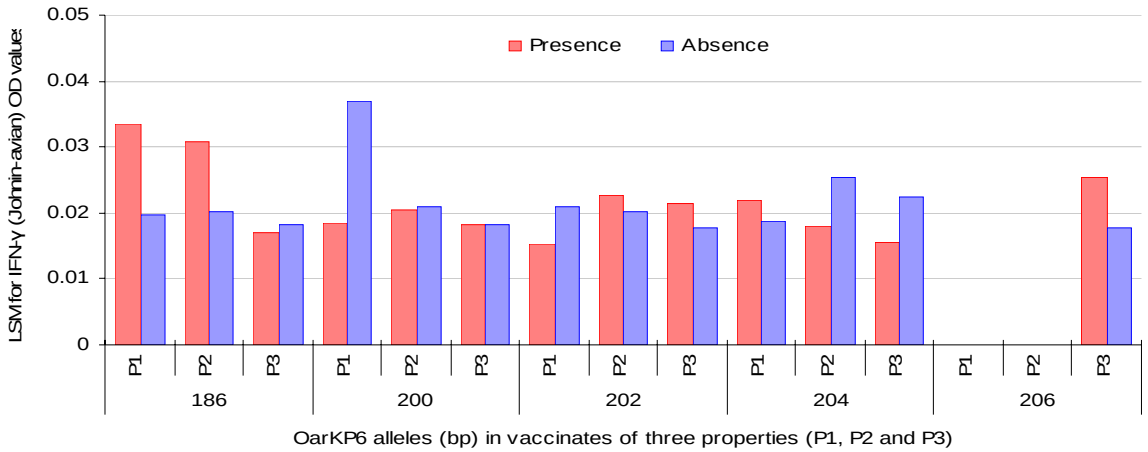

Figure 43: Effect of OarKP6 alleles on IFN-γ (Johnin-avian) production in response to Johne's vaccination in sheep
LSM = least square mean for respective genotypes in vaccinates over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. LSM values, originally in logarithmic scale were transformed to normal scale.

Vaccinated sheep with OarKP6 allele 204 had significantly low IFN-γ responses in property 2 (figure 43 and table 33). This allele possessed either increasing or decreasing effects (both approaching statistical significance), respectively, in properties 1 and 3. The remaining alleles at the marker locus had varying effects, none of which were significant.

4.4.2.9 Power analysis

Post-hoc power analysis was carried out also to determine an approximate power of the employed statistical model in detecting a significant difference in IFN-γ responses between vaccinated sheep possessing a particular allele and sheep lacking that allele, at a particular marker locus. As an illustration, power of testing the effect of allele 202 on antibody responses in property 3 vaccinates was estimated employing G*power (Faul *et al.* 2007), in a similar way as done for antibody responses (detailed in section 4.4.1.9). The estimated power value based on the observed differences in means was 0.58. Based on the observed standard deviation and differences in means, the total number of individuals required to achieve power values up to 1.00 were extrapolated (figure 44). A total sample size of around 300 would be required to achieve a power of 0.80.

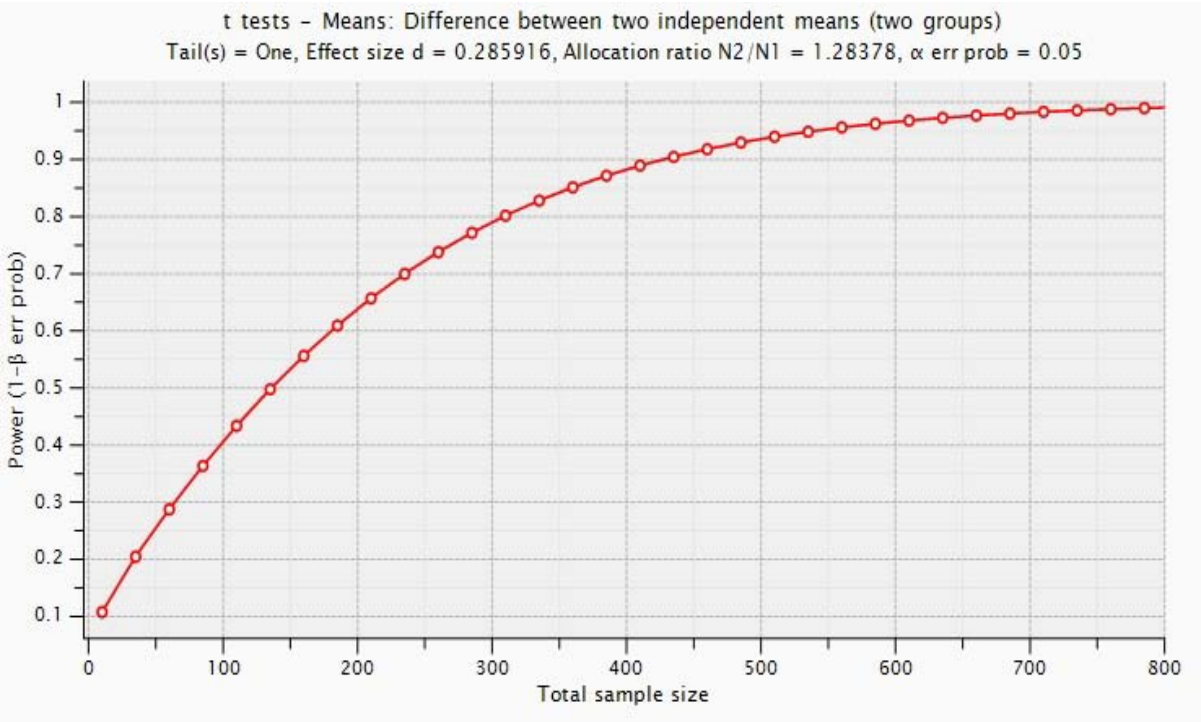

Figure 44: Sample size versus power of detecting a significant effect of allele 202 at DYMS1 locus on IFN-γ responses to Johne's vaccination

4.4.2.10 Summary

At least an allele at each of the eight investigated marker loci showed significant effect (either increasing or decreasing) on IFN-γ responses in vaccinates of one or more properties. Only the effects of three alleles (all influencing decreased IFN-γ responses), one each at DYMS1 (202), OLADRB (284) and o(IFN)-γ (124), were consistent across the three properties. The effects of the remaining alleles found to be significant were inconsistent. Alleles found to be significantly associated with either high or low responses in one or two properties either had opposite effects or were absent in the other property/properties.

4.5 EFFECT OF CHROMOSOME-WISE MARKER HAPLOTYPES ON IMMUNE RESPONSES

As mentioned in chapters 1 and 3, of the eight investigated eight markers, DYMS1, OLADRB, OLADRW and SMHCC1 were from the *Ovar-Mhc* region, located on chromosome 20, OVINRA1 and OVINRA1 from the SLC11A1 gene region on chromosome 2 and o(IFN)-γ and OarKP6 from within the IFN-γ gene region on chromosome 3. Effects of SLC11A1 (OVINRA1 - OVINRA2) and IFN-γ [o(IFN)-γ - OarKP6] haplotypes on antibody and IFN-γ responses were determined separately for control and vaccinate groups within each property. The effects of MHC haplotypes could not be determined as there were several haplotypes with quite low frequencies. The effects of SLC11A1 and IFN-γ haplotypes on both antibody and IFN-γ responses in vaccinates are detailed below.

4.5.1 Effect on antibody responses

The effects of SLC11A1 and IFN-γ haplotypes on antibody responses were insignificant in control animals of all the three properties. Haplotypes found to possess a significant effect on antibody responses in vaccinates belonging to the three properties are listed in table 34.

4.5.1.1 SLC11A1 haplotypes

Of the total 38, 28 and 23 probable SLC11A1 haplotypes in properties 1, 2 and 3 respectively (table 16), only 6, 8 and 7 haplotypes had sufficient numbers for evaluation on the respective properties. However, none of the evaluated haplotypes had significant (P>0.05) influence on antibody responses (table 34). Their effects are depicted in figure 45.

Table 34: Significant effects of OVINRA1 - OVINRA2 and o(IFN)-γ - OarKP6 haplotypes on antibody responses

Property	Haplotypes significant		Haplotypes* (bp) in comparison with		
	Haplotype (bp)	Effect			
OVINRA1 - OVINRA2 haplotypes					
Property 1	-	-	-		
Property 2	-	-	-		
Property 3	-	-	-		
o(IFN)-γ - OarKP6 haplotypes					
Property 1	-	-	-		
Property 2	124-204	▲	Others**		
	128-200	▲	Others**		
Property 3	124-204	▼	**128-200**	124-200	Others**

* Haplotypes in normal font differ significantly (P<0.05), while those in bold font differ highly significantly (P<0.01)

** Include low frequency haplotypes pooled in respective properties

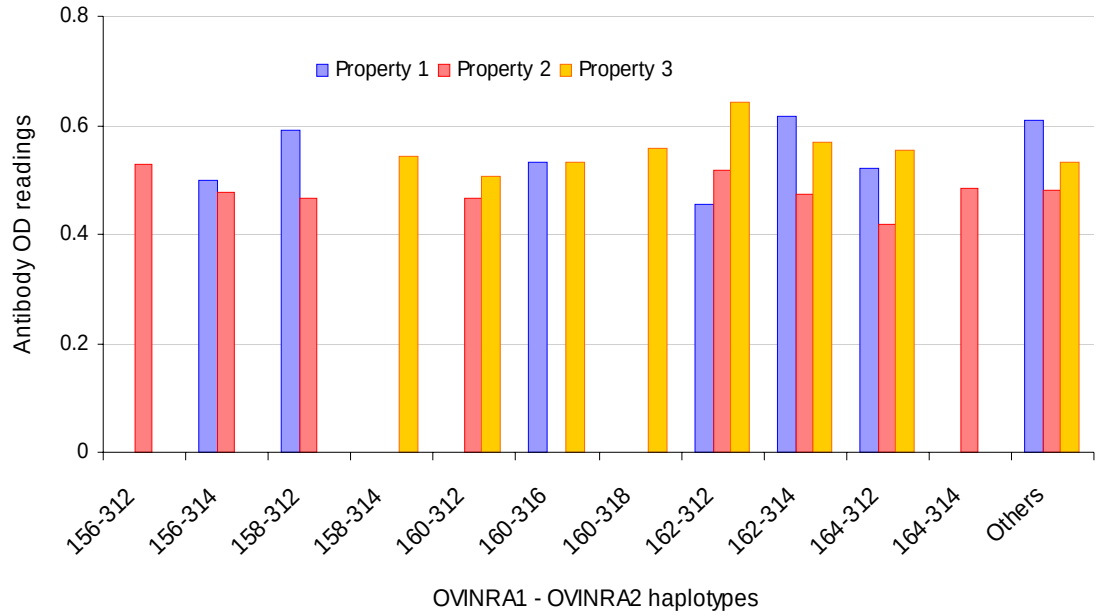

Figure 45: Effect of SLC11A1 haplotypes on antibody production in response to Johne's vaccination in sheep

Effects of haplotypes on antibody responses in vaccinates are combined effects over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. Effects originally in logarithmic scale were transformed to normal scale.

4.5.1.2 IFN-γ haplotypes

Only 3, 4 and 5 IFN- γ haplotypes had adequate frequencies for evaluation in properties 1, 2 and 3 respectively. Their effects on antibody responses in the three properties are depicted in figure 46. While no significant (*P*>0.05) effects were evident in property 1 vaccinates (table 34), two haplotypes (124-204 and 128-200) significantly (*P*<0.05) influenced high responses

compared only to the pooled haplotype group (those with low frequencies). In contrast to that seen in property 2, haplotype 124-204 had the lowest effect on antibody responses in property 3. Its effect was significantly (P<0.05) lower compared to haplotypes 128-200, 124-200 and others.

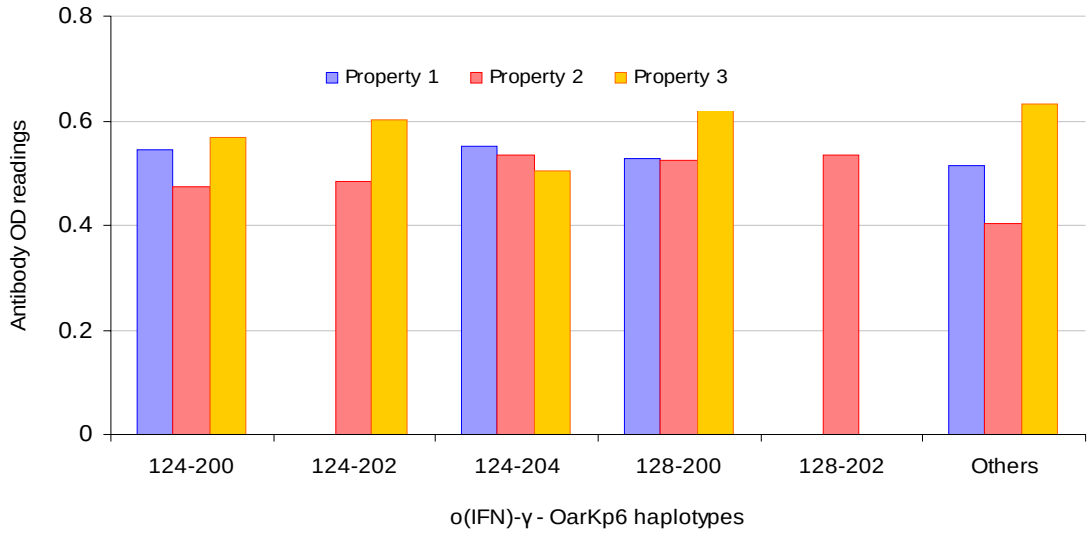

Figure 46: Effect of IFN-γ haplotypes on antibody production in response to Johne's vaccination in sheep

Effects of haplotypes on antibody responses in vaccinates are combined effects over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. Effects originally in logarithmic scale were transformed to normal scale.

4.5.2 Effect on IFN-γ responses

The effects of SLC11A1 and IFN-γ haplotypes on IFN-γ responses were insignificant in control animals on all three properties. Haplotypes found to possess a significant effect on IFN-γ responses in vaccinates belonging to the three properties are listed in table 35. IFN-γ (Johnin-nil) and IFN-γ (Johnin-avian) response-trends and levels of significance for each of the haplotypes were similar. Hence, to avoid repetition, only IFN-γ (Johnin-avian) responses for haplotypes are presented in table as well as graphical presentations.

4.5.2.1 SLC11A1 haplotypes

The effects of SLC11A1 haplotypes on IFN-γ responses in vaccinates belonging to the three properties are shown in figure 47. Haplotypes in properties 2 and 3 had varied effects, but none of them reached statistical significance (P>0.05). In property 1, haplotype 164-312 had a significantly (P<0.05) higher effect on IFN-γ responses, compared to haplotypes 162-312 and others (table 35).

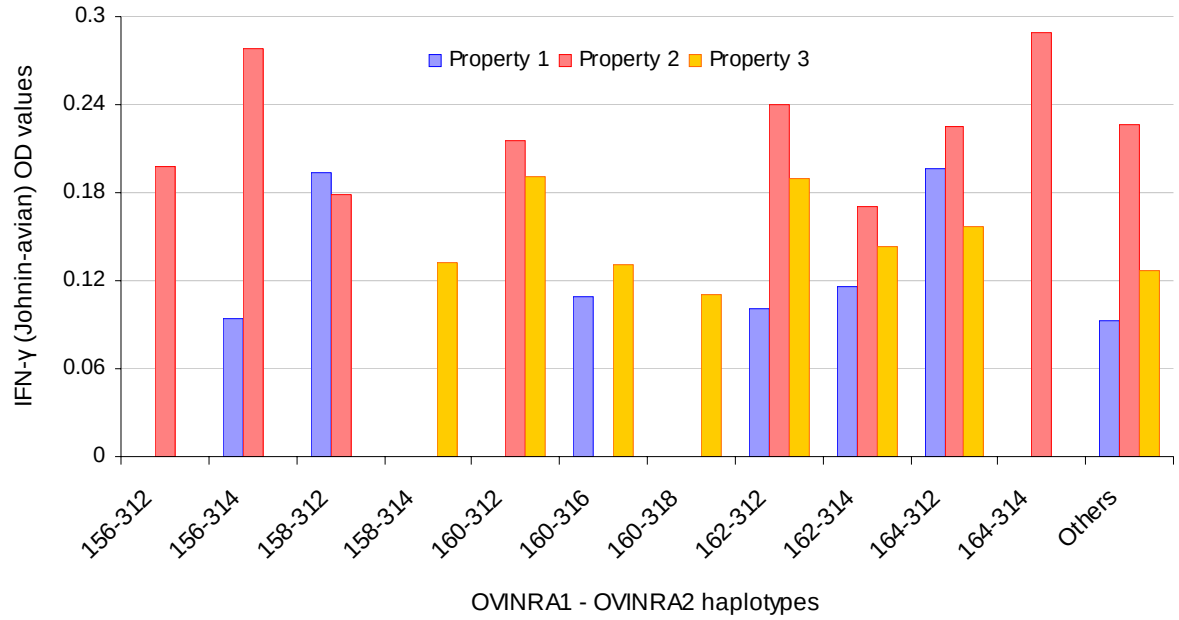

Figure 47: Effect of SLC11A1 haplotypes on IFN-γ (Johnin-avian) responses in Johne's vaccinated sheep

Effects of haplotypes on IFN-γ responses in vaccinates are combined effects over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. Effects originally in logarithmic scale were transformed to normal scale.

Table 35: Significant effects of OVINRA1 - OVINRA2 and o(IFN)-γ - OarKP6 haplotypes on IFN-γ (Johnin-avian)responses

Property	Haplotypes significant		Haplotypes* (bp) in comparison with	
	Haplotype (bp)	Effect		
OVINRA1 - OVINRA2 haplotypes				
Property 1	164-312	▲	162-312	**Others****
Property 2	-	-	-	
Property 3	-			
o(IFN)-γ - OarKP6 haplotypes				
Property 1	-	-	-	
Property 2	-	-	-	
Property 3	128-200	▲	**124-200**	**124-204**

* Haplotypes in normal font differ significantly (P<0.05), while those in bold font differ highly significantly (P<0.01)

** Include low frequency haplotypes pooled in respective properties

4.5.2.2 IFN-γ haplotypes

The effects of IFN-γ haplotypes on IFN-γ responses in vaccinates belonging to the three properties are shown in figure 48. Haplotype 128-200 had a significantly (*P*<0.01) higher effect on IFN-γ responses in vaccinates of property 3, compared to two other haplotypes

(124-200 and 124-204). This haplotype had a similar increasing but non-significant (P>0.05) effect in property 3, while having moderate influence on IFN-γ responses in property 1. The rest of the evaluated haplotypes exhibited non-significant and inconsistent effects across the three properties.

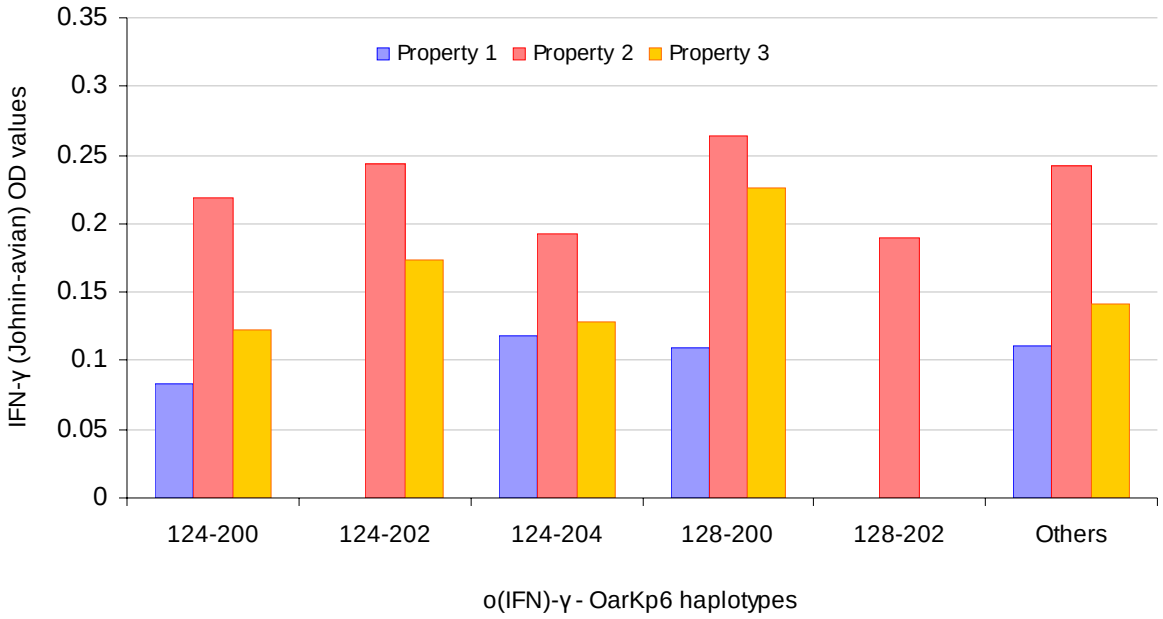

Figure 48: Effect of IFN-γ haplotypes on IFN-γ (Johnin-avian) responses in Johne's vaccinated sheep

Effects of haplotypes on IFN-γ responses in vaccinates are combined effects over different time-points for up to 54, 48 and 42 months post-vaccination in properties 1, 2 and 3 respectively. Effects originally in logarithmic scale were transformed to normal scale.

4.5.3. Summary

None of the SLC11A1 haplotypes were found to influence antibody production in response to Johne' vaccination in vaccinates of any of the properties. Two IFN-γ haplotypes, 124-204 in properties 2 and 3 and 128-200 in property 2, influenced high antibody responses. However, their effects were inconsistent across the properties. SLC11A1 haplotype 164-312 in property 1 and IFN-γ haplotype 128-200 in property 3 were found to influence high IFN-γ responses, but their effects in the other properties were inconsistent and non-significant.

4.6 OVERVIEW

This chapter reported long-term immune responses to a killed *Map* vaccine in Merino sheep on three properties, the degree of polymorphism at eight microsatellite loci, and their effects on immune responses. The investigated marker loci included four from the *Ovar-Mhc*

(DYMS1, OLADRW, OLADRB and SMHCC1) and two each from the SLC11A1 (OVINRA1 and OVINRA2) and IFNG (o(IFN)γ and OarKP6) gene regions.

Vaccination of sheep with a killed *Map* vaccine induced strong humoral and CMI responses as early as two weeks post-vaccination. Between-property differences in magnitude and trends in immune responses were evident; these effects were most likely due to the season of vaccination and the magnitude of natural infection prevalent in individual flocks. Correlations of immune responses (both antibody and IFN-γ responses) in vaccinates at a particular time-point post-vaccination with those observed at subsequent time-points were high and significant, indicating consistency of antibody and IFN-γ production in sheep in response to vaccination. Antibody and IFN-γ responses observed at each time point in vaccinates of properties 1 and 3 were found to be independent of each other, while moderate correlations were evident between the two phenotypes in vaccinates of property 2. Immune responses in control individuals on all three properties remained consistently low, except for slightly elevated IFN-γ levels at a few time points in controls of properties 2 and 3, concomitant to natural infection.

Extensive polymorphism was evident at the investigated marker loci, although allelic and genotypic numbers varied between marker loci. While only two alleles and three genotypes were found at locus o(IFN)γ, as many as 42 alleles and 137 were evident at locus OLADRW, over three properties. Markers DYMS1, OLADRB, SMHCC1 and OVINRA1 exhibited high heterozygosities (>78%), while the heterozygosities at OLADRW, OVINRA1, o(IFN)γ and OarKP6 loci ranged between 33% and 57%. Genotypes at loci DYMS1, OLADRB, SMHCC1, OVINRA1 and o(IFN)γ were in HWE, while those at OarKP6 locus were in HWE only when rare alleles (<1.0% frequency) were pooled with the closest size class. Departure from HWE, resulting from possible preferential amplification of alleles in heterozygotes, was evident at loci OLADRW and OVINRA2.

The effects of polymorphisms at each marker locus on immune responses were tested in two ways, first as genotype effects and second as allelic effects. The study revealed several genotypes/alleles at different marker loci were associated significantly with antibody and IFN-γ responses to *Map* vaccination. However, the majority of such effects were inconsistent across the three properties. The effect of a 186 bp OarKP6 allele on antibody levels was consistently low across all properties, although these were significantly low in vaccinates on

only two properties (1 and 2). The remaining genotypes and alleles showed no significant and/or consistent effects on antibody responses. Two genotypes (186/200 and 202/202) at DYMS1 and one genotype at each of the OLADRB (272/276), SMHCC1 (194/202) and OVINRA1 (156/158) were found to have a significant and consistent lowering effect on IFN-γ responses, while genotype 128/128 at the o(IFN)-γ locus influenced significantly high IFN-γ responses. Three alleles, one each at DYMS1 (202), OLADRB (284) and o(IFN)-γ (124) loci were found to significantly influence low IFN-γ responses to vaccination and their effects were consistent across the three properties.

The effects of SLC11A1 and IFN-γ haplotypes on immune responses were also tested, while those of MHC haplotypes could not be determined due to insufficient numbers of animals representing many of the haplotypes. Though a few haplotypes were found to possess significant effects on antibody as well as IFN-γ responses, none of those effects were consistent across the three properties.

Significance of the identified associations of genotypes/alleles on immune responses is discussed in detail in the ensuing chapter.

5. GENERAL DISCUSSION

This chapter provides an overall discussion of study design (employed genetic markers, techniques for genotyping and statistical analyses, study limitations), genotyping results (marker alleles and genotypes, phenotype- genotype association studies) and implications from the phenotype-genotype association studies. The chapter ends with reflections on the importance of genetic resistance/susceptibility to *Map* infection, together with suggestions for further studies.

5.1 STUDY DESIGN

5.1.1 Inherent Limitations

The current study had a few inherent limitations that were insurmountable. Before looking at those limitations, it should be emphasised that the study was not planned alongside the original long-term vaccine response study in Australia (Reddacliff *et al*. 2006), that provided inputs (phenotypes and blood samples) for the current study. Variation in the immune responses to *Map* vaccination that was evident in the original study provided an opportunity for the current study to be instigated.

Blood samples for the current study were obtained in late 2003, nearly four years after the start of the original study. By that time, several sheep especially in the control group were lost (either culled or deceased) and of the original total of 1200 sheep, blood samples could be obtained from only 934 sheep (table 4). The majority of the dead animals were diagnosed to be positive for PTB. Attempts to recover DNA from formalin-fixed tissues from those sheep were unsuccessful. Genetic information from those sheep would have made the study more effective, and eliminated possible bias

Immune responses in property 1 sheep were only recorded from 12 months post-vaccination. Consideration of immune response trends in vaccinates of properties 2 and 3 revealed that peak responses were evident between 2-8 months post-vaccination (figures 4-6). Therefore it is likely that availability of pre-12 month responses in property 1 vaccinates would have added to the accuracy of association studies in that property.

Lack of pedigree information was another limitation. The vaccination trial was undertaken in three independent commercial flocks and neither dam nor sire particulars were available for any of the individuals. Availability of pedigree details or DNA samples from sires would have enabled testing of genotype/allele effects to be undertaken using more powerful statistical tools.

Prolonged blood storage and transportation periods resulted in low DNA yields from some samples. Inadequate DNA concentrations in some of the templates led to amplification failure during PCR. Missing genotypes at one or more marker loci for some of the individuals resulted in several probable haplotypes, all with very low frequencies, rendering the haplotype-phenotype analysis ineffective.

5.1.2 Employed markers and techniques

Eight microsatellite markers were chosen after careful review of the literature concerning disease resistance studies in sheep. The four selected markers from the *Ovar-Mhc* region were implicated in resistance to different sheep diseases, predominantly gastro-intestinal nematodiasis (reviewed by Dukkipati *et al.* 2006b). Considering the importance of cytokine IFN-γ in CMI and the known role of SLC11A1 proteins in combating mycobacterial infections, two markers from each of the IFN-γ and SLC11A gene regions were selected. Alleles at the o(IFN)γ microsatellite locus had previously been shown to influence resistance to gastro-intestinal infections (Crawford and McEwen 1998; Coltman *et al.* 2001), while those at the OVINRA1 microsatellite locus were likely to be associated with susceptibility / resistance to PTB (Reddacliff *et al.* 2005).

Though microsatellites are located in the non-coding intron regions of genes, their polymorphisms often correspond to those of the adjacent functional regions (Ellegren *et al.* 1993). Initially, four markers from the coding regions of *Ovar*-DQA and DQB genes were included in the study design. However, PCR-sequencing results (data not presented) pertaining to one of those markers revealed heterozygous peaks at several locations in each sequence and assigning those differences to the two possible allelic forms in each individual was quite difficult. Discussion of the problem in AnGenMap discussion forum (available online at http://www.animalgenome.org/community/discuss.html) suggested single strand conformation polymorphism (SSCP) analysis as a possible solution to the problem. Since SSCP analysis is time-consuming, apart from being labour intensive, and considering the

study time-frame, those four sequencing markers were subsequently excluded from the study plan.

Accuracy of genotype and phenotype data is crucial in association studies. In the current study, the accuracy of automated length determination of PCR products was assessed employing the NRAMP1 microsatellite as an internal standard. As expected, NRAMP1 alleles were 56 bp larger than respective OVINRA1 alleles in all individuals studied, confirming the accuracy of the method.

Phenotypic measures obtained for the current study were collected at different time-points post-vaccination. Several individuals had a missing observation at one or more time-points for each of the phenotypes. The PROC MIXED procedure in SAS® was suggested to be an appropriate method for analyses of repeated measures data, especially with missing observations (Littell *et al*. 1998). Significant linkage between chromosome-wise markers was evident in the current study. For linked markers/genes, studying haplotype effects is more meaningful than studying individual effects of genes. However in the current study, missing genotypes at some of the marker loci and unavailability of pedigree details resulted in several probable haplotypes, all with quite low frequencies. This made the haplotype-phenotype analyses ineffective. Hence, effects of individual marker genotypes/alleles were tested employing mixed model analysis.

A possibility of testing epistatic effects among different different loci was aslo considered. However, as seen in case of haplotype analyses, there were several combinations of genotypes for each pair of markers, all with very low frequencies. As an illustration, for markers o(IFN)-γ and DYMS1 on property 1 vaccinates (169 sheep), there were 88 genotype combinations. Only 9 combinations had frequencies greater than 2%, the highest frequency being 6.6%. High allelic numbers (except in case of o(IFN)-γ), together with the occurrence of rare alleles (escpecially in the case of markers located in the MHC region) have made testing for epistatic effects virtually impossible.

An approximate statistical power (post-hoc) of the employed model in detecting a significant genotypic effect on immune responses was estimated for loci o(IFN)-γ and OVINRA1. For the respective loci, the observed power values were 0.47 and 0.86 for antibody responses and 0.68 and 0.43 for IFN-γ responses. Similarly, estimated power values for detecting the effect

of allele 202 at DYMS1 locus on antibody and IFN-γ responses were 0.55 and 0.58, respectively. For simplicity of power estimation, a one-way ANOVA model and a two-sample t-test model were assumed for genotypic and alleleic effects, respectively. However, a complex mixed model was actually employed to determine the genotypic and alleleic effects. Thus, the estimated power values are likely to be underestimates and the actual power could be much higher. Immune response is a complex phenomenon and its magnitude is highly variable, being influenced by several factors. Thus, in flocks exhibiting higher responses, the observed effect size would be higher, thereby increasing the statistical power. Also, in the current study, genotypic and allelic effects were tested separately in each property as a result of genetic dissimilarity and differences in phenotype recording intervals. However, if conditions had facilitated analyses from data pooled across the three properties, the employed statistical model would have been more powerful. In other words, as indicated from extrapolations for required sample size (figures 15, 16, 25, 26, 35 and 44), a total sample size of around 300 would result in a power of greater than 0.75, even at the current effect sizes.

5.2 PHENOTYPES, MARKER ALLELES AND GENOTYPES

Vaccination of sheep with a killed *Map* vaccine induced significant antibody and IFN-γ responses on all three properties (figures 4 and 5). The observed antibody production trend during the first 10 months post-vaccination was comparable to that seen in two Neoparasec™ vaccine response studies (Juste *et al.* 1994; Begg and Griffin 2005). None of the reviewed studies monitored antibody responses to vaccination beyond 10 months post-vaccination. Also, and consistent with the current findings, a different study (Begg and Griffin 2005) that monitored immune responses from Neoparasec vaccination for 10 months post-vaccination found IFN-γ responses to peak by 6 weeks before returning to baseline levels by 10 months. The specificity and reliability of the employed Parachek™ and Bovigam™ kits to measure antibody and IFN-γ responses, respectively, were demonstrated in several studies (Stewart *et al.* 2004; Begg and Griffin 2005; Rigden *et al.* 2006; Stewart *et al.* 2006).

In the current study, both antibody and IFN-γ responses in property 1 were consistently higher than those in vaccinates belonging to properties 2 and 3. Individuals on property 1 were vaccinated in summer (December), while those in properties 2 and 3 were vaccinated in winter (June). The difference in responses between the properties could be due to an effect of season of vaccination, with summer vaccinated sheep producing high initial responses that might have prevailed during the subsequent periods. Although no such effect of season on

immune responses to *Map* vaccination has been reported, a study in sheep (Goddard *et al.* 2000) found a significant genotype by management interaction effect on immune responses to *Map*. Scottish Blackface lambs under extensive management showed a greater immuno-reactivity than similar lambs under semi-intensive management, while the reverse was true for lambs from Texel crosses.

Genotype recovery rate in the current study varied from marker to marker. While it was >94% for markers DYMS1, OLADRB, SMHCC1, OVINRA1, o(IFN)γ and OarKP6, it was only 77% and 85% for the markers OLADRW and OVINRA2, respectively (tables 12-14). Low template DNA concentrations, variability in the ease of amplification and multiplexing of PCR reactions probably contributed to this variation in genotype recovery. The allelic bp range observed for different markers in the current study was well within or in close approximation to the range for respective markers available in the literature. Excepting the o(IFN)γ locus that had only two alleles, all other loci exhibited extensive polymorphisms with the number of alleles ranging between 9 (OVINRA2) and 42 (OLADRW), with their corresponding genotypes ranging between 24 and 137. These high numbers of polymorphisms, together with moderate to high observed heterozygosities (excepting OLADRW and OVINRA2) indicate their suitability as markers for QTL identification.

Testing for HWE is widely used as a quality control measure for large-scale genotyping and is one of the few ways to identify systematic genotyping errors (Hosking *et al.* 2004), despite indications that the test might not always reveal genotyping errors (Zou and Donner 2006). In the absence of selection, migration and mutation, it would be expected that genotypes at different loci in domestic sheep populations would be in HWE. In practice, a few exceptions might exist, for instance, if ram replacements are introduced from external sources, if natural selection occurs at immune loci or if there is a high mutation rate (in terms of allelic variation) at some of the microsatellite loci (especially those located in the MHC; Huang and Yu 2003). In the current study, genotypes at marker loci DYMS1, OLADRB, SMHCC1, OVINRA1 and o(IFN)γ were found to be in HWE, with minor exceptions (tables 12-14). Since the ewes in the three flocks have been self-replacing for several decades (Reddacliff *et al.* 2006), it is likely that these exceptions might be due to balancing selection at coding loci linked to the microsatellites. In addition, ram replacements from external sources might have contributed to departure from HWE. There was little difference (<3.2%) between observed and expected heterozygosities at these five loci.

Departure from HWE at marker locus OarKP6 was evident in all the three properties as a result of rare alleles, but when the rare alleles were pooled with the closest size class, no departures from HWE were apparent. Furthermore, the low F_{is} value together with less than 3% difference between the observed and expected heterozygosities, suggest the suitability of this marker genotype for use in phenotype association studies. In contrast, three lines of evidence: significant deviation from HWE on all the three properties, large differences (up to 30%) between observed and expected heterozygosites and quite high F_{is} value (0.246 to 0.545) suggest genotyping errors at OLADRW and OVINRA2 loci. Genotyping errors were most likely to be the result of preferential amplification of alleles in heterozygote individuals (Worley *et al.* 2006). Hence, findings from phenotype association studies involving genotypes/alleles at these two marker loci need to be cautiously interpreted.

Perusal of results from tests for allelic and genotypic differentiation of properties (summarized in table 15) suggest that each property is distinct in terms of allelic and genotypic frequencies, most likely caused by the use of different ram sources and different breeding policies. Consequently, genotypic and allelic effects on phenotypes were tested separately for each property.

5.3 EFFECT OF MARKER GENOTYPES ON IMMUNE RESPONSES

The current study revealed significant effects of several genotypes at different marker loci on antibody and IFN-γ responses to *Map* vaccination (detailed in chapter 4). However, the majority of the effects of the marker genotypes were inconsistent across the three properties. Genotypes at different marker loci possessing significant and consistent effects on IFN-γ responses are listed in table 36. They were categorised into ‘most likely’ and ‘probable’ genotypes, in terms of their effects. ‘Most likely’ genotypes had a similar (either low or high) and significant (P<0.05) effect across all the three properties. There were three such genotypes influencing IFN-γ responses. Genotypes 186/200 at the DYMS1 locus and 194/202 at the SMHCC1 locus were ‘most likely’ genotypes influencing significantly low IFN-γ responses, while genotype 128/128 at the o(IFN)-γ locus was considered ‘most likely’ to influence significantly high IFN-γ responses. ‘Probable’ genotypes had significant (P<0.05) and similar (either low or high), response effect in two properties and either a similar but non-significant (P>0.05) effect or was absent in the other property. There were only three ‘probable’ genotypes, one at each of the DYMS1 (202/202), OLADRB (276/276) and

OVINRA1 (156/158) loci, associated with significantly low IFN-γ responses to vaccination. None of the genotypes at any of the eight marker loci were significant and consistent in their effects on antibody responses across the three properties.

Table 36: Marker genotypes found to have significant and consistent effect on IFN-γ responses in vaccinates across three properties

Marker	Genotype (bp)	Effect* on IFN-γ responses			Overall effect**	
		Property 1	Property 2	Property 3	Effect	Classification
DYMS1	186/200	Low, significant	Low, significant	Low, significant	▼	Most likely
DYMS1	202/202	Genotype absent	Low, significant	Low, significant	▼	Probable
OLADRB	272/276	Low, non-significant	Low, significant	Low, significant	▼	Probable
SMHCC1	194/202	Low, significant	Low, significant	Low, significant	▼	Most likely
OVINRA1	156/158	Low, significant	Low, significant	Genotype absent	▼	Probable
o(IFN)-γ	128/128	High, significant	High, significant	High, significant	▲	Most likely

* Significance of effect (significant = $P<0.05$; non-significant = $P>0.05$; genotype absent = particular genotype had insufficient numbers and hence its effect was not evaluated)

** Overall effect was based on consistency and significance of genotype effects (Most likely = particular genotype had significant and similar, either low or high, response effect across the three properties; Probable = particular genotype had significant and similar, either low or high, response effect in two properties and either a similar but non-significant effect or was absent in the other property)

All genotypes other than those with significant and consistent effects (listed above) either had significant but inconsistent effects across properties or showed non-significant effects in the properties they were evaluated.

5.4 EFFECT OF MARKER ALLELES ON IMMUNE RESPONSES

The effects of presence versus absence of each of the alleles at different marker loci were also evaluated. In total, 8 and 17 alleles were found to have significant ($P<0.05$) effects on antibody and IFN-γ responses to vaccination, respectively, in the three properties (tables 32 and 33). Only one of the eight alleles found to influence antibody responses showed consistency of effects across the three properties. Three out of the total 17 alleles influencing IFN-γ responses had consistency in their effects across the three properties. Alleles with significant and consistent effects were categorized into 'most likely' and 'probable' alleles, in terms of their effects (table 37). 'Most likely' allele had a similar (either low or high) and significant ($P<0.05$) effect across all the three properties. A 186 bp allele at OarKP6 was considered 'probable' to have a decreasing effect on antibody responses, while two other

alleles, one each at DYMS1 (202 bp) and OLADRB (284 bp) loci were 'probable' to possess a decreasing effect on IFN-γ responses.

Table 37: Marker alleles found to have significant and consistent effect on antibody and IFN-γ responses in vaccinates across three properties

Marker	Allele (bp)	Effect* on immune responses			Overall effect**	
		Property 1	Property 2	Property 3	Effect	Classification
Antibody responses						
OarKP6	186	Low, significant	Low, significant	Low, non-significant	▼	Probable
IFN-γ responses						
DYMS1	202	Low, non-significant	Low, significant	Low, significant	▼	Probable
OLADRB	284	Low, non-significant	Low, significant	Low, significant	▼	Probable
o(IFN)γ	124	Low, significant	Low, significant	Low, significant	▼	Most likely

* Significance of effect (significant = $P<0.05$; non-significant = $P>0.05$)

** Overall effect was based on consistency and significance of genotype effects (Most likely = particular genotype had significant and similar, similar, either low or high, response effect in two properties and a similar but non-significant effect in the other property)

All alleles other than those with significant and consistent effects (listed above) either had significant but inconsistent effects across properties or showed non-significant effects in the properties they were evaluated.

5.5 EFFECT OF SLC11A1 AND IFN-γ HAPLOTYPES ON IMMUNE RESPONSES

Although there was linkage among the chromosome-wise markers employed in the study, haplotype analysis revealed a high number of probable haplotypes. The number of probable haplotypes pertaining to the four MHC markers was not less than 181 in each of the properties. This was mainly due to missing genotypes at one or more marker loci. For individuals with missing genotypes, the software lists several probable genotypes, all with low frequencies. Hence, effects of MHC haplotypes could not be tested. Although there were fewer numbers for SLC11A1 and IFN- γ haplotypes, the majority of those had non-uniform distribution of probabilities across individuals. Hence, only 6, 8 and 7 SLC11A1 and 3, 5 and 4 IFN- γ haplotypes were evaluated for their effects in properties 1, 2 and 3, respectively. Only a few significant effects of those haplotypes were evident in the three properties. The haplotype effects in most cases were inconsistent across the three properties and hence no generalized trend in the effect is evident for any of the tested haplotypes.

5.6 SIGNIFICANCE OF ASSOCIATIONS

The current study examined the influence of genetic polymorphisms at eight microsatellite loci on immune responses to *Map* vaccination in sheep. Perusal of available literature revealed that this study is the first of its kind in sheep and possibly in ruminants. Immune responses to a vaccine (containing either a live-attenuated or killed pathogens) constitute a complex phenomenon, probably influenced by several genes. The responses require the host to recognise microbial antigens in a way similar to that in any natural infection. The products of classical MHC genes, MHC molecules, play a predominant role in the presentation of foreign antigens to T lymphocytes, thereby triggering immune responses. MHC class I molecules present degradation products derived from intracellular (endogenous) proteins in the cytosol to T cell receptors of CD8+ cytotoxic T lymphocytes; the principal immune function of which is considered to be the killing of virus-infected cells and tumour cells (Rammensee *et al.* 1995). The class II molecules present peptides derived from exogenous proteins to the TCR of CD4+ helper T cells (Germain and Margulies 1993). In response to a foreign peptide, the helper T cells release cytokines that trigger the production of antibodies and CMI responses.

IFN-γ is a cytokine which has an important role in immune responses. It is produced by a TH1 subset of T cells in response to stimulation with an antigen (Mossmann and Coffman 1987) and induces expression of cell-surface class II MHC molecules (Wallach *et al.* 1982). Also, the SLC11A1 (formerly NRAMP1) protein plays an important role in protection against several intracellular pathogens including acid-fast bacteria. Apart from pleiotropic effects on macrophage activation, SLC11A1 is known to exert modulator effects on MHC class II molecules (Blackwell *et al* 2000).

The influence of genetic polymorphisms at eight genetic markers on immune responses was tested in three ways – genotype, allele and haplotype effects. While the effects of individual marker genotypes indicate additive effects of alleles, the effects of presence versus absence of alleles reveal dominance effects of the alleles. Haplotype effects are meaningful provided there is linkage between the markers. Although linkage was evident between chromosome-wise markers in the current study, missing genotypes at one or more loci in some of the individuals together with unavailability of pedigree details rendered haplotype analyses ineffective. Hence, little can be interpreted from the haplotype-phenotype study results.

The study revealed some significant and consistent effects of genotypes/alleles at six microsatellite marker loci (tables 36 and 37) on antibody and IFN-γ responses to *Map* vaccination. These marker loci included DYMS1, OLADRB and SMHCC1 from the *Ovar-Mhc*, OVINRA1 from the SLC11A1, and o(IFN)-γ and OarKP6 from IFN-γ gene regions. Considering the role of MHC, SLC11A1 and IFN-γ gene products in eliciting immune responses, these associations are not surprising. It is possible that these microsatellite loci might be in linkage disequilibrium with QTL in the adjoining coding regions. This can be substantiated by the known association of length polymorphism of microsatellite in intron 2 (OLADRW) of the expressed DRB gene with sequence polymorphism in exon 2 of the gene in various artiodactyl species (Ellegren *et al.* 1993).

In addition to the genotypes and alleles at the six marker loci listed in tables 36 and 37, a few other genotypes/alleles at those loci were also found to possess significant effects on immune responses; but, their effects were inconsistent across the three properties (detailed in chapter 5). It is possible that environmental variations (season of vaccination, prevalence of natural *Map* infection and exposure to *Mycobacterium avium* species) might have resulted in differential expression of coding regions linked to the microsatellite loci. Genotype by environment interaction has also been reported in a study on association of polymorphism of MHC class II genes with resistance/susceptibility to footrot in sheep (Escayg *et al.* 1997).

None of the marker genotypes or alleles, excepting a 186 bp allele at OarKP6 locus had significant and consistent effects on antibody responses (tables 36 and 37). In contrast, six genotypes and three alleles were considered either ‘probably’ or ‘more likely’ to influence IFN-γ responses. However, except for the genotype 128/128 at the o(IFN)-γ, the other five genotypes and three alleles were all found to be significantly associated with low IFN-γ responses.

Genotypes/alleles at three of the four investigated markers from the *Ovar-Mhc* markers were found to possess significant and consistent effects on IFN-γ responses (tables 36 and 37). Genotypes 186/200 and 202/202 at the DYMS1 locus were considered ‘most likely’ and ‘probable’, respectively, to result in low IFN-γ responses. In terms of alleles, a 202 bp allele at the locus was considered ‘probable’ to exert similar effect. Individuals with at least a single copy of this allele had significantly low IFN-γ responses. DYMS1 is located within the 5’

region of the class II *Ovar*-DYA gene and polymorphisms at the locus have been shown to be associated with gastro-intestinal nematodiasis (Buitkamp *et al.* 1996). Genotype 272/276 and allele 202 at a different MHC marker locus, OLADRB, were found to possess a 'probable' lowering effect on IFN-γ responses. This locus, though located in intron 5 of class II pseudogene *Ovar*-DRB2 (Blattman and Beh 1992), was found to be in linkage disequilibrium with the functional gene *Ovar*-DRB1 (Schwaiger *et al.* 1996). Animals with genotype 194/202 at marker SMHCC1 was found 'most likely' to elicit very low IFN-γ responses. SMHCC1 is located in the *Ovar-Mhc* I region (Groth and Wetherall 1994) and polymorphisms at the locus were found to be associated with gastro-intestinal nematodiasis in two different studies (Buitkamp *et al.* 1996; Charon *et al.* 2001).

A 'probable' association of OVINRA1 genotype 156/158 on IFN-γ responses was also evident in the study. This marker is located in the 3' untranslated region of the ovine SLC11A1 gene and a recent study revealed possible associations of alleles 162 and 160 with susceptibility and resistance, respectively, to clinical PTB (Reddacliff *et al.* 2005). However, in the current study, those two alleles had non-significant and inconsistent effects on antibody as well as IFN-γ responses.

The diallelic marker o(IFN)γ locus, located within the IFN-γ gene (Schmidt *et al.* 1996) had a significant 'most likely' effect on IFN-γ responses. Individuals with genotype 128/128 had significantly higher concentrations than those with 124/124 and 124/128. Conversely, it can be said that allele 124 had a dominant reducing effect on IFN-γ responses, which was evident in the tests for allelic effects. Positive associations of alleles at this locus with gastro-intestinal nematodiasis have been reported in three different studies. While the larger (128 bp) and smaller (124 bp) alleles at the locus were found to be associated with nematode resistance and susceptibility, respectively in a New Zealand study (Crawford and McEwen 1998), the reverse was true in two other studies involving Soay (Coltman *et al.* 2001) and Texel (Sayers *et al.* 2005) sheep. A 186 bp allele at a different marker locus (OarKP6), within the IFN-γ gene was considered 'probable' to result low antibody levels in the current study. In fact, this is the only significant association with antibody responses that was consistent across the three properties.

There were indications of some genotyping errors at OLADRW and OVINRA2 loci, as a result of preferential amplification of alleles in heterozygote individuals. Although a few

genotypes and alleles at these loci had significant effects on antibody and IFN-γ responses, none of those effects were consistent across the three properties (tables 20, 26, 29 and 33).

It is important to consider how the significant effects of markers/genotypes on immune responses might impact on the protection of sheep against natural *Map* infection. CMI responses play an important role in protection against *Map* and are usually evident relatively early in infection and remain active for months or years (Clarke 1997; Perez *et al.* 1999). Increased levels of IFN-γ (a good indicator of CMI), concomitant with infection status was evident in sheep experimentally infected with *Map* (Gowzdz and Thompson 2000, 2002; Reddacliff and Whittington 2003; Stewart *et al.* 2004; Begg *et al.* 2005). Also, IFN-γ responses vary between animals considered immune and susceptible to experimental infection with *Map* (Gwozdz *et al.* 2000). IFN-γ responses in immune sheep were consistently and significantly higher than those in susceptible sheep between weeks 9-36 post-infection, while antibody response trends were found similar between the resistant and susceptible animals. Antibody responses are generally not considered protective against *Map* and are usually not observed until late stages of infection (Clarke 1997). However, a recent study revealed that high antibody levels in vaccinated sheep under natural infection conditions were as good as IFN-γ responses in protection against *Map*, in terms of fecal shedding, infection and mortality due to PTB (Reddacliff 2005).

It is apparent from the results of earlier studies (Juste *et al.* 1994; Gowozdz *et al.* 2000) that vaccination induces higher immune responses compared to those seen in sheep experimentally infected with *Map*. However, it is unclear if animals able to elicit high or low immune responses to vaccination can also show immune responses at corresponding levels in response to natural or artificial infection. If they do, it is likely that animals able to elicit high immune responses to vaccination can more effectively combat *Map* infections, while those with poor responses are likely to be susceptible to infection.

5.7 IMPLICATIONS AND CONCLUSIONS

This study has provided an insight into possible genetic markers for immune responses to *Map* vaccination of sheep. Significant and consistent effects of six genotypes and three alleles located at six microsatellite marker loci were identified. Excepting an allele at the OarKP6 locus, the significant genotypes/alleles influenced only IFN-γ responses. Considering the significance of IFN-γ responses in protection against *Map*, it is possible that

these genotype/alleles would also influence immune responses to natural *Map* infections. On the other hand, the allele found to influence antibody responses is unlikely to influence protection against *Map* infections, as antibody responses are usually not evident until late stages of infection. Further studies, such as those listed below need to be undertaken to determine the role of the identified marker genotypes/alleles in susceptibility/resistance to PTB under natural infection conditions.

1. Testing for differences in allelic/genotypic frequencies at the identified loci in PTB-positive versus negative animals. Data from either slaughter houses or from naturally infected flocks can be utilised for such studies.
2. Faecal sheddings of *Map* were also recorded for the animals in the vaccination trial from which immunological data for the current study were obtained. It would be worthwhile to test if the identified genotypes/alleles are associated with shedding of *Map*, which is indicative of disease status in the animals.

Markers proven in such studies to be associated with resistance/susceptibility to natural PTB can then be utilised in marker-assisted selection (MAS). DNA markers facilitate screening of animals at an early stage in their life. Such screening can be used by both commercial farmers to reduce the incidence of PTB in the current generation of sheep and also by ram breeders to selectively breed resistant animals in future generations. MAS could make an important contribution to reducing the incidence of the disease. An example of the application of MAS to reduce disease incidence is provided by a selection programme employing disease susceptibility markers in the MHC region. After 5 years of selection, the prevalence of dermatophilosis in zebu Brahman cattle in Martinique (FWI) was reduced from 0.76 to 0.02 (Maillard *et al.*, 2003). However, care should be exercised to avoid either fixation or elimination of alleles, especially those from the MHC, since heterozygosity is known to confer selective advantage against multiple-strain infections (Penn *et al.* 2002).

Finally, PTB is a disease of concern also in cattle and deer. MHC, SLC11A1 and IFN-γ genes are well-conserved among ruminants and the majority of the tested markers are known to exist in either the same (as in sheep) or modified forms in cattle and deer. Hence, similar studies can be undertaken to test the identified marker genotypes/alleles for association with resistance/susceptibility to PTB in those species.

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APPENDIX

Table A1: Microsatellite marker genotypes pertaining to controls on property 1

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	124	124	198	206	194	196	478	478	276	296	198	198	-	-	-	-
2	124	124	196	200	190	192	-	-	278	278	194	200	-	-	-	-
3	124	124	204	204	192	192	508	508	276	284	192	192	160	162	312	316
4	124	124	200	204	186	196	458	458	278	286	192	200	158	162	312	312
5	124	124	200	204	200	200	558	560	270	278	186	210	160	162	312	316
6	-	-	192	200	202	202	-	-	-	-	194	202	-	-	-	-
7	124	124	204	204	186	192	458	508	284	286	192	192	160	162	312	318
8	124	124	-	-	190	192	490	490	-	-	-	-	-	-	-	-
9	124	128	200	204	176	200	500	508	270	284	186	192	158	162	312	326
10	124	128	200	204	190	196	462	462	274	288	192	194	158	162	312	326
11	128	128	186	196	194	202	480	496	270	274	188	194	162	164	326	326
12	124	124	200	204	186	192	458	480	276	286	186	192	158	160	-	-
13	-	-	200	200	-	-	496	496	270	276	192	210	158	160	-	-
14	-	-	186	200	168	168	480	480	276	296	186	202	156	166	-	-
15	-	-	200	204	192	192	-	-	278	282	192	194	162	164	-	-
16	-	-	-	-	-	-	-	-	278	284	192	194	162	162	-	-
17	124	128	200	204	-	-	-	-	276	284	192	192	160	162	-	-
18	124	124	200	204	186	196	458	566	264	288	192	194	162	162	312	312
19	124	124	186	200	-	-	490	558	270	278	-	-	-	-	-	-
20	124	128	200	204	184	190	462	478	274	296	188	194	156	164	326	326
21	124	124	204	204	190	190	556	556	284	296	202	204	160	162	312	318
22	124	128	202	204	164	200	480	480	278	286	188	192	162	162	312	312
23	124	128	200	204	184	186	480	480	274	276	188	192	158	160	312	318
24	124	124	200	204	190	204	462	496	274	276	192	194	160	162	-	-
25	124	124	202	204	194	200	556	558	278	288	192	194	158	160	312	318
26	124	124	204	204	186	200	458	480	276	288	186	192	160	162	326	326
27	-	-	200	204	-	-	480	500	270	276	186	186	158	164	312	326
28	124	128	200	204	188	192	458	480	276	288	186	192	156	160	314	316
29	124	128	-	-	188	202	480	496	270	274	188	210	156	160	-	-
30	124	128	200	204	192	200	560	588	270	278	194	194	160	160	318	318
31	124	128	200	200	188	200	480	480	274	276	188	192	156	160	314	314
32	124	128	202	204	200	200	456	458	278	286	192	200	162	164	314	314
33	124	124	200	200	184	188	480	480	276	276	186	192	158	168	312	326
34	-	-	-	-	-	-	-	-	276	284	194	210	-	-	-	-
35	124	124	200	204	184	200	478	480	274	286	188	192	158	164	312	312
36	124	128	200	200	186	200	478	478	274	276	188	192	156	156	312	314
37	-	-	-	-	-	-	480	480	274	274	192	194	164	164	-	-
38	124	124	200	200	192	192	496	496	276	276	186	192	158	164	312	312
39	124	124	200	204	192	198	490	508	-	-	190	192	156	162	312	312
40	124	124	200	204	184	200	490	494	270	270	190	192	156	160	316	316
41	124	124	204	204	192	200	456	458	276	276	-	-	160	162	-	-
42	124	124	200	204	174	192	494	508	270	284	192	192	158	160	326	326
43	-	-	-	-	190	194	490	490	270	276	190	192	156	164	314	326
44	124	124	204	204	184	198	478	558	278	296	194	198	160	164	312	318
45	124	124	192	196	192	202	558	560	276	278	192	194	160	160	-	-
46	-	-	186	194	-	-	-	-	278	278	194	198	158	160	-	-
47	124	124	200	204	186	200	478	478	-	-	192	194	160	162	312	326
48	124	128	200	204	186	186	480	480	274	278	188	198	158	162	312	312
49	124	128	200	204	188	188	480	480	270	274	188	194	156	162	314	316
50	-	-	200	204	186	200	-	-	-	-	186	194	162	164	-	-
51	124	128	200	200	190	200	480	490	276	278	198	198	162	168	312	312
52	124	128	200	200	190	190	494	494	270	276	192	194	158	164	-	-
53	124	128	200	204	200	202	500	500	276	276	194	210	162	168	-	-
54	124	124	200	204	184	204	-	-	274	276	188	194	162	164	312	312
55	124	124	200	204	186	202	488	494	270	296	194	194	160	164	312	316
56	124	128	200	200	190	198	490	496	274	274	188	210	158	162	-	-

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A1 (contd..): Microsatellite marker genotypes pertaining to controls on property 1

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
57	-	-	200	200	200	200	-	-	274	276	-	-	158	160	312	316
58	124	124	200	204	190	192	452	480	270	274	188	192	162	164	312	312
59	-	-	200	200	200	200	-	-	274	276	192	198	162	162	312	312
60	124	124	-	-	-	-	480	482	272	272	-	-	-	-	-	-
61	124	124	186	200	188	196	480	490	270	274	190	194	158	164	-	-
62	124	128	200	200	200	200	494	494	270	278	194	198	158	164	312	312
63	124	128	-	-	-	-	480	480	-	-	-	-	162	164	-	-
64	124	124	200	202	200	200	-	-	272	276	190	210	162	162	312	312
65	124	128	200	202	-	-	480	500	274	276	-	-	164	166	-	-
66	124	124	200	200	186	200	480	480	274	276	194	198	158	162	312	312
67	124	124	200	204	190	190	458	478	274	286	192	194	160	164	312	316
68	124	128	200	200	190	200	-	-	274	278	188	194	156	160	314	326
69	124	124	200	200	192	204	496	496	276	276	192	192	162	162	312	326
70	124	128	194	200	190	192	-	-	270	274	194	210	162	164	-	-
71	128	128	200	204	200	204	-	-	296	296	194	194	164	168	312	312
72	124	128	-	-	192	202	498	498	-	-	-	-	-	-	-	-
73	124	128	200	202	192	204	478	478	270	274	188	194	160	164	312	316
74	124	124	186	200	-	-	478	478	272	274	188	190	162	164	314	326
75	124	124	-	-	190	190	496	496	276	276	-	-	162	164	-	-
76	124	124	-	-	192	192	-	-	274	274	192	210	158	158	-	-
77	-	-	194	200	192	194	-	-	270	270	192	194	164	164	-	-
78	124	128	200	200	192	196	480	494	274	274	192	198	158	162	312	326
79	124	124	186	206	190	200	-	-	274	276	-	-	162	164	-	-
80	124	124	200	200	190	192	482	496	272	274	202	210	158	164	312	326
81	-	-	200	204	-	-	-	-	270	278	-	-	162	164	-	-
82	124	124	200	200	186	192	478	478	276	296	192	194	158	164	312	312
83	124	128	200	200	190	200	494	498	272	274	190	194	162	168	312	326
84	124	128	200	200	184	200	480	480	-	-	192	198	168	168	-	-
85	128	128	200	200	200	204	480	480	274	276	194	202	164	164	312	312
86	-	-	194	194	192	192	-	-	270	272	192	194	-	-	-	-
87	124	124	200	204	192	202	458	480	278	288	188	192	156	164	312	326
88	124	124	200	200	160	200	456	458	286	286	192	192	160	162	312	326
89	124	128	200	200	186	192	458	500	276	286	192	194	156	162	312	312
90	124	128	202	204	186	200	458	480	276	278	188	210	160	162	312	318
91	124	128	200	200	192	192	480	480	278	278	188	188	156	162	-	-
92	124	124	204	204	186	192	-	-	270	276	192	210	158	162	312	312
93	124	124	200	204	190	190	-	-	270	270	190	202	164	164	-	-
94	128	128	200	200	186	192	476	476	276	296	192	194	160	164	312	316
95	124	128	200	200	186	202	490	490	276	276	192	210	162	164	312	326
96	124	128	200	204	-	-	478	510	270	296	198	210	162	162	312	326
97	-	-	186	202	-	-	458	480	278	288	188	198	164	164	326	326
98	128	128	200	200	-	-	480	502	272	278	188	188	162	162	312	320
99	124	128	200	200	164	200	480	488	274	278	188	202	162	168	312	312
100	-	-	200	200	-	-	-	-	-	-	192	192	160	162	-	-
101	128	128	200	200	186	192	458	498	276	286	192	194	158	168	314	314
102	124	128	200	200	186	192	458	458	278	288	192	198	156	168	312	312
103	124	128	200	200	200	200	458	458	-	-	192	194	156	164	312	312
104	124	124	200	204	200	200	-	-	272	272	-	-	160	162	-	-
105	128	128	200	200	192	202	458	500	276	276	192	194	160	168	326	326
106	124	128	200	200	162	190	458	480	278	286	188	192	162	162	312	326
107	124	128	200	204	184	186	496	510	270	270	194	210	156	164	326	326
108	-	-	-	-	-	-	480	480	-	-	188	192	-	-	-	-
109	128	128	202	202	188	188	478	478	272	296	188	194	160	168	320	326
110	-	-	200	204	-	-	480	480	276	278	188	194	162	164	312	326
111	128	128	200	200	188	192	480	510	270	276	186	210	160	164	312	318
112	124	128	200	204	192	204	458	480	278	286	188	192	160	164	312	318
113	128	128	200	200	164	192	458	480	274	286	188	192	-	-	312	312
114	-	-	200	202	192	194	478	478	276	296	192	194	160	162	-	-
115	124	128	200	200	188	202	452	500	270	272	186	194	156	160	312	326

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A1 (contd..): Microsatellite marker genotypes pertaining to controls on property 1

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
116	128	128	200	200	192	192	452	478	272	296	194	194	162	164	312	312
117	124	124	186	190	180	192	480	490	270	274	180	188	158	164	320	326
118	124	124	200	204	-	-	-	-	-	-	-	-	158	158	-	-
119	124	128	200	200	-	-	490	490	270	276	180	210	162	164	312	326
120	124	124	200	204	188	188	478	500	-	-	188	188	162	162	326	326
121	124	124	200	204	186	192	478	500	270	296	188	194	158	164	312	326
122	-	-	-	-	-	-	-	-	-	-	198	210	-	-	-	-
123	-	-	186	200	-	-	480	590	270	276	186	194	162	162	-	-
124	124	124	200	204	186	188	476	476	270	276	192	194	162	162	320	326
125	124	128	200	204	192	200	490	490	270	276	180	192	156	156	-	-
126	128	128	-	-	-	-	478	490	-	-	180	198	-	-	-	-
127	128	128	200	200	186	190	458	480	276	288	192	210	162	168	312	326
128	124	128	200	200	186	192	478	490	270	270	180	194	158	162	312	312
129	124	124	204	204	186	192	480	500	270	274	188	188	158	164	312	326
130	124	124	200	204	192	204	490	490	270	276	180	192	158	166	312	312
131	124	128	200	204	192	200	478	490	-	-	180	188	162	166	312	312
132	-	-	200	204	200	202	500	500	270	274	188	194	158	164	312	326
133	124	124	204	204	192	204	-	-	272	272	192	198	162	164	312	312
134	124	128	200	200	-	-	480	548	270	270	188	210	158	160	-	-
135	124	128	200	200	192	192	500	500	270	278	188	200	162	162	312	312
136	-	-	204	204	200	200	500	500	-	-	188	210	162	164	312	326
137	124	124	192	200	192	192	490	496	-	-	180	194	162	168	-	-
138	124	124	188	204	176	192	-	-	-	-	188	194	162	164	326	326
139	-	-	200	204	200	202	-	-	-	-	-	-	162	162	312	312
140	-	-	-	-	-	-	490	490	-	-	180	188	-	-	-	-
141	124	124	200	204	-	-	480	480	276	276	-	-	162	162	-	-
142	124	128	200	204	196	200	480	496	270	274	190	194	158	162	312	316
143	124	128	200	200	200	200	488	488	-	-	192	194	158	162	312	312
144	128	128	200	200	188	206	480	480	274	276	194	198	158	162	312	312
145	124	128	200	204	200	204	496	508	270	284	192	194	162	162	312	326
146	128	128	-	-	-	-	458	478	288	296	192	198	162	168	326	326
147	124	128	200	200	190	200	456	458	276	276	192	194	158	164	-	-
148	124	124	200	200	188	192	490	496	270	276	194	198	158	164	312	312
149	-	-	200	200	192	198	458	494	270	270	192	194	158	160	312	316
150	124	124	-	-	190	200	-	-	276	276	192	194	162	162	-	-
151	124	124	200	200	188	190	494	494	276	278	194	200	156	162	312	326
152	124	128	200	200	188	192	-	-	276	278	194	198	162	164	312	326
153	124	124	204	204	190	200	494	494	270	276	192	194	162	164	326	326
154	128	128	200	200	188	196	490	494	270	276	194	198	150	162	312	316
155	124	124	200	200	188	200	-	-	276	276	194	210	158	162	312	312
156	124	128	200	200	200	202	480	508	274	276	194	198	158	160	312	316
157	124	124	200	204	-	-	-	-	270	272	194	196	156	158	312	312
158	124	128	204	204	186	196	476	550	276	278	180	196	160	160	312	316

* Genotype alleles are denoted as base pairs (bp) in length

Table A2: Microsatellite marker genotypes pertaining to vaccinates on property 1

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	124	124	200	204	196	196	478	488	276	276	190	198	158	168	312	320
2	124	128	200	204	188	204	480	480	276	278	186	200	158	166	312	312
3	124	124	-	-	192	202	480	480	274	274	-	-	-	-	-	-
4	124	124	200	200	186	188	478	494	270	274	188	210	158	160	312	316
5	124	124	200	204	186	196	494	510	270	270	194	210	162	162	312	320
6	124	124	204	204	196	196	498	508	270	284	186	192	160	162	312	318
7	124	128	200	202	200	204	478	478	274	296	194	198	160	164	312	312
8	124	128	200	202	188	196	480	494	270	272	190	194	160	162	312	318
9	124	128	200	204	192	200	558	560	276	278	194	194	162	162	312	312
10	124	128	200	200	186	192	480	480	274	296	188	202	158	164	312	312
11	124	128	200	200	188	196	-	-	270	276	194	200	162	162	-	-
12	124	128	200	200	184	188	452	496	272	276	192	194	162	162	312	320
13	124	128	200	200	192	196	-	-	274	278	200	210	162	162	312	312
14	128	128	200	200	194	210	490	490	276	276	194	210	162	162	320	320
15	128	128	200	200	162	190	480	500	270	278	186	188	162	164	314	314
16	124	124	200	204	186	196	476	482	272	296	194	198	162	164	314	314
17	124	128	200	200	190	190	494	494	270	270	192	194	164	164	314	320
18	128	128	200	200	192	196	490	490	270	276	180	194	162	162	312	314
19	124	128	200	200	194	196	498	500	270	278	188	200	160	162	312	314
20	124	124	200	202	200	200	480	480	276	276	186	198	162	162	-	-
21	128	128	200	200	186	200	478	488	276	278	186	192	156	158	314	314
22	124	124	200	200	186	188	478	478	276	276	186	192	158	162	312	312
23	124	128	200	204	190	192	462	462	274	284	194	204	164	164	312	316
24	124	128	200	200	180	200	490	490	270	296	180	202	156	158	-	-
25	128	128	200	200	188	200	480	480	274	274	188	210	156	164	312	314
26	124	128	200	202	192	200	-	-	276	288	192	198	156	168	312	312
27	124	128	200	200	192	200	478	478	270	274	190	194	162	162	314	314
28	124	128	200	204	184	186	488	500	276	284	194	194	160	162	-	-
29	124	124	202	204	184	186	488	510	270	278	192	210	162	164	314	314
30	124	128	202	204	184	186	488	488	284	296	192	194	162	164	312	312
31	124	128	200	204	186	192	478	478	274	296	188	194	162	164	-	-
32	124	124	200	204	184	188	488	488	270	278	190	200	156	160	320	322
33	124	124	200	204	186	196	458	488	276	276	192	194	162	164	312	312
34	124	124	204	204	194	200	558	558	276	278	194	194	162	164	-	-
35	124	128	186	202	184	192	462	462	274	286	192	194	162	162	322	322
36	124	124	200	200	186	190	480	480	274	276	188	192	158	160	-	-
37	124	128	202	204	164	200	-	-	276	278	194	198	160	160	316	316
38	128	128	186	200	186	192	480	480	274	296	188	210	156	164	-	-
39	124	124	200	204	186	188	478	478	274	296	188	194	158	162	312	312
40	124	124	200	204	192	200	-	-	276	284	192	192	160	162	312	316
41	124	128	200	200	162	186	480	502	272	274	188	188	156	162	312	316
42	124	124	200	204	190	192	456	458	274	286	192	210	158	162	312	312
43	124	124	200	204	184	202	478	494	274	296	198	210	162	162	-	-
44	124	128	200	204	184	188	480	480	276	276	186	194	156	160	314	316
45	124	128	200	204	186	198	558	560	278	284	194	198	158	162	312	312
46	124	128	200	204	190	200	488	488	284	296	194	202	162	162	314	318
47	124	124	200	200	186	186	480	480	274	278	188	198	156	158	314	322
48	124	128	200	200	184	188	480	480	274	276	188	194	156	164	312	314
49	124	128	200	200	186	186	480	488	276	276	186	192	156	162	314	314
50	124	128	200	204	190	192	458	480	278	278	188	192	156	162	314	320
51	128	128	200	200	186	190	-	-	276	276	186	210	158	160	312	316
52	124	124	190	204	190	198	508	508	276	284	192	192	160	162	314	318
53	124	128	200	200	190	192	480	498	276	278	188	194	162	164	312	312
54	124	128	200	204	184	190	494	494	270	276	192	210	158	162	314	314
55	124	124	200	204	176	176	478	478	274	276	194	198	162	162	314	314
56	124	124	200	200	192	200	494	494	270	276	192	194	156	158	314	314
57	124	128	200	200	188	192	494	494	270	296	194	202	156	162	312	314
58	124	124	200	204	192	196	480	480	276	276	198	210	162	168	312	312

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A2 (contd..): Microsatellite marker genotypes pertaining to vaccinates on property 1

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
59	124	124	186	198	186	192	478	478	276	296	192	198	162	168	-	-
60	124	128	200	200	196	200	478	480	274	296	188	194	162	164	312	312
61	124	124	204	204	184	184	488	496	276	284	192	194	162	162	-	-
62	124	128	200	200	190	192	478	498	270	274	188	188	158	162	312	312
63	124	128	200	202	190	196	496	556	276	284	186	192	162	164	314	314
64	124	128	200	200	188	190	486	486	272	276	194	208	162	162	312	312
65	124	124	200	200	186	200	476	478	270	276	198	210	168	168	314	314
66	124	124	200	204	190	190	-	-	272	274	192	210	158	158	312	312
67	124	124	200	200	184	192	496	496	276	276	192	192	162	164	312	312
68	124	128	200	200	200	200	-	-	-	-	198	210	164	168	312	312
69	124	124	200	200	196	200	458	458	276	286	192	194	158	162	312	312
70	124	124	200	200	192	202	496	496	276	276	192	192	158	162	312	312
71	128	128	200	200	186	192	480	480	274	274	188	194	158	164	312	312
72	124	124	200	200	190	196	-	-	276	276	190	198	162	162	312	312
73	124	128	200	200	200	200	474	482	272	276	194	198	162	162	-	-
74	124	128	202	204	194	196	476	478	286	296	192	194	156	160	314	314
75	124	124	200	204	196	204	480	480	274	278	194	198	160	164	-	-
76	124	128	200	200	192	196	496	502	272	276	188	192	160	162	312	318
77	124	124	200	200	190	202	496	496	276	276	192	192	158	164	-	-
78	124	124	200	204	190	196	-	-	274	274	190	210	160	162	312	316
79	124	124	200	200	198	200	490	494	270	274	190	210	162	164	-	-
80	124	124	200	200	190	196	458	480	272	286	190	192	160	162	312	318
81	124	128	200	200	184	190	494	494	270	274	-	-	158	162	314	314
82	124	124	200	200	192	204	456	458	274	286	188	192	162	164	312	312
83	124	128	200	204	184	204	480	480	274	276	194	210	158	164	312	314
84	124	124	200	200	200	202	478	488	274	276	188	198	158	164	312	312
85	124	128	200	204	162	196	480	480	278	278	188	188	162	166	312	312
86	124	124	200	204	190	196	-	-	274	276	192	210	158	162	312	312
87	124	128	200	200	192	196	480	480	272	296	190	202	160	168	312	316
88	124	124	200	204	190	196	478	496	276	296	192	194	166	170	312	312
89	128	128	200	200	196	204	478	478	274	288	194	204	160	164	312	316
90	124	124	200	204	200	200	478	478	274	274	188	210	158	160	312	312
91	124	124	200	200	196	202	498	508	274	276	188	194	162	164	314	314
92	124	124	200	204	184	196	478	478	272	296	190	198	162	168	312	312
93	124	128	200	200	190	204	480	482	274	276	188	194	164	168	312	312
94	124	124	200	204	194	200	480	488	274	276	194	198	158	162	312	312
95	124	124	200	202	186	200	480	480	274	276	194	198	160	168	312	316
96	124	128	200	200	190	204	-	-	270	276	194	194	160	164	312	312
97	124	128	200	204	184	192	478	500	270	296	188	188	158	162	314	314
98	128	128	200	200	192	200	478	480	278	296	188	194	160	168	-	-
99	124	124	200	204	186	186	510	510	270	278	196	210	164	168	312	320
100	128	128	186	202	188	192	480	480	278	296	-	-	162	164	-	-
101	124	124	200	200	164	192	458	458	276	286	192	192	160	162	-	-
102	124	124	200	200	202	202	480	480	274	274	192	194	160	168	-	-
103	124	128	200	204	162	184	480	588	270	278	188	194	162	162	-	-
104	124	128	200	200	164	186	478	478	278	296	188	198	160	168	312	312
105	124	124	200	204	192	192	-	-	276	288	192	192	162	164	312	312
106	128	128	200	200	162	164	-	-	278	278	188	192	162	164	312	312
107	124	128	200	200	200	200	458	478	274	276	186	192	160	162	-	-
108	124	128	200	204	-	-	-	-	276	278	192	194	162	164	-	-
109	124	124	200	204	190	192	450	478	270	274	188	194	160	164	312	316
110	124	124	204	204	186	196	458	458	278	288	192	200	162	164	314	320
111	124	124	200	202	184	192	480	480	270	278	188	194	162	164	-	-
112	124	124	200	204	162	184	480	488	270	278	188	190	162	162	-	-
113	124	124	200	202	192	202	478	490	272	278	190	192	164	168	-	-
114	124	128	200	200	162	202	480	498	276	278	188	188	160	168	314	318
115	124	128	200	200	192	200	490	574	276	278	196	210	160	164	-	-
116	128	128	200	200	186	192	458	458	274	288	192	194	164	168	312	312

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A2 (contd..): Microsatellite marker genotypes pertaining to vaccinates on property 1

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
117	128	128	200	200	164	188	458	458	276	288	192	192	156	162	312	312
118	124	128	200	204	186	192	-	-	272	286	192	194	156	162	312	312
119	124	128	200	200	188	192	480	480	270	276	186	210	162	164	312	312
120	124	128	200	200	194	196	478	478	276	276	186	198	164	168	312	312
121	124	124	200	204	190	192	488	496	276	276	192	192	158	162	312	312
122	124	124	204	204	190	196	480	482	272	274	190	198	160	162	-	-
123	124	124	200	200	184	192	456	494	274	274	192	210	158	164	-	-
124	124	128	186	200	188	190	478	478	276	284	194	208	156	164	-	-
125	124	128	200	204	192	200	458	458	-	-	192	210	162	162	312	312
126	124	124	200	200	192	200	490	496	276	276	180	192	162	164	314	314
127	124	124	200	204	192	200	480	480	276	276	192	198	156	168	-	-
128	124	124	200	200	190	192	480	490	278	278	188	194	162	164	312	314
129	124	128	200	204	192	192	498	500	270	270	188	192	158	162	-	-
130	128	128	200	202	190	192	-	-	270	276	188	192	156	158	-	-
131	124	128	200	200	192	192	488	488	270	270	182	190	158	162	312	320
132	124	128	202	204	186	190	480	480	276	284	194	210	156	158	-	-
133	124	128	200	204	186	198	508	508	278	284	192	200	156	162	314	314
134	124	124	200	204	188	196	-	-	270	274	194	194	162	164	-	-
135	124	128	200	202	186	200	478	500	274	284	192	194	162	168	312	312
136	128	128	200	202	190	192	480	480	272	274	188	192	158	162	312	312
137	124	124	200	204	184	194	480	480	264	270	180	190	158	162	312	312
138	124	128	200	204	192	200	490	500	270	276	180	194	160	162	-	-
139	124	128	200	204	192	196	480	480	270	276	188	202	158	158	312	312
140	124	128	200	200	192	198	482	482	272	278	194	198	156	162	312	312
141	124	128	200	200	192	192	490	498	270	276	190	192	162	164	312	314
142	124	128	202	204	192	200	458	496	276	288	192	210	162	162	314	314
143	124	124	204	204	190	192	482	482	270	276	188	194	158	160	312	316
144	124	128	200	200	186	192	496	496	274	278	200	210	162	162	312	312
145	124	128	200	204	184	200	506	508	284	284	192	192	158	162	312	312
146	124	124	200	204	188	200	480	480	276	276	186	192	158	168	314	314
147	124	128	-	-	192	200	586	588	270	276	188	194	160	162	-	-
148	124	124	202	202	186	192	490	496	270	274	-	-	158	158	-	-
149	124	124	186	202	190	192	-	-	270	270	180	186	162	162	-	-
150	128	128	200	200	194	204	476	476	270	276	188	194	158	162	-	-
151	124	128	200	204	192	200	-	-	270	296	188	202	158	166	312	312
152	124	128	200	204	190	192	490	496	276	276	192	210	162	164	314	314
153	124	124	200	200	186	190	456	478	276	286	186	192	156	158	312	314
154	124	128	200	200	192	200	480	480	276	276	194	202	162	162	-	-
155	124	124	200	200	188	200	-	-	270	276	194	210	158	162	328	328
156	124	124	200	200	188	200	584	586	270	276	194	194	158	160	-	-
157	124	128	200	200	200	206	-	-	276	278	194	200	158	162	312	312
158	124	124	200	200	184	192	478	506	278	284	188	192	162	168	312	312
159	124	124	200	200	-	-	-	-	278	278	192	196	160	162	-	-
160	124	124	200	204	186	200	478	494	270	276	186	194	162	168	-	-
161	124	128	200	200	192	200	458	458	286	286	192	192	162	162	312	312
162	124	124	200	204	184	188	-	-	276	276	194	210	156	158	312	312
163	124	124	200	200	192	200	478	478	274	278	188	198	162	162	312	312
164	124	128	200	202	186	188	458	458	270	286	192	194	160	162	312	312
165	124	128	200	200	200	204	480	480	276	276	194	202	156	162	312	312
166	124	128	200	200	186	202	478	488	276	276	186	192	156	164	312	314
167	128	128	200	200	188	200	500	500	276	284	194	194	158	164	312	322
168	124	128	200	204	196	200	480	480	274	276	188	194	158	164	312	312
169	124	124	-	-	186	200	496	496	270	276	194	210	-	-	-	-
170	124	124	204	204	192	196	480	480	264	276	190	210	160	160	314	314
171	124	124	204	204	186	200	-	-	270	272	190	196	156	164	312	312

* Genotype alleles are denoted as base pairs (bp) in length

Table A3: Microsatellite marker genotypes pertaining to controls on property 2

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	124	128	200	204	190	190	490	490	278	296	186	194	156	162	312	312
2	124	124	200	200	186	190	470	490	270	284	180	204	160	162	314	314
3	124	128	200	200	164	196	480	480	278	296	186	188	160	162	312	312
4	124	128	200	204	190	190	490	490	270	296	180	194	156	156	312	312
5	124	124	200	204	190	200	494	498	276	296	192	202	162	164	314	322
6	124	124	200	200	190	200	494	494	270	276	186	210	156	164	314	314
7	124	124	200	204	164	184	-	-	276	276	194	194	160	160	312	316
8	124	124	204	204	184	196	-	-	284	296	194	202	162	164	314	326
9	124	128	200	204	190	202	480	494	270	278	188	198	164	164	312	312
10	124	124	200	204	186	190	484	496	270	272	192	198	154	164	314	314
11	124	124	204	204	190	190	480	480	270	274	198	198	162	164	314	314
12	124	124	200	202	186	190	490	494	270	272	180	188	156	158	312	316
13	124	124	202	204	190	190	480	480	296	296	194	196	156	162	314	314
14	124	124	200	200	164	190	476	494	276	296	186	194	156	162	312	312
15	124	124	200	204	184	190	480	480	270	296	202	204	156	162	314	326
16	124	124	204	204	186	190	498	552	284	296	194	202	158	164	314	316
17	124	124	200	204	190	190	480	480	270	296	202	204	162	164	312	326
18	124	124	202	204	190	190	490	498	270	272	180	188	160	164	314	314
19	124	128	200	200	190	192	490	552	270	284	180	204	156	160	314	314
20	124	128	200	202	186	190	496	496	270	276	194	210	156	160	314	314
21	124	124	204	204	186	196	-	-	276	296	186	194	156	162	314	314
22	124	124	202	202	186	192	490	496	270	278	188	194	156	158	312	314
23	124	124	200	204	186	190	488	488	270	276	180	194	160	162	312	312
24	124	124	202	202	186	190	494	498	270	272	188	194	162	162	312	312
25	124	128	200	200	190	200	502	502	272	296	186	188	156	164	314	326
26	124	124	200	202	190	196	490	490	270	276	180	192	160	160	312	326
27	124	124	202	204	186	186	494	502	270	272	188	194	156	158	312	316
28	124	124	200	200	164	190	470	490	270	276	180	194	156	164	314	314
29	124	124	200	204	190	200	458	490	286	296	186	192	160	162	312	312
30	124	124	200	204	184	186	494	494	270	296	202	210	158	168	312	326
31	124	128	200	204	190	200	494	498	284	296	202	204	162	164	314	314
32	124	128	200	200	190	200	496	496	276	296	202	210	160	164	312	312
33	124	128	202	204	184	190	480	480	276	296	202	210	162	164	314	314
34	124	124	200	200	184	190	494	498	296	296	186	194	160	164	314	314
35	124	124	200	200	190	196	490	494	270	276	182	210	156	162	314	314
36	124	128	200	204	190	202	478	478	270	296	196	210	158	160	312	312
37	124	124	204	204	184	196	480	480	276	296	186	210	156	156	312	312
38	124	124	204	204	188	196	494	498	272	296	186	196	160	162	312	312
39	124	128	200	200	186	196	470	470	276	276	186	194	156	162	314	314
40	128	128	200	200	192	196	488	496	276	276	192	210	160	164	312	312
41	128	128	200	202	190	192	496	496	276	276	192	192	160	164	312	312
42	124	128	200	202	184	190	480	560	274	274	194	194	160	164	314	318
43	124	128	200	204	192	200	480	496	276	276	192	202	158	160	314	314
44	124	124	202	204	200	202	478	508	284	296	192	198	156	162	312	326
45	124	128	202	204	192	196	490	496	276	276	192	210	158	160	312	312
46	124	124	202	204	164	190	478	478	296	296	194	198	156	160	312	312
47	124	124	200	202	190	200	494	508	270	284	192	198	156	162	312	326
48	124	128	200	204	190	192	480	490	274	276	186	194	160	160	312	312
49	124	128	200	200	196	200	490	496	276	276	186	192	160	162	312	312
50	124	128	200	202	184	192	480	480	276	276	186	202	156	160	314	314
51	124	124	200	204	184	202	494	494	270	276	210	210	160	160	312	316
52	124	128	200	202	186	190	480	498	274	276	180	194	160	160	314	318
53	124	128	200	202	196	200	490	496	276	276	186	192	160	162	312	312
54	128	128	200	202	186	196	490	502	272	276	186	188	156	162	314	314
55	124	124	200	200	190	202	480	494	274	276	194	196	160	164	314	314
56	124	124	202	202	186	190	480	508	276	284	192	202	162	164	312	312
57	128	128	200	200	192	196	496	496	276	286	192	192	156	162	312	326
58	124	128	202	202	186	196	490	490	276	296	186	194	156	158	312	314
59	128	128	200	200	192	200	494	496	270	276	192	194	156	164	314	314
60	124	128	200	202	190	202	480	480	270	284	192	204	162	164	312	312
61	128	128	200	200	192	192	496	496	276	296	192	194	156	156	312	312

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A3 (contd..): Microsatellite marker genotypes pertaining to controls on property 2

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
62	124	128	202	204	190	192	496	496	276	276	192	194	156	156	314	314
63	124	128	202	204	176	196	484	490	272	276	186	196	160	162	312	312
64	124	128	200	200	190	192	494	496	276	276	192	210	156	164	312	312
65	124	128	200	204	196	200	490	490	276	284	186	210	156	162	314	322
66	128	128	200	200	190	192	480	490	270	276	186	204	156	156	314	314
67	128	128	200	200	186	202	480	480	272	296	188	194	162	162	312	312
68	128	128	200	200	196	196	490	498	276	276	186	192	156	160	312	312
69	124	128	200	204	192	202	442	498	272	276	188	192	158	160	312	312
70	124	128	200	202	190	190	480	488	274	276	194	196	160	160	316	318
71	128	128	200	202	192	196	490	490	276	276	186	210	156	160	312	312
72	124	128	200	202	190	196	490	496	276	276	186	192	156	156	312	312
73	124	128	200	200	196	196	496	500	276	284	192	194	156	160	312	312
74	124	124	200	204	164	190	484	484	272	296	196	202	158	160	314	314
75	124	128	202	204	184	190	490	490	276	278	210	210	164	164	312	326
76	124	128	200	204	184	190	484	484	272	276	194	196	158	160	314	316
77	124	124	204	204	184	186	508	508	278	284	192	210	162	164	312	312
78	124	128	202	204	186	190	490	494	270	278	194	198	156	164	312	314
79	124	128	200	204	186	196	494	494	276	276	194	210	158	168	312	312
80	124	124	200	204	186	200	480	480	274	296	186	194	156	162	312	312
81	124	124	200	204	190	190	494	498	272	296	188	202	158	162	314	314
82	124	124	200	204	184	186	480	480	276	276	194	202	156	160	312	316
83	124	124	200	204	186	186	480	496	274	276	194	202	160	164	312	312
84	124	124	204	204	196	196	478	494	272	276	190	194	158	162	-	-
85	124	124	202	204	186	196	442	496	274	276	194	210	164	164	314	314
86	124	124	200	202	186	190	488	488	276	296	202	210	160	160	316	318
87	124	124	200	200	164	184	470	498	276	276	194	194	158	164	312	312
88	124	128	200	204	190	192	480	496	274	276	194	202	160	162	312	326
89	124	124	200	204	186	196	478	478	296	296	198	202	158	164	312	312
90	124	124	204	204	190	192	480	480	276	296	194	202	160	160	312	316
91	124	124	202	204	184	200	496	496	270	276	194	194	158	162	312	312
92	124	124	204	204	164	190	494	496	276	296	192	202	158	160	316	318
93	124	124	202	204	184	192	478	478	276	296	198	202	158	160	314	314
94	124	124	204	204	186	196	480	480	274	278	194	210	164	164	312	326
95	124	124	200	202	176	200	480	480	270	296	194	204	156	158	312	314
96	124	124	-	-	190	202	494	498	278	296	180	202	158	164	314	322
97	124	128	200	204	186	190	480	494	270	274	194	204	158	162	314	314
98	124	128	200	204	190	196	470	494	272	296	188	202	158	158	314	316
99	124	124	200	204	196	200	-	-	276	278	194	210	160	168	312	312
100	124	124	200	204	192	196	-	-	272	296	188	202	158	160	312	326
101	124	128	200	204	190	200	490	496	274	278	194	194	162	162	314	314
102	124	128	200	200	200	200	496	496	276	296	192	194	156	158	312	312
103	124	124	200	200	184	190	478	478	276	296	194	210	160	162	312	326
104	124	124	200	204	184	184	482	482	276	288	194	198	160	160	312	316
105	124	124	204	204	196	200	480	480	296	296	196	202	162	164	312	312
106	124	124	204	204	196	202	496	496	276	276	192	196	160	162	314	314
107	124	128	200	202	190	196	480	480	270	296	202	204	156	164	312	312
108	124	128	200	200	190	196	502	554	272	284	180	188	156	156	312	312
109	124	124	200	200	196	200	494	498	276	296	194	196	162	164	314	314
110	124	128	202	204	190	196	478	494	276	296	196	198	156	164	314	314
111	124	124	204	204	192	200	478	498	274	276	194	194	158	164	314	314
112	124	124	200	202	186	190	484	496	272	276	192	196	162	164	312	312
113	124	124	202	202	192	200	478	496	276	296	192	198	162	162	312	314
114	124	124	200	204	184	190	478	494	270	276	194	210	164	164	314	314
115	124	124	200	202	190	192	496	496	276	296	180	192	164	164	314	314
116	124	124	200	204	176	190	478	478	-	-	194	198	156	166	314	316
117	124	124	200	204	188	188	480	480	276	276	192	202	158	162	312	312
118	124	124	200	204	196	196	494	498	276	296	196	202	158	164	314	314
119	124	128	200	204	186	190	494	498	270	276	186	192	156	162	312	312
120	124	128	200	200	196	202	496	502	272	276	188	192	162	162	312	312
121	124	124	204	204	176	190	480	480	274	296	198	202	156	156	312	326
122	124	124	200	202	192	192	488	496	276	276	192	196	162	164	312	312

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A3 (contd..): Microsatellite marker genotypes pertaining to controls on property 2

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
123	124	124	200	204	192	196	496	496	276	276	192	196	160	164	312	312
124	124	124	204	204	190	190	480	480	296	296	196	202	162	164	312	326
125	124	124	200	204	184	196	-	-	276	296	194	202	162	164	312	326
126	124	128	200	202	186	190	496	496	276	286	192	192	156	162	314	314
127	124	128	200	200	196	200	470	494	276	296	194	196	162	164	314	314
128	124	124	200	200	184	200	480	480	276	278	188	210	162	162	314	314
129	124	124	200	204	186	196	494	554	270	284	194	204	156	160	314	318
130	124	124	204	204	184	184	478	478	296	296	192	202	158	160	314	314
131	124	128	200	200	184	186	480	494	270	276	194	202	156	162	314	314
132	124	124	200	204	186	190	480	480	270	278	188	194	162	164	314	314
133	124	124	200	204	190	200	480	494	270	270	204	210	162	164	314	314
134	124	128	200	204	184	190	480	480	276	296	196	202	162	164	314	314
135	124	124	204	204	190	190	480	480	276	296	196	202	158	164	314	314
136	124	124	202	204	184	196	470	494	272	296	186	188	158	160	314	316
137	124	128	200	200	190	200	480	494	276	276	202	210	162	164	312	326

* Genotype alleles are denoted as base pairs (bp) in length

Table A4: Microsatellite marker genotypes pertaining to vaccinates on property 2

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	124	128	186	202	184	190	490	500	270	272	180	188	156	164	-	-
2	124	124	200	204	184	186	480	494	274	296	194	202	158	160	314	318
3	-	-	198	204	198	202	-	-	-	-	198	204	-	-	-	-
4	124	124	204	204	184	202	556	556	284	296	202	204	158	162	314	314
5	124	124	200	204	184	190	-	-	276	296	186	194	160	162	-	-
6	124	124	204	204	162	190	490	502	270	272	180	204	156	162	-	-
7	124	124	186	186	184	196	478	480	276	296	186	210	160	162	-	-
8	124	124	186	204	190	200	494	494	270	270	198	210	164	164	312	312
9	124	124	204	204	190	190	480	502	272	296	196	196	162	162	326	326
10	124	124	-	-	190	202	-	-	270	296	194	198	156	164	-	-
11	124	128	200	204	174	190	496	496	276	296	192	202	162	164	312	312
12	124	128	202	204	190	196	496	496	270	296	198	198	156	164	312	312
13	124	128	200	200	196	200	-	-	270	270	180	194	156	160	314	322
14	124	124	204	204	174	190	494	494	270	276	198	210	162	164	312	312
15	124	128	200	200	190	190	478	496	270	296	198	210	162	162	312	312
16	124	128	200	202	196	196	478	496	276	296	192	198	160	162	-	-
17	124	124	180	182	190	190	490	490	270	276	180	192	160	162	-	-
18	124	124	200	208	190	198	490	490	-	-	186	210	-	-	-	-
19	124	128	200	202	186	200	480	480	270	276	194	202	156	160	312	312
20	124	128	200	204	184	192	480	502	272	296	188	196	162	162	-	-
21	124	124	200	200	190	200	-	-	296	296	194	194	162	164	-	-
22	124	124	200	204	196	200	476	490	270	276	180	194	160	168	-	-
23	124	124	204	204	186	196	484	490	270	272	180	196	156	156	314	314
24	124	128	200	200	190	196	480	480	276	296	186	202	156	160	314	314
25	124	124	202	204	184	196	490	508	270	284	180	192	156	160	314	314
26	124	124	200	200	186	190	480	480	276	296	186	202	156	162	312	312
27	-	-	202	202	202	202	-	-	270	276	180	194	156	156	-	-
28	124	124	204	204	190	192	480	494	270	270	198	204	164	168	312	312
29	124	124	200	202	190	200	496	502	270	272	194	194	156	156	312	312
30	124	124	204	204	196	196	502	502	272	296	188	202	158	164	312	312
31	-	-	-	-	-	-	-	-	-	-	198	202	-	-	-	-
32	124	128	200	200	190	200	502	502	272	296	186	188	156	162	312	312
33	124	128	200	204	184	190	502	502	272	296	188	202	156	164	312	312
34	124	124	200	202	186	190	480	494	270	276	194	210	156	156	314	314
35	124	124	204	204	190	196	480	490	270	274	180	194	156	168	-	-
36	124	124	200	200	190	200	494	494	276	296	194	210	162	162	312	322
37	124	128	200	204	190	200	480	480	272	296	188	196	162	162	312	312
38	124	124	200	204	162	196	494	498	270	272	180	188	156	164	314	322
39	124	124	-	-	196	200	-	-	-	-	186	192	160	160	-	-
40	124	124	204	204	190	196	478	478	276	296	194	202	156	158	-	-
41	124	128	200	204	184	184	480	480	276	296	196	210	158	162	-	-
42	-	-	-	-	202	202	-	-	-	-	202	202	-	-	-	-
43	124	128	200	204	184	196	478	490	270	296	180	210	156	160	312	312
44	124	124	202	202	184	190	496	508	276	284	192	192	156	162	314	326
45	124	124	200	200	190	200	480	484	274	274	194	194	160	162	314	314
46	124	128	200	204	164	202	480	494	276	296	194	194	156	162	314	314
47	124	128	202	202	194	202	476	476	272	296	188	194	156	156	-	-
48	124	128	200	204	190	200	496	496	274	276	192	194	158	164	326	326
49	124	128	-	-	190	196	-	-	270	274	194	194	160	164	-	-
50	124	128	200	200	200	200	496	496	276	296	192	194	156	162	312	312
51	124	128	200	200	192	200	-	-	276	296	192	202	156	162	-	-
52	124	124	202	204	190	190	480	498	274	276	194	194	160	160	314	314
53	124	128	200	204	186	186	490	502	272	276	186	188	156	158	312	316
54	128	128	200	200	196	196	480	480	276	276	186	202	160	168	312	320
55	128	128	200	200	190	192	480	480	276	276	192	194	156	162	314	314
56	124	128	200	202	202	202	442	494	272	296	188	194	158	160	316	318
57	124	128	200	202	186	196	480	480	276	276	186	202	160	160	312	312
58	124	128	200	200	196	196	458	490	276	286	186	192	156	160	-	-

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A4 (contd..): Microsatellite marker genotypes pertaining to vaccinates on property 2

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
59	124	128	200	204	190	196	480	490	270	276	186	204	156	156	312	312
60	-	-	186	186	194	202	480	480	270	296	194	204	156	164	-	-
61	124	128	200	204	162	192	-	-	-	-	188	192	160	164	-	-
62	128	128	200	202	190	196	496	496	276	276	192	210	160	164	312	312
63	124	128	200	204	190	192	508	510	276	284	188	192	156	160	312	314
64	124	128	202	204	192	196	-	-	276	276	186	202	156	156	312	312
65	124	124	200	200	190	196	480	490	274	276	194	194	158	160	-	-
66	124	124	200	204	184	186	490	490	276	278	194	194	160	164	312	316
67	124	128	186	202	186	196	-	-	278	296	202	210	162	164	-	-
68	124	128	-	-	190	192	490	496	276	276	192	210	156	164	-	-
69	128	128	200	202	196	200	496	496	276	276	192	192	160	164	312	312
70	124	128	200	202	186	192	478	490	276	296	186	198	156	160	-	-
71	124	128	202	202	186	192	488	490	276	296	186	194	156	156	312	312
72	124	128	202	204	190	190	480	480	276	296	202	210	162	164	314	314
73	128	128	200	202	192	196	496	496	276	296	192	194	156	162	-	-
74	128	128	200	200	186	192	490	494	270	276	186	194	156	162	312	312
75	124	128	200	200	190	192	496	502	272	276	188	192	156	156	314	314
76	124	128	200	204	190	196	490	490	276	296	186	194	160	162	312	312
77	124	128	200	202	190	202	480	494	272	296	188	194	156	160	314	318
78	124	128	200	200	190	192	490	490	272	276	186	188	162	164	312	312
79	124	128	200	202	190	202	-	-	276	276	192	202	156	164	-	-
80	124	128	202	202	186	192	490	490	276	296	186	194	156	156	312	312
81	124	128	200	204	190	190	502	502	272	278	188	210	164	164	312	312
82	124	128	200	200	176	186	494	496	276	296	192	202	160	160	312	318
83	124	124	-	-	184	190	480	490	270	276	180	210	156	156	-	-
84	124	128	200	204	186	190	478	478	278	296	210	210	162	164	312	312
85	124	128	200	204	202	202	-	-	270	296	194	202	160	164	312	312
86	124	124	200	204	184	190	490	490	278	296	194	202	158	164	312	312
87	124	124	200	204	184	196	480	480	276	296	202	202	158	162	312	312
88	124	124	204	204	184	186	496	496	274	276	192	194	158	162	-	-
89	124	124	202	204	190	196	-	-	276	296	202	210	158	160	-	-
90	124	124	204	204	186	190	490	498	278	278	194	210	162	164	314	314
91	124	124	200	200	184	190	502	502	272	276	188	194	158	168	312	312
92	124	124	202	204	184	190	476	476	276	296	194	194	160	164	312	316
93	124	128	200	204	190	190	494	494	270	296	202	210	158	160	312	316
94	124	128	200	204	190	190	480	480	276	296	202	202	158	158	312	312
95	124	128	200	204	190	196	480	480	270	296	202	204	162	164	312	312
96	124	128	200	204	186	192	496	496	274	276	194	202	156	162	314	314
97	124	128	200	204	186	190	480	496	274	274	194	194	162	164	312	322
98	124	124	204	204	190	200	-	-	272	276	188	194	158	160	312	312
99	124	124	200	204	186	200	496	496	274	296	194	202	164	164	312	312
100	124	128	200	204	186	186	494	494	272	278	188	210	162	164	314	314
101	124	124	-	-	186	190	478	496	-	-	194	204	160	164	-	-
102	124	128	200	204	184	186	480	480	276	276	194	202	160	164	-	-
103	124	124	200	204	186	190	496	502	272	274	188	194	162	164	312	314
104	124	124	180	186	184	190	502	502	272	276	188	194	158	164	-	-
105	124	124	204	204	190	200	480	480	276	278	202	210	162	164	312	312
106	124	128	200	202	186	200	502	502	272	296	188	194	156	158	312	314
107	124	124	200	204	196	200	496	496	274	296	186	202	158	164	312	314
108	124	124	200	204	184	186	-	-	272	296	188	202	158	162	-	-
109	124	128	186	194	190	190	-	-	274	278	194	194	162	164	-	-
110	124	124	204	204	186	196	480	480	276	278	202	210	162	162	312	312
111	124	128	200	204	186	196	470	494	278	284	194	210	162	164	312	312

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A4 (contd..): Microsatellite marker genotypes pertaining to vaccinates on property 2

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
112	124	124	200	204	196	196	-	-	276	296	194	194	160	162	312	312
113	124	128	200	202	184	202	480	480	276	296	194	202	156	164	-	-
114	124	124	202	204	190	190	494	498	276	276	194	210	160	162	312	318
115	124	128	200	204	184	196	480	480	274	276	180	194	158	158	314	314
116	124	124	200	204	162	196	488	488	276	276	192	194	158	162	-	-
117	124	124	200	200	192	194	556	556	276	284	194	194	158	162	312	312
118	-	-	188	190	188	194	-	-	272	274	188	194	162	162	-	-
119	124	128	200	200	184	200	496	496	276	276	192	194	158	162	312	312
120	124	124	200	204	162	184	478	478	276	296	194	210	156	160	314	318
121	124	128	200	204	184	192	-	-	270	278	194	194	160	162	326	326
122	124	124	200	204	186	200	478	478	274	278	208	210	162	164	312	312
123	124	128	-	-	190	196	496	496	272	274	188	194	162	162	-	-
124	124	124	200	204	184	196	-	-	296	296	194	202	164	164	312	312
125	124	124	200	204	190	196	494	494	270	296	194	202	164	164	314	314
126	124	124	200	204	184	200	478	496	276	276	186	196	158	168	314	314
127	124	124	200	204	184	190	478	494	278	296	194	202	158	164	312	312
128	124	128	200	204	184	190	480	480	276	276	194	202	158	164	312	312
129	124	128	200	202	184	190	-	-	276	296	192	194	160	164	-	-
130	124	124	200	204	190	196	502	502	272	276	188	196	160	162	314	314
131	124	124	200	204	186	200	480	498	276	276	194	202	156	164	314	314
132	124	124	-	-	196	200	478	478	272	276	188	194	158	162	-	-
133	124	124	200	204	174	190	480	480	276	296	202	202	160	160	312	316
134	124	128	200	200	192	196	-	-	-	-	194	202	164	164	312	312
135	124	128	200	204	196	202	508	508	284	296	192	202	156	158	314	314
136	124	128	200	204	196	202	480	498	270	276	196	204	162	162	314	314
137	124	128	200	204	190	196	480	480	270	296	202	204	162	164	312	312
138	128	128	200	202	174	190	480	480	276	296	202	202	160	162	312	316
139	-	-	-	-	158	164	-	-	270	284	176	180	-	-	-	-
140	124	124	200	204	192	196	480	480	270	276	196	204	162	164	312	312
141	124	124	200	200	190	196	496	496	270	276	196	210	160	164	312	312
142	124	128	200	202	190	192	490	496	276	276	186	192	158	162	314	316
143	128	128	-	-	192	200	-	-	-	-	196	198	-	-	-	-
144	124	124	200	204	176	196	494	558	274	296	194	202	162	164	314	314
145	124	124	202	202	196	200	480	554	274	284	194	194	160	162	312	316
146	124	124	200	204	190	196	494	498	276	296	196	202	156	164	312	312
147	124	128	200	204	196	196	442	496	276	296	192	202	164	164	314	314
148	124	124	204	204	176	190	480	556	270	284	192	194	158	160	-	-
149	124	128	186	194	190	190	476	476	-	-	186	194	156	160	-	-
150	124	124	204	204	174	200	478	478	296	296	198	202	156	158	-	-
151	-	-	188	192	194	194	-	-	-	-	192	194	-	-	-	-
152	124	124	-	-	192	194	-	-	276	276	192	194	-	-	-	-
153	124	124	204	204	190	196	556	556	284	296	194	202	162	162	312	322
154	124	124	200	204	200	200	458	496	270	286	194	198	162	164	312	312
155	124	124	200	204	200	200	496	496	276	276	192	210	162	164	314	314
156	124	128	200	200	190	192	502	502	272	276	188	194	160	164	322	322
157	124	128	-	-	196	196	-	-	-	-	188	196	-	-	326	326
158	124	124	200	204	190	200	494	502	272	276	188	210	160	164	314	314
159	124	124	204	204	184	190	496	496	276	296	192	202	158	162	312	312
160	124	128	200	200	190	190	480	494	270	276	202	210	162	164	312	314
161	124	124	200	204	186	200	478	494	270	274	192	194	156	162	314	314
162	124	124	200	202	190	190	-	-	272	276	188	210	164	164	-	-

* Genotype alleles are denoted as base pairs (bp) in length

Table A5: Microsatellite marker genotypes pertaining to controls on property 3

Animal	o(IFN)-γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	124	124	204	204	196	200	478	478	276	296	202	202	162	162	314	314
2	-	-	200	206	200	206	-	-	276	284	192	194	162	162	314	314
3	128	128	200	200	184	202	-	-	272	274	188	198	160	162	312	312
4	124	128	200	200	184	202	508	508	-	-	192	194	164	164	312	312
5	124	124	200	204	192	202	478	494	276	296	194	210	156	164	314	316
6	124	124	200	206	184	202	478	478	276	276	-	-	162	162	312	312
7	124	124	200	202	192	196	478	478	270	276	192	194	162	162	312	312
8	124	124	200	202	176	196	-	-	276	296	192	202	162	164	-	-
9	124	128	200	200	184	200	496	496	270	296	194	202	160	164	312	312
10	124	128	200	204	190	200	490	502	270	272	180	188	156	160	312	312
11	124	124	200	204	184	196	494	494	270	276	192	194	158	162	312	312
12	124	124	-	-	184	202	494	494	-	-	194	194	-	-	-	-
13	124	124	200	204	190	202	478	490	284	296	192	194	162	162	312	312
14	124	124	200	200	192	196	478	490	270	296	180	194	164	170	314	314
15	124	124	204	204	196	196	490	502	270	272	180	188	162	162	312	312
16	124	128	200	202	184	202	494	508	270	284	192	194	160	160	318	318
17	124	124	200	200	196	200	488	488	270	296	194	202	162	164	314	314
18	124	128	200	200	184	202	-	-	270	272	188	194	160	160	314	314
19	124	124	200	204	186	196	478	480	274	296	194	208	162	164	312	312
20	124	128	200	204	196	200	494	494	270	276	194	210	160	162	312	312
21	124	128	200	200	184	202	496	496	270	276	194	210	160	168	312	312
22	124	128	200	200	186	202	478	502	272	296	188	198	160	164	312	312
23	124	128	200	204	176	202	490	502	272	278	188	194	160	164	312	312
24	124	128	200	204	184	196	490	494	270	276	194	194	156	160	314	318
25	124	124	200	200	184	190	494	508	284	296	192	198	160	164	-	-
26	124	124	200	200	188	202	490	490	270	276	180	194	162	164	312	312
27	124	124	200	204	188	196	476	488	270	296	-	-	162	164	312	312
28	124	124	200	202	196	196	496	496	276	276	192	192	156	162	312	312
29	128	128	204	204	188	188	478	494	270	296	194	198	158	162	-	-
30	124	124	200	204	196	202	480	502	272	274	188	198	162	164	314	314
31	124	124	204	206	186	188	478	478	274	276	194	208	160	162	312	312
32	124	128	200	200	188	196	490	496	270	276	210	210	156	164	-	-
33	124	124	200	200	184	184	490	508	276	284	190	192	160	168	312	312
34	124	124	200	200	196	200	478	480	276	296	192	194	162	164	-	-
35	124	124	200	206	188	202	494	494	270	276	194	210	164	166	312	312
36	124	124	200	200	184	196	478	478	270	296	194	194	162	162	312	312
37	124	124	200	200	202	202	478	478	296	296	194	198	160	162	312	312
38	124	128	-	-	192	202	-	-	270	276	192	194	160	162	-	-
39	124	124	204	204	184	202	496	502	272	276	188	198	160	162	312	312
40	124	124	200	204	184	196	502	508	272	284	188	192	158	164	312	312
41	124	124	200	204	196	202	-	-	272	284	188	192	158	164	314	314
42	124	124	200	200	164	188	478	496	270	296	198	210	162	164	312	312
43	124	124	200	200	190	202	478	478	276	296	194	198	162	164	314	314
44	124	124	200	204	188	190	496	496	270	270	198	210	162	164	312	312
45	124	124	200	204	188	196	490	494	270	270	180	210	162	162	314	314
46	124	128	200	200	186	202	496	496	276	276	192	194	162	162	314	314
47	124	124	198	202	188	192	494	494	270	270	198	210	162	168	-	-
48	124	124	200	204	184	202	496	508	270	284	192	210	164	168	312	312
49	124	124	200	200	184	188	480	480	274	276	192	194	156	162	312	314
50	124	128	200	202	186	202	490	502	270	272	180	188	160	168	312	312
51	124	128	200	200	192	202	480	494	270	274	194	198	160	162	312	312
52	124	124	200	206	188	200	-	-	270	276	210	210	156	162	-	-
53	124	128	200	200	186	202	478	502	272	296	188	198	160	162	312	318
54	124	128	200	202	188	192	478	478	276	276	194	194	164	166	312	312
55	124	128	200	200	186	202	502	502	272	272	188	188	160	162	314	314
56	124	128	200	204	196	200	496	496	270	296	194	198	160	162	312	318
57	124	124	200	206	188	192	530	532	264	276	180	194	164	164	314	314
58	124	128	200	206	186	202	492	496	270	270	194	210	162	162	312	312
59	124	124	200	202	196	196	478	496	276	296	192	194	162	164	312	312
60	124	128	200	200	196	202	496	502	272	276	188	192	162	164	312	312
61	124	124	200	204	190	202	478	490	270	296	180	198	158	162	312	312

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A5 (contd.): Microsatellite marker genotypes pertaining to controls on property 3

Animal	o(IFN)-γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
62	124	124	200	200	186	196	490	494	270	276	194	198	166	168	312	312
63	124	124	200	204	202	202	502	508	272	284	188	208	164	166	314	314
64	124	124	-	-	184	202	-	-	296	296	-	-	-	-	-	-
65	124	124	202	204	188	196	478	478	276	296	192	198	162	162	312	312
66	124	124	200	204	190	202	502	508	272	284	188	192	158	162	312	312
67	124	124	204	204	190	196	478	494	276	296	198	210	158	164	312	314
68	124	124	200	200	196	202	-	-	276	296	194	202	162	164	312	314
69	124	124	198	200	192	202	478	494	264	296	194	198	162	164	-	-
70	124	128	200	202	190	192	488	490	270	276	194	210	162	164	312	312
71	124	124	200	204	192	202	-	-	274	296	-	-	156	164	312	314
72	124	124	200	204	186	188	480	480	274	276	194	198	156	162	312	314
73	124	124	202	204	192	196	478	478	274	276	192	208	162	162	-	-
74	124	124	200	204	196	200	478	508	284	296	192	198	162	164	312	312
75	124	124	202	204	190	196	508	508	276	284	192	192	162	162	312	312
76	124	124	204	206	190	202	490	490	270	276	180	194	156	164	312	312
77	124	124	204	204	188	196	-	-	276	296	192	202	162	162	314	314
78	124	128	200	200	184	196	478	478	274	296	198	198	162	168	312	312
79	124	128	-	-	186	202	478	496	276	296	192	194	162	162	-	-
80	124	124	198	204	176	202	-	-	272	296	188	198	-	-	-	-
81	124	128	200	204	192	202	494	502	270	272	188	194	160	164	314	314
82	124	124	200	204	190	196	-	-	276	296	194	210	162	164	312	312
83	124	128	200	202	184	202	478	478	296	296	198	198	158	162	314	314
84	124	124	204	206	186	202	496	496	270	276	194	194	164	166	312	312
85	124	124	200	204	186	196	478	508	284	296	192	194	162	162	312	312
86	-	-	-	-	-	-	-	-	272	296	-	-	-	-	-	-
87	124	128	200	200	202	202	494	494	270	276	194	198	162	164	312	312
88	124	124	200	200	186	202	450	452	270	276	194	194	162	164	312	312
89	124	124	204	204	184	184	478	494	270	296	194	198	160	162	312	318
90	124	124	200	200	176	192	482	490	276	278	202	210	158	162	312	312
91	124	124	196	206	-	-	-	-	276	276	192	192	-	-	-	-
92	124	128	200	200	184	184	478	508	270	284	192	194	158	164	314	314
93	124	124	200	200	184	186	494	508	270	284	192	194	-	-	-	-
94	124	124	200	200	186	196	478	490	274	276	208	210	158	164	-	-
95	124	128	200	204	202	202	478	478	296	296	198	198	158	164	312	312
96	124	124	204	204	184	196	494	508	270	284	192	194	160	162	314	314
97	124	128	-	-	184	184	-	-	284	296	-	-	-	-	-	-
98	124	124	200	204	188	202	478	480	274	296	194	198	156	160	312	318
99	124	124	188	192	184	202	502	508	272	284	188	192	158	160	-	-
100	124	124	200	202	188	196	502	502	272	276	188	194	160	162	314	314
101	124	124	200	204	186	202	478	522	276	296	194	198	158	162	312	312
102	124	124	204	204	192	202	-	-	270	296	194	198	158	164	-	-
103	124	128	200	204	186	196	478	510	264	296	198	198	158	164	312	312
104	124	124	200	204	186	196	-	-	270	296	194	198	158	168	-	-
105	124	124	200	204	196	200	478	478	264	296	194	198	158	162	-	-
106	124	124	200	202	184	186	478	522	276	296	194	198	160	162	314	314
107	124	124	200	204	186	196	478	494	270	296	198	210	162	164	312	312
108	124	124	200	200	196	202	478	494	270	296	198	210	158	162	312	312
109	124	124	200	200	184	196	-	-	272	284	188	198	158	160	312	312
110	124	124	200	200	186	202	-	-	272	296	188	198	160	162	312	318
111	124	124	200	204	196	200	478	508	284	296	-	-	158	160	312	316
112	124	124	200	204	188	202	478	496	270	296	198	210	158	162	312	312
113	124	124	200	200	190	196	478	496	270	296	198	198	158	160	312	318
114	124	124	200	200	190	202	478	494	276	296	198	210	160	168	312	318
115	124	124	200	200	184	202	478	494	270	296	194	198	160	164	314	318
116	124	124	200	204	200	202	478	530	264	296	194	198	160	162	-	-
117	124	128	200	202	188	202	-	-	272	276	188	194	158	162	314	314
118	124	124	200	204	184	200	508	530	264	284	192	194	160	168	314	318
119	124	124	200	204	184	202	-	-	272	294	-	-	160	160	316	316
120	124	124	200	200	184	202	478	496	276	296	192	198	160	160	318	318
121	124	128	200	204	192	192	480	480	274	296	194	198	158	168	312	312
122	124	124	204	204	200	202	-	-	284	296	192	198	160	162	314	318

* Genotype alleles are denoted as base pairs (bp) in length

Table A6: Microsatellite marker genotypes pertaining to vaccinates on property 3

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	124	124	200	204	176	188	494	494	270	296	202	210	156	162	312	312
2	124	124	200	204	190	192	478	494	276	296	198	210	160	162	314	318
3	124	128	200	204	200	202	496	496	270	276	192	194	158	160	312	326
4	124	124	200	204	176	184	490	490	276	296	190	202	160	168	312	312
5	124	124	-	-	186	196	478	490	278	296	194	198	160	160	-	-
6	124	128	200	202	196	200	478	496	-	-	198	198	158	162	312	312
7	124	124	202	204	184	196	494	494	270	276	192	210	158	162	312	312
8	124	128	200	204	176	202	478	490	270	296	182	198	162	164	314	314
9	124	124	204	206	188	192	478	496	270	296	198	210	164	164	312	312
10	124	124	200	206	188	192	478	478	276	296	194	198	164	164	312	326
11	124	128	-	-	188	192	494	496	276	276	192	210	-	-	-	-
12	128	128	200	200	192	200	494	494	270	276	200	210	160	164	312	316
13	124	124	200	202	176	184	502	502	272	296	188	202	158	160	312	312
14	124	124	204	204	184	196	508	508	276	284	192	192	158	160	312	312
15	124	124	200	206	186	188	478	478	276	296	194	198	162	162	314	314
16	124	128	200	204	186	200	480	494	270	274	192	194	160	164	314	318
17	124	128	200	206	186	188	-	-	270	296	198	210	160	162	312	312
18	128	128	200	200	184	200	478	494	270	296	194	198	160	164	314	318
19	124	124	200	204	196	202	478	496	276	296	192	198	156	160	314	318
20	124	124	200	204	186	188	478	494	-	-	-	-	160	162	312	312
21	124	124	200	204	186	192	478	488	276	276	186	198	158	160	-	-
22	124	124	200	204	184	196	494	500	270	272	188	210	164	168	312	312
23	124	124	204	204	184	196	508	508	276	284	192	192	162	162	314	314
24	124	124	202	204	200	202	478	490	276	296	198	210	160	162	312	312
25	128	128	200	200	186	202	494	508	270	284	192	194	160	162	312	314
26	124	128	200	200	176	200	490	494	270	270	180	194	160	162	312	312
27	124	124	-	-	190	202	490	502	270	272	188	210	156	162	-	-
28	124	124	200	200	186	196	478	478	296	296	194	198	162	168	312	314
29	128	128	200	200	186	202	478	494	270	296	194	198	160	162	314	314
30	124	124	200	204	184	196	494	494	270	276	192	194	162	162	312	312
31	124	124	200	200	188	196	496	496	270	270	210	210	156	164	314	316
32	124	124	200	204	192	202	478	478	296	296	198	198	160	164	312	318
33	124	124	-	-	184	184	478	490	-	-	190	210	160	162	-	-
34	124	124	200	204	176	196	478	502	272	296	188	198	162	164	312	312
35	124	124	-	-	190	200	490	490	270	276	-	-	164	164	-	-
36	124	124	200	204	184	202	496	502	272	276	210	210	156	162	312	316
37	124	128	200	200	186	202	-	-	270	272	188	194	162	162	314	314
38	124	124	204	204	200	200	478	478	296	296	198	202	158	162	312	314
39	124	124	200	200	184	188	490	502	270	272	180	188	160	162	312	312
40	124	124	204	204	202	202	478	490	270	296	180	198	162	164	312	312
41	124	124	200	206	176	188	490	490	276	278	194	194	162	164	312	312
42	124	124	200	204	184	202	478	478	274	296	194	208	156	162	312	314
43	124	128	200	200	200	200	502	502	272	276	188	194	160	162	314	314
44	124	124	200	200	186	196	494	502	272	276	188	210	162	164	312	312
45	124	124	204	206	184	188	494	508	270	284	192	210	162	164	312	312
46	124	128	200	200	190	202	494	494	270	276	194	198	162	162	312	314
47	124	128	200	200	196	200	494	494	270	270	194	194	160	168	312	318
48	124	128	200	200	188	190	490	490	270	270	180	210	162	164	-	-
49	124	128	200	204	186	196	478	502	270	272	188	194	160	162	314	318
50	124	124	204	204	186	202	494	502	270	272	188	194	158	162	314	314
51	124	124	200	202	196	202	488	490	-	-	180	192	162	162	312	326
52	124	128	200	200	184	202	478	494	270	276	194	198	162	164	312	312
53	124	128	200	204	186	200	478	502	272	274	188	210	160	160	318	318
54	124	124	200	204	184	196	488	496	270	276	192	194	162	164	314	314
55	124	124	200	200	192	196	478	480	276	296	194	202	164	168	314	314
56	124	124	204	206	196	202	478	494	270	296	200	210	162	164	312	312
57	124	124	200	204	186	196	478	478	296	296	194	198	164	164	312	312
58	124	128	200	200	186	202	478	502	272	296	188	198	160	164	312	312

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A6 (contd.): Microsatellite marker genotypes pertaining to vaccinates on property 3

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele	Allele
59	124	124	200	200	186	202	478	478	296	296	198	198	162	162	314	314
60	124	124	200	200	184	186	478	508	284	296	192	198	158	160	312	316
61	124	124	202	202	196	196	478	478	296	296	198	198	156	158	314	316
62	124	124	200	200	196	196	478	480	274	296	194	198	160	160	318	318
63	124	124	200	204	196	202	494	502	270	272	188	210	162	162	314	314
64	124	124	200	204	190	202	502	502	272	272	188	202	156	162	312	312
65	124	124	200	206	184	202	508	508	276	284	192	194	160	162	312	318
66	124	124	204	204	200	200	496	500	-	-	188	198	162	162	-	-
67	124	124	200	202	188	202	478	490	270	296	180	198	158	162	314	314
68	124	128	200	200	196	202	494	494	270	276	194	198	164	164	312	312
69	124	124	200	200	186	188	-	-	276	296	-	-	162	164	312	312
70	124	128	186	200	188	202	496	502	272	276	186	210	160	162	-	-
71	124	128	200	200	184	202	478	478	296	296	198	202	160	162	312	318
72	124	124	204	204	184	202	496	502	270	272	188	194	164	164	312	312
73	124	124	202	204	192	196	494	494	270	276	192	194	158	162	312	312
74	124	124	186	186	176	202	478	496	276	296	192	198	160	162	-	-
75	124	124	200	204	184	190	478	490	270	296	180	198	160	160	316	316
76	124	124	202	204	200	202	478	494	270	296	198	198	158	162	314	314
77	124	124	-	-	184	186	494	502	270	272	188	194	162	164	-	-
78	124	128	200	206	188	192	480	480	276	276	194	202	162	164	312	326
79	124	128	200	200	184	202	478	502	270	272	188	194	160	162	314	318
80	124	128	200	204	196	200	494	496	270	276	192	194	160	160	316	316
81	124	128	200	204	196	202	-	-	-	-	192	210	158	158	312	312
82	124	124	200	206	188	190	490	490	270	276	182	194	164	164	312	312
83	124	124	200	204	184	188	494	494	270	270	198	210	162	164	312	312
84	124	128	200	204	184	202	478	496	270	296	194	198	160	162	312	312
85	124	124	-	-	192	196	478	494	276	296	194	210	162	164	-	-
86	124	128	200	202	188	202	478	494	270	274	194	208	160	164	314	314
87	124	128	200	206	184	188	494	494	270	270	194	210	162	168	312	312
88	124	124	200	200	188	196	480	480	274	276	194	202	162	164	312	312
89	124	124	-	-	184	196	-	-	270	296	-	-	162	168	-	-
90	124	128	200	204	200	202	496	502	272	276	188	198	162	168	312	314
91	124	124	200	202	188	196	490	494	270	270	180	210	162	162	314	314
92	124	124	204	204	192	196	496	496	276	276	192	192	160	162	314	318
93	124	124	200	204	186	202	-	-	278	296	198	202	162	164	312	312
94	124	128	204	204	192	202	494	502	272	276	188	210	162	162	310	310
95	124	128	-	-	190	200	502	508	272	284	188	192	160	162	-	-
96	124	124	200	202	188	190	490	494	270	270	180	210	162	164	312	312
97	124	124	200	204	202	202	490	490	276	296	194	210	158	160	312	318
98	124	124	204	204	190	196	490	490	270	276	180	192	162	162	314	314
99	124	124	204	206	184	202	494	494	276	276	194	210	162	162	312	312
100	124	124	200	204	184	202	494	494	276	296	194	210	156	158	312	314
101	124	124	200	200	196	202	478	508	284	296	192	194	158	164	312	312
102	124	124	204	204	192	202	494	502	272	276	188	210	158	162	312	312
103	124	128	200	200	186	200	478	494	270	296	194	198	160	162	312	312
104	124	124	202	204	196	202	478	496	276	296	192	198	156	162	314	314
105	124	128	200	200	184	202	502	508	272	284	188	192	160	160	-	-
106	124	124	200	204	186	200	478	502	272	274	188	198	162	164	312	312
107	124	124	200	206	188	200	508	508	276	284	192	194	162	162	312	326
108	124	124	202	204	190	202	494	494	270	276	192	198	162	164	312	312
109	124	124	200	200	190	202	490	490	270	276	180	194	162	164	312	312
110	124	124	200	200	196	202	480	494	270	274	198	210	160	164	312	318
111	124	124	204	204	184	202	494	502	272	276	188	210	158	162	314	314
112	124	124	200	202	184	202	508	508	276	284	192	192	162	162	312	312
113	124	128	200	200	196	202	508	508	-	-	194	198	156	162	314	314
114	124	124	200	200	188	202	490	490	270	276	180	194	162	164	314	314
115	124	124	200	204	192	196	496	502	272	276	188	210	158	164	312	312
116	124	124	200	204	184	202	502	508	272	284	188	192	156	160	314	316

* Genotype alleles are denoted as base pairs (bp) in length

(contd..)

Table A6 (contd..): Microsatellite marker genotypes pertaining to controls on property 3

Animal	o(IFNG)γ*		KP6*		DYMS1*		OLADRW*		OLADRB*		SMHCC1*		OVINRA1*		OVINRA2*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
117	124	128	200	204	188	192	494	494	270	270	194	210	158	160	312	316
118	124	124	204	204	186	202	478	478	270	296	198	198	158	162	314	314
119	124	124	204	204	200	202	478	478	274	296	198	198	158	162	312	312
120	124	124	200	200	200	202	478	502	272	296	188	198	158	162	312	312
121	124	124	200	202	188	202	494	496	270	276	192	210	160	164	312	312
122	124	124	200	200	202	202	478	502	272	296	188	198	158	164	312	312
123	124	124	204	204	184	184	496	508	276	284	192	210	160	160	318	326
124	124	124	200	204	184	184	-	-	276	284	192	192	158	162	-	-
125	124	124	200	204	184	186	478	496	270	296	194	198	156	160	-	-
126	124	124	200	204	184	202	508	508	284	284	192	192	160	162	314	318
127	124	128	200	202	186	190	490	520	270	276	180	194	160	162	312	318
128	124	124	204	204	184	196	496	508	276	284	192	192	160	162	312	316
129	124	124	204	204	184	188	494	494	270	276	192	210	156	158	-	-
130	124	124	186	204	202	202	-	-	276	296	186	204	160	160	-	-
131	124	124	202	204	188	202	494	502	270	272	188	210	158	158	314	314
132	124	124	200	204	184	200	480	508	274	284	192	194	158	168	-	-
133	124	124	200	200	184	202	478	478	276	296	198	202	158	162	312	312
134	124	128	200	200	186	192	490	494	270	276	194	210	158	162	312	312
135	124	128	200	204	184	202	502	508	272	284	188	192	160	160	-	-
136	124	124	204	204	184	202	478	508	284	296	192	198	158	160	312	316
137	124	128	200	204	184	190	478	490	270	296	180	198	158	160	312	316
138	124	128	200	204	200	202	478	490	270	296	180	198	160	162	314	318
139	124	124	200	204	184	184	478	508	284	296	192	198	158	162	312	312
140	124	124	204	206	186	186	-	-	270	276	194	198	158	164	314	314
141	124	124	204	204	184	202	490	508	270	284	180	192	160	162	312	318
142	124	128	200	200	184	186	494	508	270	284	192	198	158	162	314	314
143	124	124	200	200	184	202	478	478	296	296	198	198	160	162	314	318
144	124	124	200	204	196	202	490	490	276	284	192	192	160	162	-	-
145	124	124	200	204	184	192	478	490	270	296	180	198	160	168	312	316
146	124	124	200	204	184	196	478	508	284	296	192	198	158	160	314	318
147	124	124	200	200	202	202	508	508	276	284	192	192	156	160	316	318
148	124	124	200	204	176	184	478	490	270	296	180	198	160	168	312	318
149	124	124	200	204	186	196	478	560	274	296	194	198	158	162	312	326
150	124	124	204	204	188	200	452	494	270	270	-	-	158	162	312	312
151	124	124	200	204	184	196	478	508	284	296	192	198	160	162	314	318
152	124	124	200	200	192	202	478	488	276	296	198	210	158	160	312	318
153	124	124	204	204	184	202	478	494	270	296	194	198	160	162	-	-
154	124	128	200	200	184	190	478	508	-	-	192	198	160	162	314	318
155	124	128	200	202	186	188	478	522	276	296	194	198	162	162	312	312
156	124	128	200	200	184	190	496	508	276	284	192	192	160	162	314	318
157	124	124	200	202	186	196	522	522	276	296	194	202	158	168	312	314
158	124	124	-	-	196	202	496	508	276	284	192	192	160	164	-	-
159	124	124	186	202	184	202	494	508	270	284	192	194	158	168	-	-
160	124	124	204	204	184	192	494	508	270	284	210	210	156	160	314	316
161	124	124	186	186	184	192	478	494	276	296	198	210	158	164	-	-
162	124	124	200	204	186	202	478	502	272	296	188	198	158	162	-	-
163	124	124	200	202	190	200	478	490	270	296	180	198	160	162	312	316
164	124	124	200	204	184	184	478	478	296	296	188	198	158	160	312	316
165	124	124	200	200	186	202	478	496	270	296	194	198	160	162	312	318
166	124	124	200	204	188	196	478	496	270	296	198	210	162	162	312	312
167	124	124	202	204	186	196	502	522	272	276	188	194	158	160	314	318
168	124	124	200	202	188	202	494	502	270	272	188	210	158	166	314	314
169	124	124	204	204	186	200	478	522	276	296	194	198	162	164	312	312

* Genotype alleles are denoted as base pairs (bp) in length

Table A7: Immune response data pertaining to controls on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
1	0.141	0.061	0.054	0.056	-	-	0.000	0.007	0.089	0.003	0.008	-	0.002	0.004	0.088	0.007	0.001	-
2	-	0.067	0.053	0.053	0.063	0.054	-	0.000	0.000	0.021	0.017	0.022	-	0.005	0.015	0.002	0.012	0.005
3	0.122	0.188	0.084	0.151	0.220	-	0.076	0.013	0.026	0.151	0.008	-	0.052	0.003	0.022	0.110	0.000	-
4	0.172	0.059	0.059	0.148	0.075	0.051	0.001	0.006	0.036	0.000	0.018	0.005	0.000	0.002	0.005	0.004	0.000	0.003
5	0.106	0.138	0.056	0.053	0.073	0.066	0.000	0.026	0.000	0.005	0.000	0.016	0.000	0.027	0.000	0.000	0.000	0.009
6	0.083	0.059	0.054	0.063	0.057	0.054	0.063	0.000	0.067	0.019	0.000	0.001	0.045	0.000	0.043	0.000	0.000	0.012
7	0.100	0.070	0.055	0.087	0.096	-	0.000	0.000	0.004	0.007	0.250	-	0.000	0.000	0.008	0.001	0.182	-
8	0.170	0.152	0.100	0.124	0.116	0.066	-	0.001	0.036	0.006	0.003	0.018	-	0.000	0.013	0.004	0.000	0.017
9	0.096	0.266	0.068	0.167	0.215	-	0.017	0.000	0.000	0.003	0.049	-	0.005	0.016	0.002	0.007	0.030	-
10	0.088	0.079	0.043	0.070	0.057	0.091	0.002	0.002	0.055	0.006	0.000	0.000	0.004	0.011	0.021	0.006	0.005	0.007
11	0.116	0.091	0.079	0.068	0.060	-	0.136	0.031	0.003	0.063	0.000	-	0.084	0.035	0.018	0.027	0.000	-
12	0.157	0.077	0.053	0.053	0.058	0.075	0.080	0.000	0.002	0.010	0.002	0.015	0.073	0.000	0.000	0.010	0.003	0.002
13	0.141	0.184	0.102	0.232	0.083	-	0.320	0.215	-	0.113	0.016	-	0.207	0.149	-	0.022	0.008	-
14	0.120	0.162	0.073	0.089	0.073	0.067	0.000	0.000	0.004	0.000	-	0.000	0.000	0.000	0.005	0.000	-	0.000
15	0.127	0.136	0.071	0.087	0.091	0.064	0.000	0.000	0.000	0.009	0.000	0.007	0.006	0.000	0.030	0.011	0.000	0.003
16	0.114	0.087	0.095	0.089	0.092	-	0.263	0.000	0.000	0.040	0.000	-	0.186	0.000	0.006	0.015	0.000	-
17	0.064	0.061	0.051	0.066	0.080	0.076	0.000	0.000	0.015	0.000	0.006	0.014	0.000	0.000	0.005	0.000	0.002	0.000
18	0.088	0.074	0.055	0.051	0.094	0.054	-	0.000	0.109	0.007	0.005	0.000	-	0.010	0.052	0.014	0.013	0.002
19	0.131	0.057	0.060	0.074	0.065	0.060	0.000	0.001	0.005	0.002	0.010	0.018	0.000	0.000	0.000	0.000	0.010	0.016
20	0.094	0.077	0.062	0.179	0.082	0.064	0.001	0.000	0.018	0.008	0.000	0.000	0.001	0.000	0.009	0.000	0.006	0.000
21	0.096	0.106	0.056	0.066	0.089	0.072	0.000	0.000	0.017	0.136	0.000	0.012	0.000	0.011	0.024	0.079	0.000	0.011
22	0.071	0.071	0.048	0.060	0.048	0.052	0.000	0.014	0.027	0.003	0.008	0.000	0.000	0.006	0.020	0.001	0.010	0.000
23	0.099	0.087	0.068	0.112	0.054	0.058	0.036	0.007	0.004	0.082	0.087	0.047	0.026	0.000	0.009	0.023	0.061	0.041
24	0.149	0.177	0.072	0.068	0.248	-	-	-	0.009	0.009	0.000	-	-	-	0.002	0.021	0.000	-
25	0.103	0.065	0.061	0.057	-	-	0.008	0.004	0.000	0.006	0.017	-	0.000	0.010	0.001	0.009	0.018	-
26	0.147	-	0.149	0.174	0.096	-	0.000	0.000	0.031	0.002	0.011	-	0.002	0.000	0.028	0.001	0.010	-
27	0.089	0.156	0.055	0.082	0.071	0.070	0.008	0.000	0.021	0.000	0.000	0.016	0.008	0.001	0.017	0.012	0.011	0.011
28	0.087	0.144	0.054	0.062	0.058	0.062	0.000	0.000	0.000	0.016	0.025	0.038	0.000	0.000	0.000	0.004	0.015	0.018
29	0.064	0.121	0.055	0.069	0.076	0.069	0.015	0.000	0.013	0.014	0.000	0.016	0.013	0.000	0.015	0.001	0.001	0.006
30	0.078	0.064	0.057	0.059	0.066	0.052	0.009	0.000	0.000	0.012	0.000	0.013	0.013	0.000	0.000	0.000	0.000	0.000
31	0.075	0.074	0.057	0.080	0.062	0.062	0.110	0.021	0.000	0.000	0.003	0.000	0.068	0.001	0.000	0.000	0.006	0.000
32	0.064	0.060	0.064	0.060	0.052	-	0.000	0.005	0.000	0.008	0.016	-	0.000	0.000	0.002	0.007	0.006	-
33	0.065	0.193	0.104	0.186	0.075	0.058	0.003	0.000	0.000	0.002	0.031	0.064	0.006	0.001	0.001	0.000	0.004	0.041
34	0.072	0.080	0.054	0.052	0.068	0.075	0.000	0.000	0.003	0.013	0.000	0.012	0.000	0.000	0.005	0.006	0.001	0.005
35	0.082	0.059	0.061	0.063	0.047	-	0.011	0.000	0.019	0.047	0.031	-	0.007	0.010	0.007	0.039	0.008	-
36	0.083	0.199	0.090	0.091	0.082	0.061	0.000	0.001	0.000	0.002	0.005	0.020	0.002	0.000	0.000	0.000	0.003	0.017
37	0.134	0.099	0.056	0.066	0.106	0.062	0.000	0.000	0.018	0.008	0.000	0.022	0.000	0.001	0.009	0.008	0.000	0.008
38	0.102	0.067	0.057	0.071	0.056	0.063	0.000	0.000	0.011	0.024	0.003	0.013	0.000	0.000	0.009	0.015	0.014	0.009
39	0.173	0.130	0.088	0.125	0.146	0.087	0.000	0.010	0.000	0.037	0.000	0.020	0.002	0.008	0.000	0.012	0.001	0.018
40	0.090	0.081	0.057	0.095	0.087	0.055	0.077	0.000	0.000	0.000	0.000	0.008	0.065	0.000	0.000	0.000	0.000	0.000
41	0.123	0.055	0.061	0.089	0.066	0.055	0.166	0.000	0.014	0.000	0.000	0.000	0.053	0.000	0.006	0.000	0.000	0.000
42	0.081	0.089	0.056	0.060	0.085	0.052	0.005	0.022	0.033	0.011	0.000	0.004	0.005	0.020	0.009	0.001	0.000	0.006

Table A7 (contd..): Immune response data pertaining to controls on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
43	0.157	0.075	0.057	0.070	-	0.060	0.012	0.011	0.011	0.000	-	0.014	0.008	0.007	0.007	0.000	-	0.008
44	0.114	0.133	0.059	0.101	0.064	0.056	0.000	0.005	0.000	0.000	0.011	0.018	0.000	0.011	0.000	0.011	0.003	0.016
45	0.075	-	0.135	0.065	0.057	-	0.004	0.000	0.000	0.011	0.002	-	0.008	0.000	0.000	0.011	0.005	-
46	0.069	0.091	0.056	0.080	0.125	0.061	0.000	0.013	0.000	0.007	0.010	0.082	0.003	0.014	0.000	0.000	0.011	0.064
47	0.208	0.096	0.071	0.079	0.070	0.059	0.000	0.000	0.001	0.000	0.006	0.000	0.003	0.001	0.000	0.000	0.004	0.000
48	0.080	0.079	0.096	0.095	0.094	-	0.017	0.039	0.053	-	0.000	-	0.012	0.029	0.053	-	0.001	-
49	0.091	0.093	0.056	0.058	0.061	0.073	0.001	0.007	0.036	0.071	0.005	0.000	0.002	0.009	0.040	0.019	0.002	0.001
50	0.066	0.125	0.047	0.062	0.050	0.082	0.000	0.013	0.000	0.000	0.000	0.008	0.000	0.010	0.000	0.052	0.000	0.005
51	0.140	0.064	0.055	0.057	0.065	0.083	0.035	0.000	0.036	0.000	0.012	0.007	0.016	0.000	0.023	0.000	0.023	0.001
52	0.077	0.054	0.046	0.063	0.048	0.059	0.006	0.000	0.002	0.011	0.000	0.018	0.006	0.000	0.004	0.007	0.000	0.011
53	0.069	0.098	0.052	0.056	0.062	0.056	0.000	0.000	0.014	0.122	0.012	0.014	0.000	0.000	0.017	0.110	0.009	0.010
54	0.101	0.094	0.109	-	0.162	0.096	0.000	0.000	0.144	0.008	0.009	0.000	0.000	0.016	0.080	0.000	0.005	0.000
55	0.082	0.069	0.059	0.058	0.054	0.057	0.077	0.000	0.028	0.000	0.013	0.100	0.064	0.000	0.007	0.005	0.011	0.032
56	0.075	0.058	0.060	0.052	0.079	0.067	0.000	0.005	0.019	0.003	0.006	0.005	0.001	0.000	0.011	0.004	0.000	0.002
57	0.078	-	0.063	0.068	0.055	0.064	0.003	0.008	0.067	0.023	0.002	0.000	0.005	0.005	0.047	0.016	0.003	0.000
58	0.083	0.053	0.085	0.060	0.061	0.052	0.059	0.000	0.003	0.022	0.010	0.032	0.025	0.000	0.006	0.014	0.004	0.001
59	0.091	0.119	0.091	0.055	0.055	0.074	0.000	0.004	0.033	0.019	0.001	0.003	0.001	0.008	0.026	0.001	0.004	0.000
60	0.136	0.139	0.059	0.067	0.068	0.070	0.008	0.000	0.000	0.000	0.018	0.007	0.000	0.000	0.020	0.000	0.010	0.000
61	0.172	0.085	0.048	0.073	0.059	0.067	0.003	0.000	0.019	0.098	0.014	0.014	0.000	0.001	0.017	0.015	0.015	0.015
62	0.066	0.080	0.061	0.124	0.164	0.088	0.015	0.000	0.003	0.000	0.000	0.024	0.010	0.000	0.026	0.000	0.000	0.017
63	0.063	0.063	0.048	0.199	0.078	0.062	0.000	0.000	0.000	0.000	0.300	0.017	0.002	0.000	0.034	0.000	0.134	0.000
64	0.087	0.180	0.068	0.104	0.084	0.060	0.024	0.019	0.009	0.004	0.003	0.027	0.022	0.010	0.000	0.007	0.003	0.013
65	0.076	0.098	0.101	0.097	0.068	-	0.000	0.003	0.000	0.000	0.000	-	0.000	0.000	0.000	0.010	0.003	-
66	0.094	0.115	0.092	0.261	0.119	0.068	0.023	0.017	0.012	0.018	0.000	0.024	0.000	0.010	0.002	0.002	0.000	0.008
67	0.074	0.057	0.054	0.080	0.056	0.071	0.000	0.006	0.006	0.000	0.003	0.000	0.003	0.000	0.005	0.004	0.002	0.000
68	0.120	0.127	0.064	0.070	0.060	0.063	0.000	0.000	0.076	0.000	0.008	0.004	0.000	0.001	0.070	0.002	0.007	0.006
69	0.073	0.077	0.055	0.083	0.079	0.075	0.000	0.006	0.010	0.000	0.090	-	0.000	0.003	0.002	0.000	0.066	-
70	0.090	0.072	0.091	0.063	0.076	-	0.000	0.004	0.000	0.058	0.004	-	0.002	0.000	0.000	0.020	0.001	-
71	0.086	0.182	0.056	0.061	0.057	0.063	0.000	0.018	0.000	0.051	0.000	0.013	0.001	0.042	0.000	0.007	0.000	0.003
72	0.108	0.060	0.077	0.068	0.065	0.065	0.000	0.000	0.032	0.003	0.037	0.079	0.000	0.000	0.007	0.003	0.039	0.069
73	0.109	0.101	0.092	0.131	0.075	0.063	0.095	0.009	0.033	0.030	0.000	0.020	0.066	0.004	0.020	0.000	0.009	0.011
74	0.107	0.062	0.054	0.070	0.081	-	0.030	0.009	0.007	0.018	0.020	-	0.025	0.007	0.004	0.014	0.008	-
75	0.076	0.068	0.069	0.079	0.067	-	0.001	0.000	0.130	0.002	0.057	-	0.001	0.004	0.118	0.000	0.045	-
76	0.075	-	0.058	0.070	0.134	0.068	0.016	-	0.074	0.000	0.000	0.000	0.011	-	0.057	0.000	0.000	0.015
77	0.108	0.067	0.056	0.060	0.065	0.072	0.000	0.021	0.008	0.009	0.012	0.000	0.000	0.013	0.007	0.005	0.007	0.000
78	0.091	0.073	0.048	0.081	0.085	-	0.001	0.003	0.080	0.034	0.000	-	0.001	0.000	0.061	0.035	0.012	-
79	0.097	0.060	0.067	0.063	0.052	0.107	0.019	0.009	0.034	0.043	0.002	0.012	0.004	0.013	0.019	0.000	0.006	0.002
80	0.060	0.062	0.072	0.111	0.060	0.060	0.000	0.002	0.097	0.034	0.011	0.217	0.005	0.000	0.078	0.000	0.017	0.018
81	0.067	0.057	0.056	0.058	0.077	0.071	0.000	0.000	0.000	0.000	0.013	0.039	0.000	0.000	0.000	0.001	0.009	0.033

Table A7 (contd..): Immune response data pertaining to controls on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
82	0.101	0.069	0.061	0.073	0.059	0.082	0.000	0.000	0.013	0.000	0.039	0.023	0.000	0.000	0.019	0.000	0.002	0.001
83	0.110	0.088	0.063	0.077	0.075	0.075	0.000	0.029	0.028	0.003	0.002	0.000	0.003	0.019	0.000	0.009	0.000	0.021
84	0.110	0.077	0.054	0.066	0.063	0.069	0.000	0.000	0.036	0.030	0.000	0.005	0.000	0.004	0.000	0.028	0.000	0.003
85	0.377	0.216	0.161	0.145	0.101	0.078	0.084	0.007	0.008	0.008	0.003	0.005	0.040	0.007	0.003	0.003	0.004	0.004
86	0.081	0.070	0.058	0.077	0.075	0.084	0.001	0.000	0.008	0.025	0.024	0.027	0.007	0.000	0.018	0.016	0.012	0.012
87	0.103	0.244	0.091	0.108	0.079	0.066	-	0.000	0.008	0.006	0.019	0.025	-	0.000	0.015	0.004	0.010	0.007
88	0.153	0.085	0.052	0.065	0.068	0.065	0.000	0.000	0.110	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.026
89	0.064	0.062	0.063	0.050	-	0.067	0.007	0.018	0.000	0.012	-	0.005	0.009	0.004	0.000	0.006	-	0.008
90	0.064	0.064	0.060	0.156	0.117	-	0.006	0.000	0.010	0.016	0.068	-	0.004	0.002	0.006	0.008	0.059	-
91	0.198	0.087	0.067	0.074	0.081	0.055	0.002	0.088	0.000	0.044	0.009	0.004	0.006	0.000	0.000	0.018	0.001	0.012
92	0.101	0.090	0.068	0.064	0.084	0.074	0.128	0.005	0.022	0.040	0.012	0.013	0.113	0.008	0.037	0.000	0.000	0.010
93	0.115	0.087	0.063	-	0.054	-	0.008	-	0.000	-	0.000	-	0.009	-	0.000	-	0.000	-
94	0.098	0.087	0.063	0.084	0.065	0.055	0.000	0.006	0.013	0.000	0.012	0.044	0.000	0.001	0.009	0.000	0.017	0.025
95	0.095	0.072	0.055	0.066	0.056	0.068	0.004	0.000	0.014	0.084	0.000	0.000	0.003	0.005	0.009	0.077	0.003	0.000
96	0.086	0.071	0.063	0.057	0.064	0.063	0.005	0.015	0.000	0.007	0.000	0.011	0.014	0.001	0.000	0.002	0.000	0.003
97	0.076	0.101	0.057	0.094	0.061	0.062	0.005	0.000	0.005	0.000	0.000	0.005	0.002	0.008	0.019	0.005	0.002	0.002
98	0.274	0.133	0.156	0.548	1.016	0.338	-	0.003	0.022	0.016	0.039	0.004	-	0.007	0.019	0.007	0.009	0.009
99	0.101	-	0.117	0.062	0.062	-	0.019	0.000	0.000	0.000	0.015	-	0.012	0.000	0.000	0.000	0.020	-
100	0.132	0.092	0.074	0.082	0.077	0.076	0.000	0.000	0.009	0.004	0.005	0.000	0.004	0.000	0.000	0.006	0.000	0.002
101	0.105	0.076	0.053	0.069	0.097	0.066	0.042	0.000	0.013	0.007	0.001	-	0.035	0.003	0.020	0.001	0.000	-
102	0.165	0.137	0.103	0.156	0.096	0.074	0.095	0.006	0.087	0.139	0.000	0.034	0.058	0.017	0.067	0.077	0.003	0.029
103	0.160	0.067	0.066	0.057	0.096	0.096	0.131	0.012	0.009	-	0.000	0.034	0.032	0.005	0.000	-	0.000	0.004
104	0.081	0.158	0.076	0.073	0.054	-	0.008	0.000	0.001	0.020	0.000	-	0.002	0.000	0.000	0.017	0.001	-
105	0.135	0.077	0.068	0.076	0.072	0.058	0.004	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.006	0.003	0.000	0.004
106	0.102	0.141	0.053	0.125	0.146	0.118	0.011	0.000	0.003	0.002	0.033	0.017	0.011	0.000	0.000	0.000	0.022	0.013
107	0.077	0.061	0.053	0.053	0.059	0.050	0.009	0.000	0.019	0.004	0.000	0.000	0.000	0.000	0.026	0.003	0.000	0.000
108	0.247	0.124	0.083	0.082	0.146	0.120	0.239	0.002	0.014	0.007	0.046	0.028	0.204	0.000	0.005	0.011	0.038	0.018
109	0.132	0.106	0.057	0.080	0.057	0.057	0.000	0.019	0.012	0.000	0.000	0.024	0.001	0.014	0.016	0.000	0.000	0.022
110	0.074	0.166	0.095	0.054	0.060	0.063	0.302	0.000	0.033	0.008	0.000	0.032	0.155	0.000	0.000	0.001	0.000	0.028
111	0.151	0.067	0.057	0.074	0.054	-	0.073	0.012	0.016	0.094	0.052	-	0.037	0.008	0.005	0.023	0.002	-
112	0.082	0.067	0.137	0.071	0.099	0.093	0.007	0.019	0.003	0.030	0.025	-	0.007	0.006	0.013	0.030	0.028	-
113	0.107	0.078	0.059	0.058	0.050	0.059	0.003	0.023	0.040	0.000	0.000	0.002	0.002	0.007	0.005	0.000	0.000	0.000
114	0.165	0.184	0.074	0.095	0.090	0.075	0.006	0.001	0.001	0.007	0.000	0.000	0.008	0.003	0.003	0.006	0.000	0.000
115	0.118	0.066	0.058	0.120	0.079	0.065	0.016	0.000	0.024	0.001	0.002	0.002	0.011	0.000	0.018	0.001	0.001	0.009
116	0.072	0.060	0.071	0.167	0.078	0.071	0.004	0.013	0.000	0.013	0.001	0.011	0.001	0.009	0.000	0.016	0.000	0.003
117	0.148	-	0.077	0.114	0.249	0.069	-	-	0.026	0.012	0.000	0.003	-	-	0.005	0.009	0.000	0.000
118	0.092	0.199	0.073	0.093	0.261	0.157	0.064	0.000	0.006	0.021	0.350	0.007	0.037	0.000	0.010	0.009	0.202	0.000
119	0.096	0.148	0.064	0.066	0.055	0.055	0.014	0.008	0.023	0.120	0.009	0.028	0.015	0.000	0.018	0.076	0.006	0.022
120	0.274	0.145	0.115	0.073	0.104	0.065	0.000	0.000	0.014	0.004	0.009	0.004	0.002	0.000	0.007	0.004	0.005	0.000
121	0.105	0.080	0.068	0.072	0.058	0.059	0.008	0.000	0.002	0.033	0.014	0.089	0.000	0.000	0.000	0.015	0.003	0.044
122	0.122	0.200	0.066	0.117	0.058	-	0.021	0.013	0.015	0.004	0.003	-	0.020	0.020	0.008	0.014	0.000	-

Table A7 (contd..): Immune response data pertaining to controls on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
123	0.078	0.143	0.091	0.107	0.066	0.058	0.001	0.000	0.006	0.022	0.035	-	0.000	0.004	0.008	0.015	0.027	-
124	0.085	0.106	0.058	0.072	0.066	0.068	0.000	0.006	0.008	0.006	0.000	0.004	0.002	0.013	0.007	0.000	0.000	0.000
125	0.145	0.071	0.058	0.057	0.060	0.067	0.002	0.001	-	0.000	0.013	0.004	0.003	0.000	-	0.000	0.005	0.000
126	0.119	0.331	0.116	0.193	0.139	0.069	0.146	0.007	0.069	0.028	0.077	0.067	0.061	0.000	0.038	0.013	0.000	0.024
127	0.075	0.067	0.058	0.048	0.063	0.075	0.016	0.000	0.012	0.012	0.046	0.005	0.006	0.005	0.014	0.000	0.024	0.000
128	0.094	0.066	0.055	0.173	0.063	-	0.006	0.000	0.029	0.019	0.014	-	0.003	0.008	0.034	0.022	0.006	-
129	0.130	0.106	-	0.055	0.069	-	0.119	0.003	-	0.026	0.021	-	0.061	0.003	-	0.019	0.007	-
130	0.093	0.075	0.059	0.059	0.085	0.062	0.009	0.006	0.009	0.003	0.025	0.008	0.010	0.006	0.008	0.001	0.020	0.014
131	0.130	0.068	0.051	0.067	0.064	0.053	0.018	0.032	0.049	0.008	0.107	0.103	0.000	0.014	0.029	0.000	0.066	0.018
132	0.067	0.071	0.058	0.063	0.064	0.063	0.104	0.000	0.029	-	0.020	0.031	0.059	0.006	0.031	-	0.017	0.030
133	0.067	0.056	0.052	0.070	0.068	0.065	0.000	0.005	0.002	0.007	0.013	0.018	0.000	0.003	0.003	0.002	0.007	0.006
134	0.197	0.079	0.113	0.122	0.077	0.055	0.001	0.004	0.016	0.007	-	0.015	0.000	0.002	0.023	0.014	-	0.011
135	0.087	0.109	-	0.070	0.058	-	0.001	0.008	-	0.000	0.004	-	0.003	0.015	-	0.000	0.004	-
136	0.081	0.095	0.063	0.069	0.079	0.062	0.003	0.035	0.026	0.014	0.022	0.151	0.003	0.022	0.021	0.021	0.019	0.063
137	0.062	0.148	0.048	0.076	0.050	-	0.010	0.029	0.012	0.006	0.012	-	0.000	0.017	0.000	0.000	0.000	-
138	0.088	0.081	0.053	0.071	0.073	0.055	0.021	0.012	0.046	0.019	0.008	0.052	0.013	0.008	0.037	0.013	0.007	0.026
139	0.080	0.116	0.081	0.061	0.050	0.076	0.018	0.000	0.020	0.045	0.003	0.140	0.014	0.000	0.000	0.026	0.004	0.101
140	0.095	0.093	0.064	0.079	0.063	0.067	0.093	0.000	0.072	0.091	0.005	0.017	0.061	0.000	0.020	0.000	0.005	0.000
141	0.106	0.087	-	0.068	0.066	0.063	0.088	0.005	0.028	0.016	0.000	0.006	0.047	0.006	0.024	0.011	0.000	0.006
142	0.107	0.084	0.057	0.051	0.192	0.061	0.023	0.000	0.073	0.072	0.000	0.078	0.011	0.000	0.041	0.045	0.000	0.009
143	0.208	0.130	0.074	0.068	0.108	0.065	-	0.000	-	0.024	0.000	0.008	-	0.000	-	0.024	0.000	0.011
144	-	0.264	0.134	0.160	0.114	0.070	-	0.000	0.015	0.006	0.012	0.017	-	0.016	0.002	0.000	0.000	0.015
145	0.186	0.200	0.114	0.100	0.155	0.093	-	0.048	-	0.014	0.012	0.042	-	0.028	-	0.005	0.001	0.020
146	0.078	0.228	0.052	-	0.110	0.067	0.001	0.024	0.050	0.017	0.000	0.000	0.000	0.003	0.051	0.012	0.004	0.000
147	0.388	0.598	0.217	0.191	0.158	0.084	0.015	0.115	-	0.071	0.095	0.054	0.007	0.145	-	0.048	0.074	0.071
148	0.078	0.059	0.052	0.139	0.057	-	0.150	0.007	0.010	0.000	0.000	-	0.102	0.009	0.004	0.000	0.000	-
149	0.174	0.136	0.074	0.081	0.089	0.063	0.272	0.009	0.000	0.025	0.023	0.016	0.168	0.002	0.000	0.011	0.016	0.014
150	0.089	0.163	0.059	0.082	0.054	0.064	0.009	0.000	0.001	0.065	0.000	0.007	0.000	0.000	0.010	0.053	0.000	0.007
151	0.268	0.361	0.175	0.212	0.222	0.104	-	0.009	-	-	0.084	0.061	-	0.003	-	-	0.050	0.051
152	0.131	0.088	0.078	0.093	0.088	0.069	0.022	0.003	0.020	0.000	0.000	0.013	0.019	0.000	0.000	0.000	0.000	0.003
153	0.093	0.069	0.059	0.051	0.081	0.098	0.000	0.004	0.002	0.020	0.012	0.000	0.000	0.005	0.007	0.026	0.020	0.001
154	0.077	0.088	0.056	0.061	0.067	-	0.012	0.003	0.001	0.007	0.000	-	0.003	0.000	0.003	0.013	0.000	-
155	0.077	0.073	0.082	0.129	0.058	0.062	0.015	0.001	0.029	0.020	0.000	0.035	0.011	0.011	0.002	0.016	0.009	0.022
156	0.092	0.060	0.051	0.065	0.065	0.061	0.000	0.000	0.047	0.026	0.010	0.018	0.003	0.001	0.035	0.020	0.018	0.010
157	0.099	0.107	0.096	0.070	0.093	0.075	0.007	0.012	0.000	0.005	0.000	0.015	0.006	0.023	0.000	0.003	0.000	0.000
158	0.085	0.174	0.058	0.058	0.057	0.055	0.000	0.010	0.011	0.012	0.000	0.000	0.001	0.002	0.010	0.000	0.013	0.000

Table A8: Immune response data pertaining to vaccinates on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
1	0.246	0.177	0.162	0.101	0.123	-	0.319	0.029	0.124	0.219	0.087	-	0.266	0.025	0.100	0.174	0.074	-
2	0.346	0.048	0.086	0.108	0.113	0.060	0.422	0.779	0.429	0.591	0.012	0.060	0.377	0.754	0.385	0.491	0.010	0.044
3	0.411	0.194	0.145	0.149	0.192	0.064	0.474	0.030	0.223	0.089	0.117	0.011	0.371	0.014	0.206	0.064	0.088	0.000
4	0.224	0.123	0.121	0.108	0.118	0.042	1.502	0.109	0.361	0.084	0.253	0.024	0.967	0.103	0.300	0.050	0.161	0.007
5	2.097	1.334	0.397	0.383	0.374	0.140	0.151	0.077	0.091	0.261	0.000	0.048	0.110	0.071	0.038	0.186	0.000	0.041
6	0.624	0.320	0.146	0.189	0.332	0.076	0.091	0.005	0.013	0.027	0.021	0.001	0.076	0.000	0.019	0.012	0.002	0.000
7	1.638	0.619	0.251	0.313	0.471	0.193	0.195	0.035	0.134	0.009	0.000	0.000	0.171	0.036	0.063	0.003	0.006	0.002
8	0.118	0.133	0.070	0.082	0.128	0.049	0.029	0.006	0.015	0.012	0.004	0.020	0.034	0.002	0.006	0.004	0.008	0.011
9	0.279	0.114	0.078	0.135	0.174	-	0.757	0.083	0.149	0.091	0.010	-	0.650	0.075	0.146	0.062	0.006	-
10	1.308	0.516	0.404	0.282	0.445	0.155	-	0.093	0.627	0.570	0.314	0.038	-	0.069	0.490	0.417	0.196	0.013
11	0.370	0.230	0.128	0.178	0.242	0.076	0.007	0.007	0.019	0.012	0.015	0.002	0.011	0.008	0.017	0.013	0.007	0.004
12	0.685	0.249	0.229	0.098	0.128	0.066	0.113	0.018	0.094	0.065	0.000	0.000	0.096	0.014	0.089	0.052	0.003	0.000
13	0.370	0.157	0.088	0.084	0.104	0.050	0.122	0.013	0.057	0.122	0.000	0.037	0.104	0.009	0.038	0.101	0.000	0.026
14	0.903	0.528	0.235	0.288	0.329	0.141	0.388	0.159	0.343	0.351	0.038	0.030	0.332	0.172	0.309	0.371	0.036	0.027
15	0.855	0.349	0.289	0.280	0.362	0.099	0.124	0.009	0.021	0.020	0.020	0.017	0.058	0.012	0.019	0.044	0.021	0.012
16	0.553	0.223	0.119	0.124	0.250	0.085	0.318	0.051	0.076	0.033	0.006	0.015	0.245	0.035	0.065	0.038	0.002	0.011
17	3.225	1.849	0.656	0.928	1.041	0.494	0.007	0.003	0.000	0.000	0.002	0.006	0.000	0.007	0.000	0.000	0.000	0.000
18	-	0.281	0.160	0.206	0.788	0.190	-	0.011	0.079	0.045	0.000	0.013	-	0.017	0.082	0.060	0.000	0.048
19	0.134	0.129	0.123	0.068	0.110	0.026	0.222	0.025	0.040	0.013	0.260	0.067	0.208	0.020	0.038	0.001	0.213	0.067
20	2.774	1.710	0.382	0.547	0.700	0.283	0.812	0.009	0.092	0.062	0.001	0.004	0.778	0.001	0.079	0.052	0.000	0.004
21	0.276	0.153	0.140	0.109	0.098	0.050	0.238	0.008	0.040	0.032	0.033	0.037	0.209	0.009	0.043	0.031	0.013	0.026
22	0.843	0.615	0.338	0.163	0.235	0.083	0.121	0.003	0.036	0.000	0.004	0.000	0.102	0.001	0.020	0.004	0.016	0.000
23	1.272	0.627	0.212	0.258	0.364	0.143	0.345	0.025	0.275	0.000	0.068	0.068	0.280	0.024	0.263	0.000	0.069	0.067
24	0.567	0.256	0.221	0.160	0.167	0.070	0.084	0.000	0.155	-	-	0.000	0.059	0.004	0.119	-	-	0.000
25	0.885	0.414	0.166	0.238	0.272	0.104	2.158	0.085	1.030	-	0.505	-	1.742	0.066	0.923	-	0.430	-
26	1.162	0.480	0.247	0.326	0.357	0.187	0.774	0.000	0.103	0.252	0.008	0.011	0.689	0.013	0.087	0.200	0.005	0.002
27	0.926	0.445	0.198	0.204	0.236	0.040	-	0.000	0.373	0.341	0.308	0.002	-	0.004	0.315	0.289	0.000	0.011
28	0.084	0.074	0.098	0.102	0.064	-	0.000	0.009	0.023	0.030	0.006	-	0.003	0.008	0.016	0.013	0.014	-
29	4.300	-	0.755	1.143	1.372	0.632	0.753	-	0.153	0.043	0.054	0.014	0.657	-	0.142	0.032	0.033	0.009
30	0.462	0.220	0.100	0.107	0.089	0.054	0.601	0.073	0.237	0.331	0.218	0.031	0.514	0.076	0.222	0.304	0.136	0.033
31	1.004	0.597	0.227	0.345	0.353	0.119	0.320	0.059	0.060	0.050	0.149	0.004	0.262	0.046	0.060	0.034	0.113	0.000
32	0.810	0.420	0.442	0.260	0.217	0.100	0.028	0.012	0.061	0.128	0.007	0.000	0.018	0.012	0.049	0.084	0.005	0.000
33	0.561	0.252	0.202	0.178	0.250	-	1.211	0.041	0.249	0.234	0.631	-	0.975	0.038	0.200	0.084	0.527	-
34	0.782	0.405	0.159	0.158	0.247	0.095	0.394	0.017	0.120	0.044	0.020	0.024	0.349	0.023	0.027	0.034	0.020	0.022
35	0.044	0.085	0.115	0.054	-	-	0.000	0.000	0.000	-	-	-	0.006	0.001	0.000	-	-	-
36	-	0.234	-	0.213	0.327	0.113	-	0.105	-	0.315	0.024	0.035	-	0.075	-	0.219	0.015	0.036
37	1.295	0.425	0.171	0.206	0.212	-	0.697	0.043	0.537	-	0.013	-	0.627	0.044	0.459	-	0.005	-
38	0.593	0.270	0.108	0.188	0.175	0.057	1.529	0.372	0.155	0.635	0.070	0.114	1.342	0.366	0.127	0.519	0.067	0.088
39	4.300	2.086	0.807	0.973	1.397	0.744	0.345	0.013	0.154	0.044	0.033	0.020	0.240	0.014	0.147	0.020	0.028	0.016
40	0.133	0.120	0.102	0.057	0.122	-	0.002	0.017	0.054	0.005	0.011	-	0.000	0.030	0.048	0.000	0.016	-
41	1.165	0.658	-	0.238	0.297	0.121	0.288	0.000	-	0.017	0.011	0.008	0.222	0.000	-	0.012	0.015	0.008
42	0.506	0.244	0.133	0.140	0.169	0.072	0.128	0.020	0.119	0.214	0.031	0.113	0.113	0.015	0.115	0.191	0.023	0.080
43	2.458	1.320	0.375	0.465	0.541	0.145	0.250	0.001	0.000	-	0.011	0.007	0.222	0.000	0.000	-	0.015	0.012

Table A8 (contd..): Immune response data pertaining to vaccinates on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
44	1.549	0.421	0.167	0.249	0.182	0.112	0.743	0.028	0.105	0.580	0.010	0.019	0.680	0.021	0.095	0.524	0.012	0.011
45	0.572	0.258	0.119	0.176	0.249	0.096	0.193	0.023	0.040	0.017	-	0.016	0.170	0.019	0.035	0.014	-	0.008
46	3.409	1.514	0.570	0.722	0.767	0.335	0.142	0.003	0.085	0.006	0.000	0.019	0.058	0.000	0.057	0.004	0.004	0.016
47	0.587	0.289	0.117	0.149	0.183	0.060	1.081	0.021	0.022	0.032	0.053	0.000	0.825	0.018	0.000	0.035	0.032	0.000
48	1.241	0.549	0.428	0.292	0.364	0.105	0.833	0.044	0.039	0.000	0.059	0.011	0.737	0.038	0.044	0.000	0.056	0.009
49	-	1.512	0.751	0.820	0.925	0.360	1.016	0.098	0.312	0.139	0.000	0.022	0.903	0.093	0.427	0.134	0.000	0.013
50	0.626	0.257	0.229	0.140	0.173	0.079	0.092	0.020	0.013	0.059	0.031	0.051	0.083	0.022	0.026	0.053	0.027	0.042
51	1.494	0.635	0.203	0.317	0.637	0.437	0.701	0.062	0.306	0.755	0.076	0.247	0.552	0.069	0.252	0.532	0.065	0.168
52	1.836	1.031	0.322	0.417	0.496	0.181	0.482	0.030	0.170	0.055	0.140	0.033	0.378	0.019	0.071	0.042	0.148	0.027
53	0.231	0.204	0.071	0.084	0.168	0.104	0.027	0.013	0.042	0.087	0.075	0.004	0.030	0.013	0.034	0.064	0.048	0.000
54	2.877	1.224	0.480	0.607	0.862	0.383	0.472	0.099	0.401	0.362	0.035	0.028	0.388	0.089	0.339	0.285	0.025	0.034
55	0.513	0.292	0.207	0.132	0.134	0.060	0.268	0.000	0.080	0.004	0.001	0.010	0.225	0.000	0.076	0.003	0.011	0.003
56	0.640	0.787	0.682	0.426	0.419	0.154	0.413	0.000	0.070	-	0.000	0.029	0.306	0.000	0.029	-	0.000	0.029
57	1.908	1.138	0.270	0.397	0.556	0.205	0.370	0.016	0.013	0.001	0.016	0.024	0.167	0.006	0.008	0.000	0.008	0.000
58	-	1.152	0.219	0.525	0.539	0.255	-	0.013	0.018	0.003	0.000	0.000	-	0.010	0.015	0.009	0.000	0.000
59	0.243	0.135	0.089	0.101	0.103	0.055	0.508	0.171	0.229	0.135	0.009	0.019	0.388	0.123	0.184	0.061	0.015	0.012
60	0.464	0.348	0.198	0.230	0.412	0.118	0.298	0.000	0.164	0.113	0.016	0.045	0.248	0.000	0.132	0.091	0.015	0.045
61	0.423	0.218	0.130	0.143	0.226	0.070	1.166	0.087	0.273	0.135	0.009	0.117	0.759	0.091	0.248	0.114	0.042	0.118
62	0.841	0.529	0.160	0.241	0.435	0.211	0.099	0.000	0.005	-	0.050	0.034	0.076	0.000	0.014	-	0.036	0.032
63	0.616	0.390	0.151	0.234	0.366	0.175	0.148	0.004	0.004	0.009	0.012	0.000	0.120	0.001	0.000	0.000	0.008	0.000
64	0.233	0.136	0.145	0.086	0.101	0.039	0.283	0.010	0.032	0.032	0.005	0.028	0.258	0.000	0.026	0.027	0.006	0.025
65	1.363	0.433	0.183	0.245	0.224	0.113	0.014	0.011	0.046	0.030	0.004	0.008	0.005	0.003	0.040	0.025	0.007	0.009
66	0.199	0.236	0.109	0.072	0.060	0.031	0.107	0.000	0.040	0.200	0.000	0.001	0.099	0.000	0.033	0.150	0.000	0.022
67	0.236	0.135	0.190	0.136	0.114	0.027	0.714	0.030	0.021	-	0.037	0.080	0.486	0.022	0.015	-	0.009	0.039
68	2.567	0.919	0.410	0.381	0.751	0.210	0.178	0.012	0.019	0.183	0.180	0.011	0.149	0.009	0.014	0.185	0.167	0.004
69	1.641	0.700	0.337	0.438	0.529	-	0.165	0.000	0.101	0.368	0.020	-	0.122	0.003	0.095	0.333	0.017	-
70	0.737	-	0.723	1.111	1.094	0.453	0.302	0.013	0.013	0.023	0.012	0.017	0.282	0.018	0.022	0.056	0.019	0.009
71	0.253	0.334	0.177	0.098	0.112	0.031	0.530	0.055	0.179	0.953	0.096	0.273	0.431	0.047	0.197	0.685	0.082	0.220
72	0.778	0.486	0.260	0.218	0.165	0.050	0.849	0.004	0.084	0.042	0.069	0.049	0.701	0.004	0.071	0.032	0.045	0.044
73	0.194	0.111	0.283	0.072	0.086	0.051	0.246	0.001	0.122	0.220	0.027	0.021	0.210	0.000	0.077	0.153	0.025	0.016
74	1.475	0.580	0.832	0.293	0.658	0.169	0.226	0.009	0.041	0.034	0.000	0.003	0.194	0.005	0.027	0.024	0.000	0.008
75	1.442	0.589	0.277	0.259	0.454	0.131	0.040	0.000	0.019	0.016	0.000	0.003	0.027	0.002	0.015	0.022	0.003	0.000
76	4.117	1.485	0.619	0.707	0.828	0.330	0.111	0.012	0.074	0.094	0.032	0.005	0.103	0.011	0.048	0.093	0.023	0.006
77	0.114	0.083	0.055	0.110	0.067		1.132	0.040	0.034	0.076	0.015	0.012	0.749	0.039	0.014	0.070	0.008	0.003
78	0.425	0.344	0.108	0.133	0.123	0.024	0.238	0.000	0.059	0.111	0.011	0.129	0.176	0.003	0.034	0.070	0.004	0.081
79	0.937	0.337	0.162	0.205	0.185	0.099	0.172	0.088	0.144	0.000	0.008	0.025	0.147	0.076	0.131	0.037	0.005	0.028
80	0.621	0.491	0.289	0.358	0.327	0.082	1.715	0.465	0.078	-	0.038	-	1.449	0.429	0.075	-	0.045	-
81	1.481	0.602	0.528	0.352	0.539	0.189	0.374	0.010	0.068	0.042	0.000	0.004	0.296	0.011	0.059	0.055	0.000	0.007
82	3.006	1.321	0.727	0.849	0.862	0.378	0.410	0.024	0.017	0.137	0.003	0.059	0.275	0.022	0.027	0.139	0.001	0.045
83	0.511	0.356	0.170	0.269	0.320	-	0.222	0.010	0.070	0.091	0.003	-	0.204	0.000	0.059	0.067	0.000	-
84	1.361	0.722	0.258	0.439	0.617	0.207	1.431	0.087	0.255	0.083	0.007	0.021	1.145	0.077	0.231	0.078	0.022	0.016
85	1.083	0.346	0.189	-	0.370	0.116	0.578	0.005	0.083	-	0.020	0.021	0.441	0.004	0.059	-	0.021	0.002

Table A8 (contd..): Immune response data pertaining to vaccinates on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
86	0.896	0.376	0.210	0.183	0.355	0.078	0.121	0.042	0.155	0.033	0.000	0.055	0.088	0.027	0.127	0.022	0.000	0.024
87	1.417	0.555	0.360	0.366	0.517	0.178	0.116	0.000	0.014	0.050	0.003	0.000	0.090	0.000	0.028	0.030	0.001	0.007
88	0.130	0.107	0.164	0.071	0.087	0.027	0.431	0.015	0.050	0.145	-	0.014	0.393	0.015	0.051	0.086	-	0.009
89	2.575	2.052	0.368	0.613	0.762	0.404	0.486	0.000	0.156	0.000	0.000	0.038	0.384	0.000	0.101	0.000	0.000	0.038
90	4.300	2.052	3.387	1.519	2.042	1.103	0.275	0.000	0.112	0.019	0.039	0.193	0.237	0.001	0.041	0.019	0.006	0.184
91	0.782	0.530	0.234	0.228	0.274	0.096	0.077	0.002	0.000	0.027	0.009	0.000	0.064	0.008	0.022	0.028	0.017	0.009
92	1.149	0.633	0.170	0.229	0.422	0.140	0.618	0.000	0.026	0.156	0.012	0.042	0.471	0.000	0.020	0.128	0.025	0.023
93	2.056	0.922	0.276	0.289	0.338	0.138	0.581	0.003	0.288	0.597	0.037	0.029	0.472	0.000	0.168	0.491	0.034	0.027
94	1.698	0.702	0.242	0.359	0.698	0.192	0.109	0.000	0.038	0.127	0.017	0.005	0.130	0.011	0.034	0.081	0.012	0.005
95	2.964	1.429	0.389	0.615	1.139	0.456	0.202	0.000	0.020	0.132	0.000	0.006	0.171	0.000	0.013	0.083	0.000	0.006
96	0.238	0.151	0.265	0.099	0.121	0.083	1.241	0.014	0.078	0.493	0.021	0.058	1.048	0.014	0.053	0.422	0.004	0.051
97	2.934	0.963	0.269	0.305	0.497	0.231	0.150	0.008	0.026	0.115	0.028	0.013	0.102	0.004	0.018	0.002	0.000	0.000
98	0.633	0.373	0.303	0.190	0.201	0.105	0.764	0.026	0.259	0.269	0.029	0.060	0.681	0.031	0.201	0.240	0.005	0.054
99	1.266	0.421	0.460	0.241	0.316	0.146	0.080	0.005	0.061	0.190	0.080	0.039	0.073	0.003	0.065	0.186	0.074	0.026
100	1.501	0.525	0.218	0.205	0.277	0.095	0.676	0.032	0.086	0.059	0.090	0.024	0.522	0.037	0.089	0.058	0.077	0.025
101	0.330	0.238	0.161	0.138	0.182	0.058	0.007	0.062	0.082	0.005	0.002	0.021	0.000	0.043	0.081	0.010	0.000	0.014
102	1.696	0.625	0.265	0.314	0.522	0.201	0.112	0.012	0.020	0.011	0.003	0.025	0.096	0.007	0.026	0.003	0.000	0.017
103	0.885	0.406	0.396	0.267	0.252	0.108	0.274	0.025	0.117	0.062	0.104	0.054	0.238	0.032	0.118	0.056	0.080	0.045
104	1.029	0.495	0.171	0.221	0.305	0.105	0.022	0.043	0.052	0.000	0.000	0.000	0.017	0.036	0.014	0.006	0.002	0.000
105	0.122	0.096	0.161	0.132	0.087	0.048	0.068	0.017	0.037	0.030	0.000	0.002	0.051	0.020	0.000	0.000	0.000	0.004
106	0.254	0.196	0.106	0.158	0.137	0.080	0.019	0.014	0.084	0.057	0.006	0.035	0.028	0.013	0.065	0.097	0.009	0.031
107	1.734	0.630	0.493	0.290	0.369	0.102	0.068	0.007	0.056	0.056	0.064	0.058	0.083	0.004	0.059	0.045	0.070	0.000
108	0.410	0.209	0.079	0.102	0.116	0.056	0.134	0.004	0.082	0.056	0.005	0.028	0.096	0.002	0.073	0.062	0.001	0.027
109	0.580	0.367	0.171	0.167	0.807	0.193	0.256	0.029	0.153	0.084	0.066	0.040	0.233	0.001	0.136	0.088	0.042	0.035
110	2.538	1.243	0.579	0.704	1.056	0.458	-	0.182	1.189	0.578	0.051	0.049	-	0.175	1.101	0.492	0.037	0.054
111	1.660	0.544	0.605	0.381	0.338	0.190	0.463	0.255	0.100	-	0.077	0.017	0.420	0.237	0.079	-	0.070	0.007
112	1.068	0.367	0.197	0.243	0.333	0.094	0.569	0.029	0.105	0.346	0.032	0.014	0.420	0.029	0.095	0.307	0.037	0.012
113	0.299	0.175	0.200	0.313	0.144	-	0.422	0.027	0.156	0.274	0.092	-	0.394	0.025	0.073	0.249	0.070	-
114	0.521	0.297	0.207	0.227	0.545	0.161	0.071	0.012	0.004	0.004	0.000	0.001	0.054	0.008	0.006	0.009	0.000	0.000
115	1.756	0.888	-	0.550	1.060	0.442	0.231	0.025	-	0.051	0.004	0.047	0.195	0.022	-	0.020	0.006	0.044
116	0.203	0.218	0.084	0.120	0.109	0.048	0.544	0.015	0.145	0.159	0.013	0.022	0.472	0.013	0.120	0.145	0.015	0.018
117	2.172	1.086	0.280	0.348	0.374	-	0.110	0.024	0.270	0.130	0.011	-	0.105	0.027	0.219	0.123	0.005	-
118	0.405	0.235	0.107	0.126	0.144	0.085	0.316	0.072	0.047	0.058	0.011	0.010	0.272	0.064	0.000	0.000	0.011	0.008
119	1.910	0.586	0.384	0.245	0.266	0.082	0.930	0.049	0.116	-	0.001	0.058	0.695	0.052	0.102	-	0.000	0.031
120	0.936	0.582	0.364	0.243	0.309	0.126	0.755	0.082	0.458	1.212	0.190	0.023	0.668	0.080	0.417	1.142	0.197	0.009
121	1.876	0.554	0.263	0.231	0.310	0.108	0.077	0.008	0.006	0.035	0.062	0.002	0.040	0.015	0.000	0.027	0.049	0.001
122	0.881	0.429	0.435	0.238	0.266	0.132	0.698	0.027	0.054	0.043	0.027	0.025	0.553	0.046	0.023	0.034	0.033	0.025
123	2.113	0.733	0.298	0.271	0.249	0.123	0.508	0.000	0.204	-	0.000	0.000	0.436	0.000	0.177	-	0.036	0.033
124	0.558	0.335	0.194	0.197	0.202	0.056	0.383	0.053	0.107	0.105	0.008	0.033	0.307	0.033	0.084	0.044	0.017	0.026
125	0.773	0.349	0.204	0.210	0.202	0.104	0.139	0.016	0.050	0.331	0.000	0.118	0.117	0.012	0.043	0.270	0.000	0.098
126	1.308	0.585	0.408	0.266	0.284	0.115	1.326	0.105	0.111	0.177	0.128	0.067	1.080	0.092	0.075	0.159	0.110	0.041
127	0.262	0.157	0.216	0.144	0.107	0.057	0.366	0.074	0.317	0.192	0.042	0.062	0.241	0.059	0.198	0.114	0.029	0.035

Table A8 (contd..): Immune response data pertaining to vaccinates on property 1

Animal	Antibody OD values at months post-vaccination						IFN-γ (Johnin-nil) OD values at months post-vaccination						IFN-γ (Johnin-Avian) OD values at months post-vaccination					
	12	18	24	30	42	54	12	18	24	30	42	54	12	18	24	30	42	54
128	0.836	0.308	0.155	0.137	0.160	0.050	0.058	0.012	0.021	-	0.000	0.016	0.043	0.013	0.013	-	0.005	0.045
129	3.030	1.019	0.931	0.627	0.918	0.362	0.149	0.025	0.032	0.015	0.037	0.004	0.070	0.021	0.021	0.018	0.033	0.007
130	0.300	0.156	0.160	0.131	0.128	-	0.447	0.017	0.089	0.018	0.000	-	0.408	0.002	0.085	0.017	0.000	-
131	1.720	0.475	0.757	0.418	0.365	0.165	0.339	0.000	0.131	0.036	0.130	0.017	0.241	0.023	0.104	0.034	0.113	0.020
132	1.388	0.477	0.463	0.272	0.493	0.121	0.114	0.010	0.046	0.450	0.008	0.017	0.088	0.007	0.027	0.198	0.013	0.013
133	0.633	0.336	0.141	0.182	0.205	0.093	0.596	0.004	0.063	0.021	0.024	0.040	0.479	0.001	0.041	0.020	0.014	0.034
134	0.222	0.141	0.066	0.088	0.071	0.030	0.195	0.000	0.101	0.113	0.023	0.010	0.162	0.000	0.094	0.083	0.025	0.009
135	0.104	0.077	0.110	0.071	0.071	0.022	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.000	0.000	0.003	0.018	0.000
136	0.710	0.135	0.105	0.109	0.149	0.068	0.129	0.086	0.012	0.361	0.059	0.088	0.117	0.066	0.010	0.321	0.054	0.086
137	0.538	0.287	0.121	0.224	0.271	-	0.287	0.012	0.134	0.123	0.024	-	0.185	0.007	0.065	0.104	0.017	-
138	2.633	1.377	0.463	0.553	0.626	0.307	0.263	0.029	0.197	0.296	0.022	0.034	0.202	0.033	0.159	0.257	0.022	0.041
139	0.840	0.418	0.174	0.225	0.334	0.105	0.846	0.001	0.063	0.032	0.010	0.001	0.696	0.002	0.056	0.024	0.007	0.003
140	4.016	1.450	0.516	0.693	0.653	0.415	0.350	0.010	0.101	0.207	0.011	0.043	0.306	0.006	0.050	0.165	0.011	0.036
141	1.098	0.309	0.380	0.286	0.220	0.158	0.083	0.000	0.057	0.066	0.017	0.032	0.076	0.000	0.040	0.054	0.007	0.031
142	0.663	0.379	0.255	0.169	0.279	0.121	0.349	0.062	0.092	0.101	0.006	0.061	0.289	0.064	0.040	0.084	0.008	0.054
143	0.160	0.139	0.181	0.091	0.071	0.033	1.252	0.411	0.407	0.729	0.037	0.097	0.968	0.379	0.382	0.630	0.035	0.076
144	0.249	0.130	0.127	0.148	0.103	0.058	2.132	0.107	0.230	0.364	0.026	0.083	1.534	0.103	0.165	0.307	0.030	0.078
145	1.339	0.703	0.448	0.249	0.451	0.146	0.267	0.002	0.226	0.055	0.016	0.058	0.250	0.014	0.207	0.042	0.016	0.046
146	1.726	0.757	0.350	0.393	0.452	0.137	0.212	0.058	0.498	0.191	0.030	-	0.192	0.055	0.460	0.172	0.031	-
147	0.293	0.153	0.149	0.135	0.121	0.044	0.286	0.047	0.083	0.121	0.008	0.012	0.240	0.054	0.027	0.089	0.014	0.015
148	1.788	0.780	0.288	0.289	0.458	0.223	0.472	-	0.398	0.420	0.099	0.040	0.337	-	0.211	0.326	0.077	0.028
149	1.533	0.922	0.536	0.260	0.281	0.085	0.072	0.025	0.042	0.073	0.050	0.017	0.056	0.019	0.044	0.072	0.044	0.013
150	0.986	0.439	0.188	0.242	0.364	0.077	0.544	0.051	0.154	-	0.013	0.012	0.391	0.046	0.131	-	0.014	0.005
151	0.660	0.530	0.178	0.306	0.329	0.156	0.441	0.039	0.237	0.102	0.000	0.007	0.363	0.031	0.219	0.100	0.008	0.006
152	1.902	0.140	0.624	0.477	0.476	0.236	0.501	0.036	0.222	0.283	0.014	0.038	0.431	0.052	0.167	0.225	0.026	0.031
153	0.992	0.439	0.191	0.256	0.346	0.124	1.051	0.000	0.058	0.074	0.000	0.031	0.657	0.000	0.057	0.056	0.005	0.029
154	0.069	0.078	0.050	0.070	0.062	0.021	0.000	0.000	0.013	0.008	0.014	0.023	0.000	0.000	0.014	0.006	0.005	0.014
155	3.111	1.804	0.666	1.002	1.538	-	1.399	0.756	0.369	0.724	0.326	-	1.258	0.732	0.298	0.732	0.285	-
156	0.356	0.352	0.105	0.197	0.160	0.069	0.104	0.007	0.890	0.698	0.000	0.030	0.102	0.005	0.836	0.684	0.072	0.026
157	1.110	0.429	0.173	0.241	0.304	0.102	1.295	0.000	0.096	0.136	0.017	0.204	0.893	0.004	0.035	0.082	0.014	0.188
158	1.273	0.511	0.558	0.224	0.370	0.078	0.431	0.000	0.038	0.138	0.072	0.016	0.351	0.003	0.033	0.127	0.057	0.019
159	0.322	0.204	0.093	0.176	0.280	0.106	0.000	0.018	0.003	0.049	0.005	0.031	0.028	0.010	0.002	0.031	0.000	0.000
160	0.489	0.275	0.207	0.206	0.180	0.075	0.270	0.007	0.097	0.257	0.013	0.073	0.221	0.005	0.076	0.258	0.000	0.066
161	0.302	0.179	0.130	0.085	0.110	0.033	1.005	0.066	0.744	0.681	0.025	0.165	0.765	0.061	0.683	0.649	0.035	0.149
162	2.040	0.602	0.194	0.228	0.410	-	0.746	0.007	0.522	1.396	0.041	-	0.726	0.000	0.483	1.255	0.032	-
163	1.512	0.658	0.263	0.295	0.943	0.461	1.218	0.006	0.315	0.373	0.035	0.036	0.941	0.001	0.304	0.329	0.038	0.044
164	0.623	0.405	0.138	-	0.208	-	0.643	0.141	0.163	-	0.000	-	0.472	0.127	0.132	-	0.007	-
165	2.633	1.008	0.531	0.494	0.932	0.330	0.328	0.000	0.058	0.046	0.020	0.012	0.236	0.000	0.053	0.044	0.019	0.010
166	1.474	1.365	0.260	0.395	0.413	0.123	0.147	0.001	0.031	0.099	0.041	0.006	0.137	0.000	0.030	0.062	0.052	0.003
167	1.132	0.559	0.265	0.294	0.409	0.191	0.237	0.007	0.063	0.129	0.006	0.008	0.207	0.019	0.023	0.110	0.000	0.010
168	1.045	0.389	0.417	0.213	0.401	0.111	0.367	0.006	0.289	0.119	0.391	0.081	0.271	0.009	0.275	0.081	0.342	0.074
169	2.421	1.616	0.506	0.420	1.058	-	0.585	0.012	0.252	0.660	0.006	-	0.479	0.009	0.209	0.665	0.000	-
170	0.787	0.611	0.326	0.258	0.258	0.107	0.111	0.014	0.049	-	0.015	0.022	0.054	0.013	0.027	-	0.015	0.022
171	1.174	0.458	0.278	0.196	0.289	0.148	0.228	0.036	0.042	0.172	0.000	0.015	0.206	0.028	0.037	0.105	0.000	0.007

Table A9: Immune responses data pertaining to controls on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
1	0.072	0.067	0.056	0.053	0.113	0.076	0.107	0.048	0.000	0.004	0.000	0.003	-	0.031	-	0.007	0.000	0.003	0.000	0.000	-	0.012	-	0.008
2	0.071	0.051	0.093	0.073	0.157	0.110	0.097	0.045	0.001	0.003	0.009	0.011	-	0.029	0.120	0.000	0.001	0.004	0.007	0.006	-	0.018	0.062	0.001
3	0.174	0.075	0.040	0.079	0.061	0.084	0.068	0.025	0.002	0.000	0.004	0.000	0.999	0.385	0.281	0.035	0.000	0.000	0.004	0.014	0.604	0.141	0.000	0.031
4	0.097	0.055	0.039	0.066	0.114	0.082	0.079	0.038	0.000	0.000	0.000	0.015	-	0.112	0.019	0.136	0.000	0.000	0.000	0.012	-	0.077	0.003	0.017
5	0.068	0.085	0.042	0.077	0.055	0.071	0.061	0.080	0.010	0.000	0.000	0.002	0.014	0.000	0.007	0.000	0.006	0.001	0.000	0.000	0.005	0.002	0.000	0.000
6	0.053	0.074	0.049	0.075	0.092	-	0.073	0.094	0.005	0.001	0.003	0.000	0.084	0.016	0.000	0.000	0.003	0.002	0.007	0.000	0.063	0.022	0.004	0.000
7	0.135	0.075	0.055	0.071	0.066	0.102	0.073	0.049	0.002	0.000	0.000	0.000	0.020	0.000	0.072	0.005	0.000	0.006	0.009	0.000	0.000	0.000	0.069	0.000
8	0.060	0.057	0.038	0.053	0.141	0.072	0.077	0.058	0.002	0.000	0.023	0.003	0.002	0.000	0.049	0.026	0.002	0.005	0.023	0.000	0.004	0.000	0.014	0.017
9	0.064	0.238	0.043	0.064	0.055	0.065	0.116	0.039	0.000	0.000	0.004	0.001	0.024	0.000	-	-	0.000	0.003	0.016	0.003	0.011	0.000	-	-
10	0.061	0.064	0.039	0.057	0.058	0.070	0.134	-	0.002	0.003	0.000	0.012	0.027	0.024	0.091	-	0.000	0.003	0.000	0.010	0.014	0.014	0.024	-
11	0.063	0.054	0.039	0.053	0.056	0.102	0.309	-	0.000	0.000	0.000	0.001	0.033	0.035	0.000	-	0.001	0.002	0.002	0.005	0.014	0.027	0.012	-
12	0.079	0.062	0.044	0.053	0.057	0.054	0.068	0.040	0.000	0.003	0.000	0.000	0.130	0.045	0.000	0.000	0.000	0.005	0.001	0.000	0.125	0.027	0.017	0.000
13	0.067	0.128	0.064	0.081	0.065	0.071	0.077	0.069	0.015	0.000	0.017	0.032	0.020	0.006	0.000	0.000	0.006	0.001	0.017	0.002	0.000	0.011	0.015	0.000
14	0.082	0.050	0.039	0.049	0.046	0.063	0.062	0.030	0.000	0.000	0.000	0.000	0.064	0.040	0.000	0.009	0.003	0.000	0.001	0.000	0.044	0.014	0.036	0.006
15	0.084	0.090	0.059	0.118	0.084	0.074	0.068	0.043	0.005	0.007	0.001	0.000	0.053	0.008	0.009	0.000	0.007	0.006	0.002	0.000	0.052	0.004	0.000	0.000
16	0.091	0.071	0.048	0.059	0.068	0.061	0.080	0.037	0.000	0.000	0.018	0.000	0.047	0.023	0.000	0.011	0.000	0.006	0.008	0.001	0.026	0.017	0.000	0.000
17	0.077	0.062	0.040	0.072	0.053	0.063	0.078	0.120	0.003	0.001	0.002	0.002	0.004	0.029	0.113	0.019	0.004	0.000	0.004	0.008	0.000	0.017	0.119	0.017
18	0.053	0.053	0.040	0.060	0.051	0.064	0.059	0.064	0.000	0.007	0.000	0.007	0.107	0.052	0.022	0.021	0.000	0.004	0.000	0.004	0.075	0.049	0.000	0.008
19	0.059	0.063	0.045	0.104	0.192	0.198	0.151	0.078	0.007	0.000	0.000	0.000	0.218	0.057	0.165	0.008	0.000	0.006	0.003	0.000	0.152	0.021	0.054	0.010
20	0.064	0.056	0.047	0.056	0.068	0.089	0.152	-	0.006	0.000	0.000	0.008	0.205	0.253	0.092	-	0.006	0.004	0.000	0.008	0.089	0.210	0.076	-
21	0.191	0.148	0.046	0.136	0.102	0.126	0.115	0.061	0.000	0.016	0.216	0.007	0.195	0.082	-	0.011	0.004	0.009	0.141	0.013	0.143	0.069	-	0.091
22	0.089	0.070	0.046	0.066	0.057	0.086	0.053	0.023	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.012	0.012	0.011	0.000
23	0.063	0.065	0.041	0.058	0.144	0.188	0.151	0.130	0.027	0.007	0.000	0.003	-	0.037	0.029	0.051	0.028	0.011	0.000	0.000	-	0.027	0.012	0.037
24	0.064	0.062	0.053	0.066	0.057	0.067	0.081	0.026	0.000	0.000	0.007	0.000	0.022	0.003	0.000	0.004	0.000	0.010	0.005	0.000	0.009	0.000	0.000	0.003
25	0.059	0.067	0.039	0.242	0.169	0.312	0.376	0.121	0.009	0.000	0.007	0.000	0.364	0.103	0.017	0.000	0.007	0.006	0.002	0.000	0.210	0.088	0.050	0.004
26	0.057	0.052	0.052	0.061	0.055	0.065	0.068	0.051	0.002	0.000	0.020	0.000	0.067	0.020	0.015	0.009	0.005	0.000	0.017	0.012	0.043	0.021	0.021	0.006
27	0.138	0.067	0.057	0.070	0.055	0.106	0.090	0.064	0.001	0.000	0.004	0.011	0.141	0.098	0.000	0.000	0.000	0.000	0.002	0.007	0.070	0.050	0.017	0.000
28	0.065	0.072	0.044	0.068	0.078	0.077	0.085	0.152	0.007	0.003	0.001	0.000	-	-	0.024	0.018	0.008	0.004	0.000	0.000	-	-	0.018	0.008
29	0.064	0.054	0.044	0.064	-	0.063	0.096	0.020	0.003	0.000	0.000	0.000	-	0.114	0.018	0.007	0.003	0.002	0.000	0.000	-	0.077	0.019	0.004
30	0.126	0.075	0.045	0.066	0.063	0.071	0.088	0.090	0.001	0.012	0.001	0.003	0.191	0.317	0.212	0.149	0.008	0.000	0.001	0.003	0.123	0.189	0.205	0.080
31	0.056	0.096	0.055	0.079	0.079	0.073	0.075	-	0.000	0.009	0.006	0.004	0.711	0.221	0.096	-	0.002	0.003	0.002	0.003	0.219	0.127	0.035	-
32	0.090	0.069	0.040	0.071	0.057	0.066	0.060	0.055	0.007	0.000	0.027	0.011	0.001	0.025	0.026	0.013	0.010	0.002	0.023	0.008	0.018	0.024	0.014	0.007
33	0.108	0.079	0.052	0.067	0.108	0.058	0.163	-	0.000	0.008	0.005	0.000	0.027	0.007	-	-	0.005	0.001	0.000	0.000	0.000	0.011	-	-
34	0.117	0.059	0.066	0.117	0.101	0.119	0.142	0.095	0.006	0.000	0.000	0.000	0.173	0.036	0.046	0.086	0.004	0.001	0.000	0.000	0.119	0.017	0.014	0.065
35	0.053	0.066	0.104	0.051	0.139	0.107	0.127	0.038	0.000	0.000	0.015	0.002	0.053	0.008	0.006	0.000	0.002	0.007	0.007	0.001	0.029	0.010	0.000	0.001
36	0.072	0.051	0.045	0.078	0.088	0.067	0.074	0.069	0.005	0.226	0.022	0.018	0.061	0.040	0.024	0.018	0.000	0.210	0.007	0.006	0.039	0.025	0.025	0.005
37	0.063	0.049	0.041	0.056	0.087	0.100	0.117	0.088	0.000	0.000	0.000	0.000	0.946	1.056	0.375	0.358	0.002	0.000	0.002	0.000	0.692	0.676	0.244	0.166
38	0.064	0.057	0.085	0.061	0.077	0.113	0.099	0.051	0.006	0.000	0.027	0.000	0.481	0.322	0.476	0.052	0.008	0.000	0.027	0.000	0.145	0.177	0.298	0.037
39	0.096	0.087	0.089	0.132	0.141	0.124	0.110	0.062	0.001	0.000	0.000	0.000	-	0.098	0.000	0.017	0.002	0.000	0.000	0.000	-	0.058	0.035	0.014
40	0.065	0.096	0.046	0.072	0.060	0.075	0.306	0.153	0.000	0.007	0.000	0.006	0.260	0.040	0.000	0.005	0.000	0.005	0.000	0.000	0.162	0.054	0.000	0.003
41	0.063	0.067	0.052	0.133	0.068	0.062	0.069	0.065	0.000	0.000	0.005	0.000	0.043	0.026	0.000	0.014	0.000	0.000	0.006	0.000	0.029	0.025	0.005	0.007
42	0.061	0.079	0.060	0.066	0.054	0.059	0.077	0.050	0.006	0.017	0.047	0.000	0.054	0.095	0.081	0.066	0.003	0.016	0.021	0.007	0.031	0.066	0.032	0.018

Table A9 (contd..): Immune response data pertaining to controls on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
43	0.062	0.054	0.052	0.072	0.071	0.078	0.093	0.067	0.002	0.000	0.058	0.017	0.097	0.000	0.000	0.040	0.004	0.007	0.025	0.010	0.026	0.010	0.028	0.019
44	0.061	0.066	0.043	0.070	0.062	0.083	0.147	-	0.003	0.000	0.022	0.000	0.062	-	0.050	-	0.000	0.008	0.018	0.000	0.029	-	0.021	-
45	0.074	0.088	0.044	0.054	0.053	0.068	0.058	0.166	0.000	-	0.000	0.004	0.007	0.037	0.011	0.009	0.002	-	0.005	0.012	0.000	0.017	0.022	0.000
46	0.188	0.091	0.102	0.189	0.170	0.111	0.125	0.053	0.000	0.032	0.000	0.000	0.088	0.000	0.050	0.021	0.000	0.032	0.006	0.000	0.064	0.000	0.028	0.013
47	0.103	0.058	0.049	0.089	0.068	0.093	0.098	0.050	0.000	0.000	0.013	0.000	0.026	0.023	0.002	0.032	0.000	0.000	0.004	0.005	0.019	0.005	0.022	0.019
48	0.099	0.074	0.047	0.090	0.079	0.081	0.082	0.075	0.019	0.000	0.034	0.000	-	-	0.065	-	0.006	0.000	0.011	0.000	-	-	0.017	-
49	0.050	0.054	0.047	0.072	0.065	0.092	0.105	0.074	0.000	0.001	0.007	0.004	0.023	-	-	0.029	0.000	0.000	0.005	0.002	0.006	-	0.216	0.019
50	0.050	0.075	0.043	0.080	0.078	0.090	0.122	0.084	0.000	0.000	0.007	0.003	0.135	0.065	0.001	0.000	0.001	0.019	0.000	0.005	0.100	0.035	0.014	0.000
51	0.055	0.060	0.040	0.057	0.056	0.064	0.062	0.068	0.006	0.003	0.000	0.004	0.073	0.016	0.000	0.011	0.000	0.000	0.007	0.006	0.043	0.017	0.008	0.008
52	0.111	0.080	0.073	0.057	0.077	0.068	0.062	0.067	0.001	0.000	0.001	0.000	0.061	0.029	0.000	0.000	0.000	0.000	0.000	0.003	0.036	0.041	0.000	0.006
53	0.055	0.085	0.046	0.052	0.066	0.103	0.101	0.085	0.032	0.000	0.002	0.000	0.134	0.057	0.014	0.052	0.029	0.005	0.000	0.000	0.111	0.035	0.011	0.016
54	0.125	0.083	0.059	0.103	-	-	-	-	0.000	0.036	0.004	0.000	-	-	-	-	0.000	0.029	0.001	0.002	-	-	-	-
55	0.049	0.066	0.042	0.059	0.051	0.089	0.136	-	0.012	0.008	0.000	0.001	0.554	0.825	0.155	-	0.000	0.000	0.000	0.000	0.248	0.371	0.086	-
56	0.088	0.068	0.107	0.047	0.099	0.117	0.122	0.067	0.000	0.018	0.144	0.026	0.940	-	0.078	0.149	0.001	0.016	0.079	0.033	0.674	-	0.034	0.087
57	0.050	0.068	0.045	0.062	0.081	0.076	0.101	0.089	0.023	0.005	0.015	0.006	0.090	0.063	-	0.033	0.015	0.000	0.011	0.004	0.059	0.034	-	0.019
58	0.063	0.057	0.046	0.095	0.409	0.224	0.215	0.045	0.014	0.003	0.007	0.005	-	-	0.000	0.012	0.005	0.000	0.010	0.003	-	-	0.012	0.003
59	0.055	0.061	0.045	0.087	0.095	0.070	0.082	0.068	0.000	0.000	0.000	0.000	0.166	0.093	0.024	0.031	0.007	0.000	0.001	0.004	0.120	0.101	0.004	0.006
60	-	0.067	0.045	0.063	0.055	0.058	0.066	0.035	-	0.008	0.015	0.003	0.081	0.000	0.034	0.000	-	0.011	0.018	0.002	0.053	0.000	0.032	0.000
61	0.065	0.064	0.098	0.175	0.160	0.286	0.314	0.096	0.005	0.000	0.003	0.006	-	0.168	0.070	0.019	0.000	0.000	0.006	0.005	-	0.040	0.000	0.000
62	0.063	0.060	0.041	0.073	0.050	0.109	0.306	0.193	0.000	0.017	0.004	0.023	0.054	0.063	0.016	0.001	0.005	0.018	0.000	0.018	0.031	0.020	0.000	0.000
63	0.101	0.076	0.051	0.367	0.226	0.213	0.175	0.099	0.010	0.000	0.007	0.000	0.054	-	0.012	0.016	0.000	0.001	0.004	0.005	0.032	-	0.010	0.003
64	0.094	0.069	0.041	0.082	0.165	0.333	0.342	0.183	0.000	0.009	0.007	0.014	0.091	0.122	0.000	0.042	0.003	0.001	0.003	0.014	0.060	0.053	0.011	0.005
65	0.090	0.077	0.047	0.066	0.069	0.061	0.068	-	0.040	0.006	0.011	0.000	0.082	0.009	0.000	-	0.032	0.007	0.010	0.001	0.070	0.007	0.008	-
66	0.094	0.102	0.058	0.073	0.053	0.067	0.074	0.067	0.000	0.000	0.000	0.000	0.019	0.183	0.000	0.013	0.000	0.002	0.000	0.000	0.020	0.139	0.004	0.015
67	0.056	0.058	-	0.054	0.055	0.080	0.066	0.053	0.007	0.005	0.105	0.000	0.109	0.180	0.021	0.079	0.008	0.006	0.092	0.013	0.075	0.118	0.047	0.052
68	0.108	0.063	0.041	0.059	0.092	0.150	0.108	0.089	0.000	0.001	0.003	0.000	0.000	0.109	0.011	0.022	0.000	0.003	0.005	0.003	0.000	0.076	0.018	0.016
69	0.062	0.081	0.135	0.095	0.094	0.208	0.204	0.058	0.000	0.000	0.000	0.004	0.509	0.110	0.269	0.120	0.003	0.002	0.000	0.011	0.152	0.060	0.172	0.081
70	0.094	0.074	0.041	0.066	0.052	0.065	0.066	0.079	0.000	0.000	0.044	0.009	0.449	0.114	0.185	0.093	0.001	0.004	0.020	0.004	0.180	0.075	0.126	0.059
71	0.052	0.078	0.046	0.054	0.093	0.122	0.159	0.105	0.003	0.000	0.021	0.000	0.127	0.246	0.186	0.010	0.005	0.000	0.015	0.000	0.099	0.167	0.099	0.005
72	0.071	0.212	0.056	0.082	0.079	0.074	0.064	0.065	0.016	0.022	0.030	0.000	0.224	0.346	0.000	0.014	0.026	0.012	0.024	0.000	0.151	0.215	0.011	0.005
73	0.071	0.057	0.048	0.083	0.073	0.086	0.102	0.051	0.004	0.000	0.000	0.002	0.422	0.104	0.123	0.036	0.008	0.003	0.000	0.001	0.215	0.055	0.093	0.019
74	0.119	0.063	0.040	0.075	0.057	0.072	0.193	-	0.053	0.002	0.000	0.000	0.066	0.019	0.157	-	0.033	0.002	0.005	0.002	0.046	0.025	0.075	-
75	0.054	0.069	0.054	0.065	0.055	0.062	0.069	0.040	0.003	0.018	0.000	0.000	0.024	0.009	0.024	-	0.000	0.017	0.002	0.005	0.012	0.011	0.027	-
76	0.049	0.082	0.055	0.065	0.096	0.072	0.070	0.038	0.023	0.000	0.007	0.011	0.067	0.000	-	0.002	0.008	0.000	0.000	0.007	0.066	0.000	-	0.000
77	0.050	0.062	0.041	0.057	0.051	0.118	0.126	0.123	0.000	0.000	0.000	0.000	0.091	-	0.089	0.018	0.000	0.000	0.002	0.005	0.058	-	0.005	0.000
78	0.163	0.063	0.044	0.082	0.050	0.066	0.064	0.071	0.020	0.005	0.175	0.002	-	0.084	0.015	0.081	0.030	0.000	0.125	0.009	-	0.064	0.038	0.070
79	0.050	0.075	0.057	0.064	0.081	0.068	0.081	0.065	0.006	0.017	0.000	0.000	0.046	0.000	0.015	0.000	0.000	0.010	0.001	0.000	0.013	0.000	0.005	0.005
80	0.048	0.059	0.048	0.061	0.069	0.072	0.065	0.065	0.003	0.000	0.002	0.000	0.092	0.031	0.000	0.019	0.015	0.000	0.002	0.001	0.058	0.032	0.008	0.012
81	0.050	0.092	0.041	0.063	0.056	0.062	0.121	0.060	0.000	0.006	0.000	0.000	0.013	0.029	-	-	0.000	0.001	0.000	0.003	0.000	0.025	-	-
82	0.074	0.084	0.040	0.072	0.102	0.076	0.122	-	0.009	0.000	0.002	0.000	0.147	0.074	0.000	-	0.000	0.000	0.000	0.001	0.100	0.060	0.018	-
83	0.055	0.061	0.050	0.054	0.076	0.067	0.082	0.073	0.008	0.000	0.003	0.026	0.053	0.033	0.021	0.000	0.006	0.001	0.001	0.023	0.017	0.020	0.016	0.000
84	0.056	0.059	0.053	0.054	0.051	0.154	0.075	0.065	0.050	0.003	0.000	0.041	-	0.163	0.154	0.201	0.047	0.000	0.000	0.048	-	0.078	0.023	0.062

Table A9 (contd..): Immune response data pertaining to controls on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
85	0.055	0.066	0.048	0.059	0.057	0.076	0.068	0.065	0.027	0.007	0.002	0.000	0.042	-	0.063	0.026	0.000	0.000	0.000	0.000	0.021	-	0.015	0.009
86	0.061	0.075	0.044	0.048	0.084	0.101	0.094	0.063	0.019	0.000	0.000	0.001	0.142	0.183	0.060	0.057	0.024	0.014	0.000	0.000	0.056	0.130	0.000	0.015
87	0.060	0.076	0.054	0.071	0.077	0.059	0.060	0.016	0.000	0.005	0.008	0.007	0.016	0.021	0.048	0.000	0.004	0.005	0.000	0.004	0.013	0.000	0.034	0.000
88	0.106	0.105	0.040	0.093	0.109	0.282	0.448	0.328	0.029	0.002	0.000	0.000	-	0.022	0.000	0.005	0.018	0.000	0.000	0.001	-	0.019	0.000	0.000
89	0.080	0.073	0.055	0.084	0.096	0.070	0.069	0.057	0.000	0.006	0.000	0.000	0.017	0.006	0.006	0.024	0.003	0.003	0.000	0.001	0.016	0.008	0.014	0.031
90	0.049	0.091	0.152	0.071	0.064	0.067	0.109	0.071	0.007	0.000	0.000	0.000	0.008	0.035	0.132	0.063	0.005	0.004	0.000	0.009	0.002	0.030	0.085	0.000
91	0.052	0.059	0.077	0.074	0.086	0.273	0.290	0.167	0.005	0.009	0.103	0.028	0.267	0.032	0.035	0.008	0.021	0.006	0.047	0.028	0.141	0.015	0.008	0.000
92	0.076	0.060	0.044	0.086	0.049	0.080	0.081	0.047	0.013	0.000	0.037	0.000	-	0.068	-	0.050	0.012	0.002	0.013	0.000	-	0.000	-	0.020
93	0.059	0.062	0.054	0.055	0.084	0.106	0.101	0.045	0.000	0.003	0.016	0.000	-	-	0.064	0.007	0.000	0.002	0.005	0.000	-	-	0.031	0.004
94	0.103	0.070	0.054	0.063	0.081	0.085	0.134	0.176	0.000	0.026	0.004	0.000	0.008	0.000	0.019	0.005	0.000	0.019	0.005	0.000	0.013	0.000	0.002	0.008
95	0.087	0.071	0.043	0.057	0.053	0.063	0.057	0.051	0.000	0.004	0.010	0.003	0.056	0.000	0.057	0.011	0.000	0.000	0.000	0.000	0.018	0.000	0.027	0.009
96	0.067	0.073	0.063	0.073	0.065	0.073	0.078	0.036	0.000	0.000	0.003	0.005	0.001	0.029	0.000	0.003	0.000	0.001	0.000	0.002	0.000	0.000	0.000	0.000
97	0.065	0.096	0.039	0.090	0.067	0.065	0.065	0.056	0.006	0.011	0.000	0.000	0.177	0.031	0.035	0.043	0.007	0.011	0.000	0.000	0.145	0.029	0.011	0.011
98	0.048	0.080	0.044	0.233	0.150	0.182	0.383	-	0.035	0.015	0.000	0.003	0.027	0.090	0.000	-	0.028	0.014	0.000	0.000	0.017	0.030	0.000	-
99	0.061	0.067	0.062	0.081	0.069	0.089	0.088	0.073	0.000	0.001	0.000	0.000	0.181	0.161	0.020	0.046	0.014	0.001	0.003	0.000	0.108	0.121	0.013	0.036
100	0.067	0.055	0.041	0.057	0.053	0.071	0.088	0.079	0.003	0.008	0.004	0.000	0.001	0.043	0.004	0.027	0.003	0.010	0.006	0.000	0.011	0.034	0.007	0.014
101	0.056	0.074	0.050	0.064	0.058	0.097	0.087	0.020	0.000	0.008	0.023	0.000	0.359	0.303	0.022	0.098	0.015	0.022	0.029	0.003	0.263	0.148	0.000	0.083
102	0.050	0.067	0.041	0.082	0.063	0.071	0.067	0.023	0.044	0.000	0.008	0.000	0.008	0.119	0.027	0.000	0.035	0.000	0.005	0.002	0.005	0.095	0.000	0.000
103	0.055	0.071	0.046	0.061	0.071	0.121	0.101	0.027	0.009	0.000	0.000	0.007	0.071	0.085	-	0.057	0.006	0.000	0.000	0.013	0.065	0.053	-	0.059
104	0.053	0.064	0.042	0.078	0.071	0.080	0.087	0.060	0.000	0.000	0.000	0.000	-	0.109	0.010	0.089	0.008	0.000	0.000	0.002	-	0.031	0.003	0.024
105	0.169	0.144	0.075	0.138	0.115	0.113	0.141	0.062	0.000	0.000	0.003	0.000	0.511	0.174	-	0.246	0.001	0.001	0.000	0.000	0.262	0.108	-	0.070
106	0.181	0.068	0.041	0.072	0.108	0.068	0.067	0.062	0.004	0.000	0.015	0.000	0.015	0.000	0.025	0.037	0.000	0.002	0.013	0.000	0.021	0.000	0.016	0.041
107	0.378	0.117	0.052	0.072	0.088	0.078	0.072	0.044	0.000	0.006	0.004	0.000	0.027	0.021	0.097	0.059	0.003	0.022	0.003	0.000	0.015	0.030	0.019	0.035
108	0.048	0.064	0.134	0.052	0.069	0.106	0.121	0.063	0.011	0.007	0.006	0.000	0.216	0.279	0.073	0.011	0.015	0.003	0.012	0.000	0.143	0.210	0.084	0.000
109	0.050	0.062	0.043	0.057	0.057	0.184	0.198	0.081	0.003	0.000	0.034	0.000	0.771	0.891	0.252	0.011	0.010	0.004	0.020	0.002	0.606	0.514	0.137	0.001
110	0.167	0.094	0.056	0.097	0.173	0.180	0.152	0.068	0.000	0.002	0.016	0.040	-	0.121	0.147	0.259	0.000	0.001	0.000	0.032	-	0.084	0.082	0.200
111	0.063	0.076	0.041	0.066	0.177	0.246	0.257	-	0.002	0.000	0.003	0.013	0.272	-	0.041	-	0.004	0.007	0.004	0.009	0.174	-	0.000	-
112	0.111	0.052	0.043	0.069	0.085	0.102	0.114	0.058	0.002	0.000	0.000	0.016	-	-	-	-	0.004	0.000	0.005	0.028	-	-	-	-
113	0.057	0.055	0.054	0.058	0.067	0.176	0.409	-	0.017	0.000	0.048	0.006	0.771	-	0.715	-	0.016	0.007	0.041	0.000	0.358	-	0.212	-
114	0.353	0.065	0.047	0.056	0.078	0.074	0.092	0.066	0.046	0.004	0.018	0.001	-	0.015	0.000	0.001	0.056	0.006	0.012	0.000	-	0.010	0.015	0.003
115	0.174	0.083	0.054	0.090	0.060	0.061	0.110	0.033	0.000	0.000	0.000	0.007	0.033	0.154	-	0.122	0.000	0.000	0.000	0.014	0.021	0.098	-	0.111
116	0.328	0.085	0.042	0.103	0.091	0.074	0.095	0.040	0.000	0.000	0.000	0.001	0.045	0.101	0.080	0.081	0.000	0.000	0.000	0.000	0.014	0.052	0.043	0.072
117	0.062	0.070	0.063	0.096	0.085	0.136	0.111	0.066	0.000	0.000	0.000	0.000	0.165	-	0.028	0.008	0.000	0.000	0.000	0.011	0.110	-	0.010	0.007
118	0.092	0.067	0.043	0.068	0.068	0.071	0.079	0.082	0.000	0.021	0.006	0.000	0.218	-	0.148	0.101	0.003	0.002	0.000	0.005	0.191	-	0.071	0.031
119	-	0.420	0.046	0.061	0.181	0.277	0.260	0.139	0.000	0.000	0.000	0.003	0.179	0.068	0.000	0.043	0.000	0.023	0.000	0.008	0.073	0.033	0.025	0.023
120	0.675	0.072	0.039	0.064	0.082	0.074	0.110	0.104	0.003	0.000	0.000	0.000	0.099	0.026	0.000	0.000	0.000	0.000	0.001	0.000	0.094	0.014	0.022	0.001
121	0.155	0.101	0.052	0.063	0.052	0.067	0.068	0.055	0.007	0.001	0.000	0.007	0.017	0.000	0.013	0.019	0.012	0.006	0.000	0.001	0.018	0.008	0.021	0.019
122	0.175	0.091	0.042	0.065	0.071	0.057	0.078	0.056	0.027	0.000	0.054	0.008	0.097	0.014	0.000	0.028	0.028	0.005	0.035	0.004	0.052	0.021	0.005	0.017

Table A9 (contd..): Immune response data pertaining to controls on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
123	0.046	0.068	0.077	0.080	0.072	0.064	0.066	0.056	0.005	0.000	0.000	0.006	0.050	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.004	0.008	0.020
124	0.636	0.143	0.040	0.164	0.107	0.128	0.196	0.153	0.007	0.009	0.042	0.006	0.730	0.184	-	0.119	0.005	0.001	0.019	0.000	0.246	0.101	-	0.066
125	0.477	0.134	0.143	0.159	0.152	0.196	0.208	-	0.012	0.156	0.174	0.013	-	0.250	0.111	-	0.004	0.150	0.116	0.008	-	0.130	0.000	-
126	0.208	0.054	0.064	0.097	0.049	0.065	0.068	0.051	0.001	0.000	0.017	0.005	0.041	-	0.000	0.015	0.004	0.000	0.014	0.000	0.024	-	0.005	0.014
127	0.406	0.087	0.056	0.070	0.091	0.126	0.118	0.079	0.001	0.000	0.004	0.000	0.096	0.139	-	0.034	0.003	0.000	0.000	0.000	0.077	0.087	-	0.031
128	0.052	0.064	0.041	0.167	0.084	0.073	0.075	0.053	0.000	0.006	0.000	0.002	0.050	0.022	0.054	0.001	0.002	0.005	0.000	0.001	0.018	0.029	0.009	0.005
129	0.060	0.056	0.046	0.057	0.050	0.059	0.063	0.057	0.001	0.000	0.001	0.000	0.089	0.107	0.000	0.072	0.002	0.000	0.000	0.005	0.057	0.058	0.006	0.036
130	0.101	0.060	0.043	0.065	0.053	0.064	0.068	0.064	0.006	0.000	0.003	0.000	0.019	0.004	0.071	0.058	0.002	0.000	0.008	0.001	0.013	0.004	0.018	0.034
131	0.105	0.071	0.040	0.145	0.055	0.061	0.064	0.030	0.016	0.000	0.015	0.003	0.023	0.017	0.000	0.000	0.011	0.001	0.022	0.003	0.012	0.018	0.000	0.000
132	0.056	0.069	0.044	0.065	0.085	0.101	0.099	0.065	0.001	0.000	0.000	0.000	0.405	0.356	0.017	0.042	0.000	0.000	0.007	0.001	0.320	0.276	0.028	0.026
133	0.072	0.058	0.040	0.060	0.087	0.103	0.107	0.042	0.000	0.000	0.004	0.005	-	-	0.049	0.009	0.000	0.000	0.018	0.000	-	-	0.030	0.000
134	0.051	0.058	0.041	0.114	0.050	0.069	0.069	0.061	0.013	0.000	0.000	-	0.027	0.000	0.023	0.008	0.010	0.000	0.000	-	0.010	0.000	0.004	0.004
135	0.067	0.088	0.056	0.088	0.095	0.175	0.179	0.048	0.000	0.001	0.000	0.000	0.145	0.047	0.006	0.005	0.000	0.000	0.005	0.000	0.075	0.005	0.010	0.003
136	0.122	-	0.046	0.080	0.106	0.190	0.186	-	0.003	-	0.000	0.011	0.304	0.135	0.059	-	0.001	-	0.003	0.010	0.218	0.111	0.014	-
137	0.093	0.112	0.043	0.061	0.078	0.085	0.253	0.135	0.000	0.004	0.002	0.014	0.080	0.062	0.061	0.000	0.000	0.002	0.001	0.015	0.046	0.055	0.057	0.000

Table A10: Immune response data pertaining to vaccinates on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
1	0.163	0.215	0.154	0.118	0.096	0.088	0.088	0.037	0.000	0.769	0.521	0.000	0.294	0.317	0.097	0.055	0.001	0.696	0.402	0.005	0.312	0.316	0.079	0.057
2	0.058	0.504	0.495	0.469	0.219	0.252	0.148	0.087	0.000	0.358	0.413	0.001	0.000	0.133	0.317	0.098	0.000	0.339	0.359	0.001	0.021	0.121	0.302	0.074
3	0.060	0.299	0.372	0.406	0.257	0.362	0.211	0.104	0.014	0.056	0.030	0.014	0.027	0.062	0.058	0.047	0.011	0.050	0.028	0.015	0.023	0.054	0.045	0.036
4	0.158	0.128	0.656	0.481	0.233	0.551	0.329	0.486	0.000	0.016	0.003	0.000	0.033	0.017	0.087	0.048	0.001	0.009	0.003	0.002	0.035	0.033	0.061	0.040
5	0.062	1.474	1.534	2.044	0.918	1.149	1.090	0.598	0.024	0.059	0.054	0.008	0.028	0.006	0.022	0.037	0.023	0.063	0.044	0.001	0.038	0.000	0.027	0.023
6	0.058	0.147	0.516	0.488	0.280	0.251	0.236	0.121	0.000	0.278	0.287	0.011	0.174	0.150	0.273	0.059	0.000	0.241	0.219	0.011	0.164	0.165	0.231	0.064
7	0.066	0.438	0.142	0.204	0.125	0.150	0.127	0.077	0.014	-	0.085	0.006	0.141	0.000	0.040	0.002	0.011	-	0.088	0.008	0.125	0.033	0.017	0.005
8	0.058	0.350	0.174	0.181	0.093	0.108	0.106	0.068	0.000	0.839	0.442	0.021	0.206	0.026	0.041	0.011	0.001	0.739	0.387	0.027	0.190	0.032	0.033	0.012
9	0.052	0.381	0.070	0.242	0.130	0.159	0.191	0.085	0.016	-	0.091	0.011	0.066	0.042	0.026	0.038	0.013	-	0.069	0.009	0.056	0.000	0.007	0.028
10	0.062	0.310	0.392	0.480	0.250	0.308	0.239	0.114	0.000	-	0.025	0.003	0.084	0.035	0.015	0.014	0.003	-	0.029	0.006	0.097	0.041	0.018	0.013
11	0.056	0.232	0.228	0.303	0.123	0.196	0.095	0.047	0.000	-	0.087	0.019	0.071	0.006	0.031	0.056	0.000	-	0.089	0.008	0.067	0.013	0.021	0.049
12	0.105	1.269	1.199	0.653	0.253	0.345	0.355	0.177	0.000	-	0.051	0.000	0.093	0.022	0.003	0.026	0.003	-	0.038	0.000	0.078	0.006	0.001	0.029
13	0.056	0.634	0.362	0.219	0.126	0.116	0.131	0.076	0.002	0.445	0.189	0.069	0.468	0.131	0.149	0.019	0.000	0.394	0.119	0.062	0.390	0.108	0.123	0.024
14	-	0.141	0.756	0.735	0.189	0.310	0.225	0.377	0.000	0.577	0.148	0.000	0.532	0.158	0.193	0.020	0.002	0.531	0.090	0.000	0.475	0.120	0.153	0.037
15	-	0.142	1.829	1.787	1.060	0.816	0.486	0.137	-	0.276	0.112	0.007	0.261	0.189	0.265	0.015		0.236	0.096	0.000	0.233	0.177	0.202	0.024
16	0.053	0.348	0.322	0.261	0.173	0.198	0.172	0.070	0.000	0.456	0.647	0.092	0.376	0.239	0.396	0.184	0.003	0.406	0.505	0.097	0.348	0.226	0.354	0.188
17	0.058	0.549	1.166	0.363	0.217	0.215	0.188	0.053	0.000	1.842	0.392	0.000	0.157	0.189	0.241	0.144	0.000	1.548	0.342	0.000	0.126	0.179	0.188	0.158
18	0.107	0.528	0.887	0.233	0.136	0.156	0.140	0.079	0.002	0.300	0.149	0.000	0.141	0.107	0.077	0.000	0.002	0.269	0.133	0.000	0.123	0.107	0.061	0.003
19	0.055	0.141	0.609	0.421	0.344	0.354	0.293	0.172	0.005	0.076	0.000	0.002	0.095	0.157	0.104	0.009	0.004	0.067	0.000	0.009	0.095	0.139	0.105	0.010
20	0.080	0.211	1.307	0.651	0.413	0.481	0.383	0.104	0.021	0.040	0.050	0.001	0.127	0.000	0.038	0.004	0.016	0.039	0.048	0.000	0.118	0.018	0.038	0.005
21	0.060	0.165	0.465	0.495	0.298	0.315	0.260	-	0.004	0.246	0.000	0.004	-	0.039	0.057	-	0.002	0.238	0.200	0.024	-	0.029	0.035	-
22	0.063	0.570	0.240	0.248	0.149	0.188	0.146	0.090	0.000	0.484	0.358	0.019	0.201	0.098	0.191	0.000	0.000	0.390	0.296	0.010	0.154	0.069	0.139	0.010
23	0.061	0.782	1.375	-	-	1.385	0.259	0.532	0.003	0.945	1.301	-	-	0.212	0.261	0.107	0.006	0.864	1.086	-	-	0.194	0.200	0.098
24	0.073	0.279	0.178	0.234	0.191	0.095	0.135	0.042	0.019	0.538	0.195	0.042	0.575	0.147	0.137	0.045	0.019	0.474	0.208	0.033	0.505	0.146	0.102	0.034
25	0.073	0.591	1.161	0.566	0.446	0.452	0.320	0.148	0.000	-	0.231	0.011	0.208	0.219	0.146	0.009	0.000	-	0.176	0.017	0.198	0.203	0.109	0.012
26	0.070	0.754	2.116	0.898	-	0.429	0.340	0.131	0.005	-	0.106	0.005	-	0.129	0.126	0.140	0.006	-	0.061	0.000	-	0.117	0.110	0.123
27	0.061	0.271	0.185	0.206	0.129	0.179	0.111	0.053	0.001	0.204	0.042	0.000	0.366	0.005	0.228	0.020	0.003	0.178	0.028	0.000	0.293	0.037	0.222	0.021
28	0.087	0.609	1.073	0.469	-	0.244	0.218	0.114	0.011	0.349	0.070	0.008	-	0.010	0.020	0.032	0.005	0.295	0.059	0.011	-	0.035	0.017	0.031
29	0.096	0.088	0.175	0.168	0.113	0.166	0.120	0.224	0.000	0.355	0.180	0.029	0.372	0.206	0.100	0.049	0.003	0.320	0.157	0.032	0.329	0.168	0.108	0.029
30	0.070	0.434	1.783	1.972	0.814	1.007	0.593	0.399	0.010	0.356	0.346	0.037	0.605	0.156	0.155	0.021	0.010	0.311	0.282	0.042	0.548	0.140	0.157	0.029
31	0.048	0.213	0.415	0.195	0.157	0.161	0.188	0.076	0.002	0.083	0.039	0.009	0.127	0.077	0.038	0.014	0.002	0.094	0.032	0.012	0.075	0.053	0.022	0.013
32	0.066	0.106	0.622	0.373	0.271	0.351	0.324	0.116	0.001	0.233	0.173	0.027	0.250	0.162	0.085	0.007	0.000	0.223	0.156	0.032	0.220	0.122	0.063	0.013
33	0.071	0.586	1.788	1.123	0.864	0.866	0.644	0.387	0.007	0.187	0.734	0.156	0.085	0.083	0.055	0.045	0.002	0.173	0.606	0.149	0.083	0.059	0.039	0.027
34	0.063	0.083	0.093	0.150	0.087	0.096	0.120	0.033	0.000	0.163	0.272	0.025	0.163	0.057	0.092	0.028	0.000	0.149	0.194	0.018	0.162	0.052	0.069	0.014
35	0.088	0.559	0.332	0.373	0.188	0.219	0.164	0.123	0.000	0.945	0.075	0.013	0.356	0.157	0.060	0.018	0.002	0.842	0.048	0.008	0.321	0.141	0.067	0.015
36	0.089	0.105	0.270	0.262	0.161	0.263	0.191	0.099	0.001	0.658	0.234	0.051	0.538	0.079	0.063	0.045	0.001	0.600	0.190	0.038	0.476	0.055	0.061	0.045
37	0.111	0.128	0.975	0.452	0.207	0.300	0.255	0.203	0.040	0.185	0.059	0.000	0.086	0.233	0.546	0.278	0.039	0.144	0.039	0.000	0.088	0.188	0.324	0.242
38	0.129	0.087	1.323	0.574	0.379	0.373	0.274	0.179	0.000	0.747	0.183	0.000	0.314	0.000	0.062	0.017	0.000	0.686	0.106	0.000	0.283	0.007	0.036	0.016
39	0.055	0.596	1.294	0.219	0.443	0.537	0.477	0.251	0.005	0.514	0.043	0.004	0.424	0.103	0.112	0.058	0.002	0.493	0.045	0.010	0.354	0.067	0.048	0.028
40	0.057	0.621	0.582	0.516	0.255	0.227	0.226	0.130	0.000	0.562	0.104	0.060	0.587	0.053	0.075	0.329	0.001	0.497	0.080	0.049	0.562	0.048	0.000	0.259
41	0.052	0.429	0.254	0.241	0.168	0.165	0.137	0.084	0.000	0.141	0.201	0.000	0.093	0.036	0.040	0.013	0.000	0.119	0.167	0.003	0.089	0.020	0.030	0.016
42	0.076	0.316	0.208	2.031	1.067	1.485	1.029	0.587	0.001	0.087	0.114	0.007	0.087	0.000	0.007	0.011	0.000	0.083	0.103	0.007	0.065	0.029	0.010	0.015

Table A10 (contd..): Immune response data pertaining to vaccinates on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
43	0.199	0.426	0.833	0.349	0.177	0.177	0.151	0.102	0.018	0.235	0.388	0.004	0.361	0.255	0.198	0.019	0.020	0.223	0.328	0.000	0.321	0.222	0.179	0.020
44	0.092	0.665	0.154	0.934	0.359	0.562	0.360	0.120	0.000	0.073	0.059	0.018	0.281	0.148	0.515	0.596	0.001	0.081	0.016	0.008	0.212	0.076	0.225	0.478
45	0.069	0.429	0.440	0.166	0.166	0.151	0.092	0.040	0.000	0.314	0.542	0.074	0.580	0.436	0.254	0.150	0.000	0.288	0.456	0.058	0.468	0.353	0.157	0.139
46	0.103	0.085	0.179	0.160	0.107	0.129	0.121	0.041	0.000	0.060	0.051	0.013	0.011	0.028	0.031	0.171	0.002	0.055	0.029	0.009	0.027	0.025	0.023	0.188
47	0.131	0.141	0.146	0.805	0.599	0.642	0.357	0.136	0.004	0.130	0.081	0.020	0.066	0.005	0.032	0.052	0.003	0.113	0.076	0.012	0.046	0.011	0.025	0.047
48	0.206	0.525	1.176	1.233	0.688	0.851	0.091	0.197	0.000	0.191	0.034	0.035	0.140	0.102	0.005	0.049	0.002	0.169	0.014	0.030	0.112	0.094	0.010	0.046
49	0.055	1.639	2.126	0.651	0.798	0.979	0.611	0.258	0.001	1.132	0.601	0.083	0.620	0.306	0.165	0.047	0.000	0.923	0.413	0.078	0.574	0.293	0.157	0.051
50	0.087	0.816	1.256	1.165	0.626	0.871	0.353	0.123	0.030	0.208	0.069	0.007	0.348	0.544	0.084	0.019	0.000	0.197	0.054	0.006	0.264	0.407	0.061	0.017
51	0.053	0.090	0.082	0.161	0.075	0.112	0.101	0.188	0.003	0.037	0.019	0.000	0.033	0.206	0.164	0.005	0.003	0.019	0.000	0.002	0.030	0.088	0.109	0.019
52	0.138	0.288	0.136	0.137	0.102	0.114	0.109	0.046	0.007	0.219	0.533	0.000	0.261	0.072	0.001	0.006	0.009	0.207	0.397	0.008	0.231	0.058	0.013	0.002
53	0.056	0.393	3.551	1.774	1.057	0.979	1.057	0.647	0.000	0.063	0.061	0.000	-	0.076	0.014	0.015	0.000	0.060	0.059	0.000	-	0.057	0.016	0.021
54	0.117	0.082	0.192	0.147	0.101	0.281	0.288	0.105	0.001	0.067	0.042	0.012	0.104	0.014	0.045	0.033	0.013	0.084	0.023	0.012	0.092	0.030	0.038	0.028
55	0.071	0.214	0.767	0.238	0.184	0.278	0.239	0.074	0.000	0.599	0.207	0.009	0.124	0.229	0.337	0.020	0.000	0.572	0.097	0.014	0.094	0.187	0.201	0.017
56	0.055	0.379	0.664	0.737	0.256	0.331	0.301	0.173	0.035	0.443	0.021	0.001	0.078	0.037	0.017	0.000	0.023	0.396	0.034	0.002	0.082	0.033	0.001	0.000
57	0.053	0.786	0.531	0.317	0.183	0.234	0.219	0.104	0.007	0.584	0.493	0.000	-	0.214	0.271	0.220	0.009	0.515	0.394	0.003	-	0.232	0.174	0.177
58	0.271	0.387	0.521	0.456	0.347	0.368	0.236	0.098	0.020	0.490	0.122	0.000	0.181	0.077	0.090	0.016	0.027	0.439	0.103	0.003	0.182	0.067	0.094	0.016
59	-	0.087	0.238	0.168	0.173	0.117	0.122	0.041	0.018	0.108	0.051	0.001	0.049	0.028	0.000	0.025	0.002	0.106	0.043	0.000	0.049	0.032	0.015	0.022
60	0.110	0.093	0.132	0.317	0.093	0.092	0.093	0.047	0.000	0.105	0.086	0.021	0.231	0.158	0.027	0.007	0.002	0.082	0.095	0.013	0.198	0.141	0.008	0.013
61	0.051	0.251	0.317	0.271	0.159	0.212	0.125	0.091	0.000	0.381	0.032	0.000	0.088	0.017	0.046	0.005	0.006	0.341	0.026	0.000	0.046	0.020	0.015	0.006
62	0.082	0.095	1.603	0.964	0.468	0.549	0.531	0.275	0.000	0.150	0.105	0.018	0.059	0.152	0.109	0.075	0.000	0.138	0.079	0.025	0.055	0.133	0.081	0.067
63	0.078	0.133	0.705	0.828	0.617	0.838	0.533	0.251	0.000	0.177	0.174	0.005	0.161	0.042	0.049	0.015	0.004	0.160	0.126	0.004	0.148	0.035	0.042	0.016
64	0.059	0.422	0.319	0.241	0.202	0.239	0.234	-	0.000	0.057	0.020	0.004	0.027	0.046	0.055	-	0.003	0.049	0.024	0.000	0.021	0.044	0.043	-
65	0.106	0.404	0.422	0.183	0.124	0.107	0.072	0.038	0.021	0.125	0.280	0.005	0.081	0.044	0.000	0.000	0.008	0.119	0.255	0.003	0.073	0.060	0.000	0.012
66	0.051	0.575	1.062	0.787	0.288	0.520	0.418	0.156	0.005	0.209	0.040	0.008	0.012	0.043	0.080	0.028	0.006	0.201	0.025	0.007	0.025	0.041	0.078	0.031
67	0.051	0.227	0.653	0.461	0.284	0.198	0.179	0.114	0.013	0.049	0.193	0.000	0.148	0.047	0.038	0.074	0.009	0.045	0.168	0.001	0.132	0.034	0.027	0.059
68	0.132	0.377	0.817	0.504	0.440	0.632	0.263	0.353	0.006	0.571	0.706	0.055	0.413	0.279	0.014	0.024	0.004	0.472	0.453	0.041	0.331	0.244	0.017	0.013
69	0.069	0.190	0.160	0.308	0.086	0.114	0.090	0.061	0.017	0.235	0.037	0.009	0.100	0.030	0.017	0.014	0.014	0.215	0.025	0.019	0.090	0.000	0.004	0.024
70	0.065	0.614	0.333	0.359	0.239	0.218	0.177	0.071	0.003	0.250	0.005	0.014	0.211	0.000	0.037	0.006	0.004	0.205	0.000	0.022	0.174	0.010	0.021	0.017
71	0.053	0.588	1.227	0.458	0.335	0.395	0.347	0.110	0.005	0.646	0.281	0.000	0.239	0.036	0.077	0.000	0.003	0.588	0.112	0.000	0.210	0.046	0.128	0.003
72	0.115	0.234	0.497	0.318	0.153	0.215	0.119	0.057	0.003	0.362	0.064	0.013	0.145	0.033	0.012	0.026	0.002	0.337	0.053	0.012	0.128	0.006	0.008	0.023
73	0.090	0.245	2.042	0.147	0.160	0.218	0.137	0.071	0.000	1.245	0.337	0.013	0.155	0.370	0.154	0.126	0.002	1.117	0.240	0.005	0.131	0.342	0.114	0.111
74	0.063	0.424	2.880	2.129	1.635	1.739	1.294	0.408	0.019	1.587	0.824	0.011	1.291	0.286	0.525	1.152	0.010	1.193	0.620	0.013	1.027	0.285	0.482	1.034
75	0.060	0.247	0.565	0.246	0.128	0.160	-	0.339	0.000	0.280	0.245	0.003	0.441	0.281	0.083	0.055	0.004	0.262	0.192	0.000	0.318	0.201	0.018	0.044
76	0.136	0.456	0.305	0.290	0.235	0.221	0.179	0.116	0.020	-	0.023	0.001	-	0.053	0.040	0.009	0.015	-	0.019	0.003	-	0.049	0.046	0.012
77	0.060	0.404	0.371	0.365	0.183	0.225	0.181	0.072	0.001	-	0.147	0.011	0.174	0.027	0.082	0.000	0.003	-	0.130	0.006	0.160	0.035	0.076	0.005
78	0.050	0.144	0.309	0.285	0.172	0.333	0.148	0.054	0.000	0.019	0.136	0.010	0.155	0.046	0.070	0.028	0.015	0.019	0.093	0.013	0.145	0.048	0.063	0.017
79	0.047	0.713	0.353	0.673	0.476	0.459	0.260	0.129	0.000	-	0.105	0.023	0.236	0.091	0.060	0.043	0.000	-	0.090	0.033	0.209	0.086	0.039	0.034
80	0.101	0.116	0.199	0.423	0.122	0.184	0.117	0.070	0.000	0.443	0.196	0.011	0.123	0.062	0.025	0.000	0.000	0.396	0.133	0.013	0.089	0.042	0.012	0.004
81	0.054	0.110	0.224	0.274	0.167	0.175	0.172	0.105	0.000	0.089	0.019	0.009	0.175	0.118	0.424	0.011	0.000	0.098	0.027	0.012	0.139	0.118	0.207	0.010
82	0.051	0.162	0.843	0.364	0.220	0.246	0.159	0.099	0.000	0.465	0.608	0.009	0.240	0.039	0.030	0.004	0.000	0.419	0.531	0.018	0.224	0.047	0.023	0.014
83	0.048	0.264	0.131	0.191	0.107	0.132	0.106	0.089	0.026	-	0.110	0.004	0.505	0.164	0.073	0.070	0.028	-	0.100	0.003	0.447	0.129	0.067	0.054
84	0.047	0.422	1.750	0.536	0.249	0.345	0.274	0.201	0.032	0.073	0.094	0.001	0.212	0.047	0.003	0.004	0.020	0.073	0.085	0.000	0.175	0.053	0.002	0.011

Table A10 (contd..): Immune response data pertaining to vaccinates on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
85	0.129	0.076	0.063	0.138	0.091	0.102	0.118	0.090	0.017	-	0.162	0.010	-	0.077	-	0.061	0.000	-	0.138	0.011	-	0.064	-	0.055
86	0.056	0.132	1.060	0.465	0.297	0.308	0.234	0.215	0.000	0.161	0.061	0.010	0.066	0.031	0.043	0.061	0.001	0.151	0.055	0.001	0.057	0.028	0.047	0.057
87	0.049	0.428	0.382	0.164	0.093	0.105	0.102	0.046	0.035	0.000	0.069	0.003	0.065	0.024	0.000	0.000	0.001	0.001	0.064	0.004	0.060	0.022	0.000	0.004
88	0.051	0.276	0.505	0.354	0.207	0.180	0.179	0.091	0.005	0.259	0.266	0.038	0.370	0.232	0.310	0.017	0.013	0.237	0.210	0.033	0.294	0.187	0.243	0.019
89	0.046	0.363	0.237	0.437	0.292	0.167	0.114	0.061	0.023	-	0.020	0.001	0.147	0.027	0.188	0.035	0.003	-	0.020	0.009	0.137	0.030	0.156	0.029
90	0.049	0.600	0.780	0.599	0.649	0.493	0.365	0.159	0.002	0.410	0.066	0.000	0.668	0.058	0.044	0.012	0.003	0.376	0.056	0.000	0.620	0.055	0.048	0.009
91	0.061	0.281	0.721	0.451	0.353	0.390	0.243	0.102	0.002	-	0.123	0.026	0.028	0.063	0.009	0.000	0.000	-	0.107	0.016	0.033	0.056	0.000	0.007
92	0.053	0.554	0.444	0.460	0.187	0.253	0.176	0.112	0.000	0.429	0.026	0.025	0.207	0.121	0.184	0.164	0.000	0.336	0.031	0.029	0.203	0.091	0.118	0.132
93	0.049	0.327	1.241	0.726	0.258	0.295	0.209	0.061	0.000	0.304	0.220	0.014	0.065	0.244	0.155	0.017	0.000	0.295	0.136	0.012	0.070	0.225	0.145	0.022
94	0.057	0.186	0.254	0.253	0.165	0.238	0.186	-	0.007	0.144	0.056	0.000	0.026	0.041	0.054	-	0.007	0.128	0.050	0.000	0.018	0.038	0.063	-
95	0.065	0.169	0.385	0.287	0.137	0.187	0.108	0.104	0.009	0.037	0.027	0.022	0.015	0.010	0.082	0.030	0.025	0.037	0.018	0.011	0.021	0.013	0.038	0.026
96	0.056	0.349	0.472	0.487	0.335	0.329	0.260	0.104	0.006	-	0.143	0.014	0.240	0.104	0.179	0.043	0.002	-	0.102	0.007	0.194	0.064	0.036	0.018
97	0.065	0.226	0.511	0.375	0.212	0.286	0.164	0.076	0.006	0.310	0.000	0.006	0.183	0.019	0.015	0.085	0.018	0.306	0.000	0.000	0.130	0.020	0.015	0.077
98	0.064	0.094	0.318	0.247	0.199	0.223	0.280	0.090	0.000	0.157	0.384	0.017	0.189	0.008	0.049	0.000	0.004	0.135	0.288	0.006	0.177	0.000	0.028	0.000
99	0.051	0.058	0.054	0.067	0.047	0.103	0.067	0.025	0.000	0.006	0.000	0.001	0.018	0.072	0.072	0.097	0.002	0.002	0.008	0.006	0.004	0.039	0.015	0.060
100	0.052	1.075	1.830	0.888	0.267	0.547	0.705	0.244	0.000	-	0.059	0.005	0.115	0.048	0.048	0.004	0.000	-	0.047	0.010	0.103	0.052	0.046	0.003
101	0.050	0.229	0.285	0.311	0.161	0.145	0.100	0.080	0.000	0.250	0.192	0.004	0.304	0.036	0.020	0.023	0.000	0.238	0.174	0.007	0.282	0.037	0.009	0.022
102	0.052	0.060	0.164	0.212	0.130	0.223	0.157	0.052	0.000	0.414	0.069	0.001	0.095	0.074	0.055	0.024	0.003	0.366	0.055	0.012	0.085	0.089	0.051	0.020
103	0.058	0.291	0.820	0.660	0.306	0.348	0.299	0.155	0.000	0.237	0.081	0.021	-	0.149	0.083	0.023	0.000	0.257	0.054	0.020	-	0.124	0.076	0.021
104	0.056	0.101	0.158	0.106	0.237	-	0.098	0.044	0.000	-	0.031	0.013	0.022	-	0.060	0.000	0.000	-	0.030	0.012	0.012	-	0.049	0.003
105	0.057	0.359	0.297	0.368	0.226	0.237	0.242	0.109	0.000	0.018	0.000	0.000	0.084	0.022	0.003	0.012	0.001	0.020	0.013	0.001	0.082	0.001	0.022	0.008
106	0.081	0.384	1.420	0.202	0.100	0.140	0.114	0.044	0.008	0.054	0.063	0.000	0.055	0.007	0.000	0.019	0.009	0.066	0.050	0.010	0.054	0.016	0.000	0.017
107	0.053	0.795	0.323	0.186	0.109	0.164	0.115	0.052	0.002	1.754	0.525	0.035	0.604	0.155	0.167	0.090	0.005	1.352	0.455	0.032	0.542	0.139	0.138	0.061
108	0.080	0.275	3.951	1.639	1.082	1.013	0.677	0.564	0.006	0.219	0.111	0.018	0.080	0.062	0.022	0.041	0.009	0.211	0.085	0.017	0.068	0.038	0.013	0.052
109	0.087	0.348	0.278	0.080	0.067	0.074	0.073	-	0.013	0.758	0.152	0.010	0.214	0.066	0.117	-	0.010	0.658	0.141	0.008	0.185	0.043	0.090	-
110	0.047	0.750	2.780	1.065	0.471	0.672	0.274	0.129	0.036	0.136	0.176	0.010	0.801	0.274	0.098	0.031	0.035	0.134	0.117	0.000	0.742	0.222	0.087	0.035
111	0.058	0.215	0.220	0.197	0.148	0.170	0.155	0.107	0.006	0.044	0.114	0.002	0.257	0.211	0.041	0.000	0.000	0.042	0.088	0.005	0.203	0.130	0.030	0.080
112	0.054	0.135	0.147	0.266	0.162	0.155	0.159	0.111	0.000	0.278	0.029	0.010	0.148	0.060	0.097	0.108	0.009	0.256	0.009	0.011	0.103	0.000	0.019	0.041
113	0.082	0.320	0.182	0.170	0.114	0.202	0.136	0.132	0.000	0.360	0.055	0.021	0.025	0.063	0.014	0.022	0.000	0.323	0.045	0.020	0.020	0.051	0.013	0.020
114	0.103	0.208	0.333	0.293	0.190	0.336	0.204	0.105	0.000	0.000	0.041	0.000	0.038	0.038	0.023	0.022	0.000	0.013	0.026	0.003	0.031	0.019	0.020	0.015
115	0.072	0.303	0.201	0.173	0.097	0.147	0.106	0.070	0.008	0.469	0.075	0.000	-	0.037	0.048	0.034	0.006	0.438	0.066	0.009	-	0.039	0.038	0.025
116	0.068	0.169	0.221	0.146	0.134	0.180	0.099	0.057	0.004	0.275	0.037	0.015	0.015	0.006	0.000	0.004	0.004	0.241	0.026	0.005	0.007	0.012	0.000	0.000
117	0.072	0.278	0.477	0.616	0.360	0.480	0.355	0.181	0.029	0.416	0.091	0.001	0.116	0.000	0.080	0.018	0.022	0.358	0.074	0.000	0.105	0.000	0.061	0.014
118	0.058	0.256	0.303	0.310	0.165	0.219	0.149	0.068	0.000	0.351	0.123	0.022	0.020	0.236	0.206	0.097	0.014	0.337	0.115	0.009	0.033	0.228	0.189	0.087
119	0.098	0.142	0.175	0.795	0.137	0.225	0.104	0.061	0.000	0.334	0.034	0.021	0.245	0.254	0.107	0.188	0.004	0.322	0.027	0.017	0.192	0.164	0.095	0.152
120	0.076	0.084	0.249	0.157	0.117	0.140	0.111	0.059	0.024	0.360	0.075	0.029	0.154	0.052	0.067	0.120	0.023	0.323	0.059	0.041	0.123	0.060	0.031	0.103
121	0.083	0.072	0.929	0.924	0.399	0.464	0.262	0.124	0.000	0.044	0.093	0.031	0.150	0.090	-	0.124	0.003	0.042	0.059	0.035	0.082	0.034	-	0.075
122	0.091	0.072	0.046	0.125	0.069	-	0.091	0.044	0.030	0.252	0.031	0.005	0.021	-	0.020	0.021	0.028	0.219	0.020	0.003	0.020	-	0.004	0.015
123	0.057	0.056	0.115	0.118	0.082	0.098	0.070	-	0.011	0.013	0.018	0.004	0.050	0.014	0.015	-	0.011	0.014	0.025	0.002	0.041	0.016	0.011	-
124	0.180	0.100	0.063	0.115	0.084	0.084	0.099	0.026	0.000	0.065	0.029	0.035	0.044	0.039	0.034	0.000	0.000	0.057	0.022	0.027	0.033	0.037	0.033	0.004
125	0.132	0.112	0.098	0.146	0.102	0.143	0.118	0.053	0.007	0.646	0.080	0.004	0.343	0.258	0.112	0.042	0.006	0.588	0.077	0.002	0.317	0.238	0.092	0.041
126	0.127	0.145	0.293	0.329	0.204	0.260	0.238	0.090	0.000	0.141	0.027	0.005	0.000	0.029	0.020	0.016	0.000	0.127	0.022	0.007	0.000	0.012	0.010	0.004

Table A10 (contd..): Immune response data pertaining to vaccinates on property 2

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48	0	2	8	12	18	24	36	48
127	0.567	0.190	0.355	0.263	0.128	0.165	0.152	0.051	0.001	0.420	0.504	0.097	0.725	0.022	0.123	0.002	0.004	0.353	0.314	0.102	0.533	0.011	0.089	0.005
128	0.101	0.081	0.128	0.125	0.085	0.099	0.077	0.041	0.000	0.519	0.184	0.000	0.335	0.400	0.106	0.010	0.000	0.478	0.154	0.009	0.299	0.351	0.111	0.013
129	0.087	0.514	2.665	1.308	0.379	0.864	0.427	0.197	0.002	0.448	0.188	0.012	0.206	0.461	0.362	0.024	0.000	0.396	0.136	0.004	0.186	0.389	0.276	0.022
130	0.442	0.154	0.160	0.144	0.099	0.117	0.110	0.041	0.000	0.019	0.040	0.014	0.050	0.110	0.083	0.000	0.001	0.029	0.029	0.026	0.048	0.108	0.071	0.007
131	0.053	0.131	0.813	0.549	0.336	0.416	0.274	-	0.011	0.099	0.035	0.012	0.040	0.066	0.376	-	0.007	0.094	0.037	0.004	0.044	0.043	0.105	-
132	0.176	0.143	1.171	0.173	0.100	0.241	-	-	0.000	0.026	0.058	0.000	0.022	-	0.007	-	0.000	0.028	0.044	0.004	0.022	-	0.000	-
133	0.053	0.090	0.148	0.259	0.199	0.316	0.330	0.151	0.046	0.116	0.012	0.011	0.132	0.006	0.045	0.014	0.040	0.109	0.015	0.000	0.123	0.013	0.024	0.009
134	-	0.283	0.625	0.386	0.180	0.169	0.236	0.085	0.000	0.015	0.063	0.000	0.044	0.029	0.082	0.112	0.005	0.022	0.049	0.001	0.032	0.028	0.000	0.079
135	0.213	0.111	1.038	1.183	0.603	-	0.669	0.272	0.000	0.109	0.000	0.016	0.059	-	0.031	0.000	0.000	0.094	0.005	0.018	0.044	-	0.034	0.001
136	0.108	0.071	0.560	0.155	0.106	0.146	0.095	0.050	0.000	-	0.037	0.000	0.100	0.045	0.018	0.003	0.000	-	0.000	0.006	0.081	0.015	0.013	0.008
137	0.192	0.070	0.317	0.335	0.164	0.206	0.164	0.078	0.000	0.029	0.079	0.006	0.260	0.081	-	0.030	0.003	0.028	0.064	0.013	0.202	0.077	-	0.020
138	0.601	0.335	0.244	0.224	0.119	0.152	0.117	0.100	0.016	0.175	0.021	0.004	0.129	0.033	0.054	0.016	0.017	0.158	0.014	0.004	0.109	0.028	0.044	0.015
139	0.060	0.401	0.195	0.832	0.493	1.085	1.069	0.450	0.046	0.008	0.037	0.003	0.086	0.330	1.198	0.526	0.038	0.026	0.020	0.000	0.072	0.183	0.000	0.134
140	0.144	0.082	0.330	0.246	0.090	0.116	0.103	0.046	0.000	0.375	0.234	0.014	0.274	0.083	0.104	0.059	0.006	0.316	0.185	0.026	0.234	0.082	0.090	0.058
141	0.152	0.104	0.230	0.352	0.170	0.194	0.126	0.059	0.011	0.160	0.013	0.000	0.061	0.142	0.026	0.020	0.011	0.164	0.001	0.000	0.055	0.123	0.024	0.015
142	0.061	0.612	1.015	1.072	0.505	0.758	0.531	0.309	0.000	0.297	0.146	0.025	1.046	0.369	0.280	0.001	0.002	0.257	0.095	0.019	0.901	0.303	0.206	0.016
143	0.067	0.087	0.220	0.251	0.161	0.211	0.140	0.060	0.001	0.235	0.018	0.010	0.154	0.040	0.002	0.010	0.000	0.234	0.018	0.004	0.100	0.036	0.016	0.010
144	0.054	0.223	0.072	0.163	0.159	0.135	0.108	0.043	0.000	0.205	0.099	0.009	0.028	0.000	0.013	0.016	0.000	0.191	0.064	0.005	0.019	0.000	0.021	0.025
145	0.416	0.147	0.176	0.189	0.120	0.119	0.377	0.060	0.022	0.200	0.069	0.001	-	0.026	0.073	0.000	0.014	0.196	0.059	0.000	-	0.030	0.047	0.009
146	0.100	0.218	0.863	0.533	0.335	0.372	0.269	0.090	0.000	0.572	0.308	0.003	0.114	0.081	0.083	0.061	0.005	0.550	0.119	0.004	0.126	0.073	0.075	0.058
147	0.346	0.119	0.549	0.338	0.223	0.392	0.137	0.074	0.000	0.050	0.327	0.008	0.814	0.361	0.018	0.043	0.000	0.054	0.191	0.008	0.728	0.318	0.021	0.041
148	0.115	0.078	0.166	0.193	0.129	0.120	0.152	0.091	0.000	0.425	0.066	0.007	0.177	0.040	0.000	0.023	0.002	0.332	0.055	0.021	0.100	0.027	0.003	0.022
149	0.367	0.099	0.139	0.141	0.114	0.101	0.130	0.071	0.000	0.233	0.048	0.005	0.194	0.231	0.027	0.377	0.002	0.225	0.023	0.006	0.129	0.121	0.000	0.216
150	0.047	0.464	1.293	0.637	0.632	0.996	0.788	-	0.000	0.148	0.006	0.016	0.033	-	0.030	-	0.000	0.128	0.000	0.016	0.024	-	0.000	-
151	0.072	0.311	0.583	0.244	0.127	0.171	0.162	0.062	0.003	0.203	0.019	0.019	0.125	0.118	0.046	0.019	0.001	0.193	0.018	0.018	0.087	0.081	0.000	0.010
152	0.076	0.371	0.226	0.251	0.161	0.171	0.124	0.067	0.000	0.262	0.130	0.011	0.309	0.098	0.067	0.042	0.000	0.256	0.116	0.023	0.255	0.087	0.046	0.036
153	0.058	0.217	0.038	0.757	0.385	0.371	0.313	0.222	0.002	-	0.000	0.000	0.022	0.045	0.023	0.056	0.000	-	0.000	0.005	0.024	0.049	0.017	0.057
154	0.055	0.113	0.270	0.386	0.170	0.258	0.234	0.127	0.006	0.221	0.550	0.022	-	0.080	0.001	0.021	0.003	0.213	0.440	0.028	-	0.080	0.012	0.019
155	0.063	0.293	2.772	0.900	0.303	0.545	0.338	0.126	0.000	1.241	0.188	0.004	0.371	0.077	0.050	0.034	0.000	1.147	0.150	0.009	0.368	0.068	0.044	0.033
156	0.090	0.074	0.118	0.089	0.088	0.092	0.110	0.099	0.017	0.029	0.031	0.006	0.089	0.020	0.022	0.014	0.016	0.025	0.032	0.004	0.092	0.003	0.016	0.006
157	0.190	0.473	0.217	0.464	0.120	0.126	0.127	0.058	0.011	0.516	0.133	0.002	0.453	0.119	0.066	0.149	0.001	0.474	0.076	0.009	0.389	0.102	0.056	0.114
158	0.054	0.122	0.084	0.155	0.111	0.104	0.168	0.045	0.002	0.031	0.008	0.000	0.005	0.011	0.053	0.033	0.003	0.034	0.016	0.000	0.024	0.009	0.032	0.011
159	0.085	0.174	0.117	0.144	0.135	0.124	0.108	0.070	0.000	0.390	0.075	0.022	0.052	0.169	0.083	0.006	0.000	0.357	0.061	0.014	0.047	0.126	0.084	0.010
160	0.051	0.204	0.133	0.135	0.092	0.127	0.105	0.127	0.000	1.357	0.130	0.009	0.425	0.459	0.178	0.070	0.000	1.180	0.090	0.005	0.304	0.226	0.070	0.037
161	0.127	-	0.824	0.596	0.325	0.403	0.331	0.113	0.000	-	0.071	0.009	0.378	0.106	0.017	0.000	0.000	-	0.051	0.013	0.339	0.107	0.059	0.000
162	0.067	0.179	0.332	0.165	0.090	0.108	0.097	0.076	0.004	-	0.041	0.033	-	0.015	0.033	0.000	0.000	-	0.040	0.020	-	0.007	0.020	0.004

Table A11: Immune response data pertaining to controls on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
1	0.072	0.066	0.040	0.019	0.055	0.058	0.102	-	0.005	0.017	0.000	0.003	0.004	0.014	0.000	-	0.000	0.000	0.000	0.009	0.000	0.004	0.000	-
2	0.101	0.100	0.039	0.033	0.065	0.053	-	-	0.000	0.006	0.025	0.000	0.000	0.014	-	-	0.000	0.016	0.020	0.000	0.002	0.012	-	-
3	0.080	0.076	0.106	0.104	0.176	0.069	0.250	-	0.001	0.001	0.030	0.042	0.000	0.045	0.074	-	0.000	0.005	0.030	0.039	0.000	0.042	0.036	-
4	0.085	0.068	-	0.060	0.053	0.068	0.073	0.066	0.000	0.003	-	0.000	0.000	0.020	0.013	0.023	0.000	0.017	-	0.002	0.000	0.014	0.003	0.016
5	0.065	0.073	0.040	0.032	0.092	0.216	0.333	-	0.006	0.000	0.006	0.005	0.056	-	0.096	-	0.002	0.000	0.006	0.005	0.027	-	0.043	-
6	0.078	0.183	0.039	0.076	0.081	0.056	0.070	0.086	0.000	0.000	0.041	0.015	0.021	0.031	1.185	-	0.011	0.009	0.027	0.009	0.018	0.022	0.257	-
7	0.100	0.099	0.132	0.068	0.068	0.088	0.079	-	0.004	0.000	-	0.004	0.001	0.108	0.112	-	0.003	0.000	-	0.003	0.005	0.102	0.037	-
8	0.081	0.063	0.052	0.027	0.056	0.059	0.072	0.054	0.012	0.000	0.000	0.001	0.000	0.012	0.003	0.000	0.010	0.000	0.006	0.000	0.000	0.007	0.003	0.006
9	0.076	0.078	0.042	0.021	0.055	0.088	0.172	-	0.000	0.025	0.015	0.020	0.000	-	-	-	0.001	0.018	0.008	0.013	0.000	-	-	-
10	0.099	0.125	-	0.059	0.057	0.051	0.059	0.057	0.013	0.016	-	0.000	0.007	0.000	0.008	0.000	0.000	0.007	-	0.005	0.005	0.003	0.028	0.000
11	0.093	0.059	0.039	0.065	0.069	0.088	0.107	-	0.000	0.000	0.000	0.006	0.000	0.024	0.088	-	0.004	0.006	0.000	0.005	0.024	0.021	0.013	-
12	0.078	0.064	0.070	0.070	0.056	0.068	0.084	0.081	0.000	0.000	0.015	0.001	0.004	0.009	-	-	0.000	0.001	0.015	0.000	0.003	0.000	-	-
13	0.068	0.076	0.041	0.057	0.070	0.053	0.156	-	0.001	0.000	0.014	0.001	0.082	0.119	0.003	-	0.000	0.000	0.003	0.000	0.083	0.067	0.008	-
14	0.065	0.062	0.066	0.089	0.053	0.051	0.057	0.056	0.001	0.003	0.025	0.000	-	0.037	0.060	0.002	0.000	0.004	0.027	0.004	-	0.026	0.013	0.002
15	0.074	0.065	0.039	0.057	0.062	0.053	0.064	0.065	0.001	0.000	0.002	0.004	0.000	0.000	0.018	0.000	0.001	0.000	0.004	0.008	0.000	0.004	0.011	0.001
16	0.075	0.098	0.041	0.059	0.048	0.059	0.131	-	0.000	0.000	0.002	0.000	0.000	0.000	0.016	-	0.000	0.001	0.002	0.002	0.000	0.002	0.006	-
17	0.084	0.077	0.076	0.061	0.055	0.082	0.086	0.080	0.000	0.000	0.000	0.001	0.000	0.468	0.408	-	0.000	0.003	0.000	0.001	0.004	0.269	0.090	-
18	0.084	0.071	0.041	0.031	0.091	0.077	0.177	-	0.000	0.017	0.029	0.014	0.002	0.000	0.088	-	0.001	0.006	0.014	0.007	0.000	0.004	0.027	-
19	0.075	0.061	0.040	0.058	0.062	0.054	0.058	0.054	0.001	0.000	0.001	0.000	0.047	0.082	0.100	0.005	0.001	0.000	0.001	0.000	0.028	0.048	0.037	0.012
20	0.088	0.098	0.042	0.059	0.050	0.080	0.061	-	0.007	0.023	0.013	0.000	0.000	-	0.011	-	0.000	0.015	0.001	0.000	0.003	-	0.008	-
21	0.085	0.046	0.060	0.079	0.053	0.059	0.099	-	0.000	0.026	0.000	0.011	0.006	0.032	0.092	-	0.001	0.022	0.001	0.004	0.000	0.018	0.046	-
22	0.056	0.062	0.059	0.056	0.048	0.062	0.059	-	0.011	0.000	0.068	0.000	0.007	0.033	0.022	-	0.010	0.001	0.061	0.000	0.005	0.017	0.000	-
23	0.069	0.068	0.062	0.071	0.053	-	0.056	0.088	0.040	0.013	0.023	0.000	0.000	0.076	-	0.078	0.010	0.010	0.015	0.001	0.000	0.043	-	0.051
24	0.069	0.060	0.109	0.101	0.094	0.068	-	-	0.030	0.000	0.005	0.003	0.006	0.022	0.000	-	0.047	0.008	0.001	0.002	0.000	0.005	0.000	-
25	0.076	0.084	0.068	0.160	0.120	0.063	0.138	0.083	0.007	0.011	0.001	0.009	0.000	0.035	0.013	0.002	0.004	0.000	0.014	0.008	0.001	0.025	0.014	0.000
26	0.119	0.169	0.046	0.135	0.089	0.069	0.062	-	0.001	0.000	0.036	0.000	0.003	0.002	0.130	-	0.004	0.004	0.032	0.006	0.001	0.008	0.020	-
27	0.073	-	0.056	0.074	-	0.312	0.096	-	0.009	0.000	0.016	0.005	0.115	0.097	-	-	0.006	0.000	0.009	0.001	0.055	0.052	-	-
28	0.092	0.076	0.041	0.034	0.060	0.063	0.058	0.088	0.005	0.000	0.003	0.002	0.006	0.010	0.091	0.003	0.011	0.000	0.001	0.000	0.011	0.015	0.000	0.000
29	0.076	0.089	0.078	0.039	0.064	0.057	0.075	0.103	0.000	0.000	0.010	0.000	0.000	0.011	0.030	-	0.005	0.008	0.000	0.000	0.000	0.000	0.019	-
30	0.068	0.095	0.065	0.099	0.059	0.058	0.092	0.075	0.000	0.006	0.000	0.010	0.023	0.043	0.071	0.048	0.005	0.003	0.002	0.007	0.016	0.023	0.034	0.035
31	0.069	0.085	0.044	0.109	0.110	0.068	0.091	-	0.001	0.000	0.010	0.006	0.000	0.000	0.139	-	0.002	0.007	0.008	0.000	0.000	0.007	0.075	-
32	0.067	0.085	0.048	0.069	0.077	0.052	0.089	-	0.007	0.000	0.033	0.001	0.018	0.016	0.000	-	0.007	0.000	0.013	0.000	0.030	0.018	0.000	-
33	0.077	0.101	0.041	0.064	0.084	-	0.160	-	0.007	0.000	0.013	0.002	-	-	0.000	-	0.006	0.008	0.017	0.004	-	-	0.000	-
34	0.124	0.050	0.039	0.061	0.100	0.074	0.066	0.069	0.005	0.002	0.000	0.000	0.000	0.042	0.010	0.013	0.000	0.000	0.003	0.000	0.000	0.036	0.017	0.007
35	0.127	0.075	0.058	0.032	0.061	0.060	0.055	-	0.000	0.003	0.000	0.006	0.001	0.026	0.000	-	0.000	0.003	0.000	0.007	0.000	0.002	0.001	-
36	0.063	0.096	0.051	0.105	0.134	0.104	0.122	0.155	0.000	0.000	0.003	0.000	0.008	0.009	0.031	0.020	0.000	0.009	0.006	0.000	0.015	0.001	0.010	0.003
37	0.063	0.044	0.040	0.119	0.119	0.104	0.150	0.106	0.000	0.000	0.009	0.001	0.020	0.084	0.028	0.009	0.000	0.010	0.000	0.003	0.004	0.053	0.004	0.000
38	0.062	0.080	0.041	0.059	0.073	0.057	0.056	0.064	0.000	0.000	0.000	0.004	0.001	0.133	0.066	0.000	0.000	0.007	0.000	0.001	0.004	0.057	0.044	0.008
39	0.061	0.080	0.078	0.061	0.055	0.055	0.128	-	0.003	0.000	0.015	0.001	0.018	0.025	0.072	-	0.000	0.000	0.012	0.008	0.016	0.022	0.034	-
40	0.064	0.055	0.042	0.145	0.051	0.061	0.067	0.141	0.000	0.012	0.023	-	0.102	0.004	0.019	-	0.000	0.008	0.020	-	0.075	0.003	0.012	-
41	0.075	0.080	0.057	0.070	0.072	0.083	0.090	-	0.000	0.000	0.000	0.000	0.048	-	0.086	-	0.001	0.000	0.000	0.000	0.029	-	0.050	-
42	0.073	0.069	0.058	0.058	0.058	0.064	0.076	0.073	0.000	0.006	0.160	0.189	0.256	0.098	0.108	0.066	0.000	0.014	0.072	0.121	0.183	0.036	0.068	0.046

Table A11 (contd..): Immune response data pertaining to controls on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
43	0.088	0.169	0.039	0.036	0.057	0.055	0.155	-	0.000	0.000	0.042	0.001	0.003	0.105	0.025	-	0.001	0.000	0.026	0.000	0.005	0.072	0.030	-
44	0.055	0.059	0.040	0.095	0.093	0.076	0.077	0.101	0.000	0.000	-	1.141	1.015	0.504	0.754	0.096	0.000	0.000	-	0.973	0.459	0.362	0.056	0.053
45	0.047	0.249	0.062	0.031	0.103	0.053	0.083	-	0.000	0.011	0.011	0.000	0.006	0.002	0.000	-	0.000	0.000	0.010	0.000	0.002	0.007	0.000	-
46	0.061	0.068	0.039	0.063	0.066	0.072	0.085	-	0.006	0.001	0.003	0.007	0.041	0.076	0.043	-	0.000	0.006	0.002	0.005	0.042	0.052	0.038	-
47	0.066	0.108	0.067	0.027	0.073	0.068	0.081	-	0.003	0.007	0.048	0.086	-	-	-	-	0.000	0.003	0.034	0.054	-	-	-	-
48	0.069	0.086	0.038	0.072	0.125	0.059	0.058	0.096	0.000	0.001	0.008	0.007	0.009	0.007	0.009	-	0.000	0.001	0.002	0.004	0.011	0.007	0.002	-
49	0.081	0.093	0.060	0.042	0.062	0.098	0.090	0.096	0.000	0.018	0.022	0.010	0.076	0.321	0.850	-	0.000	0.017	0.018	0.010	0.057	0.222	0.281	-
50	0.063	0.131	0.139	0.054	0.093	0.072	0.058	0.082	0.000	0.011	0.008	0.006	0.013	-	0.000	0.015	0.002	0.017	0.022	0.000	0.006	-	0.002	0.005
51	0.088	0.056	0.057	0.027	0.049	0.058	0.054	-	0.000	0.014	-	0.004	0.009	0.010	0.037	-	0.000	0.000	-	0.000	0.009	0.000	0.029	-
52	0.063	0.106	0.144	0.070	0.056	0.061	0.156	0.114	0.003	0.007	0.000	0.002	0.005	0.007	0.605	0.572	0.001	0.003	0.008	0.001	0.003	0.006	0.207	0.309
53	0.050	0.083	0.040	0.027	0.051	0.060	0.057	0.071	0.000	0.015	0.006	0.003	0.003	0.013	0.000	0.002	0.006	0.015	0.007	0.000	0.000	0.000	0.000	0.002
54	0.072	0.130	0.042	0.070	0.060	0.059	0.127	0.117	0.008	0.023	0.010	0.002	0.000	0.044	0.041	0.070	0.011	0.017	0.018	0.006	0.000	0.034	0.029	0.038
55	-	0.076	0.039	0.062	0.053	0.058	0.066	-	0.001	0.000	0.000	0.002	0.008	0.007	0.020	-	0.000	0.000	0.000	0.000	0.000	0.000	0.009	-
56	0.057	0.070	0.038	0.065	0.054	0.058	0.067	-	0.002	0.003	0.000	0.017	0.000	0.010	0.020	-	0.000	0.001	0.025	0.006	0.000	0.000	0.000	-
57	0.078	0.134	0.038	0.061	0.076	0.074	0.065	0.099	0.009	0.009	0.032	0.405	0.262	0.136	0.111	0.042	0.008	0.000	0.028	0.373	0.208	0.051	0.009	0.016
58	0.060	0.088	0.046	0.103	0.066	0.066	0.089	0.057	0.000	0.021	0.022	0.007	0.060	-	0.033	0.071	0.000	0.017	0.011	0.004	0.043	-	0.013	0.029
59	0.062	0.070	0.040	0.026	0.055	0.054	0.216	-	0.000	0.009	0.000	0.003	0.003	0.047	-	-	0.004	0.000	0.001	0.007	0.000	0.013	-	-
60	0.059	0.061	0.040	0.031	0.051	0.053	0.052	0.062	0.000	0.016	0.031	0.007	0.000	0.011	0.005	0.002	0.004	0.023	0.031	0.000	0.000	0.006	0.000	0.001
61	0.070	0.065	0.044	0.102	0.057	0.052	0.063	-	0.000	0.000	0.000	0.012	0.020	0.174	-	-	0.001	0.000	0.000	0.005	0.013	0.121	-	-
62	0.062	0.078	0.044	0.030	0.054	0.053	0.083	0.125	0.000	0.005	0.006	0.006	0.007	0.003	-	0.022	0.001	0.004	0.007	0.009	0.006	0.012	-	0.022
63	0.069	0.086	0.043	0.092	0.094	0.167	0.189	0.132	0.004	0.000	0.000	0.000	0.000	0.017	0.007	0.012	0.004	0.005	0.000	0.004	0.007	0.008	0.008	0.014
64	0.062	-	0.049	0.042	0.062	0.079	0.091	0.071	0.000	0.005	0.014	0.006	0.008	0.000	0.000	0.005	0.000	0.004	0.014	0.004	0.005	0.000	0.000	0.003
65	0.066	0.074	0.063	0.066	0.058	0.055	0.069	0.088	0.000	0.000	0.029	0.001	0.001	0.021	0.000	0.015	0.000	0.001	0.021	0.005	0.000	0.000	0.018	0.008
66	0.057	0.077	0.060	0.061	0.059	0.060	0.076	-	0.007	0.001	0.003	0.000	0.001	0.059	0.011	-	0.010	0.004	0.003	0.000	0.000	0.019	0.008	-
67	0.058	0.162	0.040	0.069	0.105	0.072	0.064	0.117	0.000	0.000	0.000	0.006	0.000	0.012	0.031	0.011	0.001	0.000	0.000	0.003	0.000	0.012	0.015	0.000
68	0.076	0.092	0.038	0.038	0.052	0.061	-	-	0.000	0.007	0.007	0.001	0.000	0.000	0.027	-	0.000	0.006	0.010	0.003	0.008	0.005	0.007	-
69	0.068	0.067	0.153	0.180	0.153	0.291	0.249	-	0.000	0.034	0.158	0.043	0.394	0.159	-	-	0.001	0.028	0.116	0.035	0.248	0.113	-	-
70	0.066	0.066	0.039	0.058	0.054	0.066	0.059	0.083	0.009	0.000	0.007	0.001	0.003	0.009	0.061	0.000	0.013	0.000	0.000	0.000	0.000	0.008	0.032	0.006
71	0.058	0.068	0.047	0.054	0.053	0.050	0.056	0.092	0.000	0.004	0.000	0.004	0.003	0.000	0.019	0.003	0.000	0.012	0.000	0.008	0.001	0.008	0.017	0.000
72	0.057	0.069	0.045	0.056	0.091	0.060	0.063	-	0.000	0.000	0.020	0.000	0.010	0.036	-	-	0.000	0.000	0.014	0.003	0.002	0.007	-	-
73	0.079	0.143	0.042	0.067	0.091	0.060	0.066	0.089	0.000	0.005	0.005	0.001	-	0.016	0.027	-	0.000	0.007	0.032	0.000	-	0.000	0.020	-
74	0.087	0.146	0.042	0.132	0.160	0.395	-	-	0.018	0.010	0.012	0.013	0.247	0.350	0.393	0.066	0.012	0.008	0.018	0.011	0.192	0.262	0.116	0.050
75	0.070	0.099	0.038	0.091	0.075	0.105	0.069	-	0.000	0.011	-	-	0.103	0.112	0.026	-	0.001	0.007	-	-	0.054	0.047	0.016	-
76	0.058	0.101	0.213	0.165	0.162	0.058	0.265	0.233	0.004	0.015	0.017	0.017	0.000	0.041	0.015	-	0.002	0.012	0.019	0.000	0.000	0.020	0.007	-
77	0.075	0.124	0.098	0.129	0.131	0.137	0.181	0.162	0.000	0.000	0.174	0.194	0.174	0.008	0.091	0.034	0.000	0.002	0.110	0.169	0.161	0.000	0.000	0.002
78	0.072	0.068	0.040	0.023	0.055	0.054	0.077	0.073	0.000	0.006	0.174	0.000	0.039	0.082	0.122	0.041	0.009	0.011	0.112	0.056	0.021	0.062	0.000	0.018
79	0.066	0.077	0.067	0.063	0.053	0.050	0.117	0.111	0.009	0.009	0.000	0.007	0.000	0.016	-	0.036	0.006	0.008	0.008	0.006	0.001	0.015	-	0.019
80	0.060	0.103	0.046	0.021	0.056	0.052	0.051	0.054	0.011	0.007	0.018	0.007	0.012	0.008	0.017	0.000	0.004	0.009	0.012	0.000	0.013	0.008	0.008	0.000
81	0.058	0.093	0.081	0.079	0.095	0.118	0.113	-	0.000	0.001	0.096	0.151	0.133	-	0.031	-	0.000	0.001	0.060	0.118	0.082	-	0.015	-
82	0.077	0.102	0.057	0.062	0.055	0.048	0.065	0.089	0.000	0.020	0.000	0.000	0.000	0.004	0.033	0.019	0.000	0.028	0.022	0.002	0.000	0.006	0.008	0.005
83	0.072	0.068	0.039	0.063	0.145	0.052	0.063	0.058	0.000	0.012	0.000	0.001	0.000	0.000	0.022	0.063	0.000	0.006	0.000	0.006	0.000	0.000	0.007	0.027
84	0.066	0.111	0.039	0.047	0.084	0.106	0.107	0.103	0.000	0.016	0.000	0.201	-	0.070	0.107	0.090	0.001	0.003	0.008	0.191	-	0.054	0.047	0.076

Table A11 (contd..): Immune response data pertaining to controls on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
43	0.088	0.169	0.039	0.036	0.057	0.055	0.155	-	0.000	0.000	0.042	0.001	0.003	0.105	0.025	-	0.001	0.000	0.026	0.000	0.005	0.072	0.030	-
44	0.055	0.059	0.040	0.095	0.093	0.076	0.077	0.101	0.000	0.000	-	1.141	1.015	0.504	0.754	0.096	0.000	0.000	-	0.973	0.459	0.362	0.056	0.053
45	0.047	0.249	0.062	0.031	0.103	0.053	0.083	-	0.000	0.011	0.011	0.000	0.006	0.002	0.000	-	0.000	0.000	0.010	0.000	0.002	0.007	0.000	-
46	0.061	0.068	0.039	0.063	0.066	0.072	0.085	-	0.006	0.001	0.003	0.007	0.041	0.076	0.043	-	0.000	0.006	0.002	0.005	0.042	0.052	0.038	-
47	0.066	0.108	0.067	0.027	0.073	0.068	0.081	-	0.003	0.007	0.048	0.086	-	-	-	-	0.000	0.003	0.034	0.054	-	-	-	-
48	0.069	0.086	0.038	0.072	0.125	0.059	0.058	0.096	0.000	0.001	0.008	0.007	0.009	0.007	0.009	-	0.000	0.001	0.002	0.004	0.011	0.007	0.002	-
49	0.081	0.093	0.060	0.042	0.062	0.098	0.090	0.096	0.000	0.018	0.022	0.010	0.076	0.321	0.850	-	0.000	0.017	0.018	0.010	0.057	0.222	0.281	-
50	0.063	0.131	0.139	0.054	0.093	0.072	0.058	0.082	0.000	0.011	0.008	0.006	0.013	-	0.000	0.015	0.002	0.017	0.022	0.000	0.006	-	0.002	0.005
51	0.088	0.056	0.057	0.027	0.049	0.058	0.054	-	0.000	0.014	-	0.004	0.009	0.010	0.037	-	0.000	0.000	-	0.000	0.009	0.000	0.029	-
52	0.063	0.106	0.144	0.070	0.056	0.061	0.156	0.114	0.003	0.007	0.000	0.002	0.005	0.007	0.605	0.572	0.001	0.003	0.008	0.001	0.003	0.006	0.207	0.309
53	0.050	0.083	0.040	0.027	0.051	0.060	0.057	0.071	0.000	0.015	0.006	0.003	0.003	0.013	0.000	0.002	0.006	0.015	0.007	0.000	0.000	0.000	0.000	0.002
54	0.072	0.130	0.042	0.070	0.060	0.059	0.127	0.117	0.008	0.023	0.010	0.002	0.000	0.044	0.041	0.070	0.011	0.017	0.018	0.006	0.000	0.034	0.029	0.038
55	-	0.076	0.039	0.062	0.053	0.058	0.066	-	0.001	0.000	0.000	0.002	0.008	0.007	0.020	-	0.000	0.000	0.000	0.000	0.000	0.000	0.009	-
56	0.057	0.070	0.038	0.065	0.054	0.058	0.067	-	0.002	0.003	0.000	0.017	0.000	0.010	0.020	-	0.000	0.001	0.025	0.006	0.000	0.000	0.000	-
57	0.078	0.134	0.038	0.061	0.076	0.074	0.065	0.099	0.009	0.009	0.032	0.405	0.262	0.136	0.111	0.042	0.008	0.000	0.028	0.373	0.208	0.051	0.009	0.016
58	0.060	0.088	0.046	0.103	0.066	0.066	0.089	0.057	0.000	0.021	0.022	0.007	0.060	-	0.033	0.071	0.000	0.017	0.011	0.004	0.043	-	0.013	0.029
59	0.062	0.070	0.040	0.026	0.055	0.054	0.216	-	0.000	0.009	0.000	0.003	0.003	0.047	-	-	0.004	0.000	0.001	0.007	0.000	0.013	-	-
60	0.059	0.061	0.040	0.031	0.051	0.053	0.052	0.062	0.000	0.016	0.031	0.007	0.000	0.011	0.005	0.002	0.004	0.023	0.031	0.000	0.000	0.006	0.000	0.001
61	0.070	0.065	0.044	0.102	0.057	0.052	0.063	-	0.000	0.000	0.000	0.012	0.020	0.174	-	-	0.001	0.000	0.000	0.005	0.013	0.121	-	-
62	0.062	0.078	0.044	0.030	0.054	0.053	0.083	0.125	0.000	0.005	0.006	0.006	0.007	0.003	-	0.022	0.001	0.004	0.007	0.009	0.006	0.012	-	0.022
63	0.069	0.086	0.043	0.092	0.094	0.167	0.189	0.132	0.004	0.000	0.000	0.000	0.000	0.017	0.007	0.012	0.004	0.005	0.000	0.004	0.007	0.008	0.008	0.014
64	0.062	-	0.049	0.042	0.062	0.079	0.091	0.071	0.000	0.005	0.014	0.006	0.008	0.000	0.000	0.005	0.000	0.004	0.014	0.004	0.005	0.000	0.000	0.003
65	0.066	0.074	0.063	0.066	0.058	0.055	0.069	0.088	0.000	0.000	0.029	0.001	0.001	0.021	0.000	0.015	0.000	0.001	0.021	0.005	0.000	0.000	0.018	0.008
66	0.057	0.077	0.060	0.061	0.059	0.060	0.076	-	0.007	0.001	0.003	0.000	0.001	0.059	0.011	-	0.010	0.004	0.003	0.000	0.000	0.019	0.008	-
67	0.058	0.162	0.040	0.069	0.105	0.072	0.064	0.117	0.000	0.000	0.000	0.006	0.000	0.012	0.031	0.011	0.001	0.000	0.000	0.003	0.000	0.012	0.015	0.000
68	0.076	0.092	0.038	0.038	0.052	0.061	-	-	0.000	0.007	0.007	0.001	0.000	0.000	0.027	-	0.000	0.006	0.010	0.003	0.008	0.005	0.007	-
69	0.068	0.067	0.153	0.180	0.153	0.291	0.249	-	0.000	0.034	0.158	0.043	0.394	0.159	-	-	0.001	0.028	0.116	0.035	0.248	0.113	-	-
70	0.066	0.066	0.039	0.058	0.054	0.066	0.059	0.083	0.009	0.000	0.007	0.001	0.003	0.009	0.061	0.000	0.013	0.000	0.000	0.000	0.000	0.008	0.032	0.006
71	0.058	0.068	0.047	0.054	0.053	0.050	0.056	0.092	0.000	0.004	0.000	0.004	0.003	0.000	0.019	0.003	0.000	0.012	0.000	0.008	0.001	0.008	0.017	0.000
72	0.057	0.069	0.045	0.056	0.091	0.060	0.063	-	0.000	0.000	0.020	0.000	0.010	0.036	-	-	0.000	0.000	0.014	0.003	0.002	0.007	-	-
73	0.079	0.143	0.042	0.067	0.091	0.060	0.066	0.089	0.000	0.005	0.005	0.001	-	0.016	0.027	-	0.000	0.007	0.032	0.000	-	0.000	0.020	-
74	0.087	0.146	0.042	0.132	0.160	0.395	-	-	0.018	0.010	0.012	0.013	0.247	0.350	0.393	0.066	0.012	0.008	0.018	0.011	0.192	0.262	0.116	0.050
75	0.070	0.099	0.038	0.091	0.075	0.105	0.069	-	0.000	0.011	-	-	0.103	0.112	0.026	-	0.001	0.007	-	-	0.054	0.047	0.016	-
76	0.058	0.101	0.213	0.165	0.162	0.058	0.265	0.233	0.004	0.015	0.017	0.017	0.000	0.041	0.015	-	0.002	0.012	0.019	0.000	0.000	0.020	0.007	-
77	0.075	0.124	0.098	0.129	0.131	0.137	0.181	0.162	0.000	0.000	0.174	0.194	0.174	0.008	0.091	0.034	0.000	0.002	0.110	0.169	0.161	0.000	0.000	0.002
78	0.072	0.068	0.040	0.023	0.055	0.054	0.077	0.073	0.000	0.006	0.174	0.000	0.039	0.082	0.122	0.041	0.009	0.011	0.112	0.056	0.021	0.062	0.000	0.018
79	0.066	0.077	0.067	0.063	0.053	0.050	0.117	0.111	0.009	0.009	0.000	0.007	0.000	0.016	-	0.036	0.006	0.008	0.008	0.006	0.001	0.015	-	0.019
80	0.060	0.103	0.046	0.021	0.056	0.052	0.051	0.054	0.011	0.007	0.018	0.007	0.012	0.008	0.017	0.000	0.004	0.009	0.012	0.000	0.013	0.008	0.008	0.000
81	0.058	0.093	0.081	0.079	0.095	0.118	0.113	-	0.000	0.001	0.096	0.151	0.133	-	0.031	-	0.000	0.001	0.060	0.118	0.082	-	0.015	-
82	0.077	0.102	0.057	0.062	0.055	0.048	0.065	0.089	0.000	0.020	0.000	0.000	0.000	0.004	0.033	0.019	0.000	0.028	0.022	0.002	0.000	0.006	0.008	0.005
83	0.072	0.068	0.039	0.063	0.145	0.052	0.063	0.058	0.000	0.012	0.000	0.001	0.000	0.000	0.022	0.063	0.000	0.006	0.000	0.006	0.000	0.000	0.007	0.027
84	0.066	0.111	0.039	0.047	0.084	0.106	0.107	0.103	0.000	0.016	0.000	0.201	-	0.070	0.107	0.090	0.001	0.003	0.008	0.191	-	0.054	0.047	0.076

Table A11 (contd..): Immune response data pertaining to controls on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
85	0.075	0.101	0.039	0.102	0.057	0.161	0.075	-	0.000	0.005	-	0.114	0.103	0.126	-	-	0.000	0.001	-	0.093	0.057	0.066	-	-
86	0.079	0.043	-	0.054	0.092	0.057	-	-	0.009	0.004	-	0.005	0.000	0.019	-	0.041	0.007	0.008	-	0.004	0.000	0.015	-	0.018
87	0.066	0.067	0.048	0.079	0.086	0.072	0.068	0.069	0.000	0.014	0.017	0.000	0.000	0.000	0.038	0.002	0.000	0.014	0.014	0.005	0.000	0.000	0.010	0.001
88	0.073	0.089	0.065	0.038	0.058	0.065	0.086	0.101	0.000	0.019	0.000	0.005	0.000	0.013	-	0.111	0.004	0.021	0.010	0.000	0.000	0.013	-	0.044
89	0.066	0.094	0.038	0.057	0.102	-	-	-	0.007	0.003	0.001	0.005	-	0.039	-	-	0.004	0.008	0.004	0.000	-	0.023	-	-
90	0.052	0.110	0.060	0.054	0.072	0.058	0.057	-	0.000	0.015	0.094	0.200	-	0.104	0.192	-	0.002	0.013	0.059	0.176	-	0.054	0.030	-
91	0.059	0.061	0.053	0.028	0.051	0.063	0.057	0.068	0.004	0.005	0.012	0.000	0.000	0.018	0.000	0.000	0.000	0.004	0.014	0.000	0.000	0.000	0.002	0.000
92	0.058	0.080	0.158	0.066	0.055	0.089	0.058	0.060	0.002	0.022	0.003	0.006	0.013	0.002	0.003	0.000	0.003	0.016	0.003	0.011	0.000	0.000	0.000	0.000
93	0.060	0.105	0.058	0.101	0.097	0.051	0.075	-	0.000	0.000	0.000	0.001	0.002	0.000	0.114	-	0.001	0.000	0.004	0.000	0.004	0.002	0.070	-
94	0.048	0.116	0.072	0.064	0.063	0.055	0.071	0.058	0.007	0.000	0.000	0.000	0.011	-	0.003	0.007	0.003	0.000	0.005	0.000	0.018	-	0.005	0.005
95	0.055	0.063	0.040	0.038	0.053	0.056	0.099	-	0.000	0.016	0.003	0.000	0.010	0.001	0.104	-	0.001	0.013	0.004	0.000	0.013	0.009	0.040	-
96	0.089	0.068	0.065	0.034	0.050	0.059	0.060	0.070	0.000	0.000	0.005	0.005	0.086	-	0.007	0.000	0.000	0.000	0.006	0.000	0.057	-	0.008	0.001
97	0.059	0.047	0.039	0.229	0.077	0.056	0.096	0.090	0.002	0.000	0.008	0.007	0.005	0.074	0.190	0.030	0.000	0.000	0.001	0.005	0.009	0.065	0.087	0.016
98	0.053	0.125	0.039	0.033	0.085	0.070	0.083	0.082	0.000	0.000	0.003	0.004	0.010	0.014	0.005	0.007	0.000	0.000	0.000	0.000	0.007	0.006	0.002	0.008
99	0.067	0.055	0.058	0.243	0.113	0.095	0.081	-	0.016	0.001	-	0.016	0.003	0.031	0.009	-	0.018	0.002	-	0.015	0.001	0.009	0.006	-
100	0.074	0.082	0.052	0.136	0.084	0.055	0.064	0.066	0.000	0.007	0.022	0.007	0.021	0.020	0.016	0.019	0.000	0.008	0.010	0.011	0.012	0.000	0.014	0.008
101	0.058	0.092	0.040	0.067	0.054	0.076	0.058	0.081	0.006	0.000	0.001	0.003	0.000	0.012	0.010	0.000	0.004	0.000	0.006	0.003	0.000	0.015	0.001	0.000
102	0.076	0.059	0.066	0.040	0.051	0.059	0.071	0.092	0.000	0.006	0.000	0.004	0.000	0.000	0.710	0.235	0.000	0.008	0.000	0.000	0.000	0.006	0.193	0.147
103	0.061	0.074	0.058	0.088	0.063	0.064	0.102	-	0.000	0.000	0.000	0.000	0.000	0.020	0.108	-	0.000	0.000	0.000	0.007	0.000	0.018	0.068	-
104	0.075	0.065	0.073	0.062	0.134	0.102	0.413	0.344	0.000	0.030	0.022	0.006	0.008	0.002	0.576	0.675	0.000	0.020	0.015	0.000	0.002	0.013	0.225	0.397
105	0.067	0.059	0.066	0.037	0.062	0.060	0.064	0.067	0.001	0.000	0.148	0.005	0.023	0.000	0.073	0.001	0.000	0.000	0.120	0.012	0.020	0.004	0.000	0.000
106	0.058	0.066	0.080	0.073	0.064	0.060	0.078	-	0.000	0.000	0.009	0.000	0.004	0.085	0.040	-	0.005	0.008	0.009	0.000	0.009	0.062	0.017	-
107	0.054	0.078	0.098	0.043	0.083	0.057	0.057	-	0.007	0.000	0.015	0.002	0.000	0.000	0.000	-	0.004	0.000	0.017	0.004	0.000	0.000	0.010	-
108	0.062	0.058	0.055	0.141	0.143	0.081	0.189	0.167	0.000	0.000	0.010	0.001	0.016	0.029	-	0.086	0.001	0.005	0.010	0.007	0.013	0.009	-	0.043
109	0.063	0.076	0.048	0.064	0.057	0.075	0.068	0.070	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.008	0.002	0.000	0.000	0.001	0.007	0.003	0.000	0.006
110	0.076	0.102	0.045	0.030	0.052	0.063	0.139	-	0.000	0.015	0.000	0.000	0.031	0.056	0.083	-	0.000	0.009	0.000	0.008	0.014	0.033	0.069	-
111	0.067	0.051	0.037	0.041	0.063	0.049	0.086	-	0.009	0.000	0.000	0.000	0.000	0.000	0.032	-	0.008	0.003	0.000	0.000	0.002	0.000	0.022	-
112	-	0.079	0.042	0.051	0.082	0.075	0.090	-	0.000	0.009	-	0.035	0.131	0.084	0.114	-	0.000	0.015	-	0.031	0.076	0.015	0.043	-
113	0.059	0.063	0.041	0.027	0.059	0.057	0.052	0.056	0.000	0.016	0.000	0.006	0.001	0.017	0.009	0.000	0.000	0.013	0.007	0.000	0.007	0.019	0.009	0.000
114	0.222	0.060	0.095	0.298	0.055	0.055	0.058	-	0.000	0.009	0.011	0.000	0.013	0.013	0.020	-	0.000	0.011	0.008	0.000	0.007	0.004	0.011	-
115	0.070	0.064	0.074	0.080	0.098	0.084	0.155	-	0.000	0.000	0.128	0.090	0.110	0.165	0.012	-	0.000	0.000	0.080	0.074	0.065	0.097	0.000	-
116	0.056	0.061	0.040	0.036	0.091	0.353	0.272	0.158	0.014	0.009	0.025	0.003	0.019	0.055	0.036	0.036	0.013	0.006	0.014	0.000	0.019	0.023	0.007	0.018
117	0.055	0.064	0.041	0.080	0.058	0.067	0.059	0.055	0.000	0.001	0.045	0.030	0.003	0.006	0.026	0.005	0.003	0.011	0.032	0.022	0.010	0.008	0.000	0.007
118	0.066	0.057	0.082	0.043	0.050	0.051	0.062	0.064	0.000	0.000	0.000	0.000	0.000	0.016	0.004	0.019	0.000	0.000	0.014	0.000	0.000	0.018	0.005	0.012
119	0.050	0.075	0.040	0.065	0.050	0.067	0.058	0.057	0.000	0.000	0.030	0.004	0.011	0.012	0.034	0.001	0.000	0.000	0.048	0.003	0.000	0.016	0.023	0.000
120	0.062	0.085	0.074	0.062	0.056	0.071	0.198	-	0.000	0.015	0.005	0.000	0.000	0.129	0.049	-	0.002	0.006	0.004	0.010	0.001	0.081	0.018	-
121	0.049	0.062	0.043	0.032	0.055	0.053	0.055	0.062	0.006	0.013	0.018	0.006	0.010	0.043	0.022	0.011	0.000	0.008	0.000	0.006	0.000	0.000	0.002	0.005
122	0.111	0.064	0.042	0.063	0.077	0.053	0.148	0.091	0.011	0.000	0.005	0.000	0.002	0.004	0.016	0.004	0.011	0.000	0.009	0.015	0.006	0.001	0.011	0.003

Table A12: Immune response data pertaining to vaccinates on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
1	0.079	1.047	0.775	0.810	0.516	0.327	0.574	-	0.000	1.732	0.322	0.122	0.370	0.241	0.181	-	0.000	1.382	0.273	0.120	0.344	0.213	0.160	-
2	0.071	0.471	0.218	1.819	0.257	0.198	0.160	0.145	0.000	0.412	0.071	0.051	0.022	0.071	0.050	0.017	0.000	0.391	0.043	0.048	0.031	0.063	0.013	0.013
3	0.066	1.060	0.365	0.402	0.198	0.255	0.202	0.253	0.009	0.625	0.554	0.140	0.142	0.068	0.125	0.062	0.005	0.481	0.492	0.132	0.144	0.061	0.110	0.058
4	0.075	0.228	0.164	0.145	0.183	0.092	0.099	0.134	0.001	0.639	0.049	-	0.144	0.022	0.032	0.017	0.000	0.526	0.060	-	0.143	0.013	0.013	0.013
5	0.069	0.844	0.344	0.337	0.336	0.345	0.498	0.316	0.006	0.214	0.032	0.025	0.006	0.023	0.117	0.000	0.000	0.177	0.013	0.024	0.000	0.009	0.091	0.000
6	0.087	0.925	1.046	0.868	0.532	0.461	0.596	0.732	0.000	0.542	0.082	0.002	0.029	0.158	0.101	0.032	0.002	0.416	0.073	0.006	0.098	0.122	0.060	0.025
7	0.079	2.158	1.008	0.155	0.477	1.043	0.950	-	0.000	0.238	0.086	0.173	0.035	0.062	0.019	-	0.000	0.198	0.078	0.161	0.029	0.060	0.011	-
8	0.125	0.077	0.367	0.702	0.209	0.121	0.115	0.128	0.000	0.029	0.000	0.000	0.029	0.000	0.026	0.000	0.000	0.023	0.000	0.008	0.022	0.000	0.005	0.002
9	0.069	1.388	1.241	1.071	0.865	0.797	0.676	0.855	0.000	0.192	0.100	0.094	0.027	0.029	0.020	0.006	0.000	0.156	0.083	0.081	0.029	0.023	0.008	0.004
10	0.065	0.348	0.205	0.101	0.183	0.179	0.185	-	0.009	0.498	0.231	0.091	0.106	0.139	0.024	-	0.012	0.365	0.157	0.082	0.151	0.117	0.016	-
11	0.059	1.660	1.690	2.093	1.382	1.362	1.326	1.149	0.030	0.402	0.200	0.009	0.046	0.042	0.032	0.001	0.000	0.261	0.160	0.002	0.040	0.030	0.012	0.003
12	0.084	-	0.600	0.812	0.341	-	0.207	-	0.000	-	0.491	0.429	0.096	-	0.053	-	0.000	-	0.386	0.350	0.085	-	0.037	-
13	0.136	0.302	0.145	0.182	0.137	0.107	0.090	0.121	0.006	0.238	0.016	0.084	0.023	0.099	0.075	0.018	0.005	0.245	0.006	0.082	0.018	0.085	0.059	0.019
14	0.070	-	0.088	0.065	0.052	0.097	0.068	0.102	0.016	-	0.000	0.000	0.018	0.000	0.008	0.005	0.015	-	0.003	0.000	0.010	0.009	0.004	0.002
15	-	1.014	1.363	1.225	0.832	0.725	0.816	0.706	0.004	0.429	0.060	0.036	0.014	0.012	0.000	0.038	0.007	0.324	0.016	0.034	0.010	0.011	0.006	0.029
16	0.077	0.052	0.231	0.204	0.055	0.101	-	-	0.000	0.041	0.028	0.011	0.007	0.000	0.003	-	0.000	0.026	0.004	0.000	0.001	0.000	0.000	-
17	0.068	0.745	0.262	0.482	0.187	0.262	0.245	0.250	0.000	0.845	0.358	0.648	0.047	0.058	0.040	0.142	0.000	0.653	0.309	0.577	0.036	0.060	0.039	0.132
18	0.083	0.295	0.211	-	0.225	0.118	0.191	-	0.000	1.113	0.869	0.177	0.398	0.858	0.053	-	0.003	0.860	0.603	0.144	0.362	0.742	0.022	-
19	0.089	1.090	0.494	0.526	0.378	0.617	0.402	0.084	0.000	0.460	0.205	-	0.060	0.069	0.046	0.091	0.000	0.415	0.165	-	0.056	0.061	0.044	0.055
20	0.066	1.523	0.406	0.563	0.262	0.318	0.404	0.283	0.000	0.286	0.083	0.008	0.020	0.026	0.016	0.000	0.005	0.222	0.065	0.008	0.028	0.017	0.012	0.017
21	0.062	0.303	0.134	0.130	0.075	0.091	0.098	-	0.000	0.307	0.061	0.130	0.071	0.019	0.047	-	0.000	0.298	0.051	0.121	0.068	0.011	0.032	-
22	0.114	1.560	1.007	0.864	0.392	0.384	0.239	-	0.000	0.351	0.147	-	0.051	0.150	0.226	-	0.000	0.318	0.131	-	0.053	0.148	0.219	-
23	0.156	0.302	0.393	0.433	0.305	0.802	0.555	0.387	0.006	0.564	0.315	0.078	0.132	0.057	0.095	0.029	0.006	0.520	0.287	0.076	0.130	0.052	0.077	0.027
24	0.084	0.841	0.601	0.172	0.408	0.298	0.242	0.316	0.005	0.410	0.000	0.260	0.000	0.051	0.105	0.086	0.007	0.327	0.000	0.225	0.012	0.042	0.099	0.071
25	0.101	1.902	1.438	2.303	1.067	0.977	0.962	0.903	0.006	0.808	0.768	0.296	0.082	0.081	0.531	0.252	0.000	0.665	0.594	0.268	0.089	0.074	0.420	0.224
26	0.069	2.840	1.577	0.114	1.085	0.117	2.006	0.936	0.000	0.531	0.260	0.619	0.429	0.481	0.222	0.205	0.002	0.319	0.184	0.550	0.388	0.446	0.164	0.146
27	0.075	0.437	0.138	0.162	0.110	0.108	0.112	-	0.005	0.322	0.086	0.112	0.061	0.026	0.033	-	0.003	0.266	0.072	0.114	0.061	0.036	0.034	-
28	0.071	-	1.978	0.360	1.442	1.584	1.659	1.555	0.000	0.557	0.394	0.023	0.113	0.122	0.535	0.037	0.000	0.477	0.274	0.023	0.000	0.109	0.298	0.039
29	0.085	0.443	0.218	1.310	0.159	0.259	0.219	-	0.000	-	1.584	0.839	0.796	0.375	0.943	-	0.003	-	1.402	0.795	0.729	0.344	0.826	-
30	0.075	0.669	0.427	0.421	0.228	0.316	0.225	0.300	0.006	0.529	0.162	0.041	0.007	0.054	0.042	0.041	0.007	0.419	0.158	0.033	0.006	0.037	0.031	0.036
31	0.132	0.737	0.233	0.378	0.112	0.132	0.155	0.127	0.000	0.917	0.252	0.240	0.262	0.186	0.214	0.069	0.000	0.602	0.235	0.228	0.246	0.188	0.176	0.067
32	0.068	1.816	1.031	1.398	0.891	0.898	1.162	0.916	0.011	0.236	0.012	0.032	0.000	0.022	0.018	0.007	0.018	0.190	0.022	0.026	0.007	0.016	0.011	0.007
33	0.083	0.524	0.505	0.475	0.261	0.314	0.181	0.245	0.000	0.383	0.084	0.053	0.011	0.070	0.067	0.019	0.000	0.211	0.075	0.048	0.014	0.075	0.036	0.015
34	0.070	1.797	1.823	0.337	0.954	1.177	-	-	0.000	0.338	0.159	0.097	0.133	0.146	0.259	0.124	0.002	0.284	0.054	0.079	0.111	0.095	0.127	0.063
35	0.078	0.654	0.175	1.283	0.220	0.194	0.146	-	0.000	0.083	0.251	0.004	0.002	0.000	0.029	-	0.001	0.074	0.234	0.007	0.002	0.000	0.021	-
36	0.078	0.534	0.439	0.461	0.173	0.175	0.176	0.188	0.000	0.460	0.085	0.031	0.022	0.003	0.050	0.020	0.000	0.419	0.061	0.017	0.015	0.018	0.042	0.015
37	0.077	0.433	0.599	1.095	0.430	0.480	0.446	0.579	0.006	0.192	0.023	0.026	0.199	0.032	0.007	0.024	0.007	0.161	0.016	0.018	0.176	0.037	0.009	0.021
38	0.066	0.857	0.425	0.484	0.289	0.424	0.296	-	0.003	0.330	0.135	0.220	0.009	0.115	0.060	-	0.019	0.277	0.123	0.227	0.015	0.095	0.048	-
39	0.075	0.279	0.124	0.423	0.130	0.119	0.199	-	0.002	1.307	0.707	0.107	0.027	0.088	0.103	-	0.000	0.936	0.542	0.104	0.026	0.069	0.078	-
40	0.080	0.326	0.585	0.206	0.178	0.722	0.179	0.299	0.000	0.258	0.533	0.097	0.140	0.017	0.436	0.079	0.001	0.180	0.399	0.093	0.130	0.016	0.339	0.060
41	0.063	0.619	0.417	0.881	0.369	0.562	0.226	0.488	0.002	0.296	0.514	0.250	0.029	0.110	0.092	0.021	0.007	0.276	0.389	0.228	0.023	0.099	0.078	0.015
42	0.060	0.610	1.497	0.355	0.964	0.238	0.119	-	0.000	0.023	0.118	0.037	0.000	0.028	0.004	-	0.000	0.020	0.108	0.031	0.000	0.037	0.006	-

Table A12 (contd..): Immune response data pertaining to vaccinates on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
43	0.061	1.684	1.338	0.285	2.114	1.753	2.462	2.004	0.000	0.354	0.244	0.229	0.138	0.014	0.097	0.027	0.003	0.272	0.225	0.194	0.135	0.000	0.051	0.020
44	0.067	0.315	0.136	0.404	0.121	0.123	0.116	-	0.002	0.122	0.088	0.055	0.020	0.032	0.014	-	0.000	0.105	0.070	0.049	0.020	0.027	0.015	-
45	0.063	0.664	0.970	0.987	0.958	0.540	0.581	0.523	0.001	0.701	0.579	0.133	1.053	0.700	0.410	0.120	0.000	0.575	0.433	0.128	0.994	0.658	0.370	0.109
46	0.060	0.404	0.097	0.200	0.094	1.116	0.100	0.090	0.000	0.367	0.134	0.014	0.013	0.031	0.046	0.018	0.000	0.278	0.102	0.008	0.015	0.052	0.034	0.012
47	0.056	0.629	0.405	2.240	0.235	0.313	0.310	0.332	0.006	0.817	0.384	1.132	0.414	0.320	0.213	0.045	0.006	0.599	0.321	1.052	0.370	0.289	0.180	0.038
48	0.072	2.395	1.216	0.607	1.424	1.213	1.905	-	0.000	0.437	0.191	0.062	0.074	0.026	0.073	-	0.000	0.361	0.153	0.067	0.073	0.029	0.064	-
49	0.060	0.626	0.362	0.386	0.232	0.323	0.261	0.243	0.000	0.235	0.100	0.075	0.029	0.059	0.052	0.009	0.000	0.200	0.081	0.069	0.025	0.045	0.044	0.014
50	0.070	0.074	0.367	0.070	0.064	0.075	0.183	-	0.000	0.030	0.047	0.003	0.000	0.013	0.029	-	0.001	0.022	0.041	0.002	0.000	0.010	0.013	-
51	0.066	1.281	0.513	0.480	0.346	0.309	0.530	0.355	0.001	0.154	0.455	0.076	0.164	0.017	0.083	0.087	0.003	0.107	0.384	0.105	0.147	0.026	0.065	0.075
52	0.068	-	1.083	0.305	0.881	0.174	0.707	0.537	0.000	-	0.201	0.332	0.041	0.035	0.071	0.126	0.000	-	0.160	0.306	0.043	0.028	0.036	0.052
53	0.074	0.808	0.712	0.180	0.734	0.515	0.733	0.537	0.000	0.322	0.039	0.025	0.029	0.011	0.014	0.001	0.002	0.255	0.019	0.022	0.022	0.007	0.000	0.004
54	0.077	0.309	0.123	-	0.237	0.128	0.112	-	0.001	0.040	0.192	0.068	0.101	0.018	0.251	-	0.000	0.031	0.166	0.068	0.093	0.020	0.211	-
55	0.086	0.767	0.463	0.607	0.537	0.738	0.449	0.422	0.000	0.060	0.004	-	0.000	0.000	0.009	0.004	0.002	0.061	0.010	-	0.003	0.000	0.013	0.000
56	0.102	0.471	0.074	0.932	0.083	0.089	0.085	0.133	0.008	0.473	0.106	0.081	-	0.058	0.376	0.000	0.006	0.402	0.080	0.047	-	0.058	0.283	0.000
57	0.058	0.819	0.184	0.292	0.213	0.134	0.098	0.136	0.000	0.251	0.190	0.242	0.105	0.192	0.152	0.081	0.000	0.211	0.177	0.230	0.086	0.173	0.140	0.075
58	0.063	0.478	0.138	1.403	0.095	0.072	0.098	-	0.003	0.355	0.207	0.340	0.055	0.271	0.125	-	0.008	0.288	0.181	0.337	0.059	0.248	0.090	-
59	0.071	0.295	0.134	0.225	0.163	0.123	0.114	0.139	0.004	0.073	0.232	0.031	0.005	0.022	0.008	0.014	0.003	0.061	0.198	0.011	0.007	0.036	0.006	0.010
60	0.055	0.592	0.294	0.458	-	-	-	-	0.000	0.558	0.068	0.000	-	-	-	-	0.000	0.491	0.068	0.000	-	-	-	-
61	0.052	0.997	0.581	0.442	0.454	0.755	0.466	-	0.007	1.670	0.850	-	-	0.380	0.166	-	0.004	1.256	0.560	-	-	0.293	0.135	-
62	0.115	0.883	0.751	0.571	0.390	0.757	0.770	0.189	0.003	0.182	0.182	0.004	0.028	0.024	0.025	0.009	0.001	0.178	0.151	0.000	0.016	0.022	0.013	0.009
63	0.124	0.250	0.258	0.130	0.151	0.299	0.246	-	0.000	0.510	-	0.088	0.100	0.061	0.033	-	0.000	0.428	-	0.082	0.089	0.025	0.028	-
64	0.058	0.323	0.214	0.246	0.156	0.203	0.241	0.472	0.000	0.031	0.487	0.164	0.125	0.182	0.061	0.054	0.000	0.041	0.401	0.145	0.068	0.159	0.054	0.038
65	0.064	1.726	0.791	0.784	0.917	0.603	1.051	0.638	0.009	0.203	0.324	0.047	0.004	0.045	0.039	0.024	0.000	0.168	0.248	0.043	0.003	0.017	0.015	0.013
66	0.064	0.140	0.206	0.065	0.194	0.084	0.177	0.202	0.002	0.193	0.219	0.084	0.148	0.027	0.109	0.062	0.005	0.170	0.189	0.071	0.126	0.029	0.080	0.052
67	0.078	0.181	0.364	0.414	0.203	0.215	0.204	0.195	0.007	0.708	0.124	0.069	0.090	0.155	0.068	0.032	0.006	0.460	0.084	0.074	0.086	0.091	0.022	0.000
68	0.078	0.992	0.228	0.223	0.120	0.161	0.115	-	0.000	1.134	0.264	0.102	0.050	0.072	0.030	-	0.000	0.934	0.230	0.057	0.041	0.076	0.022	-
69	0.062	1.761	0.905	1.749	0.687	1.168	1.454	0.906	0.004	0.337	0.059	0.062	0.058	0.019	0.005	0.069	0.005	0.254	0.067	0.060	0.071	0.020	0.008	0.054
70	0.074	0.318	0.156	0.164	0.111	0.117	0.146	0.143	0.000	0.728	0.250	0.093	0.000	0.012	0.048	0.031	0.000	0.540	0.206	0.085	0.000	0.000	0.042	0.029
71	0.060	0.472	0.144	1.098	0.094	0.177	0.131	-	0.000	0.296	0.229	0.177	0.024	0.077	0.004	-	0.002	0.221	0.158	0.139	0.032	0.065	0.003	-
72	0.049	0.428	0.112	0.292	0.102	0.118	0.081	0.090	0.001	0.669	0.253	0.114	0.054	0.075	0.042	0.033	0.000	0.489	0.211	0.101	0.033	0.071	0.030	0.023
73	0.066	1.312	0.798	1.225	0.444	0.430	0.476	0.517	0.000	0.405	0.228	0.185	0.058	0.075	0.077	0.021	0.000	0.335	0.192	0.176	0.052	0.000	0.059	0.024
74	0.076	2.307	1.391	0.230	0.558	0.730	0.656	0.677	0.005	0.067	0.024	0.071	0.098	0.041	0.029	0.028	0.005	0.048	0.021	0.063	0.076	0.025	0.008	0.017
75	0.097	1.832	0.532	0.674	0.336	0.255	0.401	0.413	0.000	0.279	0.176	0.034	0.011	0.018	0.048	0.003	0.004	0.217	0.150	0.046	0.015	0.021	0.039	0.001
76	0.069	0.321	0.122	0.192	0.112	0.133	0.096	0.345	0.004	0.773	0.125	0.072	0.014	0.042	0.070	0.009	0.000	0.544	0.094	0.068	0.001	0.031	0.046	0.019
77	0.054	0.571	0.200	0.152	0.131	0.192	0.246	0.157	0.002	2.844	0.907	0.245	0.043	0.060	0.040	0.064	0.000	2.194	0.704	0.229	0.035	0.051	0.033	0.046
78	0.061	1.266	0.224	0.062	0.219	0.229	0.159	-	0.014	0.172	0.167	0.019	0.037	0.033	0.029	-	0.016	0.124	0.136	0.018	0.028	0.024	0.020	-
79	0.064	0.756	0.511	0.512	0.214	0.333	0.251	0.345	0.001	1.203	0.575	0.765	0.045	0.061	0.028	0.012	0.003	0.972	0.475	0.667	0.041	0.056	0.020	0.011
80	0.063	0.289	0.353	0.209	0.128	-	0.123	-	0.006	0.601	0.133	0.368	0.117	-	0.037	-	0.003	0.541	0.087	0.340	0.096	-	0.025	-
81	0.063	0.236	0.072	0.101	0.081	0.090	0.103	-	0.000	0.184	0.059	0.003	0.011	0.000	0.036	-	0.000	0.165	0.064	0.007	0.008	0.000	0.034	-
82	0.095	0.296	0.123	0.580	0.143	0.103	0.125	0.155	0.000	0.427	0.297	0.048	0.053	0.113	0.108	0.006	0.001	0.357	0.273	0.045	0.053	0.100	0.110	0.000
83	0.057	0.459	0.182	0.226	0.123	0.174	0.140	0.134	0.007	1.120	0.451	0.112	0.122	0.093	0.050	0.026	0.005	0.933	0.428	0.139	0.115	0.093	0.055	0.024
84	0.066	0.881	1.355	2.201	0.720	0.699	0.659	0.624	0.013	0.207	0.189	0.218	0.037	0.247	0.000	0.007	0.009	0.167	0.159	0.202	0.032	0.224	0.000	0.011

Table A12 (contd..): Immune response data pertaining to vaccinates on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
85	0.053	0.443	0.445	0.314	0.296	0.353	0.440	0.320	0.000	0.075	0.097	0.028	0.055	0.044	0.025	0.015	0.000	0.063	0.083	0.020	0.007	0.031	0.018	0.006
86	0.056	1.810	1.215	1.574	1.166	1.164	0.925	0.836	0.000	0.670	0.234	0.203	0.013	0.057	0.054	0.046	0.001	0.570	0.200	0.186	0.010	0.060	0.050	0.039
87	0.060	0.395	0.271	1.444	0.408	0.205	0.242	-	0.003	0.474	0.631	0.311	0.157	0.390	0.103	-	0.002	0.377	0.505	0.282	0.133	0.329	0.095	-
88	0.064	0.232	0.224	0.713	0.148	0.100	0.131	-	0.000	0.340	0.060	0.043	0.026	0.000	0.017	-	0.017	0.277	0.045	0.031	0.023	0.012	0.000	-
89	0.084	0.331	0.156	0.109	0.120	0.165	0.144	0.122	0.007	0.201	0.248	0.039	0.019	0.024	0.069	0.015	0.006	0.172	0.190	0.037	0.019	0.017	0.013	0.007
90	0.065	0.178	0.094	0.239	0.110	0.106	0.096	0.104	0.011	0.204	0.029	0.043	0.012	0.033	0.030	0.005	0.017	0.173	0.024	0.038	0.008	0.041	0.028	0.005
91	0.067	0.105	0.103	0.111	0.140	-	0.091	-	0.000	0.000	0.049	0.020	0.000	-	0.003	-	0.000	0.000	0.052	0.014	0.000	-	0.004	-
92	0.068	0.363	0.115	0.109	0.097	0.141	0.118	-	0.000	0.422	0.490	0.373	0.288	0.165	0.138	-	0.001	0.377	0.422	0.348	0.270	0.126	0.092	-
93	0.086	0.110	-	0.729	0.614	0.671	0.615	-	0.000	0.023	-	0.022	-	0.040	0.012	-	0.000	0.025	-	0.020	-	0.042	0.007	-
94	0.057	0.637	0.251	0.232	0.190	0.212	0.282	0.265	0.000	0.043	0.009	0.029	0.019	0.000	0.037	0.007	0.000	0.014	0.009	0.052	0.009	0.000	0.034	0.000
95	0.066	1.581	0.797	0.952	0.420	0.458	0.385	0.360	0.010	0.277	0.122	0.084	0.000	0.117	0.319	0.079	0.009	0.225	0.110	0.074	0.014	0.095	0.229	0.070
96	0.070	0.270	0.791	0.715	0.112	0.301	0.104	-	0.000	2.289	0.684	0.239	0.300	0.125	0.079	-	0.001	1.799	0.581	0.245	0.282	0.110	0.062	-
97	0.071	0.923	0.619	0.991	0.518	0.391	0.457	-	0.008	0.067	0.043	0.017	0.000	0.000	0.000	-	0.005	0.064	0.041	0.012	0.000	0.015	0.004	-
98	0.067	0.783	0.648	0.318	0.241	0.344	0.394	0.350	0.000	0.238	0.029	0.068	0.074	0.025	0.040	0.001	0.000	0.130	0.046	0.096	0.066	0.019	0.045	0.004
99	0.076	1.627	1.016	1.296	0.601	-	0.586	0.446	0.008	0.134	0.000	0.036	0.035	-	0.000	0.028	0.000	0.115	0.026	0.035	0.024	-	0.017	0.026
100	0.062	0.624	0.179	0.251	0.178	0.256	0.320	-	0.019	0.064	0.084	0.014	0.013	0.016	0.016	-	0.159	0.064	0.065	0.010	0.011	0.019	0.021	-
101	0.076	1.608	0.856	0.896	0.598	0.816	0.454	-	0.000	0.217	0.050	0.007	0.000	0.145	0.003	-	0.008	0.182	0.039	0.011	0.002	0.152	0.000	-
102	0.064	0.093	0.077	0.142	0.138	0.270	-	-	0.001	0.027	0.058	-	0.046	0.636	0.001	-	0.002	0.031	0.057	-	0.036	0.339	0.000	-
103	0.066	1.009	0.690	0.646	0.329	0.435	0.301	-	0.001	0.962	0.191	0.323	0.155	0.192	0.221	-	0.005	0.586	0.142	0.293	0.153	0.186	0.188	-
104	0.060	0.198	0.193	0.250	0.193	0.162	0.165	0.165	0.000	0.535	0.246	0.144	0.066	0.021	0.140	0.032	0.000	0.499	0.213	0.118	0.058	0.025	0.085	0.029
105	0.064	0.618	0.247	0.268	0.170	-	0.334	-	0.000	0.348	0.402	0.290	0.130	-	0.158	-	0.000	0.325	0.321	0.254	0.134	-	0.147	-
106	0.081	0.303	0.327	0.119	0.212	0.141	0.179	-	0.007	0.120	0.154	0.108	0.029	0.109	0.030	-	0.003	0.118	0.064	0.101	0.033	0.103	0.023	-
107	0.073	0.968	0.414	0.288	0.290	0.241	0.306	0.279	0.000	0.287	0.096	0.036	0.000	0.047	0.014	0.037	0.000	0.223	0.062	0.032	0.005	0.031	0.017	0.011
108	0.066	1.068	0.718	0.369	0.278	0.266	0.210	0.148	0.000	0.242	0.083	0.022	0.045	0.173	0.011	0.022	0.000	0.213	0.065	0.008	0.048	0.161	0.026	0.016
109	0.064	0.413	0.399	0.171	0.260	0.199	0.182	0.152	0.000	0.080	0.054	0.050	0.006	0.006	0.005	0.001	0.000	0.062	0.033	0.053	0.004	0.009	0.000	0.003
110	0.070	0.911	0.479	0.658	0.512	0.560	0.395	0.289	0.000	0.320	0.143	0.125	0.124	0.126	0.348	0.056	0.001	0.253	0.132	0.087	0.106	0.106	0.258	0.045
111	0.059	1.716	0.677	1.607	0.762	0.722	1.000	1.105	0.000	0.079	0.092	0.052	0.036	0.179	0.099	0.025	0.000	0.065	0.066	0.050	0.033	0.152	0.090	0.021
112	0.073	0.422	0.401	0.307	0.207	0.196	0.249	-	0.000	0.268	0.187	0.016	0.079	0.031	0.056	-	0.000	0.251	0.148	0.014	0.079	0.030	0.040	-
113	0.076	0.302	0.462	0.308	-	0.371	0.373	0.247	0.000	1.201	0.175	0.023	-	0.002	0.042	0.041	0.004	0.993	0.137	0.020	-	0.002	0.028	0.021
114	0.061	0.509	0.548	0.571	0.293	0.257	0.357	0.316	0.003	1.391	0.167	0.148	0.000	0.032	0.046	0.006	0.003	1.183	0.150	0.181	0.002	0.019	0.045	0.006
115	0.059	1.516	0.249	0.421	0.150	0.186	0.126	0.112	0.000	0.317	0.216	0.037	0.048	0.008	0.020	0.000	0.000	0.263	0.193	0.027	0.053	0.025	0.015	0.000
116	0.052	0.819	0.835	0.762	0.439	0.113	0.456	-	0.000	0.445	0.123	0.050	0.000	0.022	0.012	-	0.000	0.359	0.109	0.041	0.000	0.020	0.015	-
117	0.060	0.285	0.146	0.265	0.155	0.151	0.124	0.174	0.000	0.392	0.326	-	0.080	0.068	0.216	0.011	0.004	0.294	0.275	-	0.064	0.061	0.186	0.018
118	0.063	0.980	0.469	0.419	0.208	0.301	0.250	-	0.000	0.050	0.044	0.014	0.003	0.011	0.025	-	0.000	0.036	0.037	0.012	0.011	0.018	0.011	-
119	0.086	0.723	0.685	0.543	0.174	0.342	0.179	-	0.000	0.071	0.300	0.076	0.038	0.026	0.038	-	0.000	0.059	0.266	0.055	0.048	0.021	0.037	-
120	0.065	0.226	0.277	0.557	0.116	0.152	0.056	-	0.006	0.083	0.034	0.005	0.057	0.024	0.018	-	0.006	0.070	0.012	0.009	0.054	0.023	0.014	-
121	0.063	0.983	1.290	1.586	1.005	1.032	0.853	-	0.000	0.569	0.225	0.136	0.094	0.416	0.076	-	0.000	0.472	0.202	0.131	0.083	0.367	0.063	-
122	0.055	0.836	0.192	1.505	0.170	0.243	0.212	-	0.002	0.086	0.308	0.123	0.012	0.085	0.017	-	0.000	0.083	0.220	0.117	0.012	0.073	0.009	-
123	0.075	0.299	0.120	0.384	0.170	1.176	0.311	0.386	0.011	0.191	0.109	0.013	0.273	0.608	0.094	0.012	0.006	0.165	0.096	0.004	0.244	0.472	0.077	0.013
124	0.065	1.088	0.300	0.083	0.161	0.191	-	-	0.000	0.057	0.017	-	0.011	0.019	-	-	0.001	0.071	0.009	-	0.011	0.030	-	-
125	0.065	0.157	0.086	-	0.080	0.115	0.156	-	0.007	0.193	0.248	0.263	0.032	0.027	0.040	-	0.000	0.169	0.146	0.235	0.018	0.030	0.035	-
126	0.053	0.710	0.327	0.454	0.427	0.236	0.390	0.394	0.002	0.091	0.021	0.079	0.000	0.091	0.016	0.018	0.000	0.080	0.022	0.077	0.000	0.067	0.007	0.016

Table A12 (contd..): Immune response data pertaining to vaccinates on property 3

Animal	Antibody OD values at months post-vaccination								IFN-γ (Johnin-nil) OD values at months post-vaccination								IFN-γ (Johnin-Avian) OD values at months post-vaccination							
	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42	0	2	8	12	18	24	36	42
127	0.054	1.101	0.456	0.579	0.347	0.415	0.395	0.264	0.002	0.091	0.369	0.127	0.038	0.038	0.156	0.040	0.003	0.073	0.248	0.104	0.028	0.048	0.121	0.033
128	0.052	1.224	0.485	0.761	0.194	0.231	0.392	-	0.000	0.569	0.165	0.064	0.054	0.073	0.013	-	0.000	0.466	0.167	0.047	0.046	0.071	0.007	-
129	0.064	0.352	0.320	0.200	0.248	0.329	0.599	0.709	0.000	0.189	0.171	0.033	0.015	0.016	0.155	0.068	0.000	0.164	0.141	0.030	0.026	0.015	0.122	0.054
130	0.062	0.601	0.135	0.596	0.117	0.107	0.113	-	0.000	0.394	0.065	0.054	0.026	0.069	0.017	-	0.000	0.335	0.059	0.047	0.029	0.036	0.012	-
131	0.061	-	0.363	1.142	0.170	0.176	0.097	-	0.000	0.167	0.015	0.020	0.091	0.025	0.142	-	0.001	0.117	0.022	0.013	0.081	0.006	0.110	-
132	0.061	0.706	0.119	0.133	0.118	0.206	0.092	0.099	0.000	0.661	0.062	0.068	0.024	0.048	0.079	0.010	0.000	0.577	0.074	0.086	0.025	0.039	0.065	0.009
133	0.091	0.321	0.101	0.450	0.090	0.070	0.116	-	0.005	0.082	0.144	0.075	0.059	0.014	0.003	-	0.008	0.063	0.138	0.081	0.044	0.011	0.002	-
134	0.134	0.362	0.232	0.201	0.164	0.091	0.117	-	0.003	0.461	0.354	0.004	0.070	0.006	0.055	-	0.003	0.416	0.315	0.013	0.068	0.003	0.046	-
135	0.059	0.458	0.237	0.101	0.164	0.130	0.144	0.157	0.002	0.110	0.013	0.026	0.002	0.032	0.028	0.004	0.001	0.090	0.000	0.037	0.003	0.033	0.017	0.000
136	0.067	0.889	0.244	1.671	0.178	0.143	0.159	-	0.000	0.019	0.017	0.024	0.003	0.000	0.010	-	0.002	0.030	0.008	0.008	0.013	0.004	0.010	-
137	0.058	1.671	0.876	0.129	0.430	0.487	0.394	-	0.002	0.066	0.035	0.127	0.065	0.045	0.059	-	0.000	0.072	0.015	0.114	0.065	0.035	0.060	-
138	0.075	1.081	1.066	0.335	0.619	0.612	0.657	0.480	0.000	0.291	0.221	0.054	0.029	0.025	0.055	0.017	0.002	0.230	0.181	0.043	0.020	0.013	0.037	0.005
139	0.063	1.471	1.185	0.258	0.483	0.624	0.547	0.457	0.002	0.047	0.041	0.021	0.010	0.000	0.011	0.007	0.002	0.044	0.012	0.019	0.015	0.005	0.013	0.005
140	0.055	0.661	0.707	0.599	0.386	0.542	0.734	0.709	0.000	0.170	0.048	0.026	0.026	0.009	0.000	0.002	0.002	0.145	0.030	0.020	0.014	0.005	0.002	0.001
141	0.094	0.085	0.046	0.064	0.064	0.067	0.062	-	0.000	0.022	0.022	0.006	0.073	0.017	0.033	-	0.001	0.014	0.021	0.033	0.060	0.013	0.011	-
142	0.068	2.053	0.825	0.891	0.397	0.365	0.779	0.562	0.000	0.109	0.111	0.021	0.006	0.195	0.071	0.042	0.000	0.106	0.086	0.011	0.003	0.174	0.042	0.028
143	0.066	0.298	0.654	0.483	0.239	0.424	0.283	0.329	0.000	0.017	0.031	0.000	0.001	0.023	0.053	0.000	0.003	0.014	0.021	0.000	0.005	0.020	0.016	0.000
144	0.064	0.807	0.445	0.183	0.238	0.230	0.262	0.191	0.000	0.096	0.202	0.031	0.033	0.053	0.042	0.062	0.000	0.081	0.196	0.031	0.016	0.041	0.046	0.054
145	0.061	0.409	0.238	0.911	0.148	0.180	0.144	0.128	0.000	0.247	0.127	0.015	0.035	0.079	0.063	0.005	0.001	0.203	0.112	0.025	0.027	0.085	0.064	0.009
146	0.059	0.372	0.117	0.141	0.117	0.108	0.117	-	0.001	0.111	0.073	-	0.016	0.023	0.016	-	0.001	0.095	0.047	-	0.009	0.016	0.010	-
147	0.099	0.541	0.796	0.226	0.585	0.513	0.486	-	0.001	0.120	0.090	0.020	0.000	0.000	0.003	-	0.000	0.098	0.088	0.000	0.000	0.009	0.010	-
148	0.072	0.824	0.206	0.132	0.177	0.094	0.131	-	0.000	0.432	0.045	0.049	0.050	0.031	0.007	-	0.003	0.363	0.036	0.048	0.000	0.025	0.016	-
149	0.087	0.543	0.722	0.227	0.343	0.416	0.316	-	0.005	0.301	0.332	0.118	0.132	0.232	0.099	-	0.003	0.239	0.285	0.101	0.105	0.189	0.067	-
150	0.069	1.579	0.673	0.167	0.262	0.306	0.419	0.366	0.000	0.076	0.176	0.144	0.063	0.161	0.108	0.027	0.000	0.059	0.145	0.140	0.051	0.149	0.090	0.025
151	0.059	0.347	0.095	0.166	0.073	0.108	0.120	-	0.007	0.748	0.078	0.069	0.058	0.054	0.008	-	0.009	0.661	0.059	0.059	0.056	0.052	0.008	-
152	0.074	0.934	0.626	0.815	0.235	0.817	0.231	-	0.000	0.052	0.073	0.010	0.000	0.007	0.002	-	0.001	0.050	0.049	0.008	0.000	0.010	0.002	-
153	0.060	0.282	0.194	0.658	0.159	0.436	0.154	0.170	0.006	0.467	0.206	0.109	0.025	0.037	0.121	0.102	0.003	0.397	0.171	0.113	0.019	0.028	0.077	0.086
154	0.050	0.396	0.189	1.298	0.142	0.125	0.144	0.119	0.000	0.345	0.094	0.185	0.158	0.030	0.061	0.069	0.003	0.283	0.075	0.190	0.137	0.040	0.052	0.063
155	0.060	1.299	1.178	1.531	0.675	0.773	0.861	0.460	0.004	0.504	0.407	0.035	0.036	0.074	0.083	0.033	0.003	0.442	0.293	0.025	0.036	0.058	0.072	0.032
156	0.050	1.809	0.304	0.356	0.150	0.203	0.164	0.173	0.000	0.071	0.101	0.038	0.104	0.005	0.062	0.017	0.000	0.081	0.093	0.031	0.095	0.020	0.052	0.015
157	0.063	0.217	0.262	0.369	0.283	0.439	0.910	-	0.007	0.028	0.000	0.016	0.004	0.004	0.034	-	0.002	0.018	0.008	0.009	0.000	0.001	0.020	-
158	0.075	0.456	-	0.112	0.092	0.101	0.100	0.113	0.002	0.044	-	0.008	0.000	0.040	0.066	0.000	0.000	0.043	-	0.007	0.001	0.020	0.044	0.000
159	0.059	0.403	0.391	0.240	0.129	0.158	0.134	0.146	0.009	1.081	0.058	0.250	0.281	0.061	0.083	0.014	0.002	1.053	0.026	0.241	0.271	0.054	0.080	0.017
160	0.046	0.150	0.072	0.124	0.081	0.106	0.092	0.116	0.000	0.658	0.413	0.448	0.000	0.039	0.067	0.006	0.000	0.598	0.416	0.419	0.003	0.037	0.063	0.010
161	0.046	0.729	0.578	0.289	0.265	0.291	0.197	0.201	0.014	0.054	0.214	0.013	0.030	0.024	0.028	0.016	0.010	0.040	0.137	0.014	0.023	0.028	0.018	0.014
162	0.044	0.620	0.292	0.275	0.124	-	0.097	0.103	0.000	0.328	0.117	0.024	0.013	-	0.023	0.016	0.000	0.261	0.098	0.023	0.012	-	0.019	0.010
163	0.051	0.393	0.344	0.139	0.195	0.217	0.273	0.296	0.000	0.255	0.100	0.073	0.049	0.052	0.006	0.006	0.000	0.267	0.094	0.059	0.044	0.038	0.000	0.006
164	0.049	0.864	0.237	0.485	0.159	0.197	0.251	0.243	0.001	0.175	0.142	0.019	0.028	0.008	0.020	0.019	0.000	0.156	0.106	0.006	0.023	0.010	0.015	0.014
165	0.055	0.322	0.133	0.219	0.219	0.161	0.119	0.147	0.010	0.219	0.127	0.278	0.016	0.086	0.122	0.000	0.009	0.182	0.091	0.222	0.009	0.064	0.076	0.012
166	0.069	1.628	0.999	1.649	0.728	0.434	0.605	-	0.000	0.167	0.059	0.063	0.022	0.045	0.030	-	0.000	0.140	0.041	0.056	0.011	0.024	0.014	-
167	0.053	0.216	0.140	0.149	0.081	0.095	0.081	-	0.000	0.136	0.093	0.012	0.067	0.059	0.034	-	0.000	0.132	0.092	0.018	0.068	0.067	0.021	-
168	0.048	0.362	0.312	0.546	0.336	-	0.547	-	0.008	0.422	0.296	0.315	0.179	-	0.044	-	0.000	0.370	0.245	0.302	0.157	-	0.029	-
169	0.055	1.897	1.538	0.238	0.572	0.557	0.471	-	0.031	0.556	0.062	0.022	0.000	0.079	0.014	-	0.027	0.434	0.034	0.009	0.000	0.000	0.010	-

Table A13: Frequencies of DYMS1, OLADRB and SMHCC1 alleles in three properties

Allele (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall
DYMS1				
n	*301*	*300*	*292*	*893*
158	-	0.002	-	0.001
160	0.002	-	-	0.001
162	0.013	0.008	-	0.007
164	0.013	0.017	0.002	0.011
168	0.003	-	-	0.001
174	0.002	0.008	-	0.003
176	0.007	0.012	0.022	0.013
180	0.003	-	-	0.001
184	0.057	0.103	0.170	0.109
186	0.121	0.115	0.101	0.113
188	0.073	0.007	0.098	0.059
190	0.115	0.295	0.057	0.156
192	0.218	0.085	0.069	0.124
194	0.023	0.012	-	0.012
196	0.075	0.177	0.163	0.138
198	0.015	0.003	-	0.006
200	0.179	0.107	0.077	0.122
202	0.043	0.050	0.241	0.110
204	0.033	-	-	0.011
206	0.003	-	0.002	0.002
210	0.002	-	-	0.001
OLADRB				
n	*308*	*289*	*284*	*881*
264	0.0049	-	0.0106	0.0051
270	0.1737	0.1384	0.2482	0.1862
272	0.0536	0.0917	0.1092	0.084
274	0.164	0.0692	0.0423	0.0936
276	0.2955	0.3668	0.2359	0.2997
278	0.1282	0.0484	0.0088	0.0636
282	0.0016	-	-	0.0006
284	0.0438	0.0433	0.0933	0.0596
286	0.0438	0.0087	-	0.0182
288	0.0276	0.0017	-	0.0102
294	-	-	0.0018	0.0006
296	0.0633	0.2318	0.25	0.1788
SMHCC1				
n	*313*	*302*	*280*	*895*
176	-	0.0017	-	0.0006
180	0.0256	0.043	0.0518	0.0397
182	0.0016	0.0017	0.0036	0.0022
186	0.0511	0.0811	0.0054	0.0469
188	0.1502	0.0828	0.1054	0.1134
190	0.0431	0.0017	0.0054	0.0173
192	0.2444	0.1242	0.175	0.1821
194	0.2476	0.2616	0.2375	0.2492
196	0.0096	0.0579	-	0.0229
198	0.0815	0.048	0.2339	0.1179
200	0.0224	-	0.0036	0.0089
202	0.0256	0.1573	0.0357	0.0732
204	0.0048	0.0381	0.0018	0.0151
206	0.0016	-	-	0.0006
208	0.0032	0.0017	0.0125	0.0056
210	0.0879	0.0993	0.1286	0.1045

n = number of individuals

Table A14: Frequency of OLADRW alleles in three properties

Allele (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall
n	*273*	*263*	*261*	*797*
442	-	0.0076	-	0.0025
450	0.0018	-	0.0019	0.0013
452	0.0073	-	0.0038	0.0038
456	0.0147	-	-	0.005
458	0.0934	0.0057	-	0.0339
462	0.0147	-	-	0.005
470	-	0.0171	-	0.0056
474	0.0018	-	-	0.0006
476	0.0183	0.0152	0.0019	0.0119
478	0.1392	0.0779	0.2797	0.165
480	0.2619	0.249	0.0383	0.1844
482	0.0183	0.0038	0.0019	0.0082
484	-	0.0171	-	0.0056
486	0.0037	-	-	0.0013
488	0.044	0.019	0.0153	0.0263
490	0.0769	0.1236	0.1188	0.106
492	-	-	0.0019	0.0006
494	0.0586	0.1312	0.1897	0.1255
496	0.0696	0.1711	0.1034	0.1142
498	0.022	0.0456	-	0.0226
500	0.0476	0.0038	0.0038	0.0188
502	0.0055	0.0608	0.1034	0.0558
506	0.0037	-	-	0.0013
508	0.0275	0.019	0.1092	0.0514
510	0.0128	0.0019	0.0019	0.0056
520	-	-	0.0019	0.0006
522	-	-	0.0134	0.0044
530	-	-	0.0057	0.0019
532	-	-	0.0019	0.0006
548	0.0018	-	-	0.0006
550	0.0018	-	-	0.0006
552	-	0.0038	-	0.0013
554	-	0.0057	-	0.0019
556	0.0073	0.0133	-	0.0069
558	0.0183	0.0038	-	0.0075
560	0.011	0.0038	0.0019	0.0056
566	0.0018	-	-	0.0006
574	0.0018	-	-	0.0006
584	0.0018	-	-	0.0006
586	0.0037	-	-	0.0013
588	0.0055	-	-	0.0019
590	0.0018	-	-	0.0006

n = number of individuals

Table A15: Frequencies of OVINRA1, OVINRA2, o(IFN)γ, and KP6 alleles in three properties

Allele (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall
OVINRA1				
n	*316*	*293*	*286*	*895*
150	0.0016	-	-	0.0006
154	-	0.0017	-	0.0006
156	0.0839	0.2014	0.0472	0.1106
158	0.1551	0.1177	0.1294	0.1346
160	0.1377	0.1962	0.2185	0.1827
162	0.3608	0.2457	0.3671	0.3251
164	0.1851	0.2201	0.1836	0.1961
166	0.0127	0.0017	0.0105	0.0084
168	0.0617	0.0154	0.042	0.0402
170	0.0016	-	0.0017	0.0011
OVINRA2				
n	*233*	*247*	*241*	*721*
310	-	-	0.0041	0.0014
312	0.5815	0.5	0.5643	0.5479
314	0.1545	0.3502	0.2718	0.2607
316	0.0644	0.0486	0.0477	0.0534
318	0.0365	0.0243	0.0975	0.0527
320	0.0322	0.002	-	0.0111
322	0.0107	0.0202	-	0.0104
326	0.1159	0.0547	0.0145	0.061
328	0.0043	-	-	0.0014
o(IFN)γ				
n	*304*	*294*	*292*	*890*
124	0.6711	0.733	0.8459	0.7489
128	0.3289	0.267	0.1541	0.2511
KP6				
n	*311*	*286*	*278*	*875*
180	-	0.0035	-	0.0011
182	-	0.0017	-	0.0006
186	0.0257	0.0175	0.0126	0.0189
188	0.0016	0.0035	0.0018	0.0023
190	0.0032	0.0017	-	0.0017
192	0.0048	0.0017	0.0018	0.0029
194	0.008	0.0035	-	0.004
196	0.0048	-	0.0018	0.0023
198	0.0032	0.0017	0.0054	0.0034
200	0.6383	0.4528	0.5378	0.5457
202	0.0547	0.1556	0.0737	0.0937
204	0.2524	0.3549	0.3165	0.3063
206	0.0032	-	0.0486	0.0166
208	-	0.0017	-	0.0006

n = number of individuals

Table A16: Frequency of DYMS1 genotypes in three properties

Genotype (bp)	Frequency				Genotype (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall		Property 1	Property 2	Property 3	Overall
n	*301*	*300*	*292*	*893*	*n*	*301*	*300*	*292*	*893*
158/164	-	0.003	-	0.001	186/192	0.070	0.020	0.007	0.033
160/200	0.003	-	-	0.001	186/196	0.023	0.047	0.051	0.040
162/164	0.003	-	-	0.001	186/198	0.007	-	-	0.002
162/184	0.007	0.003	-	0.003	186/200	0.043	0.023	0.017	0.028
162/186	0.003	-	-	0.001	186/202	0.010	0.003	0.072	0.028
162/190	0.007	0.003	-	0.003	188/188	0.010	0.003	0.003	0.006
162/192	-	0.003	-	0.001	188/190	0.010	-	0.017	0.009
162/196	0.003	0.007	-	0.003	188/192	0.023	-	0.031	0.018
162/202	0.003	-	-	0.001	188/194	-	0.003	-	0.001
164/184	-	0.007	-	0.002	188/196	0.017	0.003	0.034	0.018
164/186	0.003	-	-	0.001	188/200	0.023	-	0.010	0.011
164/188	0.003	-	0.003	0.002	188/202	0.007	-	0.041	0.016
164/190	-	0.017	-	0.006	188/204	0.003	-	-	0.001
164/192	0.007	-	-	0.002	188/206	0.003	-	-	0.001
164/196	-	0.003	-	0.001	190/190	0.023	0.077	-	0.034
164/200	0.010	-	-	0.003	190/192	0.063	0.057	0.007	0.043
164/202	-	0.003	-	0.001	190/194	0.003	-	-	0.001
168/168	0.003	-	-	0.001	190/196	0.027	0.083	0.017	0.043
174/190	-	0.013	-	0.005	190/198	0.007	0.003	-	0.003
174/192	0.003	-	-	0.001	190/200	0.027	0.073	0.014	0.038
174/200	-	0.003	-	0.001	190/202	0.003	0.027	0.038	0.022
176/176	0.003	-	-	0.001	190/204	0.010	-	-	0.003
176/184	-	-	0.010	0.003	192/192	0.043	0.007	0.003	0.018
176/186	-	0.003	-	0.001	192/194	0.007	0.007	-	0.005
176/188	-	-	0.007	0.002	192/196	0.027	0.033	0.027	0.029
176/190	-	0.010	-	0.003	192/198	0.010	-	-	0.003
176/192	0.003	-	0.003	0.002	192/200	0.070	0.020	0.003	0.031
176/196	-	0.007	0.007	0.005	192/202	0.023	0.003	0.041	0.022
176/200	0.003	0.003	0.003	0.003	192/204	0.020	-	-	0.007
176/202	-	-	0.014	0.005	194/194	-	0.003	-	0.001
180/192	0.003	-	-	0.001	194/196	0.013	-	-	0.005
180/200	0.003	-	-	0.001	194/200	0.010	-	-	0.003
184/184	0.003	0.010	0.031	0.015	194/202	0.003	0.007	-	0.003
184/186	0.017	0.030	0.021	0.022	194/204	0.003	-	-	0.001
184/188	0.020	-	0.021	0.013	194/210	0.003	-	-	0.001
184/190	0.010	0.073	0.017	0.034	196/196	0.007	0.037	0.017	0.020
184/192	0.020	0.013	0.010	0.015	196/200	0.013	0.047	0.038	0.033
184/194	0.003	-	-	0.001	196/202	0.003	0.013	0.058	0.025
184/196	0.003	0.037	0.058	0.033	196/204	0.007	-	-	0.002
184/198	0.003	-	-	0.001	198/200	0.003	-	-	0.001
184/200	0.013	0.013	0.014	0.013	198/202	-	0.003	-	0.001
184/202	0.003	0.010	0.127	0.046	200/200	0.050	0.013	0.010	0.025
184/204	0.007	-	-	0.002	200/202	0.017	0.003	0.031	0.017
186/186	0.017	0.013	0.003	0.011	200/204	0.017	-	-	0.006
186/188	0.017	-	0.024	0.013	200/206	0.003	-	0.003	0.002
186/190	0.017	0.077	0.003	0.033	202/202	0.007	0.013	0.031	0.017

n = number of individuals

Table A17: Frequency of OLADRB genotypes in three properties

Genotype (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall
n	*308*	*289*	*284*	*881*
264/270	0.0032	-	-	0.0011
264/276	0.0032	-	0.0035	0.0023
264/284	-	-	0.0035	0.0011
264/288	0.0032	-	-	0.0011
264/296	-	-	0.0141	0.0045
270/270	0.039	0.0138	0.0493	0.0341
270/272	0.0162	0.0346	0.0599	0.0363
270/274	0.0617	0.0173	0.0141	0.0318
270/276	0.1039	0.09	0.1408	0.1112
270/278	0.0455	0.0173	-	0.0216
270/284	0.013	0.0277	0.0387	0.0261
270/286	0.0032	0.0035	-	0.0023
270/296	0.0227	0.0588	0.1444	0.0738
272/272	0.0097	-	0.007	0.0057
272/274	0.0227	0.0104	0.0141	0.0159
272/276	0.0162	0.0623	0.0493	0.042
272/278	0.0097	0.0069	0.0035	0.0068
272/284	-	0.0035	0.0352	0.0125
272/286	0.0065	-	-	0.0023
272/294	-	-	0.0035	0.0011
272/296	0.0162	0.0657	0.0387	0.0397
274/274	0.0357	0.0104	-	0.0159
274/276	0.0812	0.0519	0.0211	0.0522
274/278	0.0292	0.0138	-	0.0148
274/284	0.0065	0.0035	0.0035	0.0045
274/286	0.0195	-	-	0.0068
274/288	0.0097	-	-	0.0034
274/296	0.026	0.0208	0.0317	0.0261
276/276	0.1136	0.1384	0.0352	0.0965
276/278	0.0552	0.0208	0.007	0.0284
276/284	0.0357	0.0208	0.0528	0.0363
276/286	0.0195	0.0104	-	0.0102
276/288	0.0227	0.0035	-	0.0091
276/296	0.026	0.1972	0.1268	0.1146
278/278	0.026	0.0035	-	0.0102
278/282	0.0032	-	-	0.0011
278/284	0.013	0.0069	-	0.0068
278/286	0.0195	-	-	0.0068
278/288	0.0162	-	-	0.0057
278/296	0.013	0.0242	0.007	0.0148
284/284	0.0032	-	0.0035	0.0023
284/286	0.0032	-	-	0.0011
284/296	0.0097	0.0242	0.0458	0.0261
286/286	0.0065	-	-	0.0023
286/296	0.0032	0.0035	-	0.0023
288/296	0.0032	-	-	0.0011
296/296	0.0032	0.0346	0.0458	0.0272

n = number of individuals

Table A18: Frequency of SMHCC1 genotypes in three properties

Genotype (bp)	Frequency				Genotype (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall		Property 1	Property 2	Property 3	Overall
n	*313*	*302*	*280*	*895*	*n*	*313*	*302*	*280*	*895*
176/180	-	0.0033	-	0.0011	190/196	0.0032	-	-	0.0011
180/186	0.0032	-	-	0.0011	190/198	0.0128	-	-	0.0045
180/188	0.0096	0.0166	0.0143	0.0134	190/200	0.0032	-	-	0.0011
180/190	0.0032	-	-	0.0011	190/202	0.0064	-	0.0036	0.0034
180/192	0.0096	0.0132	0.0107	0.0112	190/210	0.0128	-	0.0036	0.0056
180/194	0.0128	0.0298	0.0321	0.0246	192/192	0.0575	0.0166	0.05	0.0413
180/196	0.0032	0.0033	-	0.0022	192/194	0.1438	0.0563	0.1036	0.1017
180/198	0.0032	-	0.0321	0.0112	192/196	0.0032	0.0132	-	0.0056
180/202	0.0032	0.0033	-	0.0022	192/198	0.0288	0.0166	0.0571	0.0335
180/204	-	0.0099	-	0.0034	192/200	0.0128	-	-	0.0045
180/210	0.0032	0.0066	0.0143	0.0078	192/202	-	0.043	0.0071	0.0168
182/190	0.0032	-	-	0.0011	192/204	-	0.0033	-	0.0011
182/194	-	-	0.0036	0.0011	192/208	-	-	0.0036	0.0011
182/198	-	-	0.0036	0.0011	192/210	0.0447	0.0232	0.0321	0.0335
182/210	-	0.0033	-	0.0011	194/194	0.0351	0.0695	0.0286	0.0447
186/186	0.0032	-	-	0.0011	194/196	0.0032	0.0232	-	0.0089
186/188	0.0032	0.0232	-	0.0089	194/198	0.0607	0.0199	0.1429	0.0726
186/192	0.0479	0.0298	-	0.0268	194/200	0.0128	-	-	0.0045
186/194	0.016	0.0464	-	0.0212	194/202	0.0192	0.0894	0.025	0.0447
186/196	-	0.0099	-	0.0034	194/204	0.0064	0.0166	-	0.0078
186/198	0.0064	0.0033	0.0036	0.0045	194/208	0.0064	-	0.0143	0.0067
186/200	0.0032	-	-	0.0011	194/210	0.0383	0.043	0.05	0.0436
186/202	0.0032	0.0232	-	0.0089	196/196	-	0.0033	-	0.0011
186/204	-	0.0066	0.0036	0.0034	196/198	-	0.0066	-	0.0022
186/210	0.0128	0.0199	0.0036	0.0123	196/202	-	0.0232	-	0.0078
188/188	0.0288	-	0.0036	0.0112	196/204	-	0.0066	-	0.0022
188/190	0.0064	-	-	0.0022	196/210	0.0064	0.0099	-	0.0056
188/192	0.0639	0.0166	0.0321	0.038	198/198	0.0064	0.0066	0.0464	0.019
188/194	0.0895	0.0563	0.0464	0.0648	198/202	-	0.0166	0.0143	0.0101
188/196	-	0.0132	-	0.0045	198/204	-	0.0066	-	0.0022
188/198	0.0192	0.0033	0.0607	0.0268	198/210	0.0192	0.0099	0.0607	0.0291
188/200	0.0064	-	-	0.0022	200/210	0.0064	-	0.0071	0.0045
188/202	0.0128	0.0199	0.0071	0.0134	202/202	-	0.0166	0.0036	0.0067
188/206	0.0032	-	-	0.0011	202/204	0.0032	0.0232	-	0.0089
188/208	-	-	0.0036	0.0011	202/210	0.0032	0.0397	0.0071	0.0168
188/210	0.0288	0.0166	0.0393	0.0279	204/210	-	0.0033	-	0.0011
190/192	0.0192	-	0.0036	0.0078	208/210	-	0.0033	0.0036	0.0022
190/194	0.016	0.0033	-	0.0067	210/210	-	0.0099	0.0179	0.0089

n = number of individuals

Table A19: Frequency of OLADRW genotypes in three properties

Genotype (bp)	Frequency				Genotype (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall		Property 1	Property 2	Property 3	Overall
n	*273*	*263*	*261*	*797*	*n*	*273*	*263*	*261*	*797*
442/494	-	0.0038	-	0.0013	478/478	0.0806	0.0494	0.1111	0.0803
442/496	-	0.0076	-	0.0025	478/480	0.011	0.0038	0.0192	0.0113
442/498	-	0.0038	-	0.0013	478/488	0.0147	-	0.0077	0.0075
450/452	-	-	0.0038	0.0013	478/490	0.0147	0.0076	0.0613	0.0276
450/478	0.0037	-	-	0.0013	478/494	0.011	0.019	0.0805	0.0364
452/478	0.0037	-	-	0.0013	478/496	0.0037	0.019	0.0575	0.0263
452/480	0.0037	-	-	0.0013	478/498	0.0037	0.0038	-	0.0025
452/494	-	-	0.0038	0.0013	478/500	0.0147	-	-	0.005
452/496	0.0037	-	-	0.0013	478/502	-	-	0.0421	0.0138
452/500	0.0037	-	-	0.0013	478/506	0.0037	-	-	0.0013
456/458	0.022	-	-	0.0075	478/508	-	0.0038	0.0421	0.0151
456/478	0.0037	-	-	0.0013	478/510	0.0037	-	0.0038	0.0025
456/494	0.0037	-	-	0.0013	478/522	-	-	0.0153	0.005
458/458	0.0403	-	-	0.0138	478/530	-	-	0.0038	0.0013
458/478	0.011	-	-	0.0038	478/558	0.0037	-	-	0.0013
458/480	0.044	-	-	0.0151	478/560	-	-	0.0038	0.0013
458/488	0.0037	-	-	0.0013	480/480	0.1795	0.1825	0.0192	0.128
458/490	-	0.0076	-	0.0025	480/482	0.011	-	-	0.0038
458/494	0.0037	-	-	0.0013	480/484	-	0.0038	-	0.0013
458/496	0.0037	0.0038	-	0.0025	480/488	0.0147	0.0038	-	0.0063
458/498	0.0037	-	-	0.0013	480/490	0.0147	0.0228	-	0.0125
458/500	0.0073	-	-	0.0025	480/494	0.0073	0.0456	0.0115	0.0213
458/508	0.0037	-	-	0.0013	480/496	0.011	0.0152	-	0.0088
458/566	0.0037	-	-	0.0013	480/498	0.0073	0.0152	-	0.0075
462/462	0.011	-	-	0.0038	480/500	0.0147	-	-	0.005
462/478	0.0037	-	-	0.0013	480/502	0.0073	0.0076	0.0038	0.0063
462/496	0.0037	-	-	0.0013	480/508	0.0037	0.0038	0.0038	0.0038
470/470	-	0.0038	-	0.0013	480/510	0.0037	-	-	0.0013
470/490	-	0.0076	-	0.0025	480/548	0.0037	-	-	0.0013
470/494	-	0.0152	-	0.005	480/554	-	0.0038	-	0.0013
470/498	-	0.0038	-	0.0013	480/556	-	0.0038	-	0.0013
474/482	0.0037	-	-	0.0013	480/560	-	0.0038	-	0.0013
476/476	0.011	0.0114	-	0.0075	480/588	0.0037	-	-	0.0013
476/478	0.0073	-	-	0.0025	480/590	0.0037	-	-	0.0013
476/482	0.0037	-	-	0.0013	482/482	0.0073	0.0038	-	0.0038
476/488	-	-	0.0038	0.0013	482/490	-	-	0.0038	0.0013
476/490	-	0.0038	-	0.0013	482/496	0.0037	-	-	0.0013
476/494	-	0.0038	-	0.0013	484/484	-	0.0076	-	0.0025
476/550	0.0037	-	-	0.0013	484/490	-	0.0076	-	0.0025
484/496	-	0.0076	-	0.0025	496/500	-	0.0038	0.0038	0.0025
486/486	0.0037	-	-	0.0013	496/502	0.0037	0.0152	0.0268	0.0151
488/488	0.0183	0.0114	0.0038	0.0113	496/508	0.0037	0.0038	0.0192	0.0088
488/490	-	0.0038	0.0077	0.0038	496/510	0.0037	-	-	0.0013
488/494	0.0037	-	-	0.0013	496/556	0.0037	-	-	0.0013
488/496	0.0073	0.0076	0.0038	0.0063	498/498	0.0037	-	-	0.0013
488/500	0.0037	-	-	0.0013	498/500	0.0073	-	-	0.0025
488/510	0.0037	-	-	0.0013	498/508	0.0073	-	-	0.0025
490/490	0.0366	0.0532	0.0498	0.0464	498/552	-	0.0038	-	0.0013

(contd..)

Table A19 (contd..): Frequency of OLADRW genotypes in three properties

Genotype (bp)	Frequency				Genotype (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall		Property 1	Property 2	Property 3	Overall
490/494	0.011	0.0152	0.0268	0.0176	500/500	0.0183	-	-	0.0063
490/496	0.022	0.0304	0.0038	0.0188	500/508	0.0037	-	-	0.0013
490/498	0.0037	0.0114	-	0.005	502/502	-	0.038	0.0192	0.0188
490/500	0.0037	0.0038	-	0.0025	502/508	-	-	0.0307	0.01
490/502	-	0.0114	0.023	0.0113	502/522	-	-	0.0038	0.0013
490/508	0.0037	0.0038	0.0077	0.005	502/554	-	0.0038	-	0.0013
490/520	-	-	0.0038	0.0013	506/508	0.0037	-	-	0.0013
490/552	-	0.0038	-	0.0013	508/508	0.011	0.0076	0.0383	0.0188
490/558	0.0037	-	-	0.0013	508/510	-	0.0038	-	0.0013
490/574	0.0037	-	-	0.0013	508/530	-	-	0.0038	0.0013
492/496	-	-	0.0038	0.0013	510/510	0.0037	-	-	0.0013
494/494	0.033	0.038	0.0843	0.0514	522/522	-	-	0.0038	0.0013
494/496	-	0.0152	0.0115	0.0088	530/532	-	-	0.0038	0.0013
494/498	0.0037	0.0494	-	0.0176	556/556	0.0037	0.0114	-	0.005
494/500	-	-	0.0038	0.0013	556/558	0.0037	-	-	0.0013
494/502	-	0.0076	0.0383	0.0151	558/558	0.0037	-	-	0.0013
494/508	0.0037	0.0038	0.0345	0.0138	558/560	0.0183	0.0038	-	0.0075
494/510	0.0037	-	-	0.0013	560/588	0.0037	-	-	0.0013
494/554	-	0.0038	-	0.0013	584/586	0.0037	-	-	0.0013
494/558	-	0.0038	-	0.0013	586/588	0.0037	-	-	0.0013
496/496	0.033	0.1065	0.0383	0.059					

Table A20: Frequencies of OVINRA1 and OVINRA2 genotypes in three properties

OVINRA1					*OVINRA2*				
Genotype (bp)	Frequency				Genotype (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall		Property 1	Property 2	Property 3	Overall
n	*316*	*293*	*286*	*895*	*n*	*233*	*247*	*241*	*721*
150/162	0.0032	-	-	0.0011	310/310	-	-	0.0041	0.0014
154/164	-	0.0034	-	0.0011	312/312	0.412	0.4089	0.4647	0.4286
156/156	0.0063	0.0648	-	0.0235	312/314	0.0472	0.0405	0.0539	0.0472
156/158	0.0285	0.0375	0.0105	0.0257	312/316	0.0987	0.0567	0.0456	0.0666
156/160	0.0285	0.0683	0.028	0.0413	312/318	0.0515	0.0081	0.0747	0.0444
156/162	0.0443	0.0956	0.0385	0.0592	312/320	0.0258	0.004	-	0.0097
156/164	0.0411	0.0648	0.0175	0.0413	312/322	0.0043	0.0121	-	0.0055
156/166	0.0032	0.0034	-	0.0022	312/326	0.1116	0.0607	0.0249	0.0652
156/168	0.0095	0.0034	-	0.0045	314/314	0.103	0.2874	0.195	0.1969
158/158	0.0158	0.0102	0.007	0.0112	314/316	0.0129	0.0243	0.0207	0.0194
158/160	0.0506	0.0614	0.0594	0.057	314/318	0.0129	0.0243	0.0788	0.0388
158/162	0.1076	0.0546	0.1084	0.0905	314/320	0.0129	-	-	0.0042
158/164	0.0696	0.0478	0.0455	0.0547	314/322	0.0043	0.0202	-	0.0083
158/166	0.0095	-	0.0035	0.0045	314/326	0.0129	0.0162	-	0.0097
158/168	0.0127	0.0137	0.0175	0.0145	316/316	0.0086	-	0.0124	0.0069
160/160	0.0158	0.0478	0.0455	0.0358	316/318	0.0043	0.0162	0.0041	0.0069
160/162	0.0981	0.0785	0.1748	0.1162	318/318	0.0043	-	0.0166	0.0069
160/164	0.0411	0.0785	0.0524	0.057	318/326	0.0043	-	0.0041	0.0014
160/168	0.0253	0.0102	0.0315	0.0223	320/320	0.0129	-	-	0.0014
162/162	0.1361	0.058	0.1189	0.105	320/322	0.0043	-	-	0.0014
162/164	0.1487	0.1468	0.1538	0.1497	320/326	0.0472	-	-	0.0042
162/166	0.0063	-	-	0.0022	322/322	-	0.004	-	0.0028
162/168	0.0411	-	0.021	0.0212	326/326	-	0.0162	-	0.0208
164/164	0.0222	0.0478	0.035	0.0346	328/328	0.0043	-	-	0.0014
164/166	0.0032	-	0.014	0.0056					
164/168	0.0222	0.0034	0.0105	0.0123					
164/170	-	-	0.0035	0.0011					
166/168	-	-	0.0035	0.0011					
166/170	0.0032	-	-	0.0011					
168/168	0.0063	-	-	0.0022					

n = number of individuals

Table A21: Frequencies of o(IFN)γ and KP6 genotypes in three properties

Genotype (bp)	Frequency			
	Property 1	Property 2	Property 3	Overall
o(IFN)γ				
n	*304*	*294*	*292*	*890*
124/124	0.4572	0.5272	0.7123	0.564
124/128	0.4276	0.4116	0.2671	0.3697
128/128	0.1151	0.0612	0.0205	0.0663
KP6				
n	*311*	*286*	*278*	*875*
180/182	-	0.0035	-	0.0011
180/186	-	0.0035	-	0.0011
186/186	-	0.007	0.0072	0.0046
186/190	0.0032	-	-	0.0011
186/194	0.0032	0.007	-	0.0034
186/196	0.0032	-	-	0.0011
186/198	0.0032	-	-	0.0011
186/200	0.0225	-	0.0036	0.0091
186/202	0.0129	0.007	0.0036	0.008
186/204	-	0.0035	0.0036	0.0023
186/206	0.0032	-	-	0.0011
188/190	-	0.0035	-	0.0011
188/192	-	0.0035	0.0036	0.0023
188/204	0.0032	-	-	0.0011
190/204	0.0032	-	-	0.0011
192/196	0.0032	-	-	0.0011
192/200	0.0064	-	-	0.0023
194/194	0.0032	-	-	0.0011
194/200	0.0064	-	-	0.0023
196/200	0.0032	-	-	0.0011
196/206	-	-	0.0036	0.0011
198/200	-	-	0.0036	0.0011
198/202	-	-	0.0036	0.0011
198/204	-	0.0035	0.0036	0.0023
198/206	0.0032	-	-	0.0011
200/200	0.4244	0.1993	0.295	0.3097
200/202	0.0514	0.1399	0.0935	0.0937
200/204	0.3376	0.3636	0.3201	0.3406
200/206	-	-	0.0647	0.0206
200/208	-	0.0035	-	0.0011
202/202	0.0064	0.0385	0.0036	0.016
202/204	0.0322	0.0874	0.0396	0.0526
204/204	0.0643	0.1259	0.1187	0.1017
204/206	-	-	0.0288	0.0091

n = number of individuals

Table A22: Effect of DYMS1 genotypes on immune responses to Johne's vaccination in sheep on three properties

Genotype (bp)	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
174/190	-	-1.65±0.24	-	-	-3.92±0.49	-	-	-4.02±0.41	-
184/184	-	-	-1.29±0.24	-	-	-4.18±0.54	-	-	-4.22±0.51
184/186	-	-1.39±0.22	-1.49±0.28	-	-3.65±0.44	-3.88±0.61	-	-3.25±0.36	-4.44±0.59
184/188	-1.20±0.30	-	-1.34±0.24	-3.95±0.65	-	-2.52±0.53	-4.09±0.65	-	-2.85±0.51
184/190	-	-1.52±0.13	-1.26±0.27	-	-3.56±0.27	-3.85±0.59	-	-3.65±0.23	-3.73±0.57
184/192	-1.39±0.28	-	-	-4.82±0.62	-	-	-4.56±0.62	-	-
184/196	-	-1.82±0.20	-1.52±0.17	-	-4.53±0.41	-3.77±0.38	-	-4.06±0.34	-3.81±0.37
184/202	-	-	-1.32±0.12	-	-	-4.21±0.26	-	-	-4.29±0.24
186/188	-1.17±0.34	-	-0.68±0.24	-3.93±0.74	-	-3.83±0.53	-3.48±0.74	-	-3.45±0.50
186/190	-1.02±0.34	-1.52±0.18	-	-3.03±0.74	-3.78±0.38	-	-3.00±0.74	-4.18±0.31	-
186/192	-1.64±0.23	-1.29±0.22	-	-2.08±0.49	-3.71±0.44	-	-2.73±0.49	-3.57±0.37	-
186/196	-0.98±0.34	-1.31±0.22	-1.35±0.19	-2.47±0.74	-3.20±0.45	-3.81±0.42	-2.74±0.74	-3.07±0.38	-4.48±0.40
186/200	-1.29±0.26	-1.86±0.20	-1.31±0.25	-4.69±0.55	-4.74±0.41	-4.23±0.54	-5.19±0.55	-4.43±0.34	-5.19±0.51
186/202	-	-	-1.42±0.17	-	-	-3.73±0.38	-	-	-3.75±0.37
188/192	-	-	-1.15±0.24	-	-	-3.38±0.53	-	-	-3.68±0.51
188/196	-	-	-1.53±0.27	-	-	-4.94±0.60	-	-	-4.94±0.58
188/200	-0.91±0.3	-	-	-2.23±0.68	-	-	-2.26±0.67	-	-
188/202	-	-	-1.13±0.21	-	-	-3.54±0.45	-	-	-3.85±0.43
190/190	-	-1.80±0.14	-	-	-3.81±0.28	-	-	-3.98±0.23	-
190/192	-1.47±0.19	-1.42±0.17	-	-3.37±0.41	-3.46±0.35	-	-3.62±0.40	-3.37±0.29	-
190/196	-1.55±0.26	-1.67±0.11	-	-3.56±0.56	-3.96±0.23	-	-3.95±0.56	-3.79±0.20	-
190/200	-	-1.69±0.15	-	-	-3.98±0.30	-	-	-3.70±0.25	-
190/202	-	-	-1.65±0.24	-	-	-3.98±0.53	-	-	-4.28±0.50
192/192	-1.04±0.34	-	-	-4.35±0.72	-	-	-4.81±0.72	-	-
192/196	-1.01±0.26	-1.73±0.25	-1.28±0.24	-4.81±0.55	-3.92±0.50	-4.54±0.53	-4.67±0.56	-3.72±0.41	-4.70±0.51
192/200	-1.42±0.17	-	-	-3.33±0.36	-	-	-3.57±0.35	-	-
192/202	-	-	-1.20±0.27	-	-	-5.41±0.60	-	-	-5.47±0.58
196/196	-	-1.49±0.18	-	-	-3.15±0.37	-	-	-3.09±0.31	-
196/200	-	-1.71±0.18	-	-	-3.67±0.38	-	-	-3.88±0.31	-
196/202	-	-	-1.47±0.16	-	-	-4.10±0.35	-	-	-4.08±0.33
200/200	-0.61±0.30	-	-	-3.38±0.65	-	-	-4.17±0.65	-	-
200/202	-	-	-1.48±0.21	-	-	-3.73±0.45	-	-	-3.77±0.43
202/202	-	-1.54±0.24	-1.23±0.25	-	-4.35±0.51	-4.92±0.54	-	-4.45±0.42	-4.78±0.51

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A23: Effect of OLADRB genotypes on immune responses to Johne's vaccination in sheep on three properties

Genotype (bp)	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
270/270	-0.44±0.30	-	-1.31±0.18	-4.45±0.63	-	-3.09±0.39	-4.89±0.67	-	-3.06±0.35
270/272	-	-1.50±0.22	-1.52±0.16	-	-3.59±0.46	-3.39±0.36	-	-3.08±0.41	-3.69±0.32
270/274	-1.40±0.23	-	-	-3.55±0.51	-	-	-3.65±0.54	-	-
270/276	-1.20±0.16	-1.79±0.13	-1.26±0.12	-3.32±0.34	-3.79±0.28	-3.78±0.27	-3.57±0.36	-3.79±0.25	-3.92±0.24
270/278	-1.08±0.23	-	-	-3.06±0.51	-	-	-3.27±0.54	-	-
270/284	-	-	-1.35±0.23	-	-	-3.23±0.50	-	-	-3.38±0.45
270/296	-0.98±0.33	-1.63±0.15	-1.34±0.12	-4.56±0.72	-3.63±0.33	-3.60±0.25	-5.57±0.77	-3.72±0.29	-3.77±0.23
272/274	-1.60±0.33	-	-	-4.14±0.72	-	-	-4.11±0.76	-	-
272/276	-1.55±0.33	-1.77±0.15	-1.53±0.18	-4.02±0.71	-4.80±0.31	-4.29±0.39	-4.36±0.75	-4.69±0.28	-4.71±0.35
272/284	-	-	-1.29±0.28	-	-	-4.18±0.62	-	-	-4.39±0.56
272/296	-	-1.23±0.14	-1.53±0.23	-	-3.75±0.30	-3.40±0.50	-	-3.73±0.27	-3.60±0.45
274/274	-1.16±0.25	-	-	-3.44±0.55	-	-	-3.37±0.58	-	-
274/276	-1.03±0.19	-1.67±0.20	-	-3.95±0.41	-3.85±0.41	-	-4.13±0.44	-3.37±0.37	-
274/278	-1.48±0.30	-	-	-3.89±0.63	-	-	-4.15±0.67	-	-
274/296	-0.85±0.25	-2.15±0.24	-1.05±0.28	-3.40±0.54	-4.50±0.50	-4.04±0.62	-3.58±0.57	-4.07±0.45	-4.42±0.56
276/276	-1.16±0.14	-1.60±0.11	-1.29±0.25	-3.22±0.30	-3.91±0.24	-3.82±0.55	-3.52±0.32	-3.86±0.21	-3.86±0.50
276/278	-1.59±0.21	-	-	-3.30±0.46	-	-	-3.61±0.48	-	-
276/284	-1.52±0.24	-	-1.38±0.16	-3.76±0.51	-	-4.55±0.36	-4.42±0.54	-	-4.55±0.32
276/288	-1.32±0.33	-	-	-3.80±0.72	-	-	-4.38±0.76	-	-
276/296	-	-1.65±0.09	-1.16±0.13	-	-3.74±0.18	-4.39±0.28	-	-3.80±0.16	-4.40±0.25
278/278	-1.62±0.30	-	-	-4.15±0.64	-	-	-4.27±0.68	-	-
278/296	-	-1.44±0.24	-	-	-3.80±0.50	-	-	-3.74±0.45	-
284/296	-	-	-1.38±0.23	-	-	-5.32±0.52	-	-	-4.89±0.47
296/296	-	-	-1.15±0.19	-	-	-3.87±0.41	-	-	-3.96±0.37

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A24: Effect of OLADRW genotypes on immune responses to Johne's vaccination in sheep on three properties

Genotype	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
(bp)	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
458/458	-1.32±0.25	-	-	-3.14±0.58	-	-	-3.15±0.59	-	-
478/478	-0.93±0.19	-1.62±0.17	-1.19±0.13	-4.20±0.43	-3.95±0.43	-4.08±0.31	-4.07±0.44	-4.64±0.38	-4.10±0.28
478/490	-	-	-1.32±0.15	-	-	-4.29±0.34	-	-	-4.48±0.31
478/494	-	-	-1.28±0.15	-	-	-3.30±0.34	-	-	-3.35±0.31
478/496	-	-1.59±0.20	-1.04±0.17	-	-3.79±0.51	-3.63±0.39	-	-3.99±0.45	-3.79±0.36
478/502	-	-	-1.37±0.17	-	-	-3.51±0.39	-	-	-3.72±0.36
478/508	-	-	-1.42±0.20	-	-	-5.03±0.46	-	-	-4.55±0.42
480/480	-1.22±0.13	-1.74±0.08	-	-3.37±0.29	-3.95±0.21	-	-3.75±0.30	-3.74±0.19	-
480/490	-	-1.96±0.20	-	-	-4.13±0.51	-	-	-3.73±0.45	-
480/494	-	-1.88±0.16	-	-	-3.89±0.42	-	-	-3.63±0.37	-
488/488	-1.00±0.35	-	-	-3.97±0.80	-	-	-3.77±0.82	-	-
490/490	-	-1.58±0.15	-1.25±0.16	-	-4.16±0.39	-4.46±0.37	-	-3.77±0.34	-4.33±0.34
490/494	-	-	-1.53±0.26	-	-	-3.97±0.59	-	-	-3.82±0.55
494/494	-0.43±0.31	-1.61±0.16	-	-4.82±0.72	-3.51±0.42	-	-6.02±0.74	-3.44±0.36	-
494/502	-	-	-1.58±0.17	-	-	-4.05±0.40	-	-	-4.52±0.37
494/508	-	-	-1.09±0.23	-	-	-2.92±0.52	-	-	-3.17±0.48
496/496	-1.49±0.31	-1.67±0.10	-1.67±0.30	-2.71±0.72	-3.65±0.26	-2.47±0.68	-3.18±0.74	-3.74±0.22	-2.47±0.62
496/502	-	-	-1.84±0.23	-	-	-4.15±0.52	-	-	-4.51±0.48
496/508	-	-	-1.49±0.26	-	-	-4.15±0.59	-	-	-4.32±0.54
502/502	-	-1.57±0.13	-	-	-4.11±0.34	-	-	-3.93±0.30	-
502/508	-	-	-1.29±0.26	-	-	-4.20±0.59	-	-	-4.39±0.55
502/522	-	-	-2.25±0.51	-	-	-4.16±1.18	-	-	-4.18±1.10
508/508	-	-	-1.25±0.18	-	-	-4.81±0.42	-	-	-4.75±0.38

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A25: Effect of SMHCC1 genotypes on immune responses to Johne's vaccination in sheep on three properties

Genotype (bp)	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
180/194	-	-1.84±0.20	-1.11±0.27	-	-3.75±0.46	-3.67±0.58	-	-3.48±0.38	-3.62±0.59
180/198	-	-	-1.31±0.19	-	-	-3.66±0.41	-	-	-3.97±0.42
186/192	-1.08±0.24	-	-	-4.27±0.50	-	-	-4.90±0.49	-	-
186/194	-	-1.19±0.18	-	-	-3.68±0.42	-	-	-3.71±0.35	-
186/202	-	-1.66±0.18	-	-	-3.04±0.43	-	-	-3.06±0.35	-
188/188	-1.02±0.32	-	-	-4.52±0.67	-	-	-5.52±0.68	-	-
188/192	-1.11±0.24	-	-1.29±0.27	-3.42±0.50	-	-4.16±0.59	-3.47±0.50	-	-4.38±0.60
188/194	-1.29±0.17	-1.75±0.13	-1.46±0.19	-3.06±0.36	-4.59±0.30	-3.63±0.41	-3.19±0.36	-4.44±0.25	-3.91±0.42
188/198	-1.04±0.35	-	-1.63±0.18	-4.19±0.74	-	-3.40±0.39	-3.96±0.74	-	-3.63±0.40
188/210	-1.12±0.35	-1.79±0.22	-1.39±0.16	-1.91±0.76	-4.52±0.52	-3.97±0.36	-2.10±0.75	-4.53±0.43	-4.38±0.36
190/194	-1.53±0.27	-	-	-3.54±0.56	-	-	-3.83±0.56	-	-
192/192	-1.56±0.21	-	-1.52±0.16	-3.41±0.45	-	-4.61±0.36	-4.27±0.45	-	-4.58±0.36
192/194	-1.60±0.18	-1.51±0.13	-1.27±0.16	-4.65±0.38	-3.60±0.29	-3.63±0.34	-4.83±0.38	-3.77±0.24	-4.12±0.34
192/198	-	-	-1.34±0.16	-	-	-4.26±0.36	-	-	-4.18±0.36
192/202	-	-1.64±0.17	-	-	-4.25±0.39	-	-	-4.28±0.32	-
192/210	-1.07±0.24	-	-0.92±0.20	-3.78±0.50	-	-3.47±0.44	-3.52±0.50	-	-3.85±0.45
194/194	-1.91±0.27	-1.66±0.11	-	-3.58±0.57	-3.83±0.27	-	-3.59±0.56	-3.67±0.22	-
194/198	-1.10±0.22	-	-1.17±0.12	-4.44±0.47	-	-3.41±0.26	-4.31±0.47	-	-3.67±0.26
194/202	-0.97±0.35	-1.86±0.11	-1.37±0.27	-5.04±0.74	-4.14±0.25	-5.62±0.59	-5.91±0.74	-4.10±0.21	-5.39±0.60
194/210	-0.70±0.27	-1.83±0.18	-1.28±0.21	-2.54±0.57	-3.91±0.42	-4.32±0.45	-3.42±0.57	-3.77±0.34	-3.89±0.45
198/198	-	-	-1.15±0.19	-	-	-4.28±0.42	-	-	-4.20±0.42
198/210	-0.68±0.35	-	-1.10±0.18	-4.94±0.75	-	-3.95±0.39	-5.28±0.75	-	-3.92±0.39
202/202	-	-1.56±0.20	-	-	-4.82±0.46	-	-	-4.59±0.38	-
202/210	-	-1.62±0.17	-	-	-3.75±0.39	-	-	-3.75±0.32	-

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A26: Effect of OVINRA1 and OVINRA2 genotypes on immune responses to Johne's vaccination in sheep on three properties

Genotype (bp)	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
OVINRA1									
156/156	-	-1.73±0.15	-	-	-3.83±0.31	-	-	-3.71±0.26	-
156/158	-1.41±0.24	-1.02±0.22	-	-4.49±0.53	-5.07±0.47	-	-4.66±0.53	-5.21±0.40	-
156/160	-	-1.58±0.15	-1.53±0.25	-	-3.50±0.30	-4.62±0.54	-	-3.48±0.26	-4.77±0.54
156/162	-0.84±0.23	-1.38±0.14	-1.43±0.21	-3.61±0.49	-3.18±0.29	-3.85±0.46	-4.39±0.49	-3.52±0.25	-3.86±0.45
156/164	-1.24±0.28	-1.39±0.15	-	-2.93±0.60	-3.94±0.30	-	-3.21±0.60	-3.62±0.26	-
158/160	-1.27±0.28	-1.70±0.19	-1.62±0.16	-2.35±0.60	-4.57±0.38	-4.81±0.34	-2.27±0.60	-4.19±0.33	-4.52±0.34
158/162	-0.85±0.15	-1.52±0.15	-1.32±0.13	-3.57±0.32	-4.47±0.30	-3.97±0.27	-3.67±0.32	-4.27±0.26	-4.16±0.27
158/164	-1.33±0.24	-1.55±0.19	-1.12±0.25	-3.06±0.52	-3.56±0.38	-4.69±0.54	-3.27±0.52	-3.21±0.33	-4.58±0.53
160/160	-	-1.62±0.22	-1.32±0.19	-	-3.62±0.45	-3.80±0.40	-	-3.42±0.39	-4.43±0.40
160/162	-1.56±0.17	-1.59±0.14	-1.24±0.10	-3.65±0.37	-3.98±0.29	-3.63±0.21	-4.25±0.37	-3.75±0.25	-3.65±0.21
160/164	-0.86±0.28	-1.67±0.14	-1.16±0.19	-4.62±0.60	-3.72±0.28	-3.89±0.41	-4.44±0.59	-3.99±0.24	-4.02±0.40
160/168	-0.90±0.28	-	-1.62±0.28	-5.42±0.60	-	-3.19±0.61	-5.26±0.59	-	-3.54±0.60
162/162	-1.50±0.13	-1.60±0.16	-1.22±0.14	-3.84±0.29	-3.87±0.32	-4.21±0.29	-3.98±0.29	-4.10±0.28	-4.17±0.29
162/164	-1.32±0.13	-1.69±0.10	-1.27±0.12	-3.42±0.29	-4.08±0.22	-3.74±0.25	-3.69±0.29	-3.89±0.19	-3.91±0.25
162/168	-1.53±0.28	-	-1.36±0.28	-4.96±0.60	-	-3.04±0.60	-5.08±0.59	-	-3.54±0.59
164/164	-	-2.08±0.17	-1.55±0.21	-	-4.25±0.36	-3.66±0.46	-	-4.07±0.31	-3.85±0.45
164/168	-1.21±0.28	-	-	-2.46±0.60	-	-	-2.87±0.59	-	-
OVINRA2									
312/312	-1.30±0.09	-1.61±0.07	-1.25±0.08	-3.56±0.19	-3.92±0.15	-3.70±0.18	-3.68±0.20	-3.90±0.13	-3.77±0.16
312/314	-1.25±0.22	-1.63±0.23	-1.14±0.20	-3.93±0.48	-3.70±0.49	-3.95±0.47	-4.41±0.49	-3.49±0.42	-4.28±0.43
312/316	-1.27±0.21	-1.35±0.19	-1.38±0.18	-3.97±0.45	-3.96±0.42	-4.26±0.42	-4.22±0.46	-4.07±0.36	-4.33±0.39
312/318	-1.29±0.35	-	-1.22±0.17	-3.55±0.76	-	-4.21±0.40	-4.31±0.77	-	-3.98±0.37
312/320	-1.22±0.31	-	-	-3.79±0.67	-	-	-3.51±0.68	-	-
312/326	-	-	-1.36±0.23	-	-	-3.14±0.54	-	-	-3.13±0.50
314/314	-1.00±0.15	-1.61±0.10	-1.23±0.11	-3.79±0.33	-4.04±0.22	-4.32±0.27	-4.09±0.33	-3.96±0.19	-4.28±0.24
314/316	-	-	-1.48±0.29	-	-	-3.62±0.67	-	-	-3.53±0.62
314/318	-	-	-1.58±0.15	-	-	-3.99±0.34	-	-	-4.14±0.31

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A27: Effect of o(IFN)γ and KP6 genotypes on immune responses to Johne's vaccination in sheep on three properties

Genotype (bp)	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
o(IFN)γ									
124/124	-1.20±0.08	-1.63±0.06	-1.37±0.05	-3.60±0.17	-4.07±0.14	-4.05±0.12	-3.83±0.17	-3.95±0.12	-4.15±0.10
124/128	-1.27±0.08	-1.57±0.06	-1.22±0.09	-3.89±0.19	-3.90±0.12	-3.71±0.19	-4.16±0.19	-3.84±0.10	-3.85±0.23
128/128	-1.38±0.12	-1.50±0.18	-1.02±0.30	-2.95±0.34	-3.27±0.30	-2.14±0.35	-3.14±0.34	-3.46±0.35	-2.25±0.42
KP6									
200/200	-1.21±0.08	-1.59±0.10	-1.20±0.09	-3.79±0.17	-3.51±0.21	-3.82±0.20	-3.92±0.18	-3.66±0.19	-3.85±0.19
200/202	-1.36±0.21	-1.58±0.12	-1.17±0.15	-4.64±0.44	-3.79±0.25	-3.67±0.35	-4.74±0.46	-3.66±0.22	-3.88±0.33
200/204	-1.27±0.10	-1.63±0.06	-1.42±0.08	-3.58±0.20	-4.17±0.14	-4.10±0.18	-3.99±0.22	-4.05±0.12	-4.22±0.17
200/206	-	-	-1.21±0.19	-	-	-3.39±0.43	-	-	-3.49±0.41
202/202	-	-1.56±0.20	-	-	-4.23±0.42	-	-	-3.95±0.38	-
202/204	-0.97±0.29	-1.55±0.18	-1.40±0.20	-3.00±0.62	-4.32±0.37	-3.90±0.46	-3.19±0.66	-4.12±0.34	-4.12±0.43
204/204	-1.31±0.25	-1.38±0.12	-1.54±0.12	-2.82±0.53	-3.91±0.25	-4.13±0.27	-3.24±0.56	-3.94±0.22	-4.12±0.26
204/206	-	-	-0.85±0.25	-	-	-4.18±0.58	-	-	-4.02±0.55

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A28: Effect of DYMS1 alleles on immune responses to Johne's vaccination in sheep on three properties

Allele (bp)	Allelic presence	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
		Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
176	Absent	-	-	-1.33±0.05	-	-	-3.94±0.10	-	-	-4.05±0.10
	Present	-	-	-1.21±0.19	-	-	-3.70±0.43	-	-	-3.75±0.41
184	Absent	-1.25±0.06	-1.60±0.04	-1.27±0.05	-3.62±0.13	-3.93±0.09	-3.96±0.13	-3.88±0.13	-3.90±0.08	-4.07±0.12
	Present	-1.21±0.15	-1.58±0.09	-1.44±0.08	-3.85±0.33	-3.96±0.19	-3.86±0.17	-3.93±0.33	-3.77±0.17	-3.96±0.17
186	Absent	-1.24±0.06	-1.63±0.04	-1.33±0.05	-3.73±0.14	-3.95±0.09	-3.93±0.11	-3.95±0.14	-3.90±0.08	-4.00±0.11
	Present	-1.28±0.12	-1.43±0.09	-1.30±0.10	-3.43±0.26	-3.86±0.19	-3.89±0.22	-3.66±0.26	-3.78±0.17	-4.17±0.21
188	Absent	-1.24±0.06	-	-1.37±0.05	-3.71±0.13	-	-4.02±0.11	-3.94±0.13	-	-4.14±0.11
	Present	-1.31±0.15	-	-1.13±0.10	-3.35±0.33	-	-3.53±0.23	-3.58±0.33	-	-3.63±0.21
190	Absent	-1.22±0.06	-1.57±0.06	-1.32±0.05	-3.69±0.14	-4.04±0.12	-3.93±0.11	-3.89±0.14	-3.98±0.10	-4.05±0.10
	Present	-1.35±0.12	-1.62±0.06	-1.33±0.14	-3.53±0.27	-3.83±0.12	-3.90±0.31	-3.87±0.27	-3.77±0.11	-3.93±0.29
192	Absent	-1.20±0.07	-1.61±0.04	-1.32±0.05	-3.75±0.16	-3.95±0.09	-3.89±0.11	-3.93±0.16	-3.90±0.08	-4.00±0.10
	Present	-1.33±0.09	-1.52±0.10	-1.37±0.13	-3.51±0.20	-3.88±0.22	-4.17±0.29	-3.81±0.20	-3.77±0.19	-4.28±0.27
194	Absent	-1.25±0.06	-	-	-3.69±0.13	-	-	-3.92±0.13	-	-
	Present	-1.27±0.26	-	-	-3.08±0.57	-	-	-3.19±0.57	-	-
196	Absent	-1.24±0.06	-1.57±0.05	-1.31±0.05	-3.57±0.14	-3.92±0.10	-3.92±0.12	-3.83±0.14	-3.90±0.09	-4.02±0.11
	Present	-1.29±0.12	-1.64±0.07	-1.37±0.09	-3.99±0.28	-3.97±0.15	-3.94±0.19	-4.10±0.28	-3.83±0.13	-4.09±0.19
198	Absent	-1.25±0.06	-	-	-3.67±0.13	-	-	-3.90±0.13	-	-
	Present	-1.04±0.32	-	-	-3.29±0.72	-	-	-3.35±0.72	-	-
200	Absent	-1.29±0.07	-1.57±0.04	-1.33±0.05	-3.67±0.15	-3.91±0.09	-3.97±0.11	-3.94±0.15	-3.83±0.08	-4.08±0.11
	Present	-1.13±0.10	-1.69±0.09	-1.29±0.11	-3.63±0.23	-4.06±0.19	-3.66±0.25	-3.75±0.23	-4.07±0.17	-3.80±0.24
202	Absent	-1.25±0.06	-1.60±0.04	-1.30±0.06	-3.63±0.13	-3.88±0.09	-3.73±0.13	-3.86±0.13	-3.84±0.08	-3.89±0.13
	Present	-1.18±0.23	-1.57±0.13	-1.36±0.07	-4.04±0.51	-4.45±0.27	-4.17±0.15	-4.32±0.51	-4.23±0.24	-4.22±0.14
204	Absent	-1.27±0.06	-	-	-3.64±0.13	-	-	-3.86±0.13	-	-
	Present	-0.98±0.23	-	-	-3.98±0.51	-	-	-4.26±0.51	-	-

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A29: Effect of OLADRB alleles on immune responses to Johne's vaccination in sheep on three properties

Allele (bp)	Allelic presence	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
		Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
270	Absent	-1.30±0.07	-1.59±0.05	-1.30±0.06	-3.69±0.15	-4.03±0.10	-4.25±0.14	-3.9±0.15	-3.94±0.09	-4.36±0.13
	Present	-1.16±0.10	-1.60±0.08	-1.34±0.07	-3.62±0.23	-3.64±0.17	-3.55±0.15	-3.93±0.24	-3.61±0.14	-3.72±0.14
272	Absent	-1.24±0.06	-1.61±0.05	-1.27±0.05	-3.65±0.13	-3.88±0.10	-3.96±0.12	-3.88±0.13	-3.83±0.09	-4.05±0.11
	Present	-1.44±0.18	-1.53±0.09	-1.49±0.10	-3.85±0.39	-4.1±0.19	-3.77±0.22	-4.16±0.4	-3.97±0.17	-4.08±0.21
274	Absent	-1.27±0.07	-1.56±0.05	-1.32±0.05	-3.54±0.15	-3.94±0.10	-3.90±0.11	-3.85±0.15	-3.89±0.08	-4.03±0.10
	Present	-1.24±0.10	-1.79±0.11	-1.26±0.17	-3.97±0.23	-3.86±0.24	-4.21±0.40	-4.03±0.23	-3.67±0.21	-4.52±0.38
276	Absent	-1.23±0.08	-1.54±0.06	-1.34±0.06	-3.83±0.18	-3.90±0.14	-3.72±0.14	-3.99±0.18	-3.83±0.12	-3.90±0.14
	Present	-1.29±0.08	-1.64±0.06	-1.30±0.07	-3.51±0.17	-3.95±0.12	-4.17±0.16	-3.82±0.18	-3.88±0.10	-4.28±0.13
278	Absent	-1.23±0.06	-1.60±0.04	-	-3.73±0.14	-3.93±0.09	-	-4.00±0.14	-3.86±0.08	-
	Present	-1.35±0.12	-1.51±0.14	-	-3.44±0.27	-3.93±0.29	-	-3.58±0.27	-3.84±0.26	-
284	Absent	-1.24±0.06	-1.62±0.04	-1.31±0.05	-3.63±0.13	-3.88±0.09	-3.82±0.12	-3.84±0.13	-3.82±0.08	-3.99±0.11
	Present	-1.44±0.18	-1.28±0.13	-1.37±0.11	-4.05±0.39	-4.5±0.32	-4.36±0.24	-4.51±0.39	-4.36±0.26	-4.37±0.23
286	Absent	-1.25±0.06	-	-	-3.64±0.13	-	-	-3.89±0.13	-	-
	Present	-1.44±0.22	-	-	-4.11±0.50	-	-	-4.12±0.50	-	-
296	Absent	-1.28±0.06	-1.62±0.06	-1.37±0.06	-3.63±0.13	-3.92±0.12	-3.83±0.14	-3.88±0.14	-3.82±0.11	-4.00±0.13
	Present	-1.12±0.15	-1.57±0.06	-1.26±0.07	-3.91±0.34	-3.93±0.13	-4.03±0.16	-4.06±0.35	-3.91±0.12	-4.13±0.15

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A30: Effect of OLADRW alleles on immune responses to Johne's vaccination in sheep on three properties

Allele (bp)	Allelic presence	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
		Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
458	Absent	-1.20±0.06	-	-	-3.75±0.15	-	-	-3.95±0.14	-	-
	Present	-1.26±0.19	-	-	-2.89±0.25	-	-	-3.11±0.33	-	-
478	Absent	-1.21±0.07	-1.61±0.05	-1.39±0.06	-3.57±0.16	-3.97±0.10	-3.85±0.14	-3.77±0.15	-3.81±0.09	-4.00±0.14
	Present	-1.19±0.12	-1.57±0.13	-1.24±0.06	-3.97±0.28	-3.96±0.28	-3.98±0.16	-4.18±0.28	-4.20±0.25	-4.06±0.15
480	Absent	-1.20±0.07	-1.51±0.05	-1.32±0.05	-3.76±0.17	-3.94±0.12	-3.86±0.11	-3.92±0.16	-3.89±0.10	-3.98±0.10
	Present	-1.23±0.11	-1.81±0.07	-1.46±0.22	-3.46±0.24	-4.04±0.17	-4.84±0.49	-3.76±0.24	-3.80±0.15	-5.09±0.47
488	Absent	-1.22±0.06	-	-	-3.69±0.15	-	-	-3.92±0.14	-	-
	Present	-1.15±0.18	-	-	-3.44±0.42	-	-	-3.46±0.41	-	-
490	Absent	-1.22±0.06	-1.66±0.05	-1.31±0.05	-3.68±0.14	-4.02±0.11	-3.85±0.12	-3.92±0.14	-3.94±0.09	-3.99±0.11
	Present	-1.06±0.21	-1.39±0.10	-1.38±0.10	-3.45±0.48	-3.79±0.22	-4.15±0.24	-3.20±0.47	-3.51±0.19	-4.17±0.23
494	Absent	-1.23±0.06	-1.60±0.05	-1.34±0.06	-3.58±0.14	-3.96±0.11	-4.10±0.13	-3.77±0.14	-3.87±0.09	-4.18±0.12
	Present	-0.99±0.21	-1.63±0.10	-1.28±0.08	-4.63±0.54	-4.03±0.23	-3.50±0.18	-4.93±0.46	-3.82±0.20	-3.70±0.17
496	Absent	-1.20±0.06	-1.62±0.05	-1.32±0.05	-3.73±0.14	-4.09±0.11	-3.98±0.11	-3.91±0.14	-3.92±0.10	-4.07±0.11
	Present	-1.30±0.19	-1.57±0.09	-1.37±0.11	-3.12±0.43	-3.58±0.19	-3.56±0.26	-3.52±0.42	-3.66±0.18	-3.80±0.25
498	Absent	-	-1.61±0.04	-	-	-3.93±0.10	-	-	-3.84±0.09	-
	Present	-	-1.54±0.19	-	-	-4.70±0.41	-	-	-4.23±0.37	-
500	Absent	-1.20±0.06	-	-	-3.62±0.14	-	-	-3.81±0.14	-	-
	Present	-1.37±0.26	-	-	-4.39±0.58	-	-	-4.88±0.57	-	-
502	Absent	-	-1.62±0.05	-1.27±0.05	-	-3.96±0.10	-3.94±0.12	-	-3.83±0.09	-4.00±0.11
	Present	-	-1.55±0.12	-1.53±0.10	-	-4.05±0.26	-3.79±0.23	-	-4.03±0.24	-4.11±0.22
508	Absent	-	-	-1.31±0.05	-	-	-3.81±0.12	-	-	-3.96±0.11
	Present	-	-	-1.37±0.11	-	-	-4.33±0.24	-	-	-4.30±0.23

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A31: Effect of SMHCC1 alleles on immune responses to Johne's vaccination in sheep on three properties

Allele (bp)	Allelic presence	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
		Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
180	Absent	-1.27±0.06	-1.60±0.04	-1.33±0.05	-3.69±0.13	-3.98±0.09	-3.92±0.11	-3.93±0.13	-3.92±0.08	-4.05±0.11
	Present	-1.07±0.30	-1.55±0.14	-1.33±0.13	-3.62±0.67	-3.45±0.28	-3.82±0.30	-3.49±0.68	-3.47±0.25	-3.93±0.29
186	Absent	-1.28±0.06	-1.62±0.04	-	-3.71±0.13	-3.95±0.09	-	-3.91±0.13	-3.91±0.08	-
	Present	-1.10±0.17	-1.43±0.10	-	-3.44±0.38	-3.84±0.22	-	-3.99±0.38	-3.68±0.20	-
188	Absent	-1.28±0.07	-1.61±0.04	-1.29±0.05	-3.82±0.15	-3.86±0.09	-3.97±0.12	-4.09±0.15	-3.84±0.08	-4.05±0.11
	Present	-1.20±0.11	-1.53±0.09	-1.49±0.10	-3.30±0.21	-4.24±0.19	-3.70±0.22	-3.46±0.24	-4.04±0.17	-4.00±0.21
190	Absent	-1.24±0.06	-	-	-3.69±0.13	-	-	-3.95±0.13	-	-
	Present	-1.38±0.17	-	-	-3.66±0.38	-	-	-3.66±0.39	-	-
192	Absent	-1.21±0.07	-1.64±0.04	-1.34±0.05	-3.60±0.16	-4.01±0.09	-3.85±0.12	-3.80±0.16	-3.92±0.08	-3.97±0.12
	Present	-1.34±0.09	-1.45±0.09	-1.31±0.08	-3.81±0.20	-3.66±0.18	-4.06±0.19	-4.11±0.20	-3.72±0.16	-4.21±0.18
194	Absent	-1.21±0.08	-1.51±0.05	-1.38±0.06	-3.56±0.17	-3.90±0.12	-4.00±0.13	-3.81±0.17	-3.86±0.10	-4.12±0.13
	Present	-1.33±0.09	-1.69±0.06	-1.24±0.07	-3.84±0.19	-3.97±0.12	-3.75±0.17	-4.06±0.19	-3.89±0.11	-3.91±0.16
196	Absent	-	-1.59±0.04	-	-	-3.92±0.09	-	-	-3.86±0.08	-
	Present	-	-1.66±0.14	-	-	-4.11±0.29	-	-	-4.06±0.26	-
198	Absent	-1.29±0.06	-1.61±0.04	-1.36±0.06	-3.63±0.14	-3.93±0.09	-3.97±0.14	-3.90±0.14	-3.87±0.08	-4.12±0.13
	Present	-1.11±0.14	-1.49±0.14	-1.30±0.07	-3.95±0.31	-3.96±0.30	-3.84±0.15	-4.00±0.31	-3.94±0.26	-3.94±0.15
200	Absent	-1.24±0.06	-	-	-3.71±0.13	-	-	-3.95±0.13	-	-
	Present	-1.64±0.24	-	-	-3.14±0.53	-	-	-3.30±0.54	-	-
202	Absent	-1.27±0.06	-1.59±0.05	-1.32±0.05	-3.62±0.13	-3.92±0.10	-3.89±0.11	-3.86±0.13	-3.85±0.09	-4.03±0.10
	Present	-1.06±0.24	-1.61±0.07	-1.47±0.17	-4.79±0.53	-3.97±0.15	-4.17±0.38	-4.85±0.53	-3.94±0.13	-4.21±0.37
204	Absent	-	-1.58±0.04	-	-	-3.92±0.09	-	-	-3.87±0.08	-
	Present	-	-1.78±0.15	-	-	-4.13±0.32	-	-	-3.92±0.28	-
210	Absent	-1.32±0.06	-1.59±0.04	-1.35±0.05	-3.78±0.14	-3.95±0.09	-3.90±0.12	-4.03±0.14	-3.89±0.08	-4.04±0.12
	Present	-1.01±0.13	-1.64±0.09	-1.26±0.09	-3.26±0.27	-3.88±0.19	-3.94±0.20	-3.46±0.25	-3.82±0.17	-4.05±0.19

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A32: Effect of OVINRA1 and OVINRA2 alleles on immune responses to Johne's vaccination in sheep on three properties

Allele (bp)	Allelic presence	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
		Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
OVINRA1										
156	Absent	-1.28±0.06	-1.67±0.05	-1.32±0.05	-3.67±0.14	-4.05±0.11	-3.93±0.11	-3.86±0.14	-3.94±0.09	-4.04±0.10
	Present	-1.15±0.14	-1.47±0.07	-1.43±0.14	-3.72±0.31	-3.73±0.15	-3.90±0.33	-4.10±0.31	-3.73±0.13	-3.98±0.32
158	Absent	-1.30±0.07	-1.63±0.05	-1.30±0.05	-3.78±0.15	-3.81±0.10	-3.80±0.12	-4.06±0.15	-3.76±0.09	-3.93±0.12
	Present	-1.16±0.10	-1.51±0.09	-1.42±0.08	-3.44±0.23	-4.43±0.18	-4.25±0.19	-3.52±0.23	-4.24±0.16	-4.28±0.15
160	Absent	-1.26±0.06	-1.58±0.05	-1.32±0.06	-3.62±0.14	-3.99±0.11	-3.93±0.14	-3.85±0.14	-3.91±0.09	-4.04±0.13
	Present	-1.25±0.12	-1.64±0.07	-1.35±0.07	-3.89±0.26	-3.85±0.15	-3.93±0.15	-4.08±0.26	-3.77±0.13	-4.02±0.15
162	Absent	-1.23±0.09	-1.62±0.06	-1.42±0.07	-3.63±0.20	-3.96±0.12	-4.13±0.16	-3.77±0.20	-3.85±0.10	-4.25±0.16
	Present	-1.28±0.07	-1.58±0.06	-1.28±0.06	-3.71±0.16	-3.93±0.13	-3.80±0.13	-3.98±0.16	-3.89±0.12	-3.90±0.12
164	Absent	-1.28±0.07	-1.56±0.05	-1.36±0.05	-3.80±0.15	-3.96±0.11	-3.93±0.12	-4.00±0.15	-3.91±0.10	-4.04±0.11
	Present	-1.22±0.10	-1.66±0.07	-1.26±0.08	-3.42±0.22	-3.93±0.14	-3.91±0.19	-3.70±0.22	-3.80±0.12	-4.01±0.18
168	Absent	-1.26±0.06	-1.60±0.04	-1.32±0.05	-3.63±0.13	-3.93±0.09	-3.95±0.11	-3.87±0.14	-3.85±0.08	-4.04±0.10
	Present	-1.25±0.15	-1.57±0.21	-1.42±0.16	-4.02±0.33	-4.24±0.44	-3.70±0.36	-4.13±0.34	-4.20±0.39	-3.96±0.35
OVINRA2										
312	Absent	-0.99±0.12	-1.59±0.08	-1.34±0.08	-3.89±0.29	-3.94±0.18	-4.18±0.19	-4.19±0.29	-3.85±0.15	-4.26±0.18
	Present	-1.28±0.07	-1.59±0.06	-1.26±0.06	-3.66±0.17	-3.96±0.13	-3.81±0.14	-3.85±0.18	-3.92±0.11	-3.86±0.13
314	Absent	-1.28±0.08	-1.59±0.06	-1.26±0.06	-3.65±0.18	-3.95±0.13	-3.84±0.15	-3.78±0.18	-3.95±0.11	-3.90±0.13
	Present	-1.03±0.11	-1.60±0.08	-1.34±0.08	-3.88±0.27	-3.94±0.18	-4.12±0.19	-4.32±0.27	-3.79±0.15	-4.18±0.18
316	Absent	-1.20±0.07	-1.62±0.05	-1.28±0.05	-3.72±0.16	-3.95±0.11	-3.91±0.12	-3.94±0.16	-3.89±0.09	-3.98±0.11
	Present	-1.26±0.20	-1.28±0.17	-1.38±0.14	-3.74±0.46	-3.96±0.37	-4.16±0.34	-3.99±0.48	-4.00±0.31	-4.21±0.31
318	Absent	-1.22±0.07	-1.59±0.05	-1.27±0.05	-3.71±0.15	-3.92±0.11	-3.89±0.13	-3.91±0.16	-3.89±0.09	-3.95±0.12
	Present	-1.02±0.27	-1.65±0.21	-1.37±0.10	-3.98±0.63	-4.42±0.44	-4.14±0.25	-4.59±0.64	-3.96±0.38	-4.21±0.23
320	Absent	-1.22±0.07	-	-	-3.71±0.15	-	-	-3.94±0.16	-	-
	Present	-0.99±0.23	-	-	-3.81±0.52	-	-	-3.98±0.54	-	-

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively

Table A33: Effect of o(IFN)γ and KP6 alleles on immune responses to Johne's vaccination in sheep on three properties

Allele (bp)	Allelic presence	LSM* for antibody OD value			LSM* for IFN-γ (Johnin-nil) OD value			LSM* for IFN-γ (Johnin-avian) OD value		
		Property 1	Property 2	Property 3	Property 1	Property 2	Property 3	Property 1	Property 2	Property 3
o(IFN)γ										
124	Absent	-1.38±0.13	-1.50±0.18	-1.02±0.29	-2.95±0.34	-3.27±0.30	-2.21±0.65	-3.14±0.34	-3.46±0.35	-2.24±0.62
	Present	-1.24±0.06	-1.60±0.04	-1.33±0.04	-3.75±0.13	-3.98±0.09	-3.96±0.10	-3.99±0.13	-3.9±0.08	-4.08±0.10
128	Absent	-1.21±0.08	-1.63±0.06	-1.37±0.05	-3.60±0.19	-4.07±0.12	-4.05±0.12	-3.82±0.19	-3.95±0.11	-4.15±0.11
	Present	-1.29±0.07	-1.57±0.06	-1.21±0.08	-3.72±0.17	-3.82±0.12	-3.58±0.19	-3.97±0.17	-3.8±0.11	-3.72±0.18
KP6										
186	Absent	-1.24±0.06	-1.58±0.04	-1.32±0.05	-3.68±0.13	-3.96±0.09	-3.92±0.11	-3.93±0.13	-3.90±0.08	-4.00±0.10
	Present	-1.74±0.26	-2.06±0.17	-1.48±0.26	-3.67±0.67	-3.98±0.38	-3.69±0.58	-3.40±0.68	-3.48±0.33	-4.08±0.55
200	Absent	-1.24±0.16	-1.59±0.08	-1.39±0.09	-3.17±0.45	-4.00±0.16	-3.96±0.21	-3.30±0.35	-3.87±0.14	-4.01±0.19
	Present	-1.26±0.06	-1.61±0.05	-1.30±0.05	-3.76±0.13	-3.95±0.10	-3.90±0.12	-3.99±0.13	-3.88±0.09	-4.00±0.12
202	Absent	-1.25±0.06	-1.61±0.05	-1.33±0.05	-3.62±0.13	-3.96±0.10	-3.96±0.11	-3.87±0.14	-3.91±0.09	-4.03±0.11
	Present	-1.29±0.14	-1.59±0.09	-1.26±0.12	-4.15±0.35	-4.00±0.18	-3.62±0.27	-4.18±0.35	-3.79±0.16	-3.84±0.25
204	Absent	-1.26±0.07	-1.64±0.06	-1.20±0.07	-3.87±0.18	-3.75±0.13	-3.69±0.16	-3.97±0.18	-3.68±0.12	-3.80±0.15
	Present	-1.24±0.09	-1.58±0.05	-1.42±0.06	-3.43±0.15	-4.12±0.11	-4.09±0.14	-3.82±0.17	-4.02±0.10	-4.17±0.13
206	Absent	-	-	-1.35±0.05	-	-	-3.94±0.11	-	-	-4.04±0.10
	Present	-	-	-1.08±0.15	-	-	-3.67±0.35	-	-	-3.67±0.33

* Least square mean in logarithmic scale, over the post-vaccination periods 12 to 54 months, 0 to 48 months and 0 to 42 months, in properties 1, 2 and 3, respectively