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## LEPTOSPIROSIS IN NEW ZEALAND PIG HERDS

An epidemiological study and a computer simulation model  
of endemic leptospiral infection  
in New Zealand pig herds,  
with  
particular reference to  
*Leptospira interrogans* serovar *pomona*.

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### ABSTRACT

A serological survey of pig sera from six regional areas throughout New Zealand indicated that 60% had titres to *Leptospira interrogans* serovar *pomona* and 13% to serovar *tarassovi*. Pig sera from the North Island districts had higher titres to *pomona* than those from the South Island districts, however the converse was true for titres to *tarassovi*. A serological survey of bacon weight pigs at slaughter revealed that 60% had titres to *pomona*, 53% to *bratislava*, while titres to *tarassovi* were undetectable. There was no significant linear association between the magnitude of corresponding *pomona* and *bratislava* titres. *Pomona* was isolated from 53% of pigs' kidneys, however attempts to isolate *bratislava* were unsuccessful. The median prevalence of infection in bacon pigs from farms with endemic *pomona* infection, at the time of slaughter was 80%. A retrospective study of farming practices revealed that vaccination of breeding pigs had no effect on the infection status of their grower pigs at slaughter. It was also found that farms which reared their grower pigs to pork weight for slaughter were free of leptospiral infection, as were farms with less than fifty breeding sows.

Cross sectional serological and cultural prevalence studies of grower pigs on farms with endemic *pomona* infection revealed that pigs less than ten weeks of age were not leptospiruric and had low or undetectable titres to *pomona*. Pigs between ten and twenty weeks of age showed an increasing prevalence of both leptospiuria and *pomona* titres. Further prospective studies indicated that piglets acquire passive immunity from their dams, which has a half life of around sixteen days. The majority of pigs less than fourteen weeks of age appear to be resistant to infection, thereafter the level of their passive immunity wanes and they become infected and leptospiruric. The weekly incidence of leptospiuria for pigs in an infected grower house was usually between 10% and 20%. Following infection, the intensity of leptospiuria was greatest in the first three to four weeks and it lasted for at least six weeks. Infection is believed to occur by both direct and indirect transmission of leptospires between infected and susceptible pigs. It was shown that grower pigs are at the centre of the endemic cycle of infection which is perpetuated by the transmission of infection from older infected pigs to younger susceptible pigs. This cycle of endemic infection can persist independently of the breeding herd.

Experimental evidence following the artificial exposure of grower pigs to either serovar *pomona* or *bratislava* supported an hypothesis that the occurrence of *bratislava* titres were associated with early infection of pigs with serovar *pomona*. The heterologous titres were believed to be a serological cross reaction with homologous IgM antibody to which pigs had been exposed.

Experimental evidence demonstrated that leptospires could survive in droplets of less than 50  $\mu\text{m}$ , however hamsters exposed to a leptospiral aerosol containing droplets of less than 50  $\mu\text{m}$  failed to become infected. Infection via the intranasal route in both hamsters and pigs showed that the infective dose of *pomona* was between  $10^4$  and  $10^6$  leptospires, indicating the intranasal route as a natural route for infection. Transmission of infection could therefore occur directly by infective droplets lodging in the nasal cavity.

The vaccination of pigs, commencing at ten weeks of age on a farm with endemic *pomona* infection, revealed that multiple inoculations of a commercially available bacterin can be used to control the level of endemic infection within a grower pig herd. There was evidence to suggest that persistent passive immunity in young pigs could interfere with the efficacy of vaccination.

A computer simulation model of endemic *pomona* infection in a pig herd [Simulated Leptospiral Infection within a Pig herd, SLIP89] was developed using the results of investigations described in this thesis by sequentially breaking down the cycle of endemic *pomona* infection into a series of logical events. The model repeats a number of predetermined independent and dependant events for each pig within a simulated herd. The outcome of each event is randomly determined from an appropriately selected probability distribution. Each cycle of repetition represents one week in time. The outcome generated by the simulation can be used to observe varying patterns of infection which are due to either the element of chance or the alteration of key variables within the model. The results generated by the SLIP89 must be viewed with the structure and limitations of the model in mind.

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## GENERAL INTRODUCTION

Leptospirosis is a zoonotic disease of world wide importance. Human beings may be an accidental host to leptospiral infection, however they play no role in the maintenance of infection within populations of domestic or free living animals.

### *History*

Landouzy (1883), Weil (1886) and Vasilier (1888), published accounts of a severe human illness characterised by jaundice and renal involvement which was distinct from other icteric and nephritic conditions. The cause of the syndrome, named Weil's disease was not initially known. Weil's disease, also called Infectious Jaundice was a collective description for a specific disease entity which exhibited clinical signs of severe hepatic and renal dysfunction and often resulted in jaundice and renal failure.

In 1914, in Japan, Inada and Ido (1915) isolated *Leptospira interrogans* serovar *icterohaemorrhagiae* [strain Ictero No.I] which was originally named *Yamasaki*, from a patient suffering from Weil's disease (Hata *et al*, 1988). Shortly after this first Japanese isolation of a pathogenic leptospira, a second pathogenic leptospira, *Leptospira interrogans* serovar *icterohaemorrhagiae* [strain RGA] was made in Germany from a patient also suffering from Weil's disease (Uhlenhuth and Fromme, 1916).

Following these isolations, *L. interrogans* serovar *icterohaemorrhagiae* was also isolated from rats [*Rattus norvegicus*] (Ido *et al*, 1917). Since those early days, new antigenically distinct leptospiral types have continued to be isolated from an increasing number of species of animals.

Weil's disease and leptospirosis are no longer considered synonymous because the clinical signs of Weil's disease are distinct and due only to infection with *L. interrogans* serovar *icterohaemorrhagiae*, while the clinical signs of infection with other leptospiral serovars are less specific and the disease entity is termed leptospirosis.

### ***Classification***

In 1917, Noguchi defined the Genus *Leptospira*, of the family Spirochaetaceae (Noguchi, 1918). The word *Leptospira* is derived from Greek, meaning a fine coil. *Leptospira* are single, flexuous, helical cells, 6 to 20  $\mu\text{m}$  in length and 0.1  $\mu\text{m}$  in diameter (Buchanan and Gibbons, 1974). They are readily visible by dark field microscopy [DFM], and appear as motile spirochaetes with shunting, rotating, and flexing movements.

A new classification within the family of *Leptospiraceae* has been proposed by Yasuda *et al* (1987) who used deoxyribonucleic acid hybridisation to characterize leptospiral serovars. Due to the heterogeneity exhibited within the accepted species of *L. biflexa*, *L. interrogans* and *L. parva*, Yasuda *et al* (1987) proposed that at least five new species of parasitic serovars and two new species of saprophytic serovars be formed. Therefore Yasuda *et al* (1987) proposed a total of seven new species, bringing the number of species to ten. Formerly, only parasitic serovars were included in the species of *Leptospira interrogans*, and saprophytic, free living serovars, with no known hosts have belonged to the *L. biflexa* and *L. parva* species. Provisional classification of an other species, *Leptospira illini* was proposed by the taxonomic subcommittee on *Leptospira* (Faine, 1982). *Leptospira illini* differs from *L. interrogans* and *L. biflexa* in its DNA base composition, serological characteristics and on some morphological characteristics, and is now classified as *Leptonema illini* (Yasuda *et al*, 1987).

To date, 162 distinct serological varieties of *L. interrogans* have been identified, each designated as a serovar, and there are forty serovars which hold provisional status or have been incompletely identified (Kmety and Dikken, 1988). Serovars with similar agglutination characteristics are grouped into twenty three serogroups. Recently identification and categorisation has begun using Bacterial Restriction Endonuclease DNA Analysis [BRENDNA] technique (Marshall *et al*, 1981) as a complement and enhancement to the identification of leptospiral serovars and within some serovars, individual strains or types can be identified.

At the commencement of this study, before Yasuda *et al* (1987) had published their proposal for the new system of classification, the traditional nomenclature was in use and all known isolates had been classified using the traditional system. Therefore throughout this thesis *Leptospires* will be named according to the former traditional classification system used by Kmety and Dikken (1988), in which *L. interrogans* and *L. biflexa* are the two species of importance. Individual serovar names only, will be used to denote leptospires of the *Leptospira interrogans* species, the serovar indicated can be assumed to be the standard reference strain, unless otherwise stated. The standard reference strains are shown in Chapter Two (Table 2.2). For example, *Leptospira interrogans* serovar *pomona*, reference strain *pomona* will be designated as *pomona*. If an isolate other than the standard reference strain is used, it will be specified in brackets, for example the laboratory isolate T A5 identified as serovar *pomona* will be designated as *pomona* [strain T A5].

#### *Modes of transmission of leptospires*

Transmission of leptospires can be thought of as an event which results in the contact of a potential host with parasitic leptospires. Direct transmission is the result of the passage of leptospires directly from an infected host to a susceptible, uninfected host. Indirect transmission of leptospirosis involves the passage of leptospires through an intermediary medium before contact with a susceptible host. The majority of human and animal infections are believed to occur by indirect transmission (Turner, 1967).

Direct transfer of leptospires between infected and susceptible hosts can occur by ingestion or handling of infected animal tissues, biting, and behaviours associated with sucking or licking of genitalia. Direct transmission can also occur during pregnancy and possibly during coitus.

Either water, or water in soil or air may be the medium for the indirect transmission of leptospires from infected to susceptible hosts. Leptospires shed in the urine of infected animals can contaminate the environment and transmit disease to other animals and man (Diesch and McCulloch, 1966; W.H.O., 1982). The duration of survival of leptospires outside a host is primarily determined by the level of environmental moisture, the temperature

and the pH (Hellstrom, 1978). Such factors are likely to be influenced by geographical location and topographical features of the land, which may in turn influence the epidemiological characteristics of leptospiral infection within a region.

The routes of infection which result from direct or indirect transmission of leptospire to a susceptible host are reviewed in the following section.

### *Routes of infection*

The ways by which leptospire can enter the body of a host may be termed the routes of infection. The route of infection is influenced by the mode of transmission.

#### *- Cutaneous*

Throughout the literature, infection via the skin is often either stated or assumed. Artificial infection of guinea pigs with shaven and abraded skin was achieved by partially immersing them in contaminated water (Ring and Bracken, 1956; Gillispie *et al*, 1957). Intact skin can be regarded as having multiple microscopic cuts and abrasions as remarked by Edwards (1960). Water saturated skin is also considered to allow the passage of leptospire, enabling infection to occur (W.H.O., 1982). Leptospire survive well in water, to the extent that they have been termed aquatic organisms by van der Hoeden (1958). Surface waters, streams, ponds and soil can readily become contaminated with infected effluent (Diesch and McCulloch, 1966). Doherty (1967) investigated the role of contaminated water in the dissemination of *pomona* infection and found that it was difficult to precisely determine the source of contamination of the soil or water. Leptospirosis in man has commonly been associated with occupational exposure. The names of "Swineherd's disease", "Dairy farm fever", "Rice field fever", "Swamp fever" and "Cane field fever" were coined in response to documented cases of leptospirosis which were associated occupational exposure resulting from indirect transmission of leptospire from infected animals to human beings. Direct infection of human beings, by handling infected animal products is also well recognised. Occupational exposure of meat inspectors and abattoir workers is postulated to occur through handling infected carcasses and their

products (Faine and Kirschner, 1953; Tobie and McCullough, 1959; Blackmore *et al*, 1979; Kingscote, 1986b).

#### - Oral

Infection of infants has occurred by ingestion of their mothers milk, which contained leptospires (Chung *et al*, 1963; Bolin and Koellner, 1988). Excretion of leptospires in bovine milk was reported by Thiermann (1981). Mitchell *et al* (1960) and Hanson *et al* (1964) suspected that cattle had become infected after grazing contaminated pastures. However, Kenzy and Gillispie (1957) failed to show evidence of infection via the oral route in calves which were exposed to drinking water which was contaminated by artificially infected ducks, and the urine of a leptospiruric cow. Will and Diesch (1976) also failed to induce artificial infection in syrian hamsters fed corn which had been contaminated with waste slurry containing leptospires.

Infection resulting from ingestion of contaminated carcasses or leptospiral cultures has been demonstrated (Reilly *et al*, 1970; Chalquest, 1957; Shophet and Marshall, 1980). It was proposed by Reilly *et al* (1968; 1970) that leptospires survive in large boluses of tissue that are normally swallowed by carnivores, and the leptospires then penetrate the duodenal mucosa. Infection by ingestion is suspected to occur under natural conditions of prey predator relationships (Salt and Little, 1977; Shophet and Marshall, 1980). Salt and Little (1977) suggested that badgers [*Meles meles*] and free-living carnivores may become infected from eating small rodents. Shophet and Marshall (1980) demonstrated that cats can become infected with *ballum* by feeding them mice or offal infected with *ballum*. Hathaway and Blackmore (1981) investigated the prevalence of leptospiral infection in feral cats [*Felis catus*] and free-living *Mustelidae*, however they found no serological or bacteriological evidence of leptospiral infection, even though the animals came from ecosystems where *ballum* infection was endemic in rodents. They concluded that there was little evidence to support the hypothesis that predator-prey relationships resulted in the natural transmission of leptospiral infection.

The oral route of infection does not therefore appear to be a common route of natural infection for domestic animals or human beings.

- *Respiratory*

West and Whitehead (1953), Alexander *et al* (1964), Mailloux (1975), Andrew and Marrocco (1977), and Kingscote (1986b) refer to leptospiral infection as occurring via mucous membranes, and Blackmore *et al* (1976) suggested that bulls at an artificial breeding centre may have become infected following possible nasal contact with cows' genitalia. Baker and Little (1948) infected both cows and calves via the intranasal route using 10 ml of chorioallantoic fluid from eggs which had been artificially infected. However, the large volume of intranasal inoculum used by Baker and Little (1948) is not representative of the volume which would normally be presented to the nasal mucosa by droplets. Therefore, the large volume used may have overwhelmed the nasal cavity, resulting in most of the liquid entering the pharynx and being swallowed. Thus, a cautious interpretation must be placed on the practical significance of their findings.

Burnstein and Baker (1954) induced infection in piglets following intranasal inoculation with *pomona*, using as few as 100 leptospires. Intranasal inoculation of piglets with *australis* was also achieved by Inzana and Dawe (1979). Will and Diesch (1976) used *pomona* to test the intranasal, intraocular and oral routes of infection in syrian hamsters. They found that these hamsters were most sensitive to infection via the intranasal route, requiring a minimum of  $10^5$  leptospires for infection. Will and Diesch (1976) did not state the volume of inoculum used. If intranasal inoculation is to be used as a route of infection, the volume and number of leptospires used, should be stated. Blackmore and Schollum (1986, unpublished) used rats (*Rattus norvegicus*) to test probable natural routes of infection using serovar *copenhageni*. Intranasal inoculation of 0.1 ml of a culture containing  $10^8$  leptospires/ml into each nostril resulted in the infection of all exposed rats, while an inoculation dose of 0.1 ml of  $10^4$  leptospires/ml resulted in the infection of 50% of rats. When tested using urine containing approximately  $10^2$  leptospires/ml, rats did not become infected.

Infection via the respiratory route by aerosols has been suggested by Baker and Little (1948), Coghlan (1979), Faine (1982), and Will and Diesch (1976). Will and Diesch housed weaned hamsters above an oxidation pond artificially



infected with leptospires, however the hamsters failed to become infected. Leptospiral infection by aerosol has not been shown to occur.

In Chapter Five, infection via the upper and lower respiratory routes by contact with contaminated droplets and aerosols will be examined and discussed in more detail.

#### - *Conjunctival*

Conjunctival infection has been achieved by either spraying or placing leptospiral cultures onto the conjunctiva or into the conjunctival sac (Amatredjo and Campbell, 1975). Will and Diesch (1976) succeeded in infecting syrian hamsters via the conjunctival route using an infective dose of  $10^6$  leptospires of serovar *pomona*. In contrast, Blackmore and Schollum (1986, Unpublished) failed to infect rats [*Rattus norvegicus*] with *copenhageni* via the conjunctival route using approximately  $10^4$  leptospires. Under experimental conditions, when a volume of leptospiral solution is placed in the conjunctival sac, infection may occur as a result of the movement of infective material, via the nasolacrimal duct, into the nasal cavity.

#### - *Venereal*

Leptospiral infection via the venereal route has been shown to occur by Sleight and Williams (1961), who infected two heifers, one by artificial insemination using semen artificially contaminated with leptospires, and the other became infected after natural service with an infected leptospiruric bull. The potential for the transmission of infection by coitus or artificial insemination, has been demonstrated by the survival of leptospires in semen extender (Bryan and Boley, 1955; Kiktenko *et al*, 1976). Leptospires have been isolated from the reproductive tracts of non-pregnant naturally infected cattle and pigs (Ellis *et al*, 1986a; 1986b; 1986c; 1986d; Ellis and Thiermann, 1986). Kingscote (1986a), Ellis *et al* (1986c), and Sleight and Williams (1961) considered venereal transmission to be of importance as a possible mode of transmission of leptospiral infection amongst domestic animals. It has also been considered important for the maintenance of infection amongst free living animals (Ananin, 1954).

In contrast, Tammemagi *et al* (1961) failed to demonstrate the transmission of *pomona* from leptospiruric boars to non infected sows following mating, nor was the fertility of the boars affected. Blackmore *et al* (1976) did not specifically consider transmission by coitus to be important in the epidemiology of either *pomona* or *hardjo* infection of bulls at an artificial breeding centre, but suggested that "the behavioural pattern of young bulls could be an important factor in the transmission of disease". Blackmore and Schollum (1986, Unpublished) failed to infect rats [*Rattus norvegicus*] with *copenhageni* via the intravaginal route by inoculation of 0.2 ml of culture containing  $10^8$  leptospores/ml or urine with approximately  $10^4$  leptospores/ml. They also failed to show evidence of transmission of infection from infected, leptospiruric male rats to female rats.

#### - *Transplacental*

In human beings, transplacental infection has been documented as the cause of abortion or congenital leptospirosis (Chung *et al*, 1963; Gsell *et al*, 1971).

Infection of a sow during pregnancy, may result in the transmission of leptospores across the placenta to the foetus during the leptospiraemic phase of infection (Ryley and Simmons, 1954a; Fennestad and Borg-Petersen, 1966; Ryan, 1978). Infection in the last trimester of pregnancy often results in abortion, which is a major cause of economic loss to pig and dairy farmers (Ryley and Simmons, 1954b; Ryan, 1978; Fish *et al*, 1963; Fennestad and Borg-Petersen, 1966, Hellstrom 1978).

#### - *Bites and artificial infection*

Leptospiral infection in human beings has been reported to have occurred following animal bites, however the bites were not conclusively demonstrated to be the cause of infection (van der Hoeden, 1958). Schulze (1951) reported seeing leptospores by dark field microscopy [DFM] in the sputum of 50% to 100% of specimens of dogs saliva, depending on the dilution of sputum examined. These results are in doubt as only DFM was used, however, the possibility of this mode of transmission should not be excluded.

The intramuscular (Ryley and Simmons, 1954a) see Chapter Five, intraperitoneal (Hathaway, 1978; Marshall, 1973) see Chapters Two and Five, percutaneous (Hathaway, 1978) and subcutaneous (Langham *et al*, 1958; Morse *et al*, 1958) routes have been successfully used to artificially infect animals. Differences in the time of onset of leptospiraemia and leptospiruria, duration and intensity of leptospiruria, immune response to infection, and the severity of clinical signs apparently resulting from the use of different routes for artificial infection, were noted by Burnstein and Baker (1954).

*Phases of leptospiral infection in a host*

Leptospiral infection of a susceptible host has two distinct phases. Firstly, the leptospiraemic phase, and secondly the leptospiruric phase.

Leptospiraemia is the phase in which leptospires enter the blood stream, multiply and circulate throughout the body (Turner, 1968). Generally, leptospiraemia occurs within three to twenty one days of exposure of a susceptible host to leptospires, and it usually lasts for around seven days. This first phase may be accompanied by some of the acute clinical signs of leptospirosis (Turner, 1968). In human beings, specific symptoms may include the sudden onset of severe headache, muscle pains, gastrointestinal pain, fever, jaundice, abdominal or hepatic tenderness and meningitis (Faine and Kirshner, 1953). During the leptospiraemic phase, some of the leptospires will come to lodge in the renal tubules where they will persist beyond the leptospiraemic phase, and appear to be protected from increasing levels of circulating antibody (Marshall, 1973).

Following the leptospiraemic phase of infection serum antibodies increase in response to infection.

The second phase of infection is characterised by the shedding of leptospires in the host's urine, which is termed leptospiruria. In pigs, the duration of leptospiruria can vary depending on both the infecting serovar (Burnstein and Baker, 1954; Morter and Morse, 1956) and on the individual (Ryley and Simmons 1954a) see Chapters Four and Five. In general, the better the adaption between a serovar and host, the higher the level of serum antibody

and the longer the duration and intensity of leptospiruria (Chernuckha *et al*, 1974) .

### ***The detection of leptospiral infection in a host***

During the leptospiraemic phase of infection leptospires can be isolated from almost any body organ or fluid (Turner, 1968). Blood, liver, kidney and brain are used in an attempt to isolate leptospires during the leptospiraemic phase of infection. During the period of leptospiruria, the presence of infection can be monitored by culturing urine or kidney for the isolation of leptospires, or by dark field microscopy [DFM] of urine to detect leptospiruria (Turner, 1968; MacKintosh, 1981). Chronic colonisation of the renal tubules by leptospires often results in the formation of grey white foci of 1 to 2 mm in diameter which are visible on the gross examination of the kidney's surface. These "white spots" develop in the cortex and may extend down through the medulla of the renal tissue and are considered characteristic of leptospiral infection. Typically, histological examination reveals that the lesions are foci of interstitial infiltration by lymphocytes and plasma cells (Marshall, 1973).

The Microscopic Agglutination Test is an accepted method which is used to detect the maximum dilution of serum at which 50% of leptospires agglutinate (MacKintosh, 1981). The presence of agglutinating antibodies may either indicate the presence of maternal antibody, or present or past infection (Hellstrom, 1978).

The methods used for the diagnosis of leptospiral infection, and for the detection of leptospires throughout this study are outlined in Chapter Two.

### ***Maintenance hosts and populations***

#### ***- Maintenance and accidental hosts***

Blackmore and Hathaway (1980), defined a maintenance host as having the following characteristics: " a) High susceptibility of infection [low infective dose]. b) Low pathogenicity of serovar for the host. c) Long term kidney infection related to the systemic phase of infection. d) Natural transmission within the species."

Some serovars have more than one maintenance host, and some species of animal are the maintenance hosts to more than one serovar, as shown in Table 1.1.

If a host, other than a maintenance host becomes infected, it is termed an accidental host. Infection of an accidental host may occur following its entry into the ecosystem of a maintenance host, or by movement of the maintenance host from its original ecosystem into that of the accidental host (Hathaway, 1978; 1981). A maintenance host may also be an accidental host to one or more other leptospiral serovars.

*- Maintenance population*

Blackmore and Hathaway (1980) suggested that the minimum infective dose and the duration and intensity of leptospiruria were two factors which could be related to the creation of a maintenance population. They defined a maintenance population as "a population of a species of animal which acts as a continuous reservoir of infection in a specific ecosystem". An application of this concept of a maintenance population will be discussed in Chapters Four and Seven.

## LEPTOSPIRAL INFECTION IN PIGS

Ryan (1978) has extensively reviewed the literature on leptospiral infection in pigs. There shall be no attempt to repeat Dr T.J. Ryan's review, however a brief history of leptospiral infection in pigs, and a summary of leptospiral infection in pigs due to serovars *pomona*, *tarassovi* and *bratislava*, and an introduction as to means of control of leptospiral infection in pigs are given in this section of Chapter One. The epidemiology of endemic *pomona* infection within pig herds is the predominant theme of this thesis and therefore most emphasis will be given to this serovar.

### *History of leptospiral infection in pigs*

In 1935, the role of pigs as hosts of leptospiral disease was first recognised by Sander (1935) who described a disease entity in pigs which was similar to Weil's Disease of human beings, then synonymous with *icterohaemorrhagiae* infection. This was followed by the isolation of *icterohaemorrhagiae* from a jaundiced piglet by Klarenbeek and Winser (1937). Until this time leptospirosis was considered primarily a disease of human beings and rodents.

The first isolate of *pomona* was from a dairy farm worker in Queensland (Clayton *et al*, 1937), this isolate is now referred to as serovar *pomona* strain Pomona (Kmety and Dikken, 1988). The earliest isolates of *pomona* from pigs were obtained in Jakarta [Indonesia] by Mochtar (1940) and in Queensland [Australia] by Johnson (1942). By 1946, naturally occurring infections of pigs with either *pomona* or *tarassovi* (*hyos*) had been associated with leptospirosis in human beings (Alexander *et al*, 1964). Thereafter, the aetiology of "Swineherds disease" was attributed to *pomona* by Gsell (1944) who described infection in both human beings and pigs. From this time onwards many leptospiral serovars have been isolated from pigs throughout the world. The range of serovars isolated from pigs is diverse; *pomona*, *tarassovi*, *bratislava*, *icterohaemorrhagiae*, *canicola*, and *grippotyphosa* are the most commonly reported infections while *mazdok* (Kocik, 1988), *muenchen* (Hathaway *et al*, 1982), *australis*, *balcanica*, *autumnalis*, *guidae*, *javanica*, *moldaviae*, *monjakov*, *vietnam* (summarised by Ryan, 1978), *ballum*, *serjoe*, *bativae* (Hansen *et al*, 1971) and *pyrogenes* (Topacio *et al*, 1968) have been reported less frequently.

### *Serovar pomona*

*Pomona* is the most widespread and common of the leptospiral serovars reported to infect pigs (Ryan, 1978). Pigs are considered a maintenance host for *pomona* in many countries including New Zealand (Ryan, 1978), and in North America, skunks (*Melphitis melphitis*) are also responsible for maintaining this serovar (Tabel and Karstad, 1967).

Infection of pigs with *pomona*, primarily occurs through direct or indirect contact with infective urine (Buddle and Hodges, 1977) see Chapter Four. Infection is characteristically asymptomatic, in some cases a mild fever may occur which lasts a few days (Ryley and Simmons, 1954a). Transplacental transmission can occur when a pregnant sow or gilt is infected during the last trimester of its pregnancy, and may result in either abortion or the birth of weak and runty piglets (Ryley and Simmons, 1954a, 1954b; Fennestad and Borg-Petersen, 1966). Infected pigs have been known to be leptospiruric for more than a year, although they usually experience leptospiruria of high intensity for six to twelve weeks, they may also become intermittent shedders (Alexander *et al*, 1964; Morse *et al*, 1958; Hodges, 1973) see Chapter Four. The intermittent shedding of leptospires may continue for approximately ten to 120 days, with as long as sixteen months having been reported (Mitchell *et al*, 1966).

Accidental infection of other animals, including human beings, may result from direct or indirect contact with infected pigs (Diesch and McCulloch, 1966). Other species of animal from which *pomona* has been isolated, but for which the epidemiological significance is less clear are wombats [*Vombatus ursinus*] (Munday and Corbould, 1973), striped field mice [*Apodemus agrarius*] (Borg-Petersen and Fennestad, 1956), house mice [*Mus musculus*] (Whyte and Ratcliff, 1982), field voles [*Microtus agrestis*] (Hanson, 1972; Little and Salt, 1975), brown rats [*Rattus norvegicus*] (Hanson, 1972; Morales *et al*, 1978), porcupine [*Logomorpha*] (Mitchell *et al*, 1966), sheep (Hanson, 1972; Ellis *et al*, 1983), the Californian sea lion [*Zagophus californsonus*] (McIlhattan *et al*, 1971), wild guinea pigs [*Cavis aperea festina*] (Leptospiral serotype distribution lists , 1966), opossums [*Didelphis marsupialis*] (Ferris and Andrews, 1967), horses, goats, raccoons [*Procyon lotor*] (Hanson 1972), red deer [*Cervus*

*elaphus*] (Fairley *et al*, 1986), wood chuck [*Marmota monax*], red fox [*Vulpus fulva*], gray fox [*Urocyon cinereoargenteus*], bobcat [*Lynx rufus*] (Hanson, 1972), domestic cat (Harkness *et al*, 1970), feral cat (Ferris and Andrews, 1967) and dogs (Murphy *et al*, 1958; Hanson, 1972).

The cycle of *pomona* infection is perpetuated when infection is directly or indirectly transmitted to other pigs. The epidemiological features and cycle of endemic *pomona* infection in New Zealand piggeries are detailed and discussed in Chapter Four.

#### Serovar *tarassovi*

Serovar *tarassovi* [previously known as *hyos* and *mitis* - Johnson] infection of pigs also occurs worldwide, however, it does not appear to be as common as *pomona* (Ryan, 1978). In New Zealand, pigs are considered to be maintenance hosts for *tarassovi* (Ryan, 1978). This serovar has also been isolated from cattle and various wild animals (Alexander *et al*, 1964; Ryan, 1978). Naturally occurring infection in pigs is usually asymptomatic however artificial infection may result in a transient increase in temperature and inappetence (Tammemagi and Simmons, 1956; 1958). Abortion is not a consistent feature of *tarassovi* infection of sows or gilts (Tammemagi and Simmons, 1956, 1958).

#### Serovar *bratislava*

Recently, infection of pigs with serovar *bratislava* has been shown to be associated with poor reproductive performance in breeding herds (Ellis *et al*, 1985), and has been isolated from sows, boars and piglets in Ireland (Ellis *et al*, 1985; 1986a; 1986b; 1986c), and from sows in U.S.A. (Ellis and Thiermann, 1986). Previously, *bratislava* [also known as *L. esposito* and *L. erinacei-europaei*] had been primarily isolated from hedgehogs [*Erinaceus europaeus*] and foxes [*Vulpus vulpes*] (Borg-Petersen and Fennestad, 1962; Farina *et al*, 1977; Hartman *et al*, 1975). Serum agglutinating antibodies to *bratislava* have been detected in pigs from the Netherlands (Hartman *et al*, 1975), England (Hathaway *et al*, 1981), Italy (Farina *et al*, 1977) and Australia (Chung, 1968).



Artificial infection of gilts with *bratislava* has been shown to be asymptomatic, however infection of sows resulted in high fever, gastrointestinal disturbances, a decrease in their general condition, and infection was also presumed to be responsible for the expulsion of dead foetuses from some infected sows (Farina *et al*, 1977).

An investigation for evidence of infection with *bratislava* in pigs in New Zealand is described and discussed in Chapters Three and Five.

#### *Treatment, control and eradication*

There are two major ways in which leptospiral infection could theoretically be controlled in animal populations. One way is to decrease the probability of transmission of leptospire, and the other is to increase resistance to infection. For example, the probability of transmission can be decreased by implementing management changes which prevent susceptible pigs from coming in contact, either directly or indirectly with infected animals. Alternatively, increased resistance to infection can be achieved by vaccination, thus reducing the probability of infection.

Antibiotics have long been used to treat leptospirosis in animals, including human beings. They are used extensively for the treatment of leptospiral infection of domestic animals, with the intention of eliminating leptospiruria (Ferguson *et al*, 1956; Lococo *et al*, 1958; Doherty and Baynes, 1967; Stalheim, 1967; Dobson, 1971 and 1974; Fenske and Horsch, 1974; Kasimova *et al*, 1977; Edwards and Daines, 1979; Hodges *et al*, 1979; Ketterer and Dunster, 1985; Biro, 1988). The use of antibiotic treatment in attempts to control leptospiral infection in pigs will be reviewed in more detail in Chapter Six.

Transmission of infection can theoretically also be partially or totally prevented by physical segregation of infected from uninfected pigs. Buddle and Hodges (1977) reported that pigs which were penned "up-stream" from infected pigs became infected later than pigs penned "down-stream", however infection was not prevented in pigs which were not in direct contact with the effluent of infected pigs.

Infection can be prevented, as discussed above, by reducing the risk of transmission of disease. This may not be possible in many piggeries, due to poor design and management with respect to control of leptospiral infection. Prevention of infection by means of vaccination is another method for the control and possibly the eradication of leptospiral infection from pig herds. Resistance to infection can also be derived from passive immunity. Vaccination strategies for the control of leptospirosis will be discussed in Chapter Six.

Genetic resistance of pigs to leptospirosis has been reported by Przytulski and Porzeczkowska (1980). The heritability of the resistance to infection was both difficult to measure and reported to be low. Genetic resistance to infection is unlikely to play a significant role in the control of leptospirosis in piggeries in the near future. The concept of genetic variability influencing a pig's susceptibility to infection is of potential importance in the selection of pigs for trial work.

### LEPTOSPIRAL INFECTION IN NEW ZEALAND PIGS

In New Zealand, domestic pigs are considered to be maintenance hosts for serovars *pomona* and *tarassovi* (Ryan, 1978), however Daniel (1967) found no evidence of leptospiral infection in feral pigs. The first isolation of *pomona* from a pig in New Zealand was made by de Jong and Fowler (1968). Isolates of *pomona* have also been obtained from sheep (Hartley, 1952), a dog (Te Punga and Bishop, 1953), a cat (Harkness *et al*, 1970) and human beings (Jamieson and Davidson, 1970).

Other leptospiral serovars have also been isolated in New Zealand. The first pathogenic leptospires were isolated in the 1950's, serovar *copenhageni* [at the time identified as serotype *icterohaemorrhagiae*] was isolated from human beings, rats and dogs (Kirschner and Gray, 1951) and *pomona* from cattle (Anon, 1951). Since then, serovars *ballum* (Anon, 1967), *hardjo* (Lake, 1973), *tarassovi* (Ryan and Marshall, 1976) and *balcanica* (Marshall *et al*, 1976) have been isolated in New Zealand from either wild or domestic animals. Each serovar is associated with one or more maintenance host(s), as shown in Table 1.1.

**Table 1.1 Maintenance hosts for leptospiral serovars found in New Zealand.**

Leptospiral serovar	Maintenance Host(s)
<i>balcanica</i>	Possum [ <i>Trichosurus vulpecula</i> ]
<i>ballum</i>	Mouse [ <i>Mus musculus</i> ] Black rat [ <i>Rattus rattus</i> ] Hedgehog [ <i>Erinaceus europaeus</i> ]
<i>copenhageni</i>	Brown rat [ <i>Rattus norvegicus</i> ]
<i>hardjo</i>	Domestic cattle
<i>pomona</i>	Domestic pigs
<i>tarassovi</i>	Domestic pigs

In New Zealand, the term "red water fever" is used to describe a clinical disease in which haemoglobinuria is commonly a presenting sign in cattle infected with serovar *pomona*. This disease has caused up to 100% mortality in calves (Anon, 1951; Stewart, 1934; Ensor, 1953) and was often associated with pig contact, however cattle were also believed to maintain *pomona* (Salisbury, 1954). Jamieson and Davidson (1970) reported that 11% to 16% of abortions in dairy cows were due to infection with *pomona*.

In 1952, Kirschner *et al* described the symptoms of a farmer suffering from leptospirosis and demonstrated serum agglutinating antibodies to *pomona* in this farmer. The farmer's illness was associated with skinning calves which had died of "Red water fever". Leptospirosis due to *pomona* became a disease readily associated with farming, in particular with dairy farming (Kirschner *et al*, 1952; Faine and Kirshner, 1953; Salisbury, 1954; Jamieson and Davidson, 1970). It was acknowledged by Kirschner *et al* (1952) that in New Zealand, a country with a large number of dairy farmers, that cattle were a likely source of infection for human beings. Schollum and Blackmore (1982) concluded from their investigations that the occupational risk of acquiring leptospirosis was of similar magnitude for both pig farmers and dairy farmers. Pig farmers constitute only 5% of the total number of both dairy and pig farm workers, and therefore the total number of pig farmers who contract leptospirosis will be far less than the total number of dairy farmers contracting the disease. Significant positive correlations were found between the presence of agglutinating antibodies in sera from pig farmers and the number of breeding pigs and grower pigs which were kept at their farms (Schollum and Blackmore, 1982). Blackmore *et al* (1979) reported seroprevalences of 10% and 6.2% to leptospiral infection respectively in meat inspectors and meat workers, at a minimum agglutinating antibody titre of 1/24. Inspection and processing of pigs were shown to be the most important risk factors for the exposure of meat workers and inspectors to leptospiral infection. Eighty five percent of the serological reactions were to serovars *pomona* and *tarassovi*.

In 1973, *hardjo* was isolated from cattle (Lake, 1973). Until the 1970's the majority of human leptospiral infections were attributed to serovar *pomona* (Jamieson and Davidson, 1970). Subsequently, it was found that *hardjo* was

the cause of the majority of cases of leptospirosis in human beings in New Zealand, which predominantly occurred in dairy farm workers (Christmas *et al*, 1974). Cattle were found to be the maintenance hosts for *hardjo* and capable of maintaining an endemic cycle of infection within a herd (Hellstrom, 1978). In 1979, a bivalent *hardjo/pomona* vaccine for use in cattle was introduced and its use promoted by the Ministry of Agriculture and fisheries [MAF], Department of Health, and the Accident Compensation Corporation. It appears that as a result of widespread vaccination of cattle the average annual incidence of notified human cases of leptospirosis in New Zealand has fallen by more than half (Marshall, 1987).

After the isolation of *hardjo*, *pomona* became neglected and little progress was made in the investigation of leptospiral infections of pigs. Only in the last ten to twelve years have some aspects of the epidemiology leptospiral infection of pigs in New Zealand been investigated, and attempts to control infection been made.

Early serological surveys by Kirschner *et al* (1952), and Russell and Hansen (1958), and more recent serological surveys by Ryan (1978), showed the presence of agglutinating antibodies to *pomona* and *tarassovi* in adult and grower pigs. A detailed review of these serological surveys, and others conducted as part of this study are given in Chapter Three.

Attempts to control leptospiral infection in New Zealand piggeries by using either antibiotic treatment or vaccination, or a combination of both have been reported (Edwards and Daines, 1979; Hodges *et al*, 1979). Vaccination has been and still is widely used to prevent abortions in breeding stock and perinatal piglet losses (Cook, 1964). Its potential use for the control leptospirosis within piggeries was investigated by Hodges (1977) and Hodges *et al* (1976; 1985), and these will be reviewed in detail in Chapter Six.

The first epidemiological investigation into leptospirosis in pigs in New Zealand was conducted by Buddle and Hodges (1977), who studied asymptomatic *pomona* infection in young grower pigs. They found that pigs

housed in a grower house became infected and suggested that infection was maintained due to the exchange of pigs and transfer of effluent between pens.

A second epidemiological investigation was conducted by Ryan (1978), whose study of leptospiral infection in pigs led to the conclusion that pigs are the maintenance host for *pomona* and *tarassovi* in New Zealand. From a detailed study of a pig herd with endemic *pomona* infection Ryan concluded that the focus of infection was in pigs aged between six and twelve months. Pigs were also believed to be the focus for *tarassovi* infection (Ryan, 1978). Infection of pig herds with *pomona* alone was reported to occur most commonly, followed by infection with both *pomona* and *tarassovi*, and least common was infection with *tarassovi* alone. In an abortion storm monitored by Ryan (1978), 22% of mated sows aborted and *pomona* isolates were obtained from the vitreous humour of aborted piglets.

This thesis is the third major epidemiological investigation into leptospiral infection of pigs in New Zealand piggeries. This study deals predominantly with serovar *pomona*. Chapter Two outlines the materials and methods used throughout this study. Initial serological surveys are presented in Chapter Three, which is followed by detailed epidemiological investigations of infection in several pig herds, described in Chapter Four. Experimental infection of pigs with *pomona* and *bratislava*, and an investigation of aerosols and droplets as a mode for the transmission of infection are given in Chapter Five. Vaccination trials and strategies for the control of endemic *pomona* infection are described and discussed in Chapter Six. Finally, the information gained throughout this study was used to develop a computer simulation model of the endemic cycle of infection within a piggery, which is described in Chapter Seven.

### ***New Zealand piggeries***

#### ***- General structure, management and practices***

The majority of commercial piggeries in New Zealand have a breeding and a grower herd.

The breeding herd comprises breeding gilts, sows and boars. The annual replacement rate of sows is around 30%, and boars make up about 5% of the

total breeding herd. Artificial insemination is rarely used. On many farms, self replacement of breeding stock is practised. This practice involves retaining a small proportion of female pigs which have been bred and reared on the farm as replacement stock for sows culled from the breeding herd. A gilt is a sexually mature female pig which has not had a litter of pigs, thus pigs retained for placement into the breeding herd are commonly referred to as "self replacement gilts". The proportion of gilts retained for self replacement and the number purchased from other piggeries varies greatly between farms.

In modern piggeries the sows are housed indoors, and within individual pens. At the time of mating they may be penned with other sows. Sows are naturally mated twice during their period of oestrus. Pregnant sows return to individual pens. At the estimated time of farrowing, sows are moved to a farrowing house, and each sow is contained within an individual farrowing pen. Litters of piglets are most commonly weaned at around five weeks of age, however the age of weaning may vary from three to eight weeks of age.

Weaned pigs are housed in weaner sheds. These pigs are penned in groups of twenty to thirty in pens which generally have raised floors and solid walls. Pigs usually remain in the weaner pens until they are approximately ten weeks of age, at which time they are housed in a grower shed.

The design of grower pig houses varies greatly. In some piggeries there are solid concrete walls between pens, while others have pens separated by railings. Some farms have flushing effluent systems, with effluent channels below the flooring, others have open drainage pipes, which may pass through one or more pens. Feeding may be from boxes or from the floor. Pigs are generally fed grain meal, however some farmers feed their pigs whey or scrap foods. The age at which pigs are sent for slaughter is dependant on farm management. Most piggeries raise pigs to "bacon weight" [approximately 90 kg live weight, 22 to 30 weeks of age] or less commonly pigs are raised to "pork weight" [approximately 60 kg live weight, 16 to 20 weeks of age] and are commonly known as "baconers" and "porkers".

The design of buildings, the management, and husbandry practices, all have a potential influence on the likelihood of transmission of infection between pigs on a farm, and this will be discussed in Chapters Three, Four and Seven.

*- Age structure of a population of pigs in a piggery*

The pig population within most New Zealand piggeries can be divided into young grower pigs and older breeding pigs. The age structure of these populations is one of relative uniformity throughout the year. This is due to the high fecundity rate and all year round breeding of pigs. Pigs up to thirty weeks of age form a constantly large proportion [80 to 90%] of the total pig population at any given time throughout the year. This is in contrast to the semi-intensive systems of farming cattle, in which all cows calve only once a year during a short period of two to three months, and the age structure of the population varies throughout the year. The consequences of having an animal population with such a uniform age structure throughout the year is discussed in Chapter Four.

The objective of the authors work described in this thesis was to increase the understanding of the epidemiology of leptospiral infection in New Zealand pig herds with an aim to recommend measures for the control of infection in pig herds. Serological and cultural surveys were undertaken to determine the current infection status of pig herds (Chapter 3). The objectives of observational studies described in Chapter 4 were to study the naturally occurring cycle of endemic *pomona* infection of pig herds. Experimental studies were undertaken to investigate the significance of *bratislava* titres which were found to occur in grower pigs, and the possibility of *pomona* infection being spread by aerosols. The objective of a vaccination trial described in Chapter six, was to determine if a commercially available vaccine could be effectively used to reduce the prevalence of *pomona* amongst grower pigs. A further objective of the overall study was to develop a computer simulation model of endemic *pomona* infection within a pig herd. The purpose of the model was to create a simulation which would aid ones understanding of the interactions between infectious agent, host and environment, and the potential influence of control factors on the prevalence of disease in the simulated pig herd.



## Chapter Two

**MATERIALS AND METHODS**

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## INTRODUCTION

This chapter describes the materials and methods used throughout this study for the isolation of leptospires and for the detection of leptospiral infection. Included are the methods used for the collection of samples, detection of leptospires and leptospiral antibody, and the identification of isolates. Those methods for which little information is currently available have been described in more detail than those which are well documented in the literature.

The results of three experiments used to validate various methods for the detection of leptospiral infection are given at the end of this chapter. The outcome of these validation experiments subsequently influenced some of the methods used throughout this study.

## THE COLLECTION OF BLOOD, URINE AND KIDNEY SAMPLES

### *Collection and processing of samples from pigs*

#### *- Blood*

Pigs of different ages were successfully bled using a variety of techniques. Blood samples were collected for serology and allowed to clot at room temperature before being further processed.

Suckling piglets up to approximately six weeks of age, were restrained on their back and bled via the jugular vein. Restraint of the piglets was carried out with the help of an assistant, who would hold the piglet on its back, clasping the forelegs together in one hand and the hind legs in their other hand. The piglet was restrained in this extended position and its chin was held down by the person collecting the sample. The jugular vein was located by identifying the jugular groove and a 1½" [3.75 cm] 20 gauge needle vacuum containers [vacutainers]<sup>1</sup> attached to a plain vacutainer was directed at right angles into the jugular groove within 1" [2.5 cm] of the thoracic inlet.

Weaner pigs aged six to ten weeks were the most difficult to bleed, due primarily to difficulties with their restraint. Blood samples from weaners were obtained using one or more of three sites; the jugular vein, a peripheral ear

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<sup>1</sup>

Needles 1 1/2" and plain vacutainers. Venoject. Becton Dickinson, Melbourne, Australia.

vein, or the brachial vein. To obtain a sample of blood from the jugular vein or a peripheral ear vein, the pig was restrained with the aid of a rope secured around its upper jaw. An assistant would stand astride the pig and pull the rope upwards so that the pig's head was in a horizontal position. To obtain a sample of blood from a peripheral ear vein or the brachial vein, the pig was restrained by the assistant who would squat and cradle the pig in a "sitting" position while it rested on its hind quarters. The target vein was then occluded using an elastic band and clamp and a sample of blood was removed using a  $1\frac{1}{2}$ " [1.25 cm] 18 or 20 gauge needle<sup>2</sup> and syringe<sup>3</sup>.

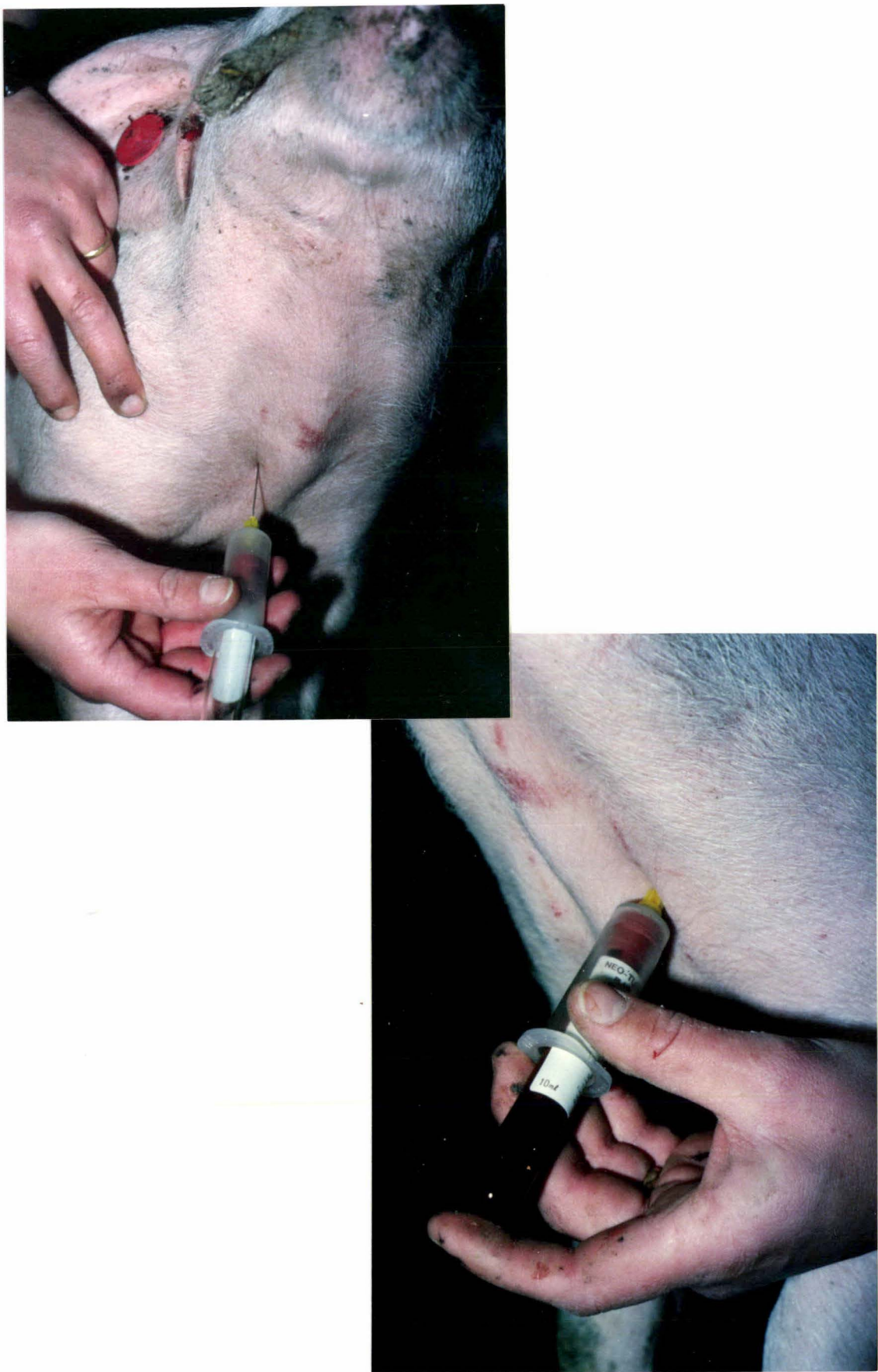
In breeding pigs and pigs aged ten weeks and older, blood samples were obtained from either the jugular vein or a peripheral ear vein. Restraint of these larger pigs for either of these methods was by the use of a rope around the pig's upper jaw, as shown in Figure 2.1. On most occasions, the assistant would restrain the pig against a wall and apply moderate pressure to the flank and shoulder regions using their knees. This technique adequately immobilised most pigs. Blood was collected from the jugular vein in a location around the middle to distal sector of the jugular groove. A  $1\frac{1}{2}$ " [3.75 cm], 18 or 20 gauge needle was directed at right angles into the jugular groove, and the vacuum container attached. In contrast to other domestic species, the jugular vein is not visible in pigs. As a result, finding the jugular vein can be difficult, and there is always a risk that the jugular vein or carotid artery can be damaged by the needle which may be fatal. However, once the technique had been mastered, collecting samples of blood from the jugular vein was found to be effective and rapid (twenty to forty samples per hour) method for sampling a large number of pigs on a regular and repetitive basis. Up to 40 samples per hour were collected using this technique. To collect blood from a peripheral ear vein of older pigs the same method as that described for weaner pigs was used.

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<sup>2</sup> Needles  $1\frac{1}{2}$ ", 18 or 20 gauge. Japan Medical Supplies. Hiroshima, Japan.

<sup>3</sup> Syringes 5 or 10 ml. Jintan. Terumo Co. Ltd. Tokyo, Japan.

**Figure 2.1**     **Collection of a blood sample by Jugular bleeding.**



Samples of blood were collected from pigs at the time of slaughter by holding a tube under the incision made by the slaughterman.

- *Urine*

All urine samples were cultured and therefore while collecting the samples, every attempt was made to maintain the highest degree of sterility possible. Fifty to 200 mg a distal loop diuretic, furosemide<sup>4</sup>, was administered intramuscularly in the anterior neck to each pig. The diuretic induced urination in most pigs within ten to thirty minutes of its administration. The diuretic was used on most pigs which were five weeks of age or older, however it was not administered to suckling piglets, or to pigs from which coincidental urine samples had been collected.

**Figure 2.2** Collection of a midstream urine sample.



<sup>4</sup>

Frudix, 50 mg/ ml furosemide. Pfizers. Auckland, New Zealand.

Samples of urine from live pigs were collected into sterile 50 ml specimen pots<sup>5</sup> or "pottles" as shown in Figure 2.2. A simple wire construction or "pottle holder" was extensively used for the collection of urine samples. This served as an effective extension of ones arm and therefore minimised direct contact with urine, and reduced the tendency for pigs to move while a sample of their urine was being collected. Gloves were worn when urine was being collected. Pottles remained sealed until the appropriate pig was urinating, the cap was then removed, and a mid-stream urine sample collected, the lid replaced, and the pottle labelled with the pigs identity number.

*- Kidneys*

One kidney from each pig was collected from the inspection tray at a local abattoir, and placed into a labelled plastic bag. The kidneys had been prepared for inspection by incision of the kidney and removal of the capsule.

*Collection of samples from mice and hamsters*

*- Blood*

Samples of blood were collected from mice and hamsters during CO<sub>2</sub> or ether anaesthesia. Collection of blood samples was by cardiac puncture or by incision of the axilla. Animals were not allowed to recover from the anaesthesia after the collection of blood samples.

*- Urine*

Urine samples were obtained from mice and hamsters by applying abdominal pressure to restrained animals. Generally, only one or two drops of urine were collected using this method, an amount sufficient for DFM however generally insufficient and unsuitable for culture. Urine samples were sometimes collected after the death of mice or hamsters and this was done by either passive release or bladder puncture.

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<sup>5</sup> Specimen pots, 50 ml. InterMed Scientific, PO Box 33268, Takapuna, Auckland



### - Kidneys

Kidneys of mice and hamsters were removed from the carcass within one hour of death. The animals were placed in posterior recumbency and the abdominal cavity opened. Sterile forceps and scalpel were used to remove left and right kidneys. When histological samples were required, half of one kidney was placed in 10% buffered formalin and the remainder used for culture.

## THE DETECTION OF LEPTOSPIRES

### *Cultural examination*

#### - Culture media

Tween-albumin bovine serum culture medium [EMJH] (Johnson and Seiter, 1977) was routinely used throughout this study. This medium was prepared in two forms, liquid and semisolid. Liquid medium, containing no agar, was used for the maintenance of reference strains of leptospires which were used in the MAT, as described in the section on The Detection of Leptospiral Antigen. Semisolid medium containing 0.2% agar was used to isolate leptospires from kidneys, urine and other samples. Semisolid medium was prepared with either 100 µg, 200 µg or 400 µg of antimicrobial 5 fluorouracil [5FU]<sup>6</sup>/ ml medium.

Tween 80/40 lactalbumin hydrolysate culture medium [T80/40LH] (Ellis and Thiermann, 1986) was prepared specifically for the isolation of serovar *bratislava*.

Stuart's basal medium [SBM] was prepared as a diluent for the preparation of kidneys and urine for culture, and a one percent bovine serum albumin diluent [BSAD] was prepared as a transport medium for samples of urine.

All media used in this study were prepared according to the formulae given in Appendix I. These media were prepared using Analar grade chemicals, double glass distilled water, and sterilised glassware. Fresh culture medium was dispensed in volumes of 5 ml into 10 ml screw cap bottles. Diluent medium [SBM] was dispensed in volumes of 4.5 ml, 10 ml or 100 ml depending on its

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<sup>6</sup>

See Appendix I (iii) for the preparation of 5FU.

intended use, and transport medium [BSAD] was dispensed into 4.5 ml aliquots. Freshly dispensed medium was incubated at 37°C for 72 hours and examined for evidence of bacterial contamination, before storage at room temperature. Each batch of new culture medium was tested for its ability to support leptospiral growth. Five bottles of medium from each batch were inoculated with a viable leptospiral culture and examined for growth five to seven days after incubation at 29°C.

*- Culture of kidneys*

Each kidney to be prepared for culture was placed on a tray and sprayed with 95% ethyl alcohol. A section of kidney [approximately 3 cm<sup>3</sup>] was removed with sterile forceps and scalpel. The tissue was again sprayed with 95% alcohol and flamed until it turned a grey-brown colour.



**Figure 2.3    Kidney culture procedure**

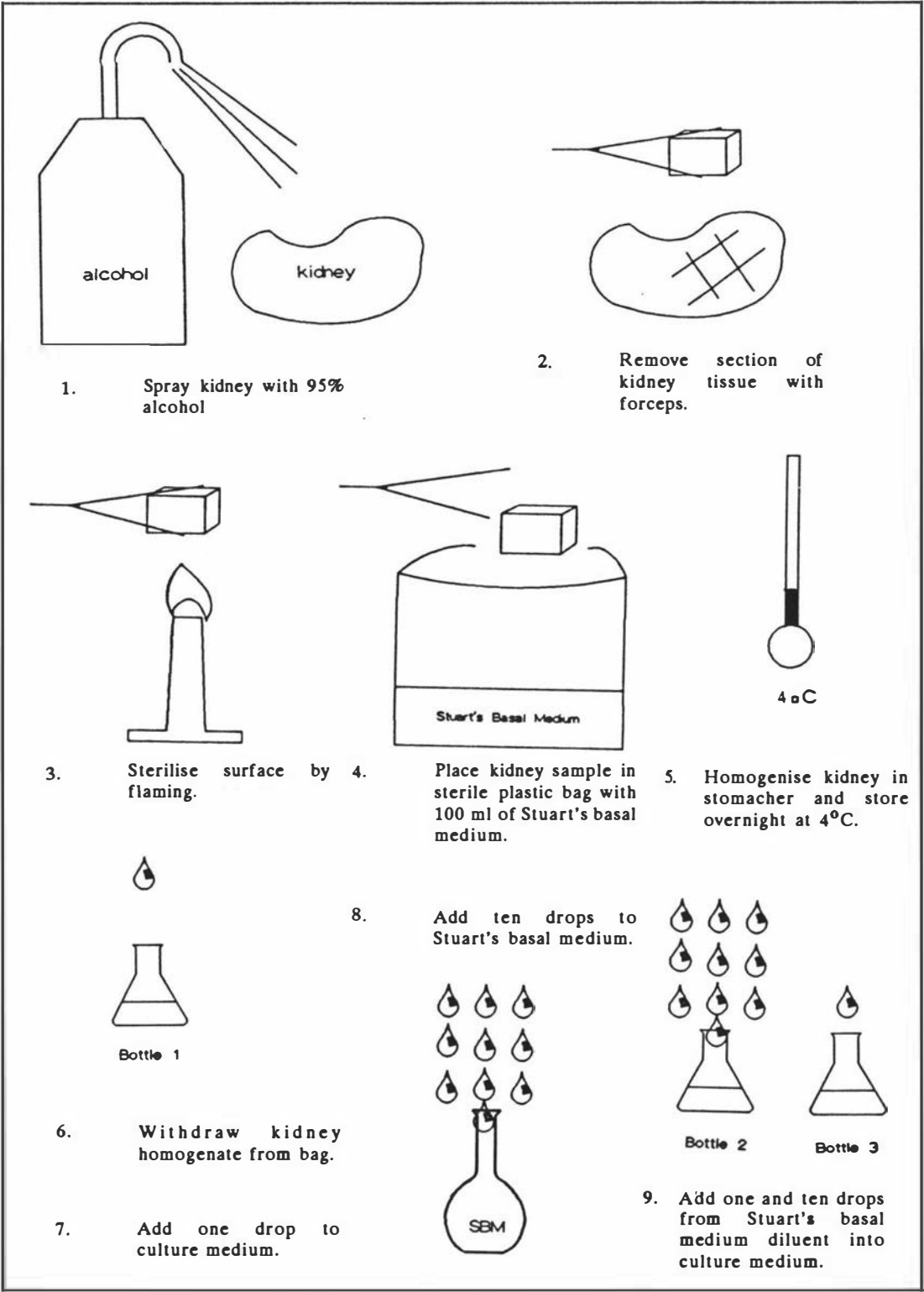
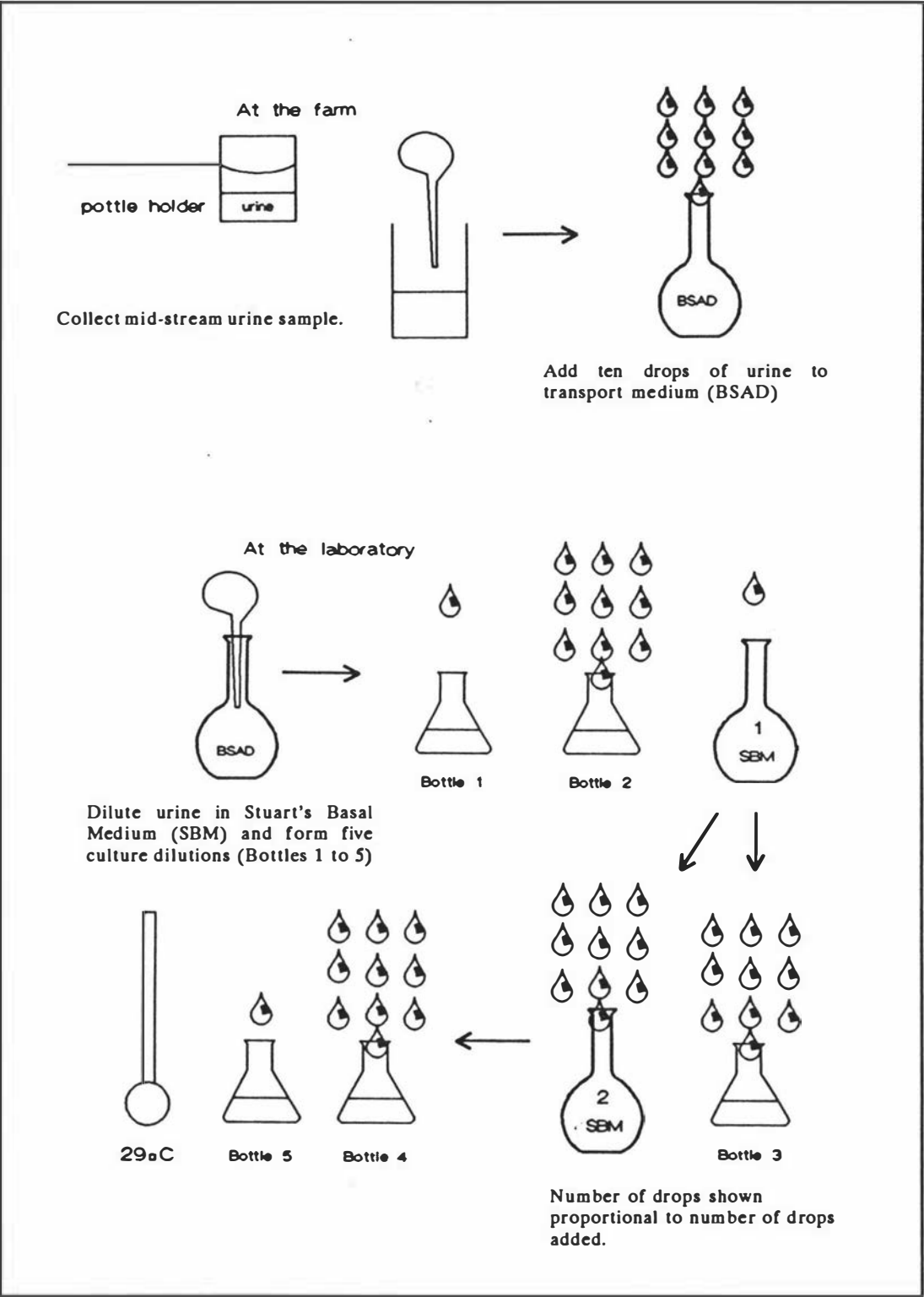


Figure 2.4 Urine culture procedure.



The section of kidney was then placed in a sterile plastic bag<sup>7</sup> and 100 ml of SBM was added. The sample of kidney in the SBM was broken down or "homogenised" using a Coleworth stomacher<sup>8</sup> for one minute. The bags containing the homogenate were then stored overnight at 4°C [see section on Validation of Some Laboratory Methods]. The following day, one drop of suspension was inoculated into 5 ml of semisolid culture medium and ten drops were diluted in 4.5 ml of Stuart's medium. One drop and ten drops of the diluted suspension were then inoculated into two other bottles containing 5 ml of culture medium. Thus, there were three final culture dilutions from each kidney, as shown in Figure 2.3. The kidney cultures were incubated at 29°C for twenty eight days and examined by DFM every fourteen days, unless otherwise specified. Isolates were subcultured into liquid medium for subsequent identification. Subculture was carried out by transferring three drops of the original culture into 5 ml of fresh culture medium.

Generally the kidneys of mice and hamsters were processed using a procedure similar to that already described, except that whole kidneys were used, and after flaming, the kidneys were homogenised by placing them in a sterile 5 ml syringe without an attached needle, and forcing them through the syringe into a bottle containing 10 ml of Stuart's medium.

#### *- Culture of urine*

To prepare urine for transportation to the laboratory, ten drops of urine were added to 4.5 ml of BSAD transport medium at the farm within an hour of collection. On return to the laboratory, this was then further diluted to a final concentration of 1/1000 and 1/10,000. From the three urine dilutions, five final culture dilutions ranging from 1/100 to 1/10<sup>6</sup> were formed, as shown in Figure 2.4. The cultures were incubated at 29°C for twenty eight days, and examined every fourteen days for evidence of leptospiral growth.

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<sup>7</sup> Gamma sterilised stomacher '400' plastic bag. A J Seward & Co. Ltd., 6 Stamford Street, London SE1, 9UE, England.

<sup>8</sup> Coleworth stomacher, A.J. Seward and Co. Ltd., 6 Stamford St, London SE1 9UE, England.

*- Culture of other samples*

The brain, lymphnodes, reproductive tract and liver from some animals were also cultured during this study. These tissues were flamed, broken down and cultured in a manner similar to that described for the culture of kidneys. Heparinized blood samples were cultured the same way as urine.

***Dark field microscopy [DFM]***

Examination of cultures and urine for the presence of leptospires was conducted using a dark field microscope<sup>9</sup> at a magnification of 125.

***Histology***

Histological sections were fixed in 10% buffered formalin. Wax embedded sections of kidney, brain, lymphnodes, reproductive tract and liver from experimentally infected animals were stained with haematoxylin and eosin. Some of these samples were specifically stained for the detection of leptospires using modified Warthin Starry method (Young, 1969). Sections were examined by light microscopy for histopathological changes and for the presence of leptospires.

## THE DETECTION OF LEPTOSPIRAL ANTIBODY

***Microscopic agglutination test [MAT]***

*- Maintenance and preparation of leptospiral antigen*

Cultures of twelve leptospiral serovars as specified in Table 2.2, were maintained for use as antigens in the MAT. Leptospiral cultures were maintained by delivering one to three drops of dense culture into liquid medium which was incubated at 29°C for three and seven days. Cultures of serovars *pomona*, *tarassovi* and *bratislava* were the most frequently used antigens. Each culture selected for use in the MAT was counted in a counting chamber<sup>10</sup> and then diluted with PBS to give a final concentration of 1 to 2

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<sup>9</sup> Olympus BHS Dark Field Microscop, Hyde Instruments, PO Box 9531, Auckland.

<sup>10</sup> Petroff-Hausser and Helber Counting Chamber, C.A. Hausser and Son, Philadelphia, U.S.A.

$\times 10^8$  leptospire / ml, which was then counted again to confirm the concentration.

*- Preparation and storage of sera*

Samples of blood were centrifuged at 300 r.p.m. for 10 to 15 minutes. The serum was transferred to individually identified wells of a serum reference plate [SRP]<sup>11</sup> by Pasteur pipette<sup>12</sup>, and sealed with cellophane tape. Any remaining serum was stored in individually identified serum containers<sup>13</sup>. The SRP's and the serum containers were stored at -20°C.

*- MAT procedure*

The MAT procedure used throughout this study was based on the method described in "Guidelines for the control of Leptospirosis" (W.H.O., 1982).

Preparation of the MAT involved five steps. These steps were (1) the initial dilution of serum samples; (2) counting of antigen; (3) serial dilution of serum samples; (4) dispensing of antigen; and (5) incubation. Sera were removed from the SRP by multidilutor heads<sup>14</sup> or micropipette<sup>15</sup> and mixed with the appropriate volume of phosphate buffered saline<sup>16</sup>. The MAT was conducted in 12 x 8 well microtitre plates<sup>17</sup>. Twenty five microlitres of PBS were

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<sup>11</sup> Flat bottomed Dynatech Microtitre Plates, Cooke Laboratory Products, 900 Slaters Lane, Alexandria, Virginia 22314, U.S.A.

<sup>12</sup> Pasture Pipette 5". InterMed Scientific, PO Box 33268, Takapuna, Auckland.

<sup>13</sup> Serum containers 5 ml Sterilin Ltd. Lampton House, Lampton Road, Hounslow, Middlesex, TW3 4EE, UK.

<sup>14</sup> Minidiluter, Cooke Engineering Co., Alexandria, Virginia, U.S.A.

<sup>15</sup> Disposable Micropipette, "Dispo pipette", Cooke Laboratory Products, Singapore.

<sup>16</sup> Preparation given in Appendix I (iv).

<sup>17</sup> "U" bottomed Microtitre Plates, Dynatech Laboratories Inc, Alexandria, Virginia, U.S.A.

delivered into each microtitre well using an automatic dispenser<sup>18</sup>. The consistency of the volumes delivered by the dispenser were visually appraised by comparing the size of drops delivered onto a plastic slide. Ten serum samples were tested per plate, the remaining two columns of wells were occupied by a positive and a negative control. The positive control contained antisera prepared against a known serovar. The negative control contained no serum but an extra 50  $\mu$ l of PBS, and therefore contained half the number of leptospires than in all the other wells. Diluted serum samples were delivered to the first row of wells of the microtitre plate by the multidilutor, two fold dilutions were of sera were serially transferred to either seven or fourteen rows of wells [1 or 2 plates]. Diluter tips were washed and dried three times between batches of serum samples. Twenty five microlitres of the appropriate concentration of antigen was added to all of the wells using a multiple dispensing pipette<sup>19</sup>. The final test dilutions of serum are shown in Table 2.1. These microtitre plates were then incubated at 37°C for one hour.

**Table 2.1**      **The final test dilutions of serum samples in the microtitre plates used for the MAT.**

Row Number	Initial serum dilution in serum reference plate		
	1 / 1	1 / 2.5	1 / 10
1	1 / 4	1 / 10	1 / 40
2	1 / 8	1 / 20	1 / 80
3	1 / 16	1 / 40	1 / 160
4	1 / 32	1 / 80	1 / 320
5	1 / 64	1 / 160	1 / 640
6	1 / 128	1 / 320	1 / 1280
7	1 / 256	1 / 620	1 / 2560

<sup>18</sup> Multidispenser, Dynatech Laboratories Inc., 900 Slater Lane, Alexandria, Virginia 22314, USA.

<sup>19</sup> Stepper 411 (Multipipette) Socorex Swiss. Renens, Switzerland.

Following incubation, a portion of the content of each well was transferred to a glass slide (Ryan, 1978) and examined for the presence of agglutination by DFM. A positive reaction was recorded for the wells in which 50% or more of the leptospire had agglutinated. Assessment of a 50% agglutination level is a subjective end point, and to aid in the detection of this end point, reference was made to the well which contained no serum, and only half of the concentration of leptospire in the well [the negative control well]. Positive control serum was used to verify that the appropriate serovar had been selected as the test antigen.

#### - Serological results

Throughout this thesis, the term "titre" will be used to express the highest serum dilution at which 50% or more of leptospire agglutinated a specific serovar. Therefore a titre of 1/5,120 to *pomona*, represents a serum sample which diluted to at least 1/5,120, will agglutinate a minimum of 50% of leptospire of serovar *pomona* using the MAT procedure as previously described. Serum samples for which agglutinin titres were recorded have been termed seropositive. Serum samples in which the level of agglutination was less than 50% in all of the test wells a titre of 0/0 was recorded, and these serum samples have been termed seronegative. Seroprevalence is used to describe the percentage of serum samples which are seropositive at a designated minimum dilution. Reciprocal titres increase exponentially with doubling dilutions. It was therefore convenient to convert these titres, using the first equation shown in Figure 2.5, into coded titre units [CTU's] which are linear in character. Following standard statistical calculations, CTU's were converted back to the titre equivalent using the second equation shown in Figure 2.5.

**Figure 2.5** Equations to convert reciprocal titre to a coded titre unit [CTU] and CTU to a reciprocal titre.

$$\text{CTU} = \frac{\text{Ln (reciprocal titre)} - 2.303}{0.6931} + 1$$

$$\text{Reciprocal titre} = 10 \times 2^{(\text{CTU} - 1)}$$

For example, a titre 1/10 is equivalent to a CTU of 1, 1/20 to 2, 1/40 to 3, and so forth, Appendix II has a conversion table of CTU's and reciprocal titres. Using CTU's, standard statistical procedures have been used to calculate the average CTU of seropositive serum samples only. The geometric mean titre [GMT] was derived by converting the average CTU to a titre. Wherever possible the results of MAT's have been documented as titres, however CTU's have been used in some tables.

## IDENTIFICATION OF LEPTOSPIRES

### *Serotyping*

Leptospiral isolates were tentatively classified as belonging to a serovar by using a simple agglutination test procedure. Antisera of known specificity and titre, obtained from reference laboratories, shown in Table 2.2, were used for the test. Two fold dilutions of 25  $\mu$ l of each selected antiserum and 25  $\mu$ l of the unknown antigen was added to each test well, this was then incubated at 37°C for one hour. The isolate was tentatively identified as belonging to the antisera with which it agglutinated at the highest serum dilution. In this study the majority of isolates were only tested by this method using antisera to the seven serovars known to occur in New Zealand [see Table 1.1] and to the antisera of serovar *bratislava*. The identity of selected isolates was confirmed by bacterial restriction endonuclease DNA analysis.

### *Bacterial restriction endonuclease DNA analysis*

Bacterial restriction endonuclease DNA analysis<sup>20</sup> [BRENDA] was used to confirm the identification of some isolates obtained throughout this study. BRENDA was also used to confirm the identity of all isolates which were used for animal inoculation studies.

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<sup>20</sup>

Conducted by Dr. Ramadas, BRENDA Laboratory, Massey University, Palmerston North, New Zealand.



**Table 2.2**      **Leptospiral serovars maintained for the Microscopic Agglutination Test.**

Serovar	Serogroup	Strain	Source
australis	Australis	Ballico	Brisbane *
autumnalis	Autunmalis	Aki Yami A	Brisbane
ballum	Ballum	Mus 127	Brisbane
bataviae	Bataviae	Swart	Brisbane
bratislava	Australis	Jezbratislava	Brisbane
canicola	Canicola	Hond Utrecht IV	Brisbane
copenhageni	Icterohaemorrhagiae	M20	Brisbane
grippotyphosa	Grippo	Moskova V	Brisbane
hardjo	Sejroe	Hardjoprajitno	Brisbane
javanica	Javanica	Veldrat	Brisbane
		Bataviae 46	
pomona	Pomona	Pomona	Brisbane
		T A5	Massey **
pyrogenes	Pyrogenes	Salinem	Brisbane
tarassovi	Tarassovi	Perepelicyn	Brisbane

\* Leptospirosis Lab., Brisbane, Queensland, Australia.

\*\* Leptospirosis Lab., Massey University, New Zealand. Field isolate from pig urine, 1987.

## VALIDATION OF SOME LABORATORY METHODS

### *Estimation of the intensity of leptospiruria*

An experiment was undertaken to determine if culturing serially diluted urine could be used as an adequate indicator for quantitatively assessing the intensity of leptospiruria.

#### *- Method*

Midstream urine samples were collected from three pigs known to be free of leptospiral infection. From each of these samples, decreasing intensities of leptospiruria were formed by the addition of a known numbers of leptospires. This was attained by making six, ten fold serial dilutions of a culture of *pomona* [strain T A5] containing  $10^9$  leptospires/ ml. One drop of each diluted leptospiral culture and nine drops of urine sample were added to 4.5 ml of BSA diluent. Five culture bottles were inoculated with each of the artificially

infected urine samples, as previously described in the section on "Culture of Urine", as shown in Figure 2.4. The theoretical number of leptospires inoculated into each culture bottle was calculated and is shown in Table 2.3.

Urine containing added leptospires diluted in BSAD transport medium and leptospires diluted in PBS, were examined by DFM. Cultures were incubated for eight weeks and were examined at two weekly intervals, cultures with leptospiral growth were discarded.

**Table 2.3      The theoretical number of leptospires inoculated into five culture dilutions of urine.**

<i>pomona</i> per ml urine	Final Dilution of Urine in Culture				
	1/10 <sup>2</sup>	1/10 <sup>3</sup>	1/10 <sup>4</sup>	1/10 <sup>5</sup>	1/10 <sup>6</sup>
10 <sup>9</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>
10 <sup>8</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>
10 <sup>7</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10
10 <sup>6</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10	1
10 <sup>5</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10	1	0
10 <sup>4</sup>	10 <sup>2</sup>	10	1	0	0
10 <sup>3</sup>	10	1	0	0	0
0	0	0	0	0	0

- Results

Leptospires were isolated from all five culture dilutions when inoculated with at least 10<sup>6</sup> leptospires. The isolation rates progressively decreased from cultures inoculated with decreasing numbers of leptospires.

For example, leptospires were isolated from all of the first three culture dilutions when inoculated with between 10<sup>4</sup> and 10<sup>6</sup> leptospires, and isolates



#### - Discussion

This method of urine culture appears to be a highly sensitive one for the detection of leptospiuria, as leptospires were isolated from culture dilutions which were inoculated with theoretically only one leptospira. However, the theoretical number of leptospires is unlikely to have reflected the true number for several reasons, one reason being the difficulty of counting, diluting and accurately dispensing leptospires to such high dilutions, as with these procedures small errors become magnified. Another reason is the likely uneven distribution of leptospires within a solution. In the authors experience, leptospires will clump together and therefore are not of uniform density in a solution.

#### - Conclusion

Serial dilution of urine samples can be used to give a quantitative estimate of the intensity of leptospiuria. Slower growing samples indicate that lower numbers of leptospires were inoculated into the culture medium. An incubation period of four weeks was found to be adequate to determine if serovar *pomona* was going to be isolated from a sample. Culturing leptospires was a more sensitive method for their detection than DFM. The level of dilution from which leptospires were cultured and the time taken for their isolation were both found to be indicators of the intensity of leptospiuria, which can be quantified into categories of high, medium and low. High is used to describe a urine sample containing greater than  $10^6$  leptospires/ ml, medium containing  $10^3$  to  $10^6$  leptospires/ ml, and low containing up to  $10^3$  leptospires/ ml.

#### *Effect of overnight storage of homogenised kidney on the isolation of leptospires*

An experiment was conducted to determine the effect of leaving homogenised kidney samples overnight, before culturing, on their subsequent culture results.

#### - Method

Ten pig kidneys were collected from a local abattoir. The samples were processed as soon as possible upon returning to the laboratory. EMJH semisolid medium containing 100  $\mu$ g 5FU/ ml medium was used as the culture

medium. One group of media bottles were inoculated with kidney homogenate within four hours of the kidneys having been collected from the abattoir. The homogenised kidney samples were retained in their sterile plastic bags and stored overnight at 4°C. Twenty four hours later, the second group of media bottles were similarly inoculated with the kidney homogenate and incubated, as shown in Figure 2.3. All of the cultures were incubated at 29°C. Cultures were examined fourteen and twenty eight days after the time of their collection.

#### *- Results*

Fourteen days after the collection of the kidneys from the abattoir, leptospires were isolated from eight of the ten kidney samples inoculated within four hours of collection, and from nine of the ten kidney samples inoculated twenty four hours after collection. Twenty eight days after incubation, leptospires were isolated from the ninth kidney sample inoculated four hours after its collection, however leptospires were not isolated from one of the kidneys inoculated either four or twenty four hours after its collection. Leptospires were isolated in all three culture dilutions in the case of four of eight kidneys cultured within four hours of collection, and from six of nine kidneys cultured twenty four hours after collection.

#### *- Discussion*

No appreciable difference could be demonstrated between cultures inoculated with kidney homogenate stored for four or twenty four hours. There was a tendency for leptospires to be detected sooner by DFM in cultures which had been inoculated with homogenate which was stored for twenty four hours. It could be hypothesised that this tendency was due to the leptospires having more time to move from the broken tissue fragments of the kidney into the surrounding SBM, which was then used for inoculation into the culture medium.

Other reports also suggest that there is no apparent change in the viability of leptospires in kidneys twenty four hours after an animals death. Hathaway (1978) demonstrated the ability of leptospires to survive in whole kidneys of possums for at least twenty four hours after their death and Ho and Blackmore (1979) reported the isolation of leptospires from kidneys which had been stored at 0°C for fourteen days. As there was no apparent disadvantage in extending

the period between processing samples and their inoculation into culture medium, and in order to streamline processing of large numbers of kidneys, the holding of kidneys was adopted as part of the standard method for culturing kidney samples throughout this study.

*- Conclusion*

There was no difference between the isolation of leptospires from cultures inoculated with kidney homogenate which had been stored for either four or twenty four hours at 4°C.

*Inoculation of mice with urine*

Inoculation of mice with pigs' urine was undertaken in an experiment to determine if the procedure could be used to provide an accurate and rapid test for the diagnosis of leptospiuria in pigs.

*- Method*

Eighty six mice, between the age of four and six weeks, were inoculated with 0.5 ml of pigs urine via the intra peritoneal route for the detection of viable leptospires in the urine samples. The urine used in this experiment was collected aseptically from the eviscerated bladders of pigs at slaughter and was cultured in the manner previously described for the culture of urine. The mice were housed in stainless steel cages and provided with sterilised sawdust bedding and unrestricted amounts of feed and water. Following inoculation the mice were kept for two weeks, they were then anaesthetized and samples of their blood collected as previously described. The sera were tested by the MAT for the presence of titres to serovar pomona.

*- Results*

Titres to *pomona* were detected at a minimum dilution of 1/4 in thirty four of sixty four mice inoculated with pigs' urine containing leptospires. Titres were not detected in any of the twenty three mice sera which were inoculated with pigs' urine from which leptospires were not isolated by culture.

- *Discussion*

A seroconversion rate of 53% for the mice inoculated with urine containing leptospire was poor. Hathaway *et al* (1983) found weaner mice to be the more susceptible to *pomona* infection than adult mice, with a minimum infective dose of 10 organisms given via the intraperitoneal route. The ratio of serological to bacteriological prevalence was found to be 1:1 for mice artificially infected with serovar *pomona* (Hathaway *et al*, 1983). Therefore, the results obtained in this study indicate either that mice are not always able to become infected when inoculated with urine containing leptospire, or that seroconversion had not occurred at the time of sampling. Had the serological examination been conducted at a later time, perhaps a higher proportion of mice inoculated with naturally infected pig's urine may have seroconverted. However, delaying the serological examination would have defeated the purpose of the test as a rapid method for the detection of leptospiuria.

- *Conclusions*

Seroconversion by weaner mice inoculated two weeks earlier with pig's urine was a less sensitive method for the detection of leptospiuria than the direct culture of diluted urine. Inoculation of mice with pig's urine did not reduce the time for diagnosis of leptospiuria. Therefore, no further use was made of this procedure for the detection of leptospiuria in pigs.

## SUMMARY AND CONCLUSIONS

1. Samples of blood were successfully collected from pigs of all ages from the jugular vein. Bleeding pigs by this route was the most rapid method for repeatedly bleeding large numbers of pigs.
2. Samples of urine suitable for culture were collected from pigs after the intramuscular administration of furosemide, a diuretic. A long handled pottle holder was frequently used to aid in the collection of urine from pigs.
3. The MAT and the culture of urine and kidneys were used throughout this study to determine the immunological and infection status of study pigs.
4. EMJH medium was routinely used for the maintenance and culture of leptospires. T80/40LH medium was specifically used for the culture of *bratislava* from field samples.
5. The intensity of leptospiuria was determined by using culture dilutions of each urine sample, and it could be categorised as low [less than  $10^3$  leptospires/ ml], medium [between  $10^3$  and  $10^6$  leptospires/ ml] or high [greater than  $10^6$  leptospires/ ml].
6. Samples of kidney were homogenized and inoculated into medium after over night storage at 4°C, the results of which were no different from inoculating freshly homogenised kidney. This delay in the procedure for the culture of kidneys facilitated the streamline processing of large numbers of kidneys for culture.
7. The intraperitoneal inoculation of weaner mice with pig's urine was a less sensitive test for the detection of leptospiuria than the direct culture of urine and therefore mice were not used to determine the infection status of pigs.



Chapter Three

PREVALENCE STUDIES OF LEPTOSPIRAL INFECTION IN PIGS

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## INTRODUCTION

In this chapter, the results of three studies on the prevalence of leptospiral infection in pigs in New Zealand are presented and discussed.

The first study [Study 1] was a serological survey to determine the seroprevalence of *pomona* and *tarassovi* titres in pigs from six veterinary districts throughout New Zealand. This study was similar to one conducted by Ryan (1978) who reported a seroprevalence of 86% to serovar *pomona* and 25% to serovar *tarassovi*, in adult pigs' sera using a minimum dilution of 1/12. Ryan's survey was preceded by serological surveys of pigs from abattoirs in New Zealand by Kirschner (1954), and Russell and Hansen (1958). Kirschner (1954) reported the prevalence of agglutinin titres of 1/300 to serovars *pomona* and *tarassovi* in adult pigs from the South Island to be 19% and 6% respectively. Russell and Hansen (1958) reported a prevalence of 5% and 4% to agglutinin titres of 1/200 to serovars *pomona* and *tarassovi* in their survey of adult pigs.

The second study [Study 2] was conducted at a local abattoir and the objective was to determine the prevalence of leptospiral infection in locally slaughtered bacon weight pigs. Ryan (1978) reported the isolation of *pomona* from the kidneys of 45% of young pigs, and from 2% of adult pigs slaughtered at the same abattoir. In contrast, *tarassovi* was isolated from only 1% of young pigs and from 3% of adult pigs. Using a titre of 1/48, Ryan reported the respective seroprevalences of *pomona* and *tarassovi* titres in young pigs to be 72% and 7%, and in adult pigs to be 58% and 5%.

Studies from overseas have recently shown *bratislava* infection in pigs to be an emerging problem, as infection with *bratislava* results in economic loss due to abortion (Ellis *et al*, 1986a). Infection of pigs with serovar *bratislava* is extensively reviewed in Chapter Five. As a result of the overseas findings, another objective of this study was to determine the seroprevalence of *bratislava* titres in bacon weight pigs, and to determine if there was evidence of infection with *bratislava* in locally slaughtered bacon weight pigs.

The third study [Study 3], also an abattoir survey, was conducted to determine if statistical associations could be demonstrated between particular farming practices, and the serological or cultural prevalence of *pomona* in bacon weight pigs.

The abattoir surveys also served to identify farms which might subsequently be visited to undertake further epidemiological investigations.

## STUDY 1

SIX REGIONAL SEROLOGICAL SURVEYS OF *POMONA*  
AND *TARASSOVI* IN PIGS*Materials and Method*

A stratified sample of five hundred and thirty sera from pigs in six MAF administrative districts of New Zealand were selected for examination, see Figure 3.1. The sera were obtained from a bank<sup>1</sup> of adult pig sera which had been collected from farms participating in the Aujeszky's disease eradication program in 1985. One hundred serum samples were obtained from the Waikato, Manawatu, Blenheim, Christchurch and Otago districts, and thirty from the Northland district. The sera from each district were stratified into farm samples which consisted of ten serum samples each. Thus, the Waikato, Manawatu, Blenheim, Christchurch and Otago regions were represented by one hundred serum samples from ten farms, whereas thirty serum samples from three farms were selected from the Northland district.

The MAT, as described in Chapter Two, was used to determine the titres to serovars *pomona* and *tarassovi* at dilutions of 1/20, 1/80, 1/320, 1/1,280 and 1/5,120. All of the serum samples were tested for agglutinins to *pomona*. One hundred and forty sera were not included in the survey for agglutinins to serovar *tarassovi* because of either contamination of the sample or insufficient sera. These sera included twenty from the Waikato district, twenty from the Manawatu district and all one hundred sera from the Christchurch district.

*Results*

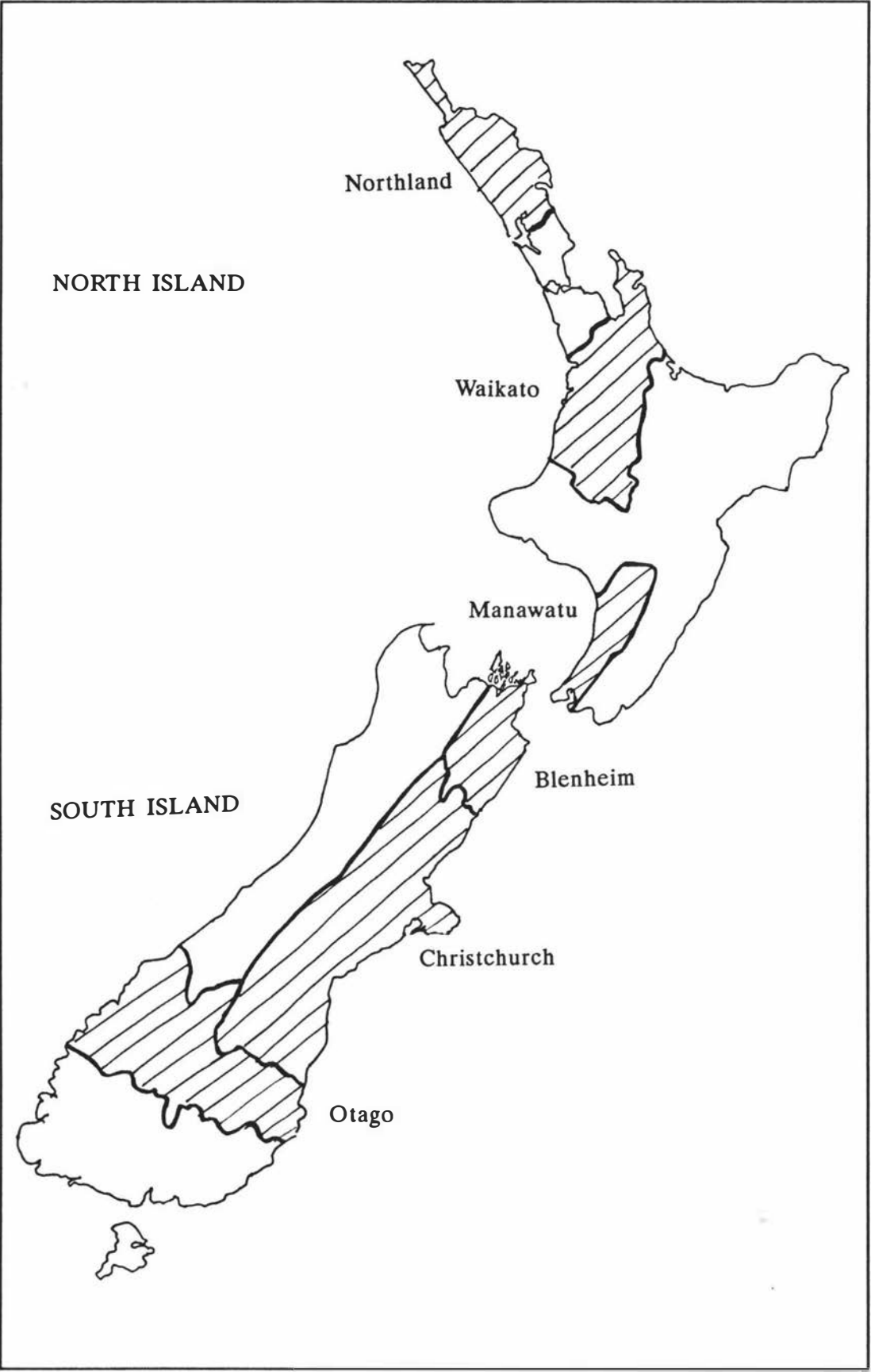
A total of three hundred and nineteen pigs [60%] had titres to *pomona*, and sixty seven pigs [13%] had titres to *tarassovi*, when a minimum serum dilution of 1/20 was used.

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1

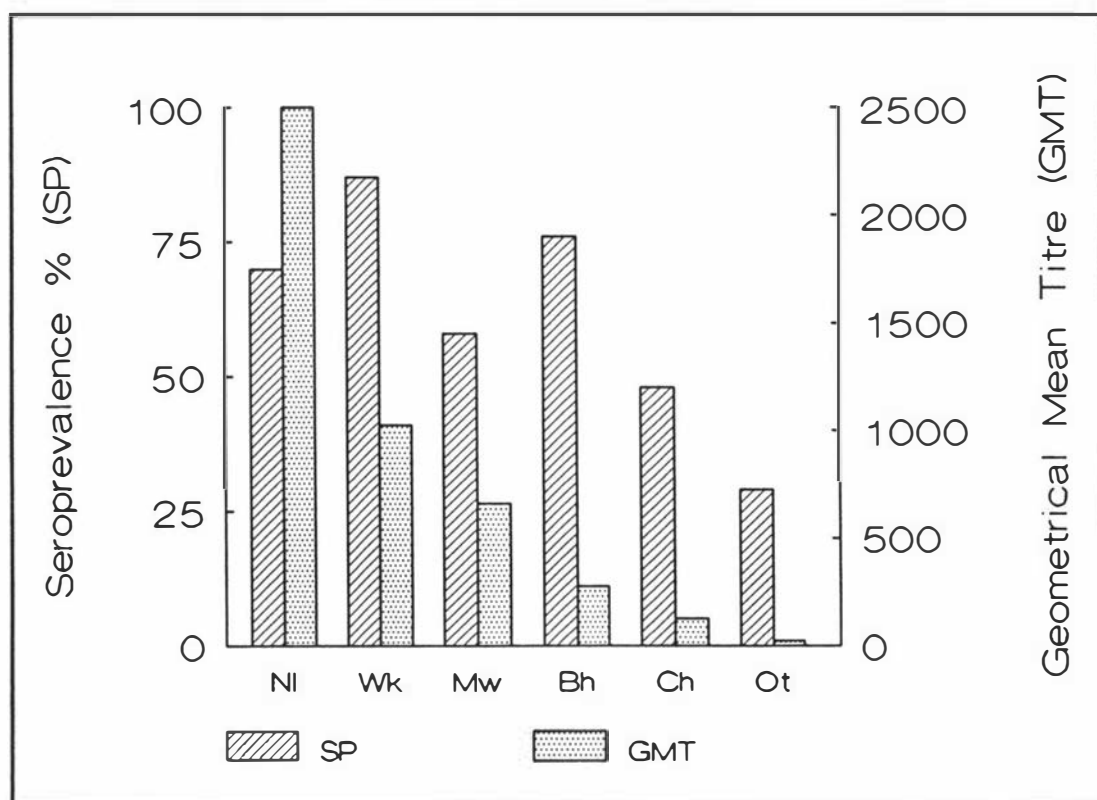
Ministry of Agriculture and Fisheries [Now MAF Qual],  
Wallaceville, Animal Research Centre, Upper Hutt, New Zealand.

**Figure 3.1**      **The six MAF administrative districts of New Zealand used in the serological survey of *pomona* and *tarassovi* titres of pigs throughout New Zealand.**



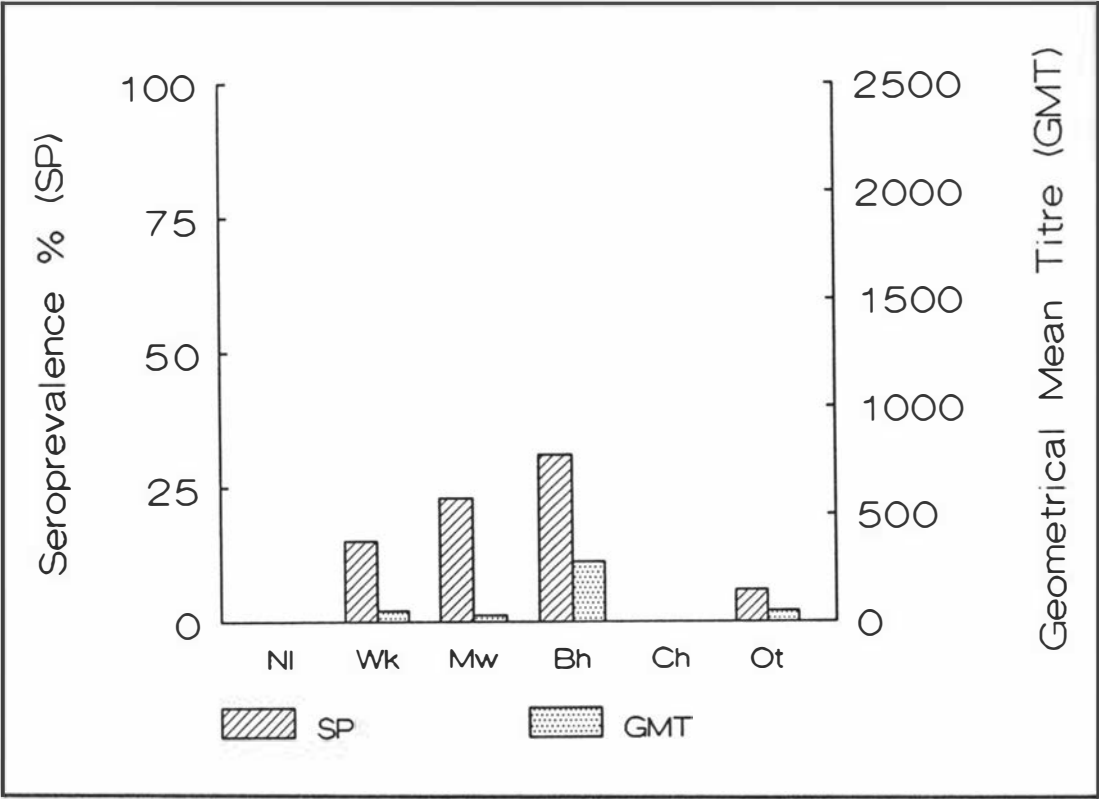
The seroprevalences and GMT's of titres to *pomona* for all serum samples tested in the serological survey are shown in Table 3.1 and Figure 3.2. The seroprevalence of *pomona* titres of pigs from five of the six districts were similar and ranged between 87% and 47%, with exception of pigs from the Otago district for which the seroprevalence was 29% (see Figure 3.2). However the GMT's of pigs in the six districts progressively decreased from a high of 1/2,646 in pigs from the Northland district to a low of 1/24 in the southern most district of Otago. The district of Waikato had the highest recorded seroprevalence of 87%, and a GMT of 1/1025 (see Figure 3.2). The majority of farms [90%] had pigs with titres to *pomona*, however the prevalence of seropositive farms was lower [70% to 80%] in the southern regions, than in the central and northern regions [100%] (see Table 3.1). The *pomona* titres of pigs from all of the three districts in the North Island were greater than those of pigs from the three districts in the South Island [ $P < 0.001$ ].

**Figure 3.2** Serological results for serovar *pomona* of pigs from six MAF administrative districts of New Zealand.



Titres to serovar *tarassovi* are given in Table 3.2, and the prevalence and GMT's of the *tarassovi* titres of the six veterinary districts are shown in Figure 3.3. Titres to *tarassovi* were most prevalent in the central districts of Blenheim [31%] and Manawatu [18%]. The highest GMT of 1/280 was obtained from the Blenheim district. The high seroprevalence of *tarassovi* titres in pigs from the Blenheim district was largely attributed to three farms, two of which had seroprevalences of 90%, and GMT's of 1/1,097 and 1/148 respectively, and a third farm which had a seroprevalence of 60% and a GMT of 1/508. The prevalence of *tarassovi* titres of pigs from other districts ranged from zero to 12%, and the GMT's were not above 1/50. Forty six percent of farms had pigs with titres to *tarassovi*. In the Manawatu district, seven of eight farms were seropositive, however in all other districts surveyed the number of seropositive farms ranged between zero and five. The GMT's of *tarassovi* titres from pigs in the three North Island districts were less than the *tarassovi* titres of pigs from the two South Island districts [ $P < 0.05$ ].

**Figure 3.3**                      **The seroprevalence and GMT of *tarassovi* agglutinins of pigs in six veterinary districts of New Zealand.**



**Table 3.1**                      **Serological results for serovar pomona of pigs from six MAF administrative districts of New Zealand.**

		FARM										
MAF		1	2	3	4	5	6	7	8	9	10	All
Northland	SP	100	30	80								70
	GMT	5120	1280	1522								2646
Waikato	SP	100	60	100	100	90	100	80	90	100	50	87
	GMT	1114	2021	2460	2220	940	278	80	806	844	139	1025
Manawatu	SP	10	50	50	40	30	20	90	100	90	100	58
	GMT	320	557	61	640	50	320	1280	557	592	2940	660
Blenheim	SP	70	60	100	50	50	100	90	100	50	90	77
	GMT	54	160	557	105	104	844	274	557	320	235	276
Christ-church	SP	100	100	90	20	30	80	0	50	0	10	47
	GMT	557	422	373	80	806	113	0	183	0	80	130
Otago	SP	40	10	10	0	0	60	60	80	30	0	29
	GMT	57	20	20	0	0	100	201	226	806	0	24

SP = Seroprevalence of *pomona* titres  
GMT = Reciprocal Geometrical Mean Titre



**Table 3.2**                      **Serological results for serovar tarassovi of pigs from six MAF administrative districts of New Zealand.**

		FARM										
MAF		1	2	3	4	5	6	7	8	9	10	All
Northland	SP	0	0	0								0
	GMT	0	0	0								0
Waikato	SP	0	30	0	20	50	0	0	0			15
	GMT	0	201	0	40	26	0	0	0			50
Manawatu	SP	10	20	60	10	40	20	0	0			24
	GMT	20	40	40	2	20	80	0	0			32
Blenheim	SP	90	60	0	50	20	0	0	0	0	90	33
	GMT	1097	508	0	61	80	0	0	0	0	148	280
Christ-church	SP											
	GMT											
Otago	SP	0	0	20	0	0	10	30	30	0	0	6
	GMT	0	0	20	0	0	20	127	127	0	0	50

SP    =   Seroprevalence of *tarassovi* titres  
GMT   =   Reciprocal Geometrical Mean Titre

## STUDY 2                      THE CULTURAL AND SEROLOGICAL PREVALENCE OF *POMONA*, *TARASSOVI* AND *BRATISLAVA* IN BACON WEIGHT PIGS AT SLAUGHTER

### *Materials and Method*

One hundred and forty blood samples were collected from bacon weight pigs at the time of slaughter as described in Chapter Two. Pigs were either Large White or Landrace, or cross breeds thereof. Sera were tested by the MAT [see Chapter Two] using serovars *pomona*, *tarassovi* and *bratislava* at dilutions of 1/25 to 1/6400.

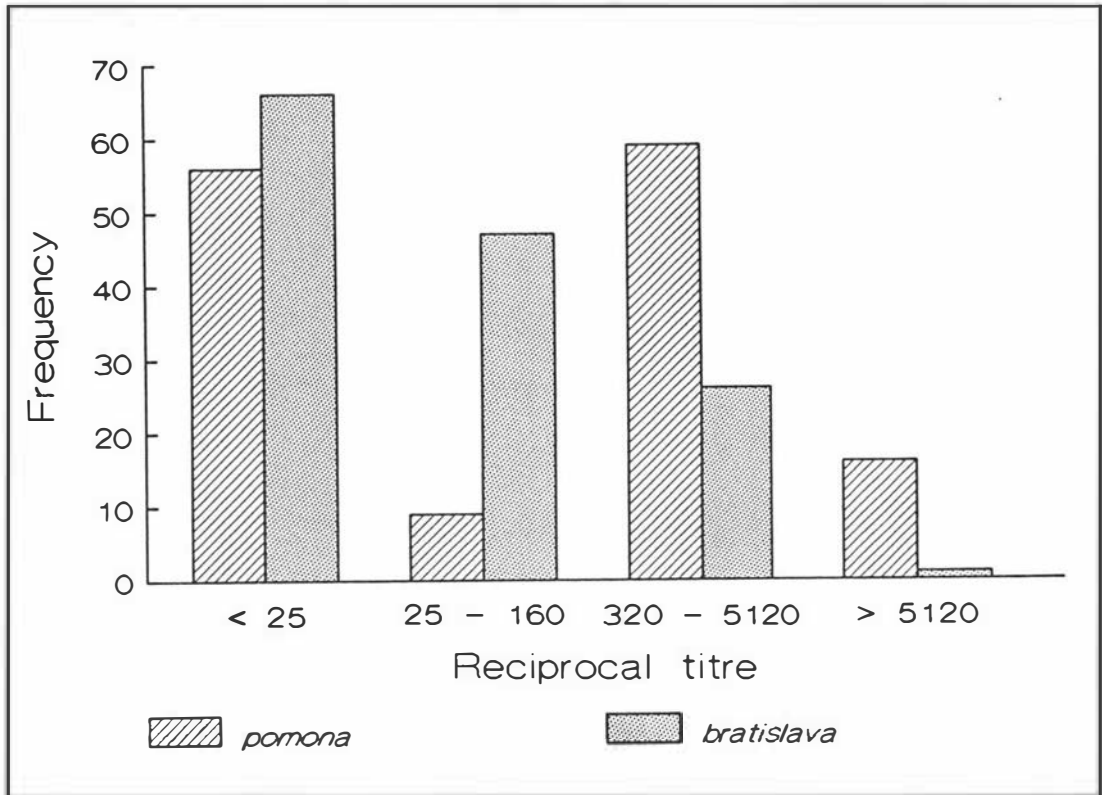
Kidneys were collected from forty seven bacon weight pigs which originated from farms with a high seroprevalence of *bratislava* titres. From twenty two of these pigs, additional samples of blood and either uterus or testes were collected for subsequent culture.

### *Results*

The prevalence of *pomona* and *bratislava* titres was 60% [84/140] and 53% [74/140] respectively. In contrast, no titres to *tarassovi* were detected at a minimum dilution of 1/25. Table 3.3 and Figure 3.4 show the distribution of titres to serovars *bratislava* and *pomona*. The majority of *bratislava* titres were less than 1/320, whereas the majority of *pomona* titres ranged between 1/320 and 1/5120, as shown in Figure 3.4. Thirty five of one hundred and forty sera [25%] tested did not have detectable titres to either *pomona* or *bratislava* at a 1/25 dilution. Fifty three pigs [38%] had titres to both *pomona* and *bratislava*. Thirty one pigs [22%] had titres to *pomona* but no detectable agglutinins to *bratislava*, and conversely twenty one pigs [15%] had titres to *bratislava* but no detectable agglutinins to *pomona*. The GMT of *pomona* titres was 1/1160 which was found to be significantly higher than the GMT of 1/153 for *bratislava* titres [ $P < 0.05$ ]. However, in fifty three pigs with both *pomona* and *bratislava* titres, there was poor correlation between the magnitude of titres to one serovar and that of the other serovar [ $r = 0.0933$ ].

Figure 3.4

The frequency distribution of *pomona* and *bratislava* titres in 140 bacon weight pigs at slaughter.



The results for which both cultural and serological examinations were conducted are shown in Table 3.4. All pigs' kidneys from which leptospires were isolated had titres to *pomona*, however, as expected, leptospires were not isolated from pigs which did not have a detectable *pomona* titre. Leptospires were isolated from seventeen of forty seven kidney samples [36%], and all of these were identified as serovar *pomona* by the simple agglutination test described in Chapter Two. Selected isolates were also identified by BRENDA to be serovar *pomona*. Leptospires were not cultured from any of the female reproductive tracts, however, one *pomona* isolate was made from the testes of a pig. This isolate was identified as a *pomona* isolate by BRENDA. Leptospires were not isolated from any of the blood samples.

**Table 3.3**                      **The distribution of pomona and bratislava titres of grower pigs at slaughter.**

Reciprocal <i>pomona</i> titre	Reciprocal <i>bratislava</i> titre										<i>pomona</i>
	0	25	50	100	200	400	800	1600	3200	6400	
0	35	4	12	3	1	1	0	0	0	0	56
25	3	0	0	1	0	0	0	0	0	0	4
50	1	0	2	0	0	0	0	0	0	0	3
100	1	0	0	0	0	1	0	0	0	0	2
200	4	0	2	0	1	3	0	1	0	0	11
400	8	0	1	2	1	3	0	0	0	0	15
800	1	1	2	3	1	0	0	0	0	1	9
1600	3	0	1	0	0	0	0	0	0	0	4
3200	4	1	4	4	2	4	0	1	0	0	20
6400	6	0	2	2	2	1	3	0	0	0	16
	66	6	26	15	8	13	3	2	0	1	140
<i>bratislava</i>											

Table 3.4

The serological and cultural results of twenty two bacon weight pigs sampled at slaughter.

Pig	Sex	Blood	Culture Results		Reciprocal of titre	
			Repro. Tract	Kidney	<i>pomona</i>	<i>bratislava</i>
1	F	-	-	-	0	160
2	F	-	-	-	0	0
3	F	-	-	-	0	80
4	F	-	-	+	> 2560	1280
5	F	-	-	-	NE	NE
6	F	-	-	+	NE	NE
7	F	-	-	+	0	320
8	F	-	-	-	0	0
9	F	-	-	-	0	0
10	F	-	-	-	0	160
11	F	-	-	-	0	160
12	F	-	-	-	0	0
13	F	-	-	-	0	0
14	M	-	-	-	0	0
15	M	-	-	-	0	0
16	M	-	-	+	> 2560	360
17	M	-	-	-	640	2560
18	M	-	-	+	> 2560	40
19	M	-	-	-	0	0
20	M	-	-	+	NE	NE
21	M	-	-	+	NE	NE
22	M	-	-	-	NE	NE

-                    Leptospires not isolated  
+                    Leptospires isolated, serovar *pomona*  
F, M                Female, Male

## STUDY 3

A STUDY OF FARMING PRACTICES AND THE  
PREVALENCE OF *POMONA* INFECTION IN GROWER  
PIGS AT SLAUGHTER*Materials and Method*

Two hundred and thirty seven blood samples and two hundred and twenty five matching kidneys were collected from slaughtered pigs originating from twelve farms. The twelve farms were selected when their pigs were slaughtered at a local abattoir, at a time when sampling was initially most convenient. Generally, ten pork or bacon weight pigs were chosen without any form of selection from pigs being slaughtered on the day. At a later date at least one other set of samples was collected from pigs of each of the selected farms.

The majority of pigs sampled were Large White, Landrace, or cross breeds thereof. The sex of the pigs was recorded, and matching blood and kidney samples were collected and processed as described in Chapter Two. The MAT was used to detect agglutinins to serovar *pomona* in sera diluted from 1/20 to 1/163,840.

Following the collection of samples, farm managers were contacted by telephone. The farmers were informed of the results obtained from examining their pigs and were asked to answer a series of predetermined questions concerning their farming practices (see Appendix III).

*Results**- Cultural and serological results*

All serological and cultural results are shown in Table 3.5. Leptospires were isolated from thirty nine percent [87/225] of the kidneys. The overall seroprevalence was forty four percent [105/237]. Seven of the twelve farms had infected pigs, the remaining five farms appeared to be free of leptospiral infection. The cultural prevalence of infection in pigs from infected farms averaged 67% and ranged from 15% to 90%, with a mode of 80%.

*- Results from the questionnaire*

All twelve farmers contacted by telephone agreed to answer the questionnaire. A summary of their farming practices, together with the infectious status of

their grower herd, including the seroprevalences and GMT's, are shown in Table 3.5. Seven of twelve farms vaccinated their breeding herd with a leptospiral vaccine, the remaining five farms did not. None of the farmers vaccinated the pigs in their grower herd. The number of breeding sows on the twelve farms varied between twenty and 650. Two of the twelve farms raised pigs to pork weight, the remainder raised pigs to bacon weight. Three of twelve farmers allowed contact between breeding and grower pigs. Ten farms engaged in the practice of self replacement of breeding pigs [see Chapter One]. Eight farms had separate effluent drainage systems for individual pens, while the drains of the remaining four farms passed through more than one pen. Eleven of the twelve farms used pressure hosing to clean pens, the only farm which did not, used a flushing system to remove effluent. Ten farms had solid concrete floors in their piggeries and the other two farms had slatted flooring.

*- Effect of vaccinating breeding pigs*

Four of the seven farms which vaccinated their breeding pigs, and three of the five farms which did not vaccinate breeding pigs, had infected grower pigs.

Leptospire were isolated from fifty of 143 pigs [35%] originating from farms which vaccinated their breeding pigs. Leptospire were isolated from thirty seven of eighty two pigs [45%] originating from farms which did not vaccinated their breeding pigs. The isolation rates of leptospire from the kidneys of grower pigs from the two groups of farms were not significantly different [ $P > 0.1$ ].

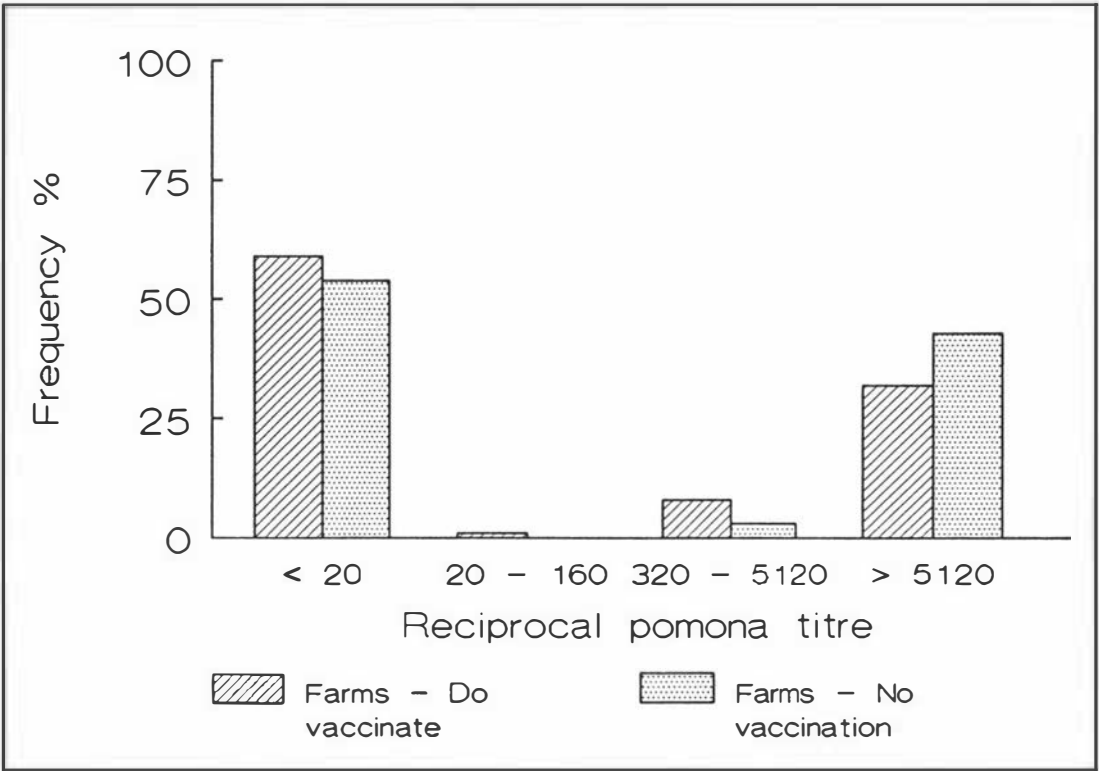
The frequency distribution of *pomona* titres from grower pigs which originated from farms which vaccinated their breeding pigs, and those which did not are shown in Figure 3.5. There was no significant difference [ $P > 0.1$ ] between the seroprevalence of *pomona* titres of grower pigs which came from farms which vaccinated their breeding pigs [41%] and those which did not [46%].

*- The effect of farm size*

The average size of farms with infected pigs was 280 breeding sows, and significantly greater than the average of 52 breeding sows for farms that did

not have infected pigs [ $P < 0.05$ ]. This was also true, irrespective of the vaccination status of breeding pigs, as shown in Table 3.6. The two farms [Farms I and K] with the herd sizes of less than fifty breeding sows were both free of infection.

**Figure 3.5**                    **The distribution of *po* titres in grower pigs at slaughter, from farms which either do or do not vaccinate their breeding pigs.**





**Table 3.5**                      **A comparison of some farm practises and the infection status of their pigs, for twelve farms.**

<b>Farm</b>	<b>Sow Num.</b>	<b>Contact</b>	<b>Floor</b>	<b>Cleaning</b>	<b>Effluent</b>	<b>Slaughter</b>	<b>Gilts</b>	<b>Vaccinate</b>	<b>Infected</b>	<b>S e r o - prevalence</b>	<b><u>1</u> GMT</b>
A	65	No	Concrete	Hose	Mixed	Pork	Yes	Yes	0%	0%	0
F	75	No	Concrete	Flush	Apart	Bacon	Yes	Yes	0%	0%	0
G	180	No	Concrete	Hose	Apart	Bacon	No	Yes	80%	95%	34130
H	70	Yes	Concrete	Hose	Apart	Bacon	Yes	Yes	0%	0%	0
J	300	No	Concrete	Hose	Apart	Bacon	Yes	Yes	15%	15%	130040
L	160	No	Concrete	Hose	Apart	Bacon	No	Yes	75%	95%	40960
N	650	No	Concrete	Hose	Mixed	Bacon	Yes	Yes	80%	85%	44440
I	20	No	Concrete	Hose	Apart	Pork	Yes	No	0%	0%	0
K	30	No	Concrete	Hose	Apart	Bacon	Yes	No	0%	0%	0
C	75	No	Concrete	Hose	Apart	Bacon	Yes	No	90%	91%	122369
M	80	No	Slats	Hose	Mixed	Bacon	Yes	No	55%	60%	130040
B	25	Yes	Slats	Hose	Mixed	Bacon	Yes	No	90%	100%	76434

Apart                      =        Effluent drainage separate  
 Mixed                    =        Moves through more than one pen  
 Gilts:                    =        Yes        = self replacement of gilts  
                                       No        = all gilts brought in from other piggeries

Table 3.6

Sizes of twelve farms in comparison with the infectious status of their grower pigs at slaughter and the vaccination status of their breeding pigs.

Farm Status	Number of farms	Average farm size	Minimum farm size	Maximum farm size
Infected	7 [B,C,G,J,L,M & N]	280 Sows	75 Sows	650 Sows
Uninfected	5 [A,F,H,I & K]	52 Sows	20 Sows	75 Sows
Vaccinate	7 [A,F,G,H,J,L & N]	215 Sows	70 Sows	650 Sows
Do Not Vaccinate	5 [B,C,I,K & M]	91 Sows	20 Sows	250 Sows
Vaccinate and Infected	4 [G,J,L & N]	322 Sows	160 Sows	650 Sows
Vaccinated and Not Infected	3 [A,F & H]	73 Sows	70 Sows	75 Sows
Not Vaccinated and Infected	3 [B,C & M]	135 Sows	75 Sows	250 Sows
Not Vaccinated or Infected	2 [I & K]	25 Sows	20 Sows	30 Sows

- *Serological results of pigs from infected and apparently non-infected farms*

The distribution of titres of pigs which came from farms where infection was present was significantly different from farms without infection [ $P < 0.001$ ], as shown in Figure 3.6. As was expected, the majority of titres from pigs on infected farms were greater than 1/5,120 [GMT = 1/51,840]. In contrast, all titres from pigs on uninfected farms were undetectable at a 1/20 dilution, except for two of the ninety three pigs which had titres of 1/320 and 1/640.

Two farms [Farms A and L] raised pigs to pork weight only, and both of these farms were apparently free of leptospiral infection.

## DISCUSSION

In the first study [Study 1] described in this Chapter, a stratified sample was used to assess the prevalence of *pomona* and *tarassovi* titres in pigs within six MAF administrative districts of New Zealand. The criterion for the selection of farms within each district was the availability of ten sera from farms participating in the Aujeszky's Disease eradication program in 1985. Therefore in contrast to the ideal situation, samples were not randomly selected and the farms that were sampled may be not represent a true cross section of farms within each district. Six districts were chosen to determine the distribution of *pomona* and *tarassovi* titres within both the North and South Islands. Although the serum bank was an excellent source of a large number of sera, the district and farm number from which the sera originally came was the only information available to the author. For the reasons outlined above, the national prevalence of pigs infected with *pomona* and *tarassovi* can not be estimated from this first survey.

Further difficulty was encountered in analyzing and determining the significance of the results of the first serological survey described in this chapter. Ryan (1978) suggested that agglutinating titres of 1/24 to *pomona* or *tarassovi* were indicative of past infection with these respective serovars, however similar titres to *pomona* and *tarassovi* have also been reported in pigs which had been vaccinated with a *pomona/tarassovi* vaccine (Dobson and Davos, 1975; Cargill and Davos, 1981). Ryan (1978) also conducted studies on two piggeries, both of which had dual *pomona* and *tarassovi* infections, and

Table 3.7

The number of farms within each district with near equal, or uneven ratios of *pomona*: *tarassovi* titres

District	High	Near equal	Low
Northland	3/3	0/3	0/3
Waikato	8/8	0/8	0/8
Manawatu	4/8	4/8	0/8
Blenheim	6/10	4/10	0/10
Christchurch	t NE	t NE	t NE
Otago	6/10	4/10	0/10

t NE = sera not tested for agglutinins to *tarassovi*

almost all of the sows on these two farms had titres to both *pomona* and *tarassovi*, in the absence of vaccination. Therefore from this study it was not possible to distinguish between farms which vaccinate their breeding pigs and those which have dual infection with *pomona* and *tarassovi*. For the reasons outlined above, the prevalence of infection with *pomona* and *tarassovi* in each district can not be estimated. In an attempt to interpret the significance of some of the titres, the ratio of *pomona* to *tarassovi* titres which occurred on individual farms was examined. Farms with an unequal ratio of *pomona* and *tarassovi* titres could be said to have leptospiral infection with the serovar exhibiting the highest seroprevalence. Table 3.7 shows the number of farms in each district which have equal and unequal ratios of *pomona* and *tarassovi* titres. By disregarding farms with an approximately equal proportion of *pomona* and *tarassovi* titres, the estimated prevalence of both serovars is lowered. However, there is still a trend indicating a higher prevalence of *pomona* in the North Island than in the South Island. There are no farms where the seroprevalence of *tarassovi* is greater than *pomona*. This may indicate either that infection with *tarassovi* occurs at a lower prevalence and always in the presence of infection with *pomona*. The number of farms with nearly equal ratios of *pomona* and *tarassovi* titres increases from none in the Northland and Waikato districts to four of eight farms in the Manawatu district, and to four of ten farms in the two South Island districts. It may be that farmers south of the Waikato district either vaccinate their breeding pigs, or that more pig herds have had dual infection with *pomona* and *tarassovi* infections. The results of a nation wide serological survey of abattoir workers by Blackmore *et al* (1979) determined that 85% of serological reactions in abattoir workers were due to serovars *pomona* and *tarassovi*.

From data (Blackmore, 1978 Unpublished) of the same serological survey it was ascertained that of five abattoir workers surveyed at the Blenheim abattoir, three had titres to *pomona* and one had a titre to *tarassovi*. This equates to the highest proportion of serological reactions to *pomona* and the second highest proportion to *tarassovi* of any group of abattoir workers surveyed throughout the country. Although their sample size for this particular abattoir was small, it did demonstrate that abattoir workers within this district had been exposed to *pomona* and *tarassovi*. As the titres of these abattoir workers are likely to have been acquired following occupational exposure to infected pigs, it follows that the high seroprevalence in this group of abattoir workers is a reflection of a high prevalence of both *pomona* and *tarassovi* in pigs within the Blenheim district. More recently in 1989, an outbreak of *tarassovi* infection occurred in abattoir workers of a newly opened pig abattoir in the Blenheim district (Marshall, pers. comm.)<sup>2</sup>. Therefore an other indication that there may be a relatively high prevalence of *tarassovi* infection in pigs within the Blenheim district. The seroprevalence of *tarassovi* titres was consistently lower than the seroprevalence of *pomona* titres, this is a finding which is consistent with all three previously conducted serological surveys (Kirschner, 1954; Russell and Hansen, 1958; Ryan, 1978). These authors also reported the seroprevalence of *pomona* to be higher in the North Island than in the in the South Island. These previous surveys also found a lower prevalence of *tarassovi* titres in the South Island, however in this study the seroprevalence of *tarassovi* in the South and North Islands were not significantly different [ $P > 0.05$ ]. Ryan (1978) suggested that the lower prevalence of both serovars in the South Island may be the result of low temperatures in the South Island, which reduce the survival time of leptospires in the environment, thereby reducing transmission. One would therefore expect to find differences in the seasonal prevalence of leptospiral infection due to changes in the ambient temperature. However, in this authors experience no difference in the seasonal prevalence of *pomona* infection in pigs has been noted. Brockie (1976) reported that the incidence of human infection with *pomona* was also relatively constant throughout the year. These results therefore indicate that the ambient temperature does not appear to markedly influence the rate of transmission of *pomona* infection within pig herds. The majority of pigs in New Zealand are housed indoors, and thus extremes of temperature are unlikely to affect the transmission of

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<sup>2</sup>. Dr R B Marshall, Massey University, Palmerston North, New Zealand. Personal communication of an outbreak of *tarassovi* in abattoir workers in the Blenheim district.

infection within a piggery. It is possible that the higher prevalence of *pomona* infection is related to a higher pig population in the North Island [290,757] compared with that of the South Island [163,705] (Anon, 1986).

The seroprevalence and prevalence of infection of 60% and 53% respectively for *pomona* in grower pigs at slaughter, as described in the second study of this chapter, are similar to those of 72% and 45% reported by Ryan (1978). Ryan (1978) also reported 7% of grower pigs with titres of 1/48 to *tarassovi*, and the isolation of *tarassovi* from a pig's kidney. In contrast to Dr T. J. Ryan's results, titres to *tarassovi* were not detected in this study, nor was *tarassovi* isolated. The proposed sporadic occurrence of *tarassovi* infection in pigs (Ryan 1978) may be the reason why it was not detected in this serological and cultural survey. Alternately, the real prevalence of *tarassovi* infection in pigs may have decreased since the time of Dr. Ryan's study.

The high prevalence of *bratislava* titres [53%] was of interest and concern as they had not previously been detected in pigs in New Zealand. The failure to detect *bratislava* to date is explained because of the omission of *bratislava* as a serological test antigen. *Bratislava* was not isolated from any of the reproductive tract, kidneys or blood samples even though they were specifically cultured in an attempt to isolate *bratislava* using the method described by Ellis and Thiermann (1986).

The high seroprevalence of mainly low *bratislava* titres in bacon weight pigs, and the failure to isolate *bratislava* could best be explained by either of three hypotheses. One hypothesis is that the pigs were infected with *bratislava*, however, the infection was of short duration and possibly asymptomatic, and therefore *bratislava* had not been isolated, and no disease entity was identified. The second hypothesis is that titres to *bratislava* are the result of a serological cross reaction, with perhaps a commonly occurring serovar, such as *pomona*. Ryan (1978), demonstrated significant serological cross reactions between *pomona* and *australis*, *autumnalis*, *bativiae*, and *grippotyphosa*, in which low agglutinating titres to the cross reacting serovars were associated with high agglutinating titres to *pomona*. Ryan concluded that although the titres were poorly correlated with *pomona* [ $r = 0.047$ ,  $r = 0.137$ , respectively], the *ballum* and *hardjo* titres were due to serological cross reactions, in addition neither *hardjo* nor *ballum* were isolated from any of the pigs in his study. In this study the correlation between *pomona*

and *bratislava* titres was also low [ $r = 0.09$ ], and may have been due to the non-linear distribution of *pomona* titres [see Figure 3.4].

The third hypothesis is that the technique used for the isolation of *bratislava* was not adequate. It was considered important to determine the significance of *bratislava* titres in grower pigs at slaughter and therefore an investigation into the first and second hypotheses were undertaken. A detailed serological and cultural investigation of two groups of five pigs artificially exposed to either *pomona* or *bratislava* is described in Chapter Five.

The *pomona* isolate obtained from the testes of a pig is possibly best explained by postulating that the pig was slaughtered while it was leptospiraemic. During the leptospiraemic phase leptospire can be isolated from a range of organs throughout the body [see Chapter One]. It was unfortunate that matching samples of blood and kidney were not obtained for culture at the same time as the testes, as these would have provided evidence corroborating either leptospiraemia or localised infection of the testes.

In the third study described in this chapter, the prevalence of infection and the seroprevalence of *pomona* in grower pigs were both slightly lower [39% and 44% respectively] than in the second study. The inclusion of the results of pork weight pigs in the third study has resulted in these lower prevalences. Leptospire were not isolated from any of the pork weight pigs, and only two of forty pork weight pigs titres to *pomona* [1/320 and 1/640], the remainder were seronegative at a 1/20 dilution. By removing the results of pork weight pigs in the third study, the prevalence of infection and seroprevalence of bacon weight pigs in the third study now becomes 47% and 53% respectively. These results are in agreement with previous studies by both the author and Ryan (1978).

The third study, attempted to investigate any association between the prevalence of leptospiral infection in grower pigs and farming practices. Results indicated that the vaccination of breeding pigs had no effect on the prevalence of leptospiral infection of grower pigs within the same piggery. In an earlier study by Schollum and Blackmore (1982), there was no association between vaccinated and unvaccinated breeding pigs and whether farmers had either a titre to *pomona* or a clinical history of leptospirosis

in their pig herd. Thus pig farmers are still at risk to occupational leptospirosis despite the vaccination of their breeding pigs, and this again indicates that the breeding pigs were not the source of most occupationally acquired leptospiral infections due to serovar *pomona*. The size of the piggery appears to be an important factor influencing the presence or absence of leptospiral infection within the piggery. Leptospiral infection was not detected in grower pigs which originated from piggeries with less than fifty breeding sows. It therefore appears that infected grower pigs are most likely to occur on farms with more than fifty breeding sows. Schollum and Blackmore (1982) also found that there was a significant association between a farmer having a titre to *pomona* and having more than fifty breeding sows [ $P < 0.05$ ]. Farming practices which were investigated in the third study are likely to be confounded by farm size. For example, the management of farms with less than fifty sows can be described as semi-intensive because of some pasture grazing by sows, whereas larger piggeries have more intensive systems with continuous indoor housing of pigs. Schollum and Blackmore (1982) demonstrated that farms with more than fifty breeding sows were more likely to house their pigs indoors [ $P < 0.001$ ]. Farm management is therefore influenced by the size of the breeding herd, and any apparent effect said to be due to management may instead be a reflection of breeding herd size. Schollum and Blackmore (1982) believed that pork or bacon pigs sold for slaughter were the major source of infection for workers. The findings from this study and studies described in Chapter Four support the hypothesis that bacon weight pigs are potentially a major source of infection for abattoir workers and pig farmers, however it would appear that pork weight pigs constitute a far lower risk to these workers.

The average prevalence of *pomona* infection in bacon weight pigs from infected farms was 67%. In order to detect at least one infected pig which is present in a population of 120 or less, and where the expected prevalence of infection at slaughter is 25%, at least ten pigs are required for examination at slaughter (Thrusfield, 1986). Therefore, the sample size of ten pigs that was used on two separate occasions should have been sufficient to detect farms with endemic leptospiral infection. The failure to isolate leptospires and the absence of *pomona* titres in all pigs sampled from some farms suggests that these farms were indeed free of *pomona* infection at the time of sampling. The isolation of leptospires from 15% of pigs from Farm J, was from one sample of ten pigs for which the serological and cultural prevalence was zero, and from a second sample of ten pigs where the prevalence was found to be 30%. Farm J had several



grower sheds, and it was possible that individual grower sheds may have had endemic *pomona* infection, while others may have been free of infection. No conclusions can be drawn about the influence of the self replacement of gilts, method of cleaning the piggery, design of the floor, and the design of effluent channels, and the mixing of pigs, on the presence or absence of leptospiral infection in grower pigs. Farms G, J, and L had effluent channels which did not pass between pig pens, did not mix pigs of different ages, and yet they all had endemic *pomona* infection. This was initially thought to be a surprising finding as the transmission of infection was regarded to be the result of direct contact between pigs or their effluent. It therefore appeared from this study that there must be other modes of transmission. Some aspects of transmission of *pomona* within piggeries were examined and are discussed in Chapter Five.

The distribution of *pomona* titres as shown in Figure 3.5, suggests that high titres are associated with current infection. Ryan (1978) suggested that the apparent prevalence of *pomona* infection could be determined from the *pomona* titres of pigs and he calculated an 85% sensitivity and specificity for titres of at least 1/384. The results from this present study showed that the absence of *pomona* titres always indicated absence of infection. Leptospire were not isolated from any pigs in the absence of detectable titres. Leptospire were isolated from 89% of pigs' kidneys with *pomona* titres greater than 1/5120, and from 60% of pigs which had titres between 1/10 and 1/5120. Thus, only the presence of high agglutinin titres in pigs can be used to indicate that pigs have come from a farm with endemic *pomona* infection, while the total absence of titres indicates that pigs have originated from a farm free of *pomona* infection.

From the results of these three studies it is clear that infection with serovar *pomona* remains the most common and important leptospiral infection of pigs in New Zealand today.

Throughout these studies several farms with endemic *pomona* infection were identified and their farm managers contacted. After obtaining their consent, epidemiological studies were conducted on Farms B, C, and D. Details of these investigations are described in Chapter Four. Vaccination trials were also conducted on Farms A and B, and these are described in Chapter Six.

## SUMMARY AND CONCLUSIONS

1. The *pomona* and *tarassovi* titres of five hundred and thirty pig's sera from six Ministry of Agriculture and Fisheries veterinary districts in New Zealand were determined using the MAT. The results showed a higher seroprevalence of *pomona* in the North Island than in the South Island, while the converse was true for *tarassovi* titres. However the prevalence of infection could not be estimated from the serological results because of insufficient information about the vaccination status of pigs from which the serum samples were obtained.
2. Blood, kidney and reproductive tract were obtained from slaughtered grower pigs at a local abattoir. The seroprevalence of *pomona*, *tarassovi* and *bratislava* titres were 60%, 0% and 53% respectively. Isolates of *pomona* were made from 53% of kidneys cultured, however no isolates of *bratislava* were made from either kidneys or reproductive tracts, despite the use of T80/40LH medium. It was concluded that the *bratislava* titres may be either due to a serological cross reaction, or due to transient infection.
3. Two hundred and twenty five matching blood and kidney samples were collected from slaughtered pork and bacon weight pigs originating from twelve piggeries. A retrospective study about each of the farming practices was conducted by telephone. It was concluded from this study that the infection status of grower pigs at slaughter was independent of the vaccination status of the breeding herd at their farm of origin. Farms raising pigs to pork weight were free of infection. Farms with less than fifty breeding sows were free of infection, and the average size of farms with infected pigs was significantly greater than the size of farms which were free of infection. Conclusions could not be drawn about the association between other farming practices due to either the influence of pig herd size on these variables, or insufficient number of farms in each category.

Chapter Four

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## INTRODUCTION

This chapter describes three epidemiological studies. These were undertaken on four farms with the aim of determining at what age grower pigs were becoming infected and the likely source(s) of their infection. The studies were conducted by observing the natural patterns of infection occurring in pigs within infected herds. In New Zealand two other major studies into the epidemiology of leptospiral infection of pig herds have been conducted. They were by Buddle and Hodges (1977) and Ryan (1978) and these were briefly reviewed in Chapter One. These and other relevant investigations are now reviewed in detail before the observational studies of this chapter are described.

Buddle and Hodges (1977) conducted a serological investigation of some aspects of the epidemiology of leptospiral infection within a pig herd. They found that there was no serological evidence of infection in weaner pigs or in young grower pigs before they entered a finisher house, at approximately ten weeks of age. However, at the time of slaughter, after pigs had been in a finisher house for several weeks, there was strong evidence from serological results that infection had occurred in the majority of these grower pigs. Buddle and Hodges conducted several studies within their overall epidemiological investigation. One study included transferring twenty six, six week old pigs and eleven, nine week old pigs at the time of weaning, directly to a finisher house. Eight of twenty four pigs transferred at six weeks of age, and five of ten pigs transferred at nine weeks of age, were infected after having been held in the finisher house for three weeks. All of the transferred pigs were infected by the time they were slaughtered. It was concluded by Buddle and Hodges (1977) that these pigs had not become infected during the period of suckling, but that the onset of infection was associated with their location, rather than the age of the pig.

Other studies by Buddle and Hodges (1977) investigated the infectiveness of effluent from pigs' pens. Hamsters which were allowed contact with contaminated effluent from pigs' pens became infected, however other hamsters which were put in contact with effluent from the same pens which had been

diluted by hosing the pen with water, failed to become infected. Susceptible pigs similarly exposed to either undiluted or diluted effluent, failed to become infected. Buddle and Hodges (1977) also found that pigs penned "down stream" from infected pigs became infected more readily than pigs housed in "up stream" pens. Finally, they noted that there was no evidence of infection in susceptible pigs which had been housed "up stream" with at least one vacant pen between them and infected pigs.

Buddle and Hodges (1977) suggested from their studies that infection had at some stage been introduced into the finishing house and that the effluent disposal system together with the exchange of pigs between pens had resulted in the infection becoming widespread and endemic within the building.

Ryan (1978) used evidence gained through a serological study to investigate the pattern of infection within both breeding and grower pig herds of the same farm. Ryan reported that an average of 53% of piglets in the farrowing house had serum agglutination levels to *pomona* of 1/12 or greater. The lowest seroprevalence of *pomona* in grower pigs of 30% occurred in pigs twelve to fourteen weeks of age. Thereafter, the seroprevalence and GMT of *pomona* titres increased, to a maximum of 100% in gilts and sows. The GMT's of gilts was 1/1230, and that of sows was 1/135. Ryan (1978) estimated the half life of *pomona* titres following infection to be approximately one year. Ryan (1978) also estimated the real prevalence of infection as distinct from the apparent prevalence, and calculated a real prevalence of 2% in suckling pigs, 0% in weaner pigs and young grower pigs, 19% in medium aged grower pigs, 74% in older grower pigs, 86% in gilts, and 18% in sows. The high titres of the neonates, which in turn lead to an estimated real prevalence of infection of 2%, were attributed by Ryan (1978) to be due to colostral antibody, rather than active infection. It was also concluded that infection had little effect on the fertility of the pig herd, as most gilts were infected before becoming pregnant. Cultural studies of the breeding herd indicated that pigs eighteen months or older were no longer shedding leptospire in their urine (Ryan, 1978).

Ryan (1978) came to the same conclusion as Buddle and Hodges (1977), namely that the endemic state of infection with *pomona* in the pig herd was

dependant on either direct or indirect contact of young susceptible pigs with older infected animals and that the focus of infection was in pigs aged six to twelve months of age.

Unsuccessful attempts to control an epidemic of infection with *pomona* in a New Zealand breeding herd has been described by Edwards and Daines (1979). It was noted in their investigation that serum agglutinating antibodies to *pomona* were not detected in the grower pigs which were the progeny of infected sows. They presumed that the grower pigs were protected from infection by maternally acquired antibodies when they were piglets.

In New Zealand there has been no evidence of rats, hedgehogs, or mice being infected with serovar *pomona*, even when these were caught in the vicinity of the piggeries (Buddle and Hodges, 1977; Ryan, 1978). It has therefore been concluded by these authors that rats, hedgehogs and mice are not important as sources of leptospiral infection within piggeries throughout New Zealand. Morales *et al* (1978) isolated *pomona* from one of 111 rats caught in Columbian piggeries, and they also concluded that rats did not play an important part in the epidemiology of infection of pig herds infected with *pomona*. Whyte and Ratcliff (1982) established that mice exposed to infective pig effluent could indeed become infected with *pomona*. However, they did not determine whether or not mice could naturally transmit *pomona* between members of its own species, and therefore the role of mice in the epidemiology of infection in pig herds was uncertain. Zieris (1987) reported that fluctuations in the prevalence of *pomona* in pig herds in the Democratic Republic of Germany could be associated with fluctuations in the population of field mice [*Apodemus agrarius*], which followed a three year cycle. In contrast to the findings of other investigators, Zieris (1987) concluded that these field mice as well as cattle were acting as sources of infection for pig herds, and that pigs were no longer the reservoir hosts for *pomona*. *Apodemus spp.* do not occur in New Zealand and there has been no evidence suggesting that cattle are reservoir hosts for *pomona* in this country.

## STUDY 1 - A SEROLOGICAL AND CULTURAL CROSS SECTIONAL STUDY OF WEANER AND GROWER PIGS IN THREE HERDS

The objective of the study was to determine the prevalence of infection in pigs of various ages, using serology and urine culture.

### *Materials and method*

Following an abattoir survey [Chapter 3, Study 2], the managers of three farms [B, D and E] were contacted and they agreed to allow a cross sectional study of the weaner and grower pigs on their farms to be conducted.

All three farms were commercial pig farms, with differing systems of management. None of the farmers vaccinated their breeding pigs with leptospiral bacterin for the prevention of abortion. The breed of pigs were Landrace, Large White or cross breeds thereof.

On all three farms, pigs between the time of weaning and slaughter were selected for examination. Samples were collected from three to five pigs in each of the pens selected for sampling. Pigs within a pen were chosen without any form of selection.

Urine was inoculated into T80/40LH and EMJH medium, and was examined by DFM every two weeks, for sixteen weeks. Three culture dilutions were made from each urine sample. Serum samples were tested against serovar *pomona* at serum dilutions of 1/10 to 1/163840. The samples were otherwise collected and processed as described in Chapter Two.

### *- Farm B*

Farm B had approximately 300 breeding sows. Piglets were weaned at four to five weeks of age and subsequently held in a weaner house in groups of twenty to thirty piglets per pen. When pigs were approximately ten weeks of age they were housed according to their gender in separate grower sheds. These sheds were spray hosed every day. Effluent channels flowed beneath metal grating floors which covered half of each pen, the other half was concrete flooring. Metal railing partitions separated the pens. The two grower sheds were of the

same design, shown in Figure 4.1. The standard of hygiene on this farm was moderate. Up to twenty, ten week old pigs were placed in pens at one end of the grower shed. As the pigs grew older, they were moved into pens further along the shed, so that the oldest grower pigs were at the opposite end of the shed to the youngest grower pigs. Grower pigs were raised to bacon weight for slaughter, and some female pigs were retained for replacement into the breeding herd.

Samples of blood and urine were collected from a total of fifty five pigs, thirty two females and twenty three males.

#### *- Farm D*

Farm D had approximately 100 sows. The general condition of pigs and the standards of hygiene on this farm was poor. Weaner and grower pigs were housed in four sheds, shown in Figure 4.2. Pig pens had both solid concrete floors and walls, and the effluent drained under the gate of each pen into a common effluent drain located in a central corridor. During the winter months pigs were fed whey and the growth rate of pigs over the winter period was low. In summer, when the price of grain was less than in winter, pigs were fed grain and "fattened" for slaughter. At the time of the study, pigs were being fed whey. Accurate records of the pigs ages were not available and pigs were selected on their relative size, and later an estimate was made of the pigs' ages. Piglets were estimated to be weaned between six and eight weeks of age.

Forty six blood and urine samples were obtained from pigs ranging in age between approximately six and twenty six weeks of age. Eighteen samples were from male pigs and twenty eight from female pigs. No more than four pigs in each pen on this farm were sampled.

#### *- Farm E*

Farm E purchased weaner pigs and reared them to slaughter. Breeding pigs were not kept on this farm. Weaner pigs were purchased from several different farms. The weaner pigs were kept in weaner boxes until they were approximately eight weeks of age. Pigs were then transferred to a large communal pen containing pigs which ranged between eight and twelve weeks



of age. Thereafter, groups of fifteen to twenty pigs were housed in pens with solid concrete floors and walls. In one building each pen had a separate effluent channel which drained into a common effluent channel in a central corridor of the building, this channel then passed through the communal pen, as shown in Figure 4.3. In other buildings, the effluent passed under the gate of each pen, or through outlet drains into common effluent channels. The general standard of hygiene on this farm was good.

A total of forty three blood and urine samples were collected from twenty males and twenty three female pigs from farm E.

Figure 4.1 Design of piggery on Farm B.

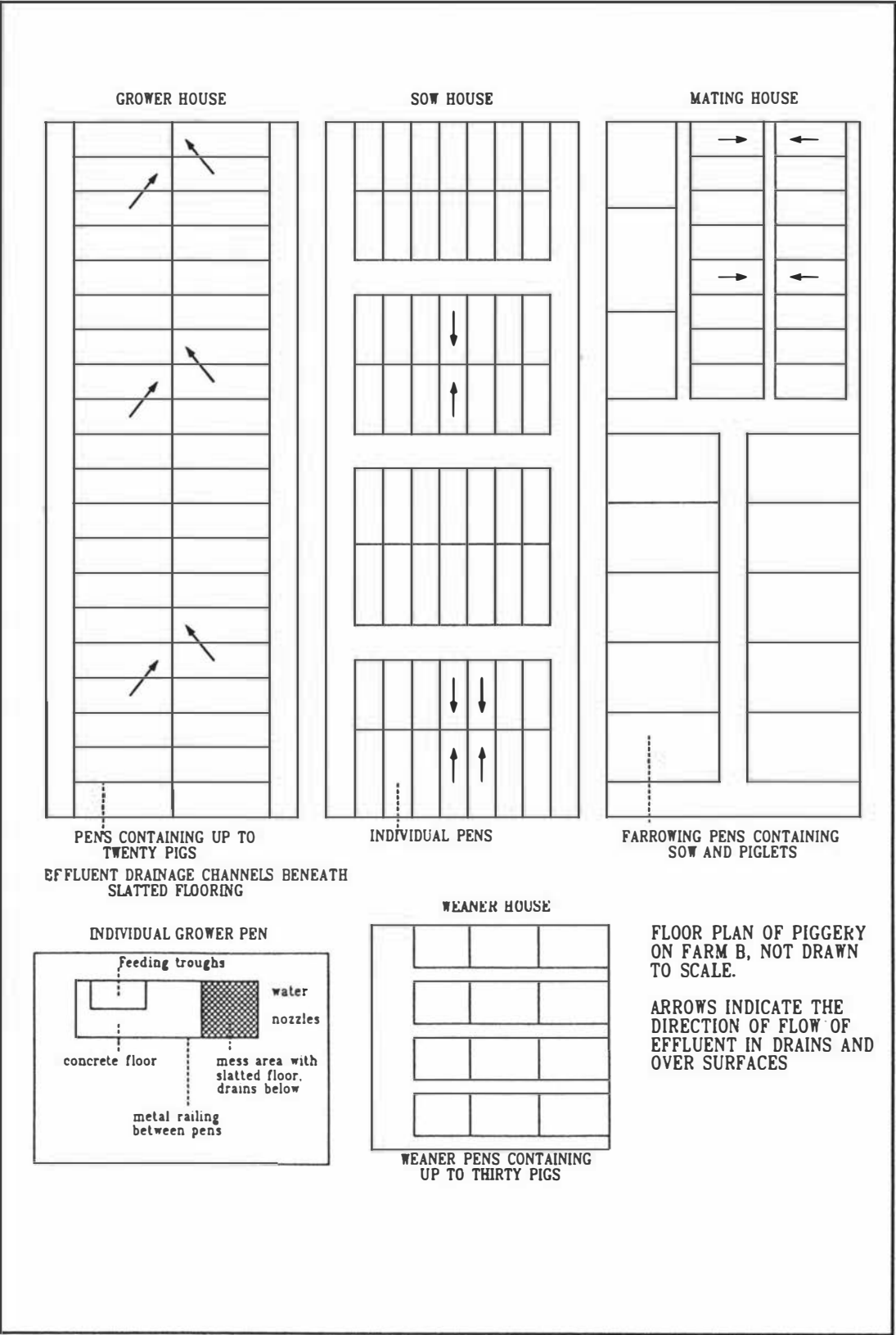


Figure 4.2                      Design of piggery on Farm D.

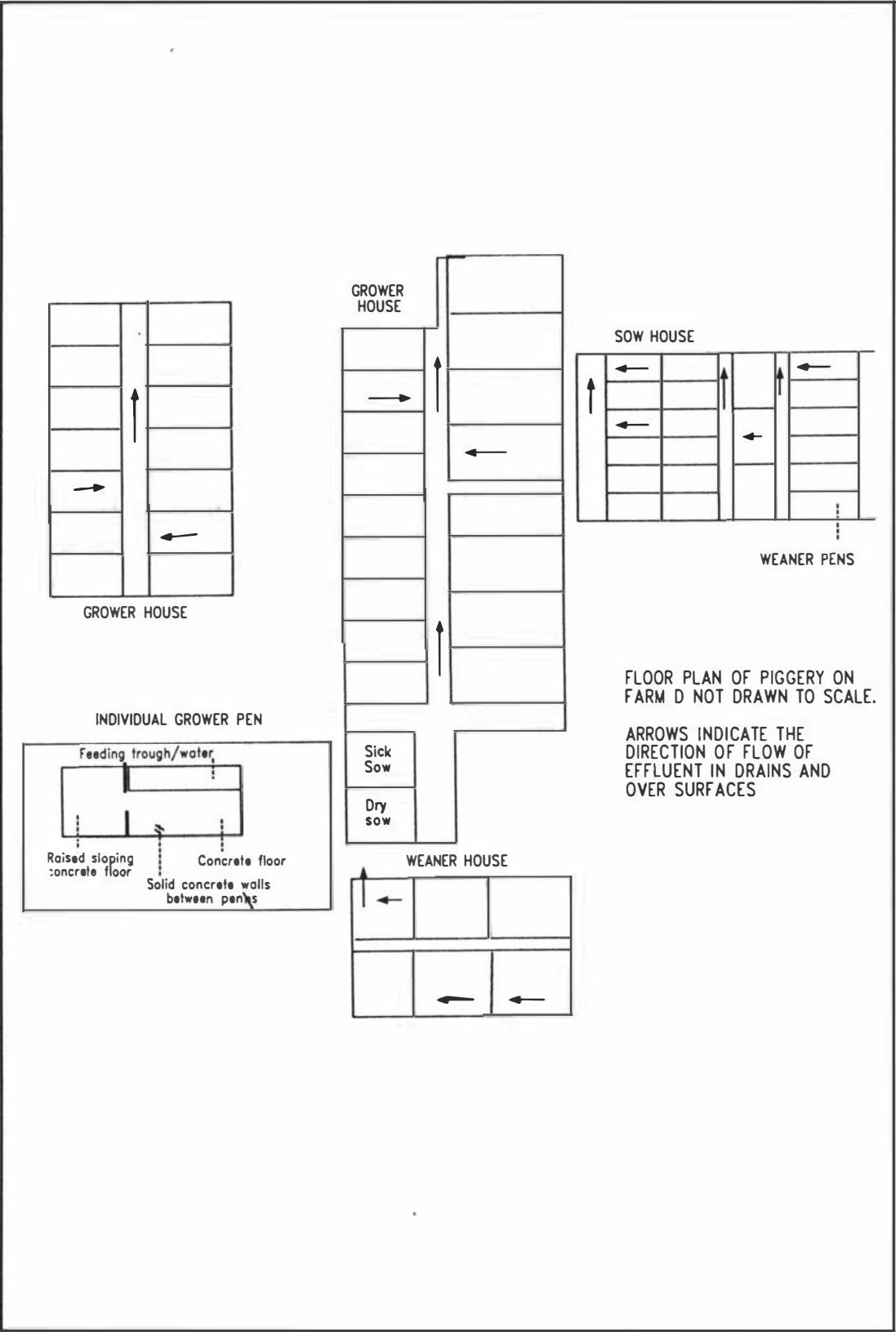
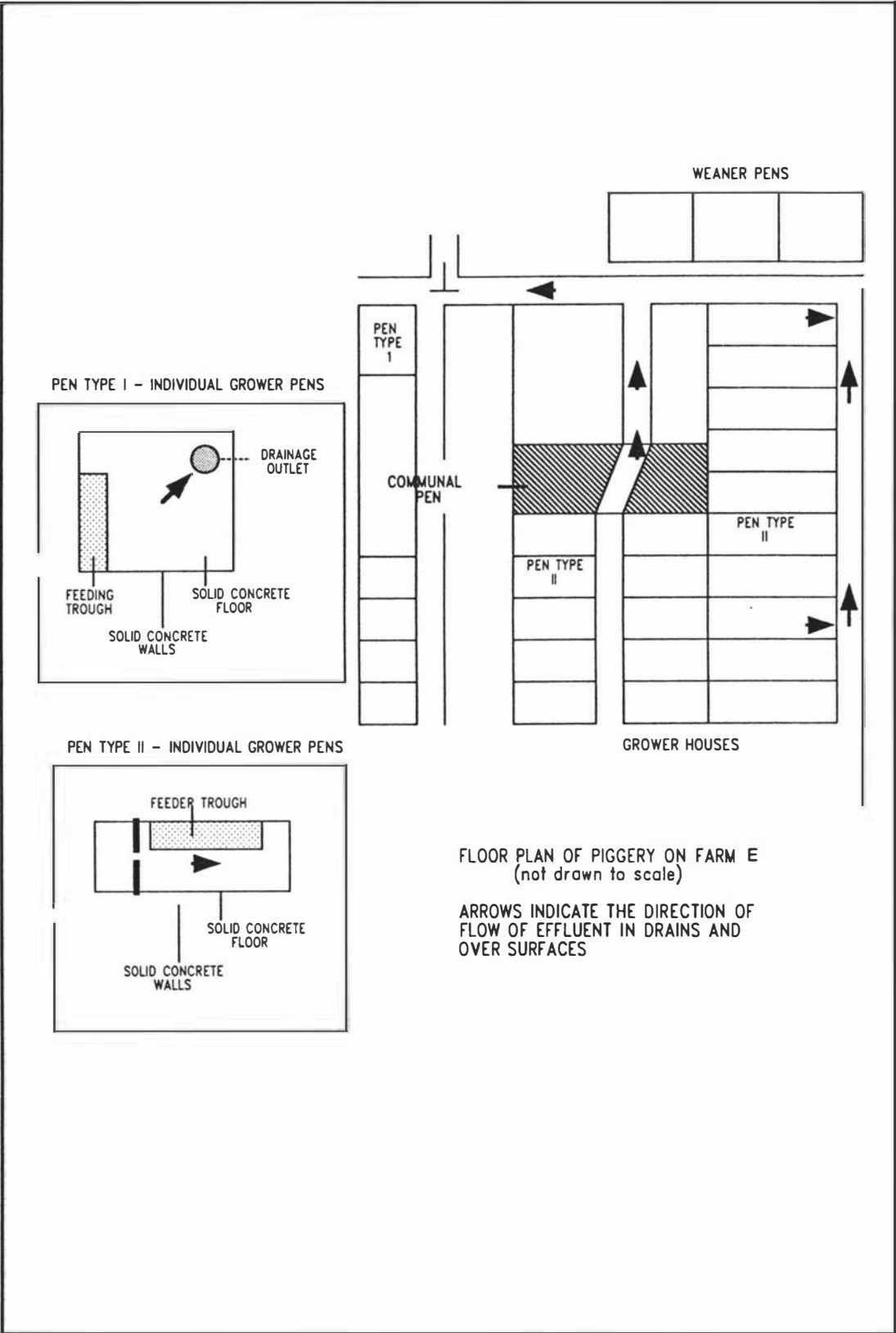


Figure 4.3                      Design of piggery on Farm E.



## **Results**

In previous abattoir surveys (see Chapter Three), between 80% and 100% of pigs sampled from either Farm B or D had titres to *pomona* and leptospires were isolated from their kidneys. However, there had been no evidence of infection in pigs from Farm E at the time of slaughter.

### **- Farm B**

The serological and cultural results of the fifty five pigs sampled on Farm B are shown in Table 4.1. Pigs of approximately fifteen weeks of age did not have detectable titres, however titres of less than 1/100 were present in pigs between four and eleven weeks of age, and higher titres were present in pigs seventeen weeks and older. Only one pen of female pigs, which were the oldest group to be examined, at 26 weeks of age, were leptospiruric and these pigs had titres which ranged between 1/10,240 and greater than 1/163840. Leptospires were not isolated from any of the male pigs and only one male pig which was older than eleven weeks of age had a detectable titre of 1/640.

### **- Farm D**

The serological and cultural results of pigs examined at Farm D are shown in Table 4.2. Leptospires were isolated from sixteen of forty six samples of urine collected. All of the pigs which had leptospires isolated from their urine had titres of 1/163,840 or greater. The infection state of individual pigs within a pen were usually the same as each other. The pigs from which leptospires were isolated ranged between approximately eleven and twenty six weeks of age. Leptospires were not isolated from the urine of pigs ten weeks of age or less, however these pigs had titres ranging between 1/10 and 1/5,120.

### **- Farm E**

Leptospires were not isolated, nor were titres to *pomona* detected in any of the forty three pigs examined on Farm E.

**Table 4.1**                      **Serological and cultural prevalence of infection with pomona in grower pigs from Farm B.**

Weeks of Age	Reciprocal <i>pomona</i> titre					Number of pigs Leptospi- ruric	$\frac{1}{\text{GMT}}$
	1	2	Pig 3	4	5		
4	1280	40	0	0	0	0	226
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
7	320	80	80	40	0	0	95
8	80	80	80	80	40	0	70
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	80	40	40	0	0	0	50
12	80	40	0	0	0	0	56
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
15	0	0	0	0	0	0	0
16	-	-	-	-	-	-	-
17	640	0	0	0	0	0	640
18	-	-	-	-	-	-	-
19	0	0	0	0	0	0	0
20	163840	20480	10	0	0	0	3225
21	-	-	-	-	-	-	-
22	0	0	0	0	0	0	0
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-
26	327680	327680	327680	20480	10240	4/5	94100

-        =        Not examined

**Table 4.2**                      **Serological and cultural prevalence of infection with pomona in grower pigs from Farm D.**

Weeks of Age	Reciprocal <i>pomona</i> titre				Number of pigs Leptospi ruric	$\frac{1}{\text{GMT}}$
	1	2	Pig 3	4		
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	10	0	0	-	0/3	10
7	10240	320	160	80	0/4	452
8	5120	5120	160	-	0/3	1613
9	0	0	0	-	0/3	0
10	0	0	0	-	0/3	0
11	327680	327680	163480	-	2/3	130000
12	327680	327680	327680	327680	4/4	327680
13	0	0	0	-	0/3	0
14	327680	327680	327680	-	3/3	327680
15	-	-	-	-	-	-
16	0	0	0	0	0/4	0
17	-	-	-	-	-	-
18	0	0	0	-	0/3	0
19	-	-	-	-	-	-
20	0	0	0	-	0/3	0
21	-	-	-	-	-	-
22	-	-	-	-	-	-
23	327680	327680	327680	327680	4/4	327680
24	-	-	-	-	-	-
25	-	-	-	-	-	-
26	327680	327680	327680	-	3/3	327680

-        =        Not examined

**STUDY 2 - A PROSPECTIVE SEROLOGICAL AND CULTURAL COHORT STUDY OF SUCKLING, WEANER AND GROWER PIGS IN A PIG HERD NATURALLY INFECTED WITH *POMONA***

Groups of pigs were examined by cultural and serological methods over a period of about sixteen weeks in a prospective cohort study. The study was aimed at gaining detailed information about some of the following aspects of the epidemiology of leptospiral infection in pig herds; the magnitude and duration of detectable maternal antibody in piglets, the onset, intensity and duration of leptospiuria in naturally infected pigs, and the prevalence and incidence of infection occurring in pigs of different ages in a herd naturally infected with *pomona*.

***Materials and method***

Previous abattoir surveys had established that a high cultural and serological prevalence of *pomona* in bacon weight pigs at slaughter, existed on Farm C. The owner of Farm C was contacted and agreed to allow an observational study of endemic infection with *pomona* to be undertaken on the farm over a period of up to four months.

Farm C had around seventy five breeding sows. Some female pigs were retained for replacement into the breeding herd, and some gilts were purchased from other farms. Breeding pigs were not vaccinated with leptospiral bacterin. Pigs were Large White, Landrace or cross breeds thereof. Sows which were due to farrow were moved from an outside concreted yard into a farrowing house. Piglets were weaned at approximately five weeks of age, and moved into the weaner shed. Weaner pigs were housed in raised pens in groups of twenty to thirty pigs per pen. At approximately ten weeks of age, they were transferred to a grower shed. In this shed, approximately twenty pigs were kept in each pen which had solid concrete floors and walls. Effluent flowed directly out of each pen into a common effluent drain on the outside of the building.

Pens were hosed down each day. The pigs were fed grain meal rations.



Blood and urine samples were collected from pigs and processed as described in Chapter Two.

Urine samples were collected from a total of twelve sows, two of these sows had their urine samples collected on three separate occasions [Sows 66 and 63] and one sow had urine collected on two occasions. Blood samples were collected from nine sows, including samples from Sows 66 and 63 which had farrowed within the previous four days. Five piglets [Pigs 56 to 60] from the litter of Sow 66, and five piglets [Pigs 71 to 75] from the litter of Sow 63, were selected as the "Suckling Cohort".

Three other cohorts of ten pigs each, were concurrently studied for eleven weeks. The "Weaner Cohort" was made up of recently weaned pigs which were five to six weeks of age. The "Young Grower Cohort" consisted of pigs which had been moved to the grower house within the last week, and they were approximately eleven weeks of age. The oldest cohort, "Old Grower Cohort", consisted of ten pigs, five of which were approximately sixteen weeks of age, and another five which were approximately eighteen weeks of age.

The original intention was to sample all pigs in the different cohorts for serological and cultural examination on a weekly basis for a period of ten weeks. Due to the difficulty and risk associated with bleeding suckling piglets, as described in Chapter Two, no attempt was made to obtain a sample of blood from piglets if they appeared to be stressed. If difficulty was being experienced whilst bleeding from the jugular vein, sampling was halted. As a consequence fewer blood samples were obtained than was originally planned. The schedule of examination of pigs in all four cohorts throughout the period of study is shown in Table 4.3. Urine samples were occasionally collected from pigs that were not members of a cohort. The frequency of examination, and the time taken to collect the samples began to interfere with farm management. For this reason either serological or cultural samples were collected from each cohort, except for pigs in "Weaner Cohort" which were not examined in the seventh, ninth or tenth weeks. In the seventh week of the study, all untagged pigs which were in the same pen which contained five pigs from the "Young Grower Cohort" were blood sampled to determine the

seroprevalence of *pomona* within the pen. From the seventh week onwards, pigs from the "Old Grower Cohort" began to be sent for slaughter. Blood and kidneys were collected from a total of fifteen pigs at the time of slaughter.

In the eighth week of the study all pigs within the two pens which contained the pigs from the "Young Grower Cohort" were treated for pneumonia with 10 to 20 mg oral tetracycline/ kg body weight for a period of one week. From this time onwards, pigs in this cohort were no longer serologically examined.

Twenty one mice [*Mus musculus*] were captured within the vicinity of the piggery of Farm C and their kidneys cultured for the presence of leptospires.

### **Results**

All titres refer to serovar *pomona*, and all leptospiral isolates obtained throughout the study were identified as *pomona* by the simple agglutination test described in Chapter Two. One isolate was also identified as *pomona* by BRENDA.

#### **- Breeding pigs**

Leptospires were not isolated from the urine of any of the twelve sows. Nine sows were serologically examined for titres to *pomona* and all sows had detectable titres. The titres of four sows were 1/640, 1/1,280, 1/10,240 and 1/163840 or greater, two sows had titres of 1/5,120, and three sows had titres of 1/320. The GMT of these titres was 1/2,195.

#### **- Suckling cohort**

Leptospires were not isolated from any of the suckling piglets, nor were leptospires isolated from the urine of their dams on three separate sampling occasions.

The serological results of piglets and their dams are shown in Table 4.4. Within one week of farrowing, the titres of Sows 66 and 63 were 1/640 and 1/10,240 respectively.

**Table 4.3**                      **Schedule of serological and cultural examination of two age cohorts over an eleven week period.**

Week of Study	Weeks of Age	Number of Blood samples	Number of Urine samples	Cohort Group
1	1	10/10	7/10 +7	Suckling Cohort
2	2	0/10	10/10	
3	3	4/10	3/10 +2	
4	4	0/10	4/10 +3	
5	5	6/10	0/10	
1	6	10/10	10/10	Weaner Cohort
2	7	10/10	10/10	
3	8	0/10	9/10	
4	9	0/10	9/10	
5	10	10/10	10/10	
6	11	9/9	9/9	
7	12	9/9	9/9	
8	13	9/9	9/9	
9	14	0/9	0/9	
10	15	0/9	0/9	
11	16	0/9	3/9 +4	

Table 4.4

Serological results to serovar *poona* of two sows and five of their piglets between five and thirty-four days of age

Pig number	Reciprocal <i>poona</i> titres		
	5 days	19 days	33 days
Sow 66	10,240	-	-
Piglet 71	1,280	-	40
Piglet 72	5,120	-	40
Piglet 73	2,560	-	40
Piglet 74	5,120	-	40
Piglet 75	2,560	-	40
Sow 63	640	-	-
Piglet 56	640	80	-
Piglet 57	640	-	-
Piglet 58	320	40	20
Piglet 59	640	80	-
Piglet 60	640	80	-

- Not examined.

In spite of infrequent serological examination of piglets a trend did emerge. At five days of age, the GMT's of the piglets from the two litters of Sows 66 and 63 were 1/557 and 1/2,940 respectively, which was similar in magnitude to their dams. The GMT of four piglets from Sow 66 at nineteen days of age was 1/67. At thirty three days of age, only one pig from the litter of Sow 66 was examined, and it had a titre of 1/20. At thirty four days of age the GMT of five piglets from the Sow 63 was 1/40. Thus, piglets had high titres at one week of age and these were similar to their dams titre at the time of farrowing. As time progressed the titres of the piglets declined, as shown in Table 4.4 and Figure 4.5.

- *Weaner Cohort*

Leptospire were not isolated from the urine of any pigs in this cohort throughout an eight week period of sampling. Neither were leptospire isolated from urine of three pigs in this cohort nor from four other pen mates from which samples of urine were collected at the eleventh week of the study.

The serological results of pigs in the Weaner Cohort are shown in Table 4.5. The seroprevalence and GMT's of pigs in the Weaner Cohort were 50% and 1/26 when they were six weeks of age and 60% and 1/32 when they were seven weeks of age. Three weeks after being transferred to the grower shed, titres were not detected in any of the pigs in this cohort.

- *Young Grower Cohort*

The cultural and serological results for the Young Grower Cohort are shown in Table 4.6.

Leptospire were not isolated from the urine of pigs in the Young Grower Cohort until the seventh week of the study, when pigs of this cohort were around seventeen weeks of age. In the seventh and eighth week of the study, only one pig was leptospiruric. From the eighth to the ninth week, pigs in this cohort were treated with oral antibiotic. In



the ninth week, the pig which had been leptospiruric in the seventh and the eighth week of the study, was apparently no longer leptospiruric. However in the tenth week, after the antibiotic treatment had ceased, leptospire were again isolated from the same pig's urine. In the following week, leptospire were isolated from five of ten pigs in this cohort.

In the first and third week of the study, when pigs were estimated to be eleven and thirteen weeks of age respectively, three of ten pigs in the Young Grower Cohort had titres to *poona*, and the GMT was 1/50. In the fifth week, only one pig had a detectable titre of 1/20. In the seventh week, the pig which was leptospiruric had a titre of 1/40,960, see Table 4.6 and Figure 4.5. Three of sixteen pen mates which were serologically examined in the seventh week, had titres of 1/20,480, 1/40960, and 1/163840 or greater. Thus, of all twenty one pigs in the pen, four had titres to *poona*. Serological results for this group were not available beyond the seventh week of the study.

Table 4.6

Serological and cultural results to serovar pomona of pigs between eleven and twenty-one weeks of age in the "Young Grower Cohort"

Age in weeks	Young Grower Cohort Pig Number										$\frac{1}{\text{GMT}}$
	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	
11	*40 o	80 o	40 o	0 o	0 o	0 o	0 o	0 o	0 o	0 o	50
12	- o	- o	- o	- o	- o	- o	- o	- o	- o	- o	-
13	40 o	40 o	80 o	0 o	0 o	0 o	0 o	0 o	0 o	0 o	50
14	- o	- o	- o	- o	- o	- o	- o	- o	- o	- o	-
15	0 o	20 o	0 o	0 o	0 o	0 o	0 o	0 o	0 o	0 o	20
16	- o	- o	- o	- o	- o	- o	- o	- o	- o	- o	-
17	0 o	0 o	0 o	0 o	40960 +	0 o	0 o	0 o	0 o	0 o	40960
18	- o	- o	- o	- o	- +	- o	- o	- o	- o	- o	-
19	- o	- o	- o	- o	- o	- o	- o	- o	- o	- o	-
20	- o	- o	- o	- o	- o	- o	- +	- o	- o	- o	-
21	- o	- o	- o	- o	- +	- +	- +	- +	- +	- o	-

\* = reciprocal *pomona* titre  
o = leptospires not isolated

+ = leptospires isolated  
Kid = results from kidney culture



- *Older Grower Cohort*

The serological and cultural results of pigs in the Older Grower Cohort are shown in Table 4.7.

In the first week of the study, leptospires were isolated from the urine of three of five eighteen week old pigs housed in one pen, but were not isolated from any of the sixteen week old pigs housed in another pen. The initial quantitative estimate of the intensity of leptospiuria in these pigs, and those which became leptospiruric during the course of the study was medium to high, that is, the intensity was estimated to be  $10^3$  or more leptospires per ml of urine, as shown in Figure 4.4. Some pigs appeared to be continually leptospiruric while others appeared to be intermittently leptospiruric and shedding leptospires at low to medium intensities, of less than  $10^3$  leptospires/ml. Leptospires continued to be isolated from all but one of the five oldest pigs [C32 to C35] in this cohort. One pig [C31], which was not leptospiruric at any time throughout the study had a consistently low titre of either 1/160 or 1/320. Leptospires were not isolated from the younger pigs of the Older Grower Cohort [C36 to C40] until the fifth week of the study, at which time these pigs were approximately twenty one weeks of age. Associated with the onset of leptospiuria, the titres of individual pigs rose to high levels, and remained high, as shown in Figure 4.5 and Table 4.7. From the seventh week onwards, the number of pigs in this cohort decreased as they were sent for slaughter. By the end of the study, leptospires had been isolated from nine of ten pigs in the Older Grower Cohort.

- *Cultural results of mice kidneys*

Leptospires were not isolated from any of the twenty one mice which were trapped within the vicinity of the piggery on Farm C.

Figure 4.4

The intensity of leptospirosis in naturally infected pigs over a ten week period of examination.

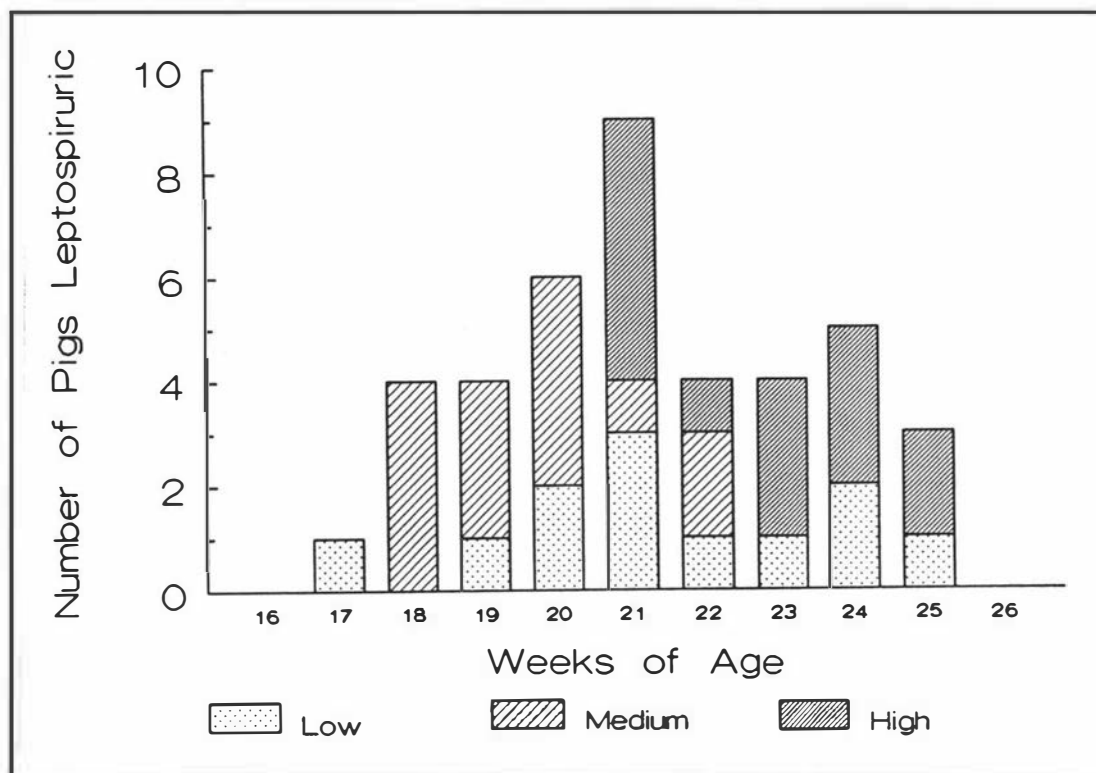
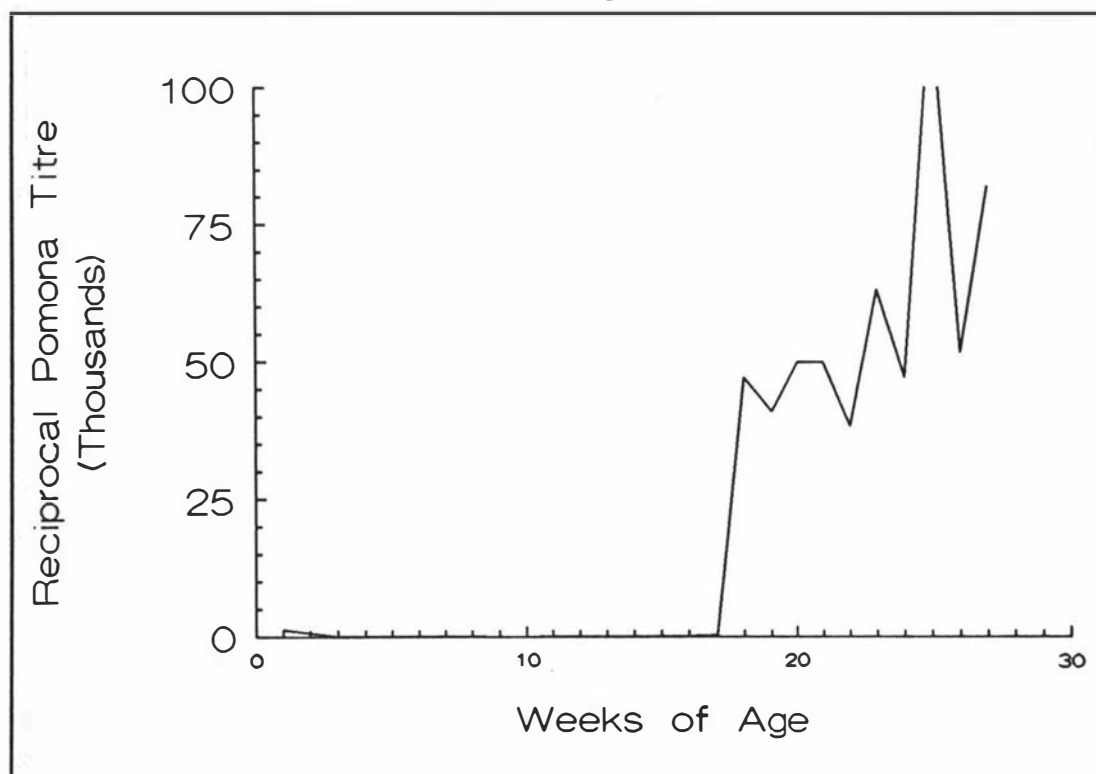


Figure 4.5

The geometric mean titre of *pomona* in growers pigs from Farm C with endemic *pomona* infection.



**Table 4.7** Serological and cultural results to serovar pomona of pigs between sixteen and twenty-six weeks of age in the "Older Grower Cohort"

Age in Weeks	Old Grower Cohort Pig number										$\frac{1}{\text{GMT}}$
	C31	C32	C33	C34	C35	C36	C37	C38	C39	C40	
16	-	-	-	-	-	*40	80	0	0	0	56
	-	-	-	-	-	o	o	o	o	o	
17	-	-	-	-	-	20	40	0	0	0	28
	-	-	-	-	-	o	o	o	o	o	
18	320	163840	163840	163840	163840	0	0	0	0	0	47050
	o	+	+	+	+	o	o	o	o	o	
19	160	163840	163840	163840	163840	0	0	0	0	40960	40960
	o	o	+	+	+	o	o	o	o	o	
20	320	163840	163840	163840	163840	40	0	163840	0	20480	49930
	o	o	+	+	+	o	o	+	o	+	
21	320	163840	163840	163840	163840	0	0	163840	0	20480	49930
	o	o	+	o	+	o	o	+	o	+	
22	640	163840	163840	163840	163840	163840	2560	163840	10240	*20480	38217
	o	o	o	o	o	Kid +	+	+	+	+	
23	640	163840	163840	163840	163840		20480	163840	163840	-	63169
	o	o	o	o	+		+	+	+	o	
24	320	163840	163840	163840	163840		-	-	-	-	47050
	Kid o	+	+	o	Kid o		+	+	+	o	
25		40960	163840	163840			-	163840	-	-	11585
		o	o	Kid o			+	Kid +	+	o	
26		-	-				163840		163840	5120	51606
		o	-				Kid +		Kid +	Kid o	
27		40960	163840								81920
		Kid o	Kid +								

\* = reciprocal *pomona* titre  
o = leptospire not isolated

+ = leptospire isolated  
Kid = results from kidney culture

### STUDY 3 - A PROSPECTIVE CULTURAL STUDY OF GROWER PIGS IN A HERD INFECTED WITH *POMONA*

It was intended in this third study to see if the grower herd on Farm B had a similar pattern of infection to that presented by the pig herd on Farm C [Study 2]. If this were the case then it could perhaps be considered to be typical of many farms in the district. In addition, the movement of pigs within the grower shed and the infection status of pigs in surrounding pens were also monitored. These two additional factors were investigated with the aim of determining their significance for the transmission of leptospiral infection within a grower herd, and throughout a piggery.

#### *Materials and method*

A description of Farm B has been given in Study 1 of this chapter, and a plan of the piggery is shown in Figure 4.1.

Two pens containing a total of twenty one pigs which were approximately eleven weeks of age, were selected and tagged for the study. All pigs in the study group had been transferred to the grower shed within the last week. Pigs were identified according to their pen locality, for example, pigs originally located in pen BB were numbered BB-1 to BB-21, and pigs in pen BG were numbered BG-1 to BG-21. Samples of urine were collected for culture every week until the pigs were sent for slaughter. Their movements were monitored by recording the pen in which they were located at the time of urine sampling. Urine was also collected from five pigs from within each of the pens adjacent to the a pen containing the majority of tagged pigs. This enabled the infection status of pigs in surrounding pens to be determined.

Urine was collected on several occasions, from twenty two self replacement gilts, over a period of up to three months. One urine collection took place while gilts were housed in the breeding shed, and on the other sampling occasions the gilts were housed in the grower shed.

Urine was collected and three dilutions of this urine were cultured as described in Chapter Two.

### *Results*

One pig [Pig BG-6], was leptospiruric in the first week of the study and it died in the fifth week, the cause of death was not known. The culture results of pigs originally in pens BB and BG are shown in Table 4.9. By the fourth week, when the pigs were approximately fourteen weeks of age, two pigs from pen BG had become leptospiruric, thereafter the prevalence of leptospiuria increased over several weeks to above 90% in the weeks before slaughter, as shown in Figure 4.8. It was not until the seventh week, when pigs were approximately seventeen weeks of age, that a pig originally from pen BB [Pig BB-19] became leptospiruric. The prevalence of leptospiuria then increased over a period of several weeks to reach almost 90% at the time of slaughter, as shown in Figure 4.8. The weekly incidence of leptospiuria was greatest in pigs aged between sixteen and twenty three weeks of age, see Figure 4.9.

It became apparent from monitoring the movement of pigs that they were frequently moved between pens and thus mixed with other pigs, as shown in Figures 4.6 and 4.7.

Throughout the period of study at least one pen, adjacent to a pen containing the pigs under study, had one or more leptospiruric pigs. Towards the end of the study, it was usual for more than one adjacent one pen to contain leptospiruric pigs, as shown in Table 4.8.

Leptospire were isolated from the urine of sixteen of twenty two gilts, as shown in Table 4.10. One gilt was leptospiruric over a period of three months, while being housed in both the grower and breeding sheds.

Table 4.8

Mixing and movement of pigs between pens, and the infectious status of pigs in adjacent pens.

Age of BG pigs in weeks	BG pigs mixed with other pigs	BG pigs moved to other pen	Surrounding pens have leptospirosis pigs
11	No	No	Yes
12	No	No	Yes
13	No	No	-
14	No	No	-
15	Yes	Yes	-
16	No	Yes	Yes
17	No	No	-
18	Yes	Yes	-
19	No	Yes	-
20	No	No	-
21	No	Yes	Yes
22	Yes	No	Yes
23	Yes	Yes	Yes
24	No	Yes	Yes
25	Yes	Yes	Yes
26	Yes	Yes	Yes

Table 4.9 The onset and duration of leptospiuria in grower pigs from Farm B.

Pig Tag BB#	Weeks of Age																	
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	o	o	o	o	o	o	o	o	-	o	o	-	o	o	o	o	+	+
2	o	o	-	-	-	-	-	-	-	-	o	-	-	-	-	-	-	-
3	o	o	-	-	-	-	-	-	-	-	o	-	o	-	+	-	-	-
4	o	o	o	o	o	o	o	o	o	o	o	-	o	o	-	-	-	-
5	o	o	o	o	o	o	o	o	-	o	o	-	o	o	o	o	o	o
6	o	o	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
7	o	o	o	o	o	o	o	+	o	+	+	-	+	-	o	-	-	-
8	o	o	o	o	o	o	o	o	o	o	+	-	+	o	-	-	-	-
9	o	o	o	o	o	o	o	o	o	o	+	-	+	o	+	+	+	+
10	o	o	o	o	o	o	o	o	-	-	-	-	-	-	-	-	-	-
11	o	o	o	o	o	o	o	o	-	o	o	-	o	o	+	+	o	+
12	o	o	o	o	o	o	o	o	-	o	o	-	o	o	o	o	-	-
13	o	o	o	o	o	o	o	o	o	o	o	-	o	+	+	+	-	-
14	o	o	o	o	o	o	o	o	-	o	o	-	o	o	o	-	-	-
15	o	o	-	-	-	-	-	-	-	+	+	-	+	o	+	+	+	+
16	-	-	o	o	o	o	o	+	+	-	+	-	-	+	+	+	+	+
17	-	-	o	o	o	o	o	o	o	o	o	-	o	o	o	+	+	+
18	-	-	o	o	o	o	o	o	o	o	o	-	+	o	+	-	-	-
19	-	-	o	o	o	o	+	+	+	-	+	-	+	+	+	-	-	-
20	-	-	o	o	o	o	Died											
21	-	-	o	o	o	o	o	+	-	o	o	-	o	o	o	-	-	-

o = Leptospire not isolated from urine.  
 + = Leptospire isolated from urine, all isolates identified as *pomona*.  
 - = Not examined

Table 4.9 ...continued

Pig Tag BG#	Weeks of Age																		
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	o	o	-	o	-	-	o	o	+	+	-	-	o	-	o	+	-	-	-
2	o	o	-	o	-	-	o	o	o	o	o	-	o	-	o	o	o	-	-
3	o	o	-	o	o	-	o	-	-	-	-	-	-	-	-	-	-	-	-
4	o	o	-	o	-	-	-	-	+	+	-	-	+	-	+	o	-	-	-
5	o	o	-	o	-	-	o	o	+	+	-	-	o	-	-	-	-	-	-
6	+	+	-	+	-	Died													
7	o	o	-	+	+	-	+	+	+	+	+	-	o	-	o	o	-	-	-
8	o	o	-	o	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
9	o	o	-	o	-	-	+	+	+	+	-	-	+	-	+	+	-	-	-
10	o	o	-	o	-	-	o	o	+	+	-	-	+	-	+	-	-	-	-
11	o	o	-	o	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
12	o	o	-	+	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+
13	o	o	-	o	o	-	+	o	+	+	-	-	+	-	+	+	-	-	-
14	o	o	-	o	+	-	+	+	+	+	-	-	+	-	+	+	+	+	-
15	o	o	-	o	-	-	-	-	-	-	-	-	o	-	o	-	-	-	-
16	o	o	-	o	-	-	o	+	o	+	+	-	+	-	+	+	-	-	-
17	o	o	-	o	-	-	o	-	o	+	+	-	o	-	+	-	-	-	-
18	o	o	-	o	-	-	o	+	+	+	+	-	o	-	+	-	-	-	-
19	o	o	-	o	o	-	o	+	+	o	+	-	o	-	+	+	+	+	+
20	o	o	-	o	-	-	o	+	+	-	+	-	o	-	o	-	-	-	-
21	o	o	-	o	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

o = Leptospire not isolated from urine.  
 + = Leptospire isolated from urine, all isolates identified as *pomona*.  
 - = Not examined



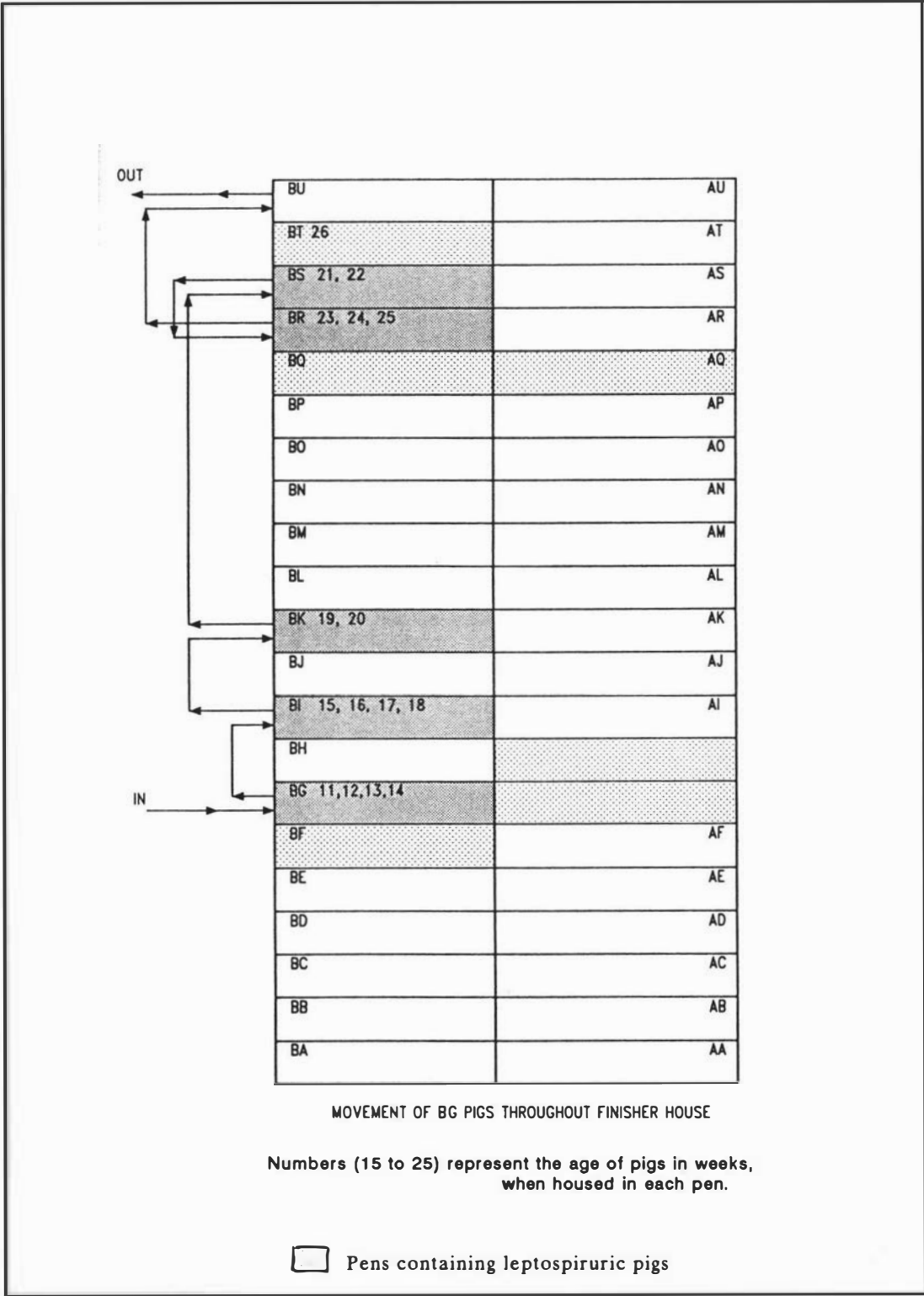
Table 4.10

Culture results and location of gilts on Farm B at the time of examination.

DAY LOCATION	1 Grower shed	3 Grower shed	31 Grower shed	45 Grower shed	72 Dry Sow shed	87 Grower shed	129 Grower shed
GILT NUMBER							
G 1	-	o	-	-	o	o	o
G 2	-	o	-	-	o	o	o
G 3	-	+	-	-	-	-	-
G 5	-	-	-	o	o	-	-
G 6	-	-	-	+	+	-	-
G 7	-	-	-	o	o	-	o
G 8	-	-	-	+	+	+	+
G 9	-	-	-	+	o	o	o
G 11	-	-	-	+	-	-	-
G 12	-	-	-	+	-	-	-
G 13	-	-	-	+	-	-	-
G 14	-	-	-	+	o	-	-
G 15	-	-	-	+	+	-	-
G 16	-	+	-	-	o	o	o
G 17	+	-	-	-	-	-	-
G 18	o	-	-	-	o	o	o
G 19	-	o	-	-	-	-	-
G 20	+	-	-	-	+	-	-
G 21	+	+	+	-	+	+	o
G 24	-	-	-	+	o	-	-
G 25	-	-	-	o	+	o	o
G 26	-	-	-	+	o	-	-

o = Leptospire NOT isolated from urine.  
+ = Leptospire isolated from urine.

**Figure 4.6**                      **Schematic representation of the movement of a group of pigs throughout the grower shed on Farm B over a period of sixteen weeks.**



**Figure 4.7** Schematic representation of the movement of two individual pigs BG-1 and BB-1 throughout the grower shed on Farm B for a period of sixteen weeks.

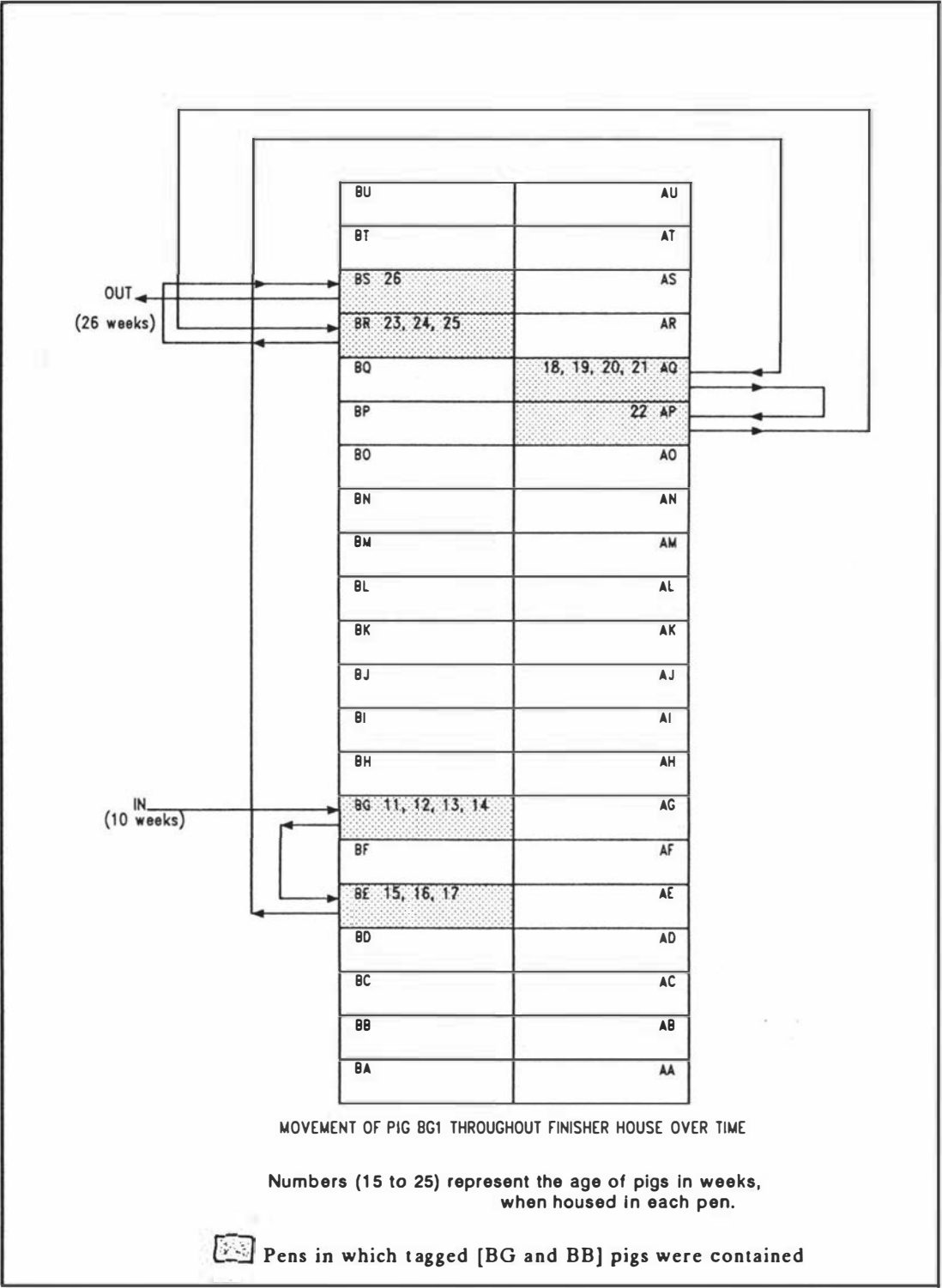
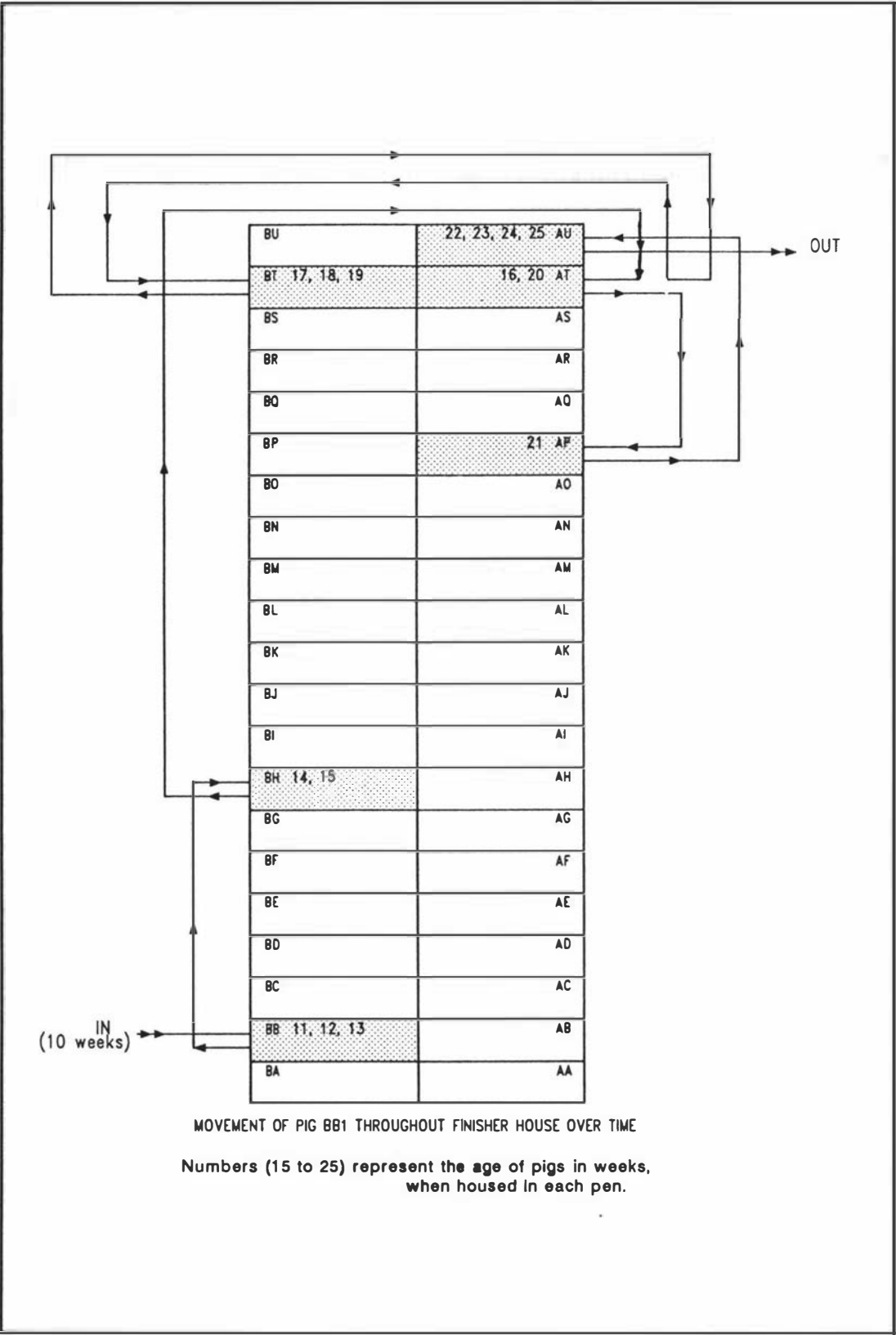
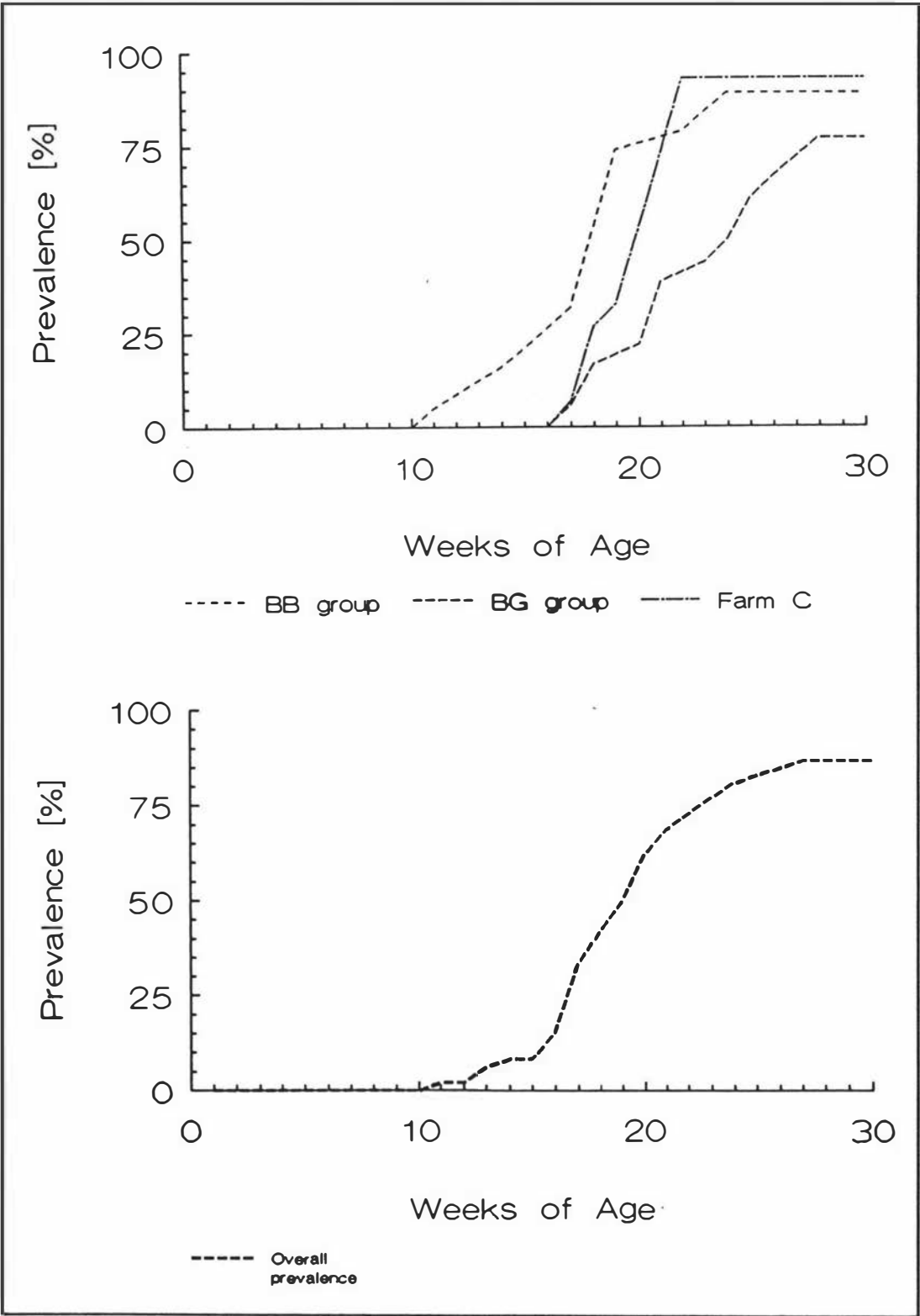


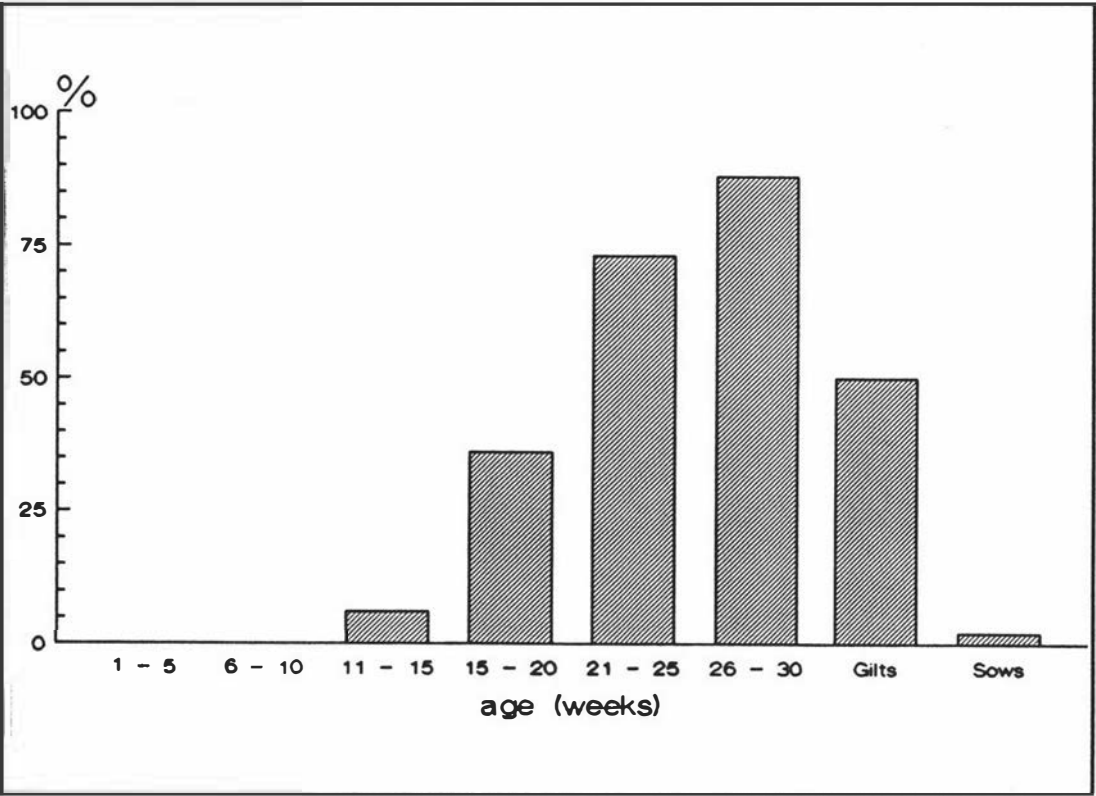
Figure 4.7 ...continued



**Figure 4.8**                      **The average prevalence of leptospirosis in three groups of naturally infected grower pigs.**



**Figure 4.9**                      **Prevalence of leptospiruria the pig herd from Farm B with endemic *pomona* infection.**



## DISCUSSION

The tendency for young suckling and weaner pigs to have detectable serum antibody titres in the absence of leptospirosis [Study 1 and 2] is an expected and explicable one. The presence of such titres have been previously described, and assumed by Ryan (1978), Buddle and Hodges (1977) and Edwards and Daines (1979) to be due to maternal antibody. It appears that piglets acquire an antibody titre similar in magnitude to that of their dams at the time of farrowing [Study 2] (Millar *et al*, 1987). The half life of maternally derived antibody in piglets was calculated to be approximately sixteen days. This calculation is in agreement with Millar *et al* (1987) who calculated the half life of maternal antibody in piglets of vaccinated dams to be 15.8 days. The result is also similar to that for calves, in which the calculated half life of colostral MAT titres to *hardjo* was fifteen to seventeen days Hellstrom (1978). Hellstrom (1978) also demonstrated that calves were refractory to experimental inoculation with *hardjo* for a period of three months. Similarly Mitchell *et al* (1966) demonstrated that piglets belonging to a leptospirotic sow did not become infected, and Chaudhary *et al* (1966a; 1966b) demonstrated that piglets artificially challenged with *poona* via the intranasal route, did not become infected. Using a half life of sixteen days, the time required for a pig's initial maternal antibody titre to decline to an undetectable level can be determined, as shown in Figure 4.10. If one examines the distribution of breeding pigs' titres (Ryan, 1978), and assumes that each piglet will acquire the same titre as that of its dam at the time of farrowing, then one can plot the time taken for the maternal antibody titres of a population of grower pigs to decline to undetectable levels, Figure 4.10. Thus, as the population of grower pigs becomes older, the proportion of pigs that become susceptible to infection becomes higher. It is interesting to note that the prevalence of grower pigs with leptospirosis, closely matches the theoretical proportion of pigs that should become susceptible to infection, Figure 4.11. Therefore the results of this epidemiological study support the hypothesis that grower pigs with detectable levels of maternal antibody are resistant to infection. The duration of persistence of maternal antibody in a population of grower pigs is therefore influenced by the immune status of the breeding sows. As a result, the immune

status of the breeding sows influences the pattern of infection within a grower pig herd.

Leptospire were not isolated from any pigs aged ten weeks or less throughout all of the studies conducted in the course of this research. The absence of infection in pigs of ten weeks of age or less is likely to be the result of both the resistance to infection resulting from the presence of maternal antibody, and to the low risk of transmission of infection to pigs after weaning and before their entry into a grower shed. On all of the farms studied, weaned pigs were housed in separate buildings and out of contact with older pigs. If pigs were not protected by maternal antibody then one might expect piglets of leptospiruric dams to become infected, and this in turn would mean that pigs within weaner houses would be infected, thus greatly an altered pattern of infection within pig herds.

**Figure 4.10**                      **Decline of maternal antibody titres in pigs.**

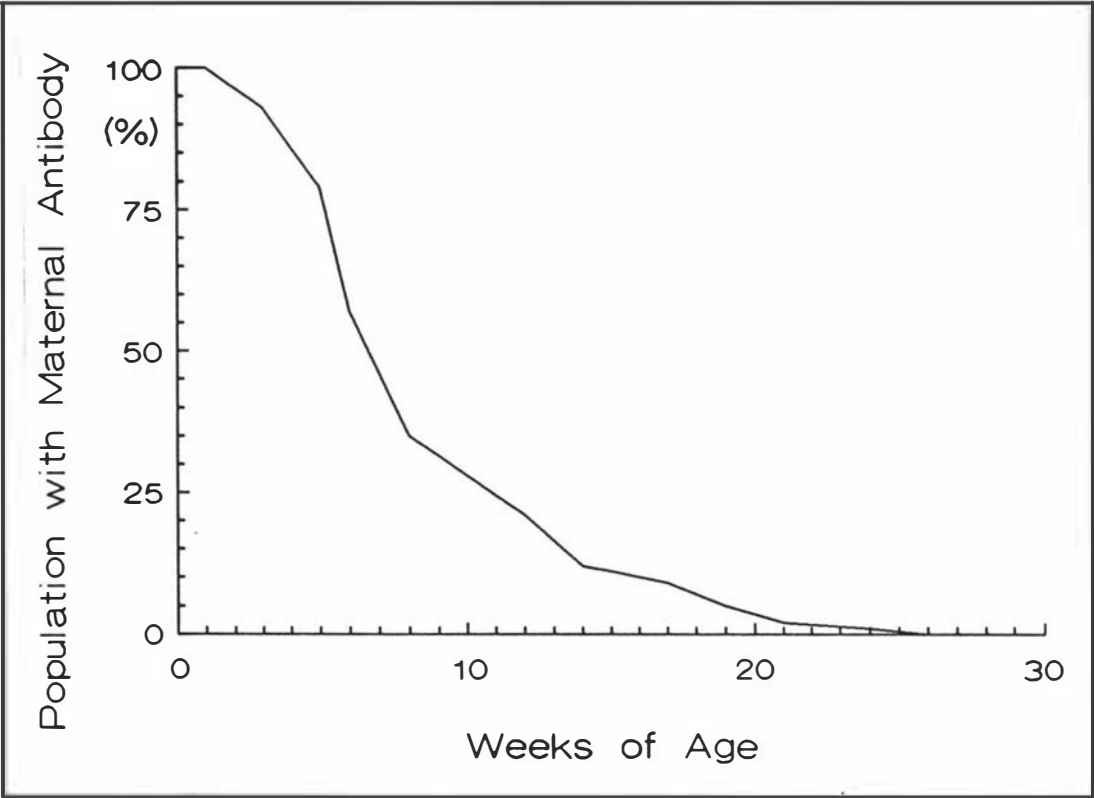
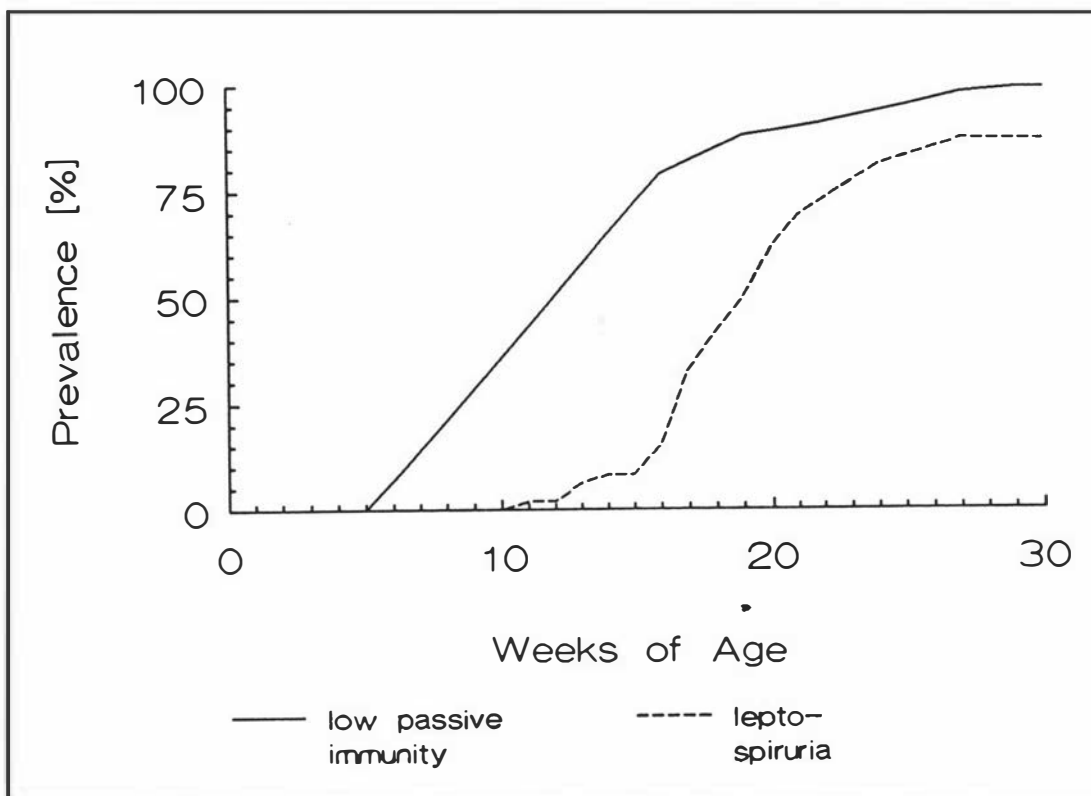




Figure 4.11

The age of onset of leptospirosis in grower pigs of Farm B and C, and the time to decline of maternal antibody for a population of grower pigs.



When the serological and cultural prevalence study on Farm B [Study 1] was first undertaken it was expected that pigs of all ages within the grower house would be found to be infected because there were no solid walls between the pens. However this was not the case, and only the oldest grower pigs were found to be leptospiruric, in spite of the potential for transmission of disease to pigs of all ages within the grower shed. During the cultural cohort study on Farm B [Study 3], it was demonstrated, for the entire duration of the study, pigs examined within the grower house were housed adjacent to pens which contained leptospiruric pigs. The delay in the onset of infection in younger grower pigs can only be explained by the presence of maternal antibody in these pigs. A more detailed study of the onset of leptospirosis in grower pigs within the grower sheds [Farm C and B, Study 2 and 3], showed that of the total number of pigs which eventually became infected, less than 15% were leptospiruric when they were sixteen weeks of age or less. A combination of resistance to infection and a low probability of direct transmission of infection from infected pigs within the same pen, could account for the lower prevalence

of infection in younger grower pigs than in the older grower pigs. This hypothesis is also supported by the failure to isolate leptospires from the kidneys of porker weight pigs. Birnaum *et al* (1972; 1974) considered that maternal antibodies influenced the carrier state of mice infected with serovar *grippotyphosa*. These authors found that the potential of mice to become infected was dependant on the immune status of their dam, and only mice born to dams which had not been infected, were susceptible to infection. They proposed that only mice infected at a very young age could maintain the disease in nature. Their hypothesis is however different from the one proposed in this chapter to explain the cycle of maintenance of infection in pig herds within piggeries, however the influence of maternal antibody on the epidemiology is central to both hypotheses.

The finding that eleven and twelve week old pigs from Farm D were leptospiruric, was different from the results of pigs from Farms B and C.

Several contributing factors may have been responsible for the high cultural and serological prevalence of infection in pigs between ten and fifteen weeks of age on Farm D. Firstly, on this farm the standard of hygiene was poor and this may have resulted in a higher level of environmental contamination, thereby increasing the likelihood of indirect transmission of leptospires between pigs. Secondly, the serological results from other pigs from Farm D suggested that some groups of young pigs had detectable maternal antibody, while others of similar age did not. A possible explanation for this finding is the variation in dams' titres which were reflected in some litters of piglets having high and longer lasting maternal antibody whereas others received low levels of maternal antibody. As a result, the susceptibility of pigs at any given age will depend on the magnitude of their initial maternal antibody titre. Thirdly, as the growth rate of pigs at Farm D was poor, the ages of pigs may have been under estimated by as much as two to three weeks. Throughout the period of study, "clusters" of infected pigs from within the same pen occurred on Farm D [see Table 4.2], this finding is similar to that observed by Ryan (1978) when he investigated the epidemiology of *pomona* infection in a pig herd. The occurrence of clusters of infected pigs is covered in more detail later in this discussion.

As the onset of leptospirosis occurs from seven to fourteen days following natural infection [see Chapter One], the age at which the pigs become infected is estimated to be one to two weeks prior to the detection of leptospirosis. Therefore the majority of pigs became infected when they were fourteen weeks of age or older. The intensity of leptospirosis in pigs from farm C appeared to be greatest in the first three to four weeks of their infection, thereafter the intensity declined and leptospirosis was often only intermittently detected. These results agree with those of Morse *et al* (1958), Alexander *et al* (1964), and Hodges (1973). Pigs continued to be sent for slaughter at twenty four weeks of age or older, therefore an estimate of the duration of detectable leptospirosis in naturally infected grower pigs was not possible. Most of the pigs which became leptospirotic during the course of the study remained so until the time of slaughter, thus indicating that the period of leptospirosis for most pigs was at least six weeks.

The weekly incidence of infection within a pen is influenced by the number of infected pigs and the intensity of their leptospirosis. As demonstrated in Study 2 and 3, once a pen contained an infected pig, an epidemic of infection within that pen occurred. As a result there was a high weekly incidence of infection within the same pen and in a few weeks the majority of pigs within that pen became infected. This resulted in "clusters" of infected pigs being detected when measuring the prevalence of infection as was seen in the serological and cultural cross sectional surveys in Study 1 on Farms B and D. The high prevalence of infection in bacon weight pigs at slaughter was reflected in a high serological and cultural prevalence of the oldest grower pigs in all of the infected herds. On Farm B, the moving of pigs within the grower shed, and the mixing of pigs between pens ensured direct or indirect contact with leptospires at some stage of the pigs' life and this can explain the pattern of infection found. On Farm C, pigs were not moved between pens, the pens had concrete floors and walls, and the effluent channels were separate, never the less cross infection between pens of pigs still occurred. It is possible that leptospires are transmitted indirectly via fomites such as the farmers boots and utensils, or by aerosols and droplets created by urinating pigs and pressure hosing of pens with water. The ability of this infection to be transmitted by droplets or aerosols was therefore further investigated and is discussed in Chapter Five.

The management systems of commercial piggeries ensures a continual supply of potentially susceptible pigs to the grower herd. Therefore, infection can continually be transmitted from infected to susceptible grower pigs, and a constant proportion of those grower pigs become infected every week. Once infection has been introduced to a grower pig herd, the herd becomes a maintenance population and thus an endemic *pomona* infection within that piggery is established. Within a grower herd, infection may continue independently of the breeding herd, as represented in Figure 4.12.

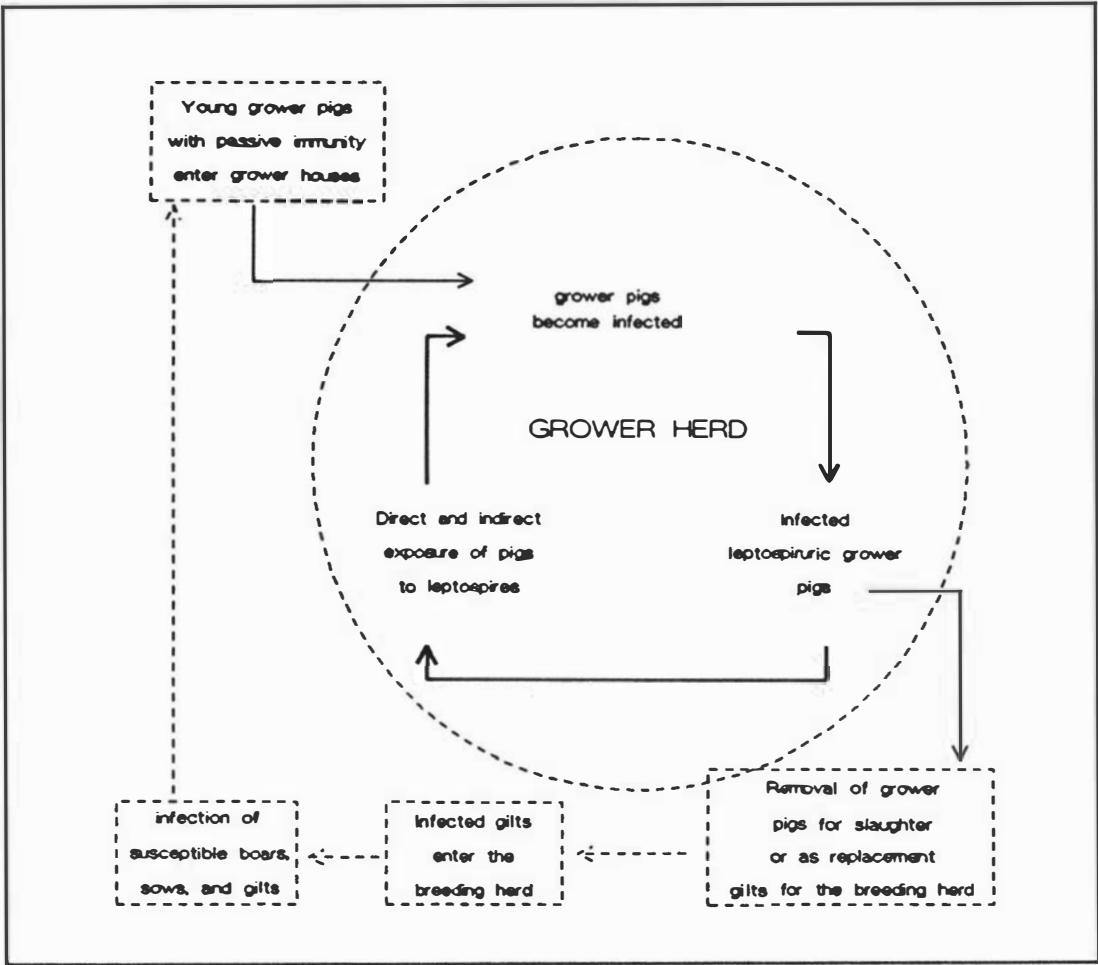
Farm E, was found to be free of infection and is likely to remain so because of the absence of any opportunity for infected pigs to be introduced into the herd. It also demonstrates that infection from dam to piglet is unlikely, otherwise infection would have been introduced via infected weaner pigs arriving at the farm.

On Farm B and less frequently on Farm C, gilts and sows were housed in the grower house. In grower pig herds it is likely that infected gilts have been responsible for the initial introduction of disease into grower herds. Grower pigs which are retained for replacement of members of the breeding herd, become infected while in the grower shed, and remain leptospiruric for a period of between six weeks to six months [Ryan, 1978; Study 3]. During their period of leptospiuria new gilts will enter the breeding herd for mating and will be housed with other sows. Therefore on farms like Farm B and C, these gilts can act as a source of infection for other breeding pigs within the breeding herd. Likewise, grower pigs may act as a source of infection for breeding pigs housed within a grower shed.

Mice did not appear to become infected with *pomona* and are therefore not important in the transmission of *pomona* within the pig herd of Farm C. This finding agrees with the authors of other epidemiological studies of leptospirosis in pigs reviewed in the introduction of this chapter.

Emphasis for the control of endemic *pomona* infection within piggeries must therefore be directed towards the prevention of infection in grower pigs.

**Figure 4.12**                      **The cycle of maintenance of infection with *pomona* in a grower pig herd.**



## SUMMARY AND CONCLUSIONS

1. The serological and cultural prevalence of infection in weaner to bacon weight pigs was studied on three farms, and two further prospective studies were conducted on two farms.
2. Infection was not detected in pigs from one farm on which breeding pigs were not kept, but where weaned pigs were purchased and reared to slaughter at bacon weight. On the two other farms where breeding pigs were kept and self replacement of gilts was practised, infection was found to commence in pigs ten weeks of age and older. Younger weaner pigs often had low titres, in the absence of infection. The majority of the oldest grower pigs sampled were infected. Individual pens of pigs of the same age had differing prevalences of infection.
3. Following infection, pigs developed high levels of immunity. The intensity of leptospirosis was greatest for the first three to four weeks of its detection, thereafter it lessened and sometimes became only intermittently detectable. The duration of leptospirosis was at least six weeks for the majority of infected pigs. Leptospirosis was detected for three months in one gilt. The use of an oral antibiotic [oxytetracycline] only temporarily suppressed leptospirosis in an infected pig.
4. Direct transmission of infection was facilitated by the mixing of grower pigs from different pens within the grower house and by the design of the piggery, especially where direct contact between pigs in different pens was possible.
5. Indirect transmission of infection appeared to occur by transfer of infective effluent from one pen to another either by communal effluent drainage systems, via fomites or by droplet formation.

6. Once a single pig within a pen became infected, a "pen epidemic" occurred with the majority of other pigs within the pen becoming infected with an incidence of infection usually between 10 and 20% per week.
7. Pigs of less than ten weeks of age were not found to be leptospiruric. The prevalence of infection increased to a maximum of between 80% and 100% for bacon weight pigs. The prevalence of leptospirosis in older pigs was less, with around 30% of gilts and 2% of sows being leptospiruric.
8. The proposed cycle of maintenance of endemic *pomona* infection in pig herds can be summarised as follows:
  - i Piglets acquire passive immunity from their dams, and very rarely become infected from their dams.
  - ii They are protected from infection until at least ten weeks of age due to their passive immunity and physical isolation from infected pigs.
  - iii When pigs are moved to infected grower houses they become exposed to infection.
  - iv The majority of pigs do not become infected until they are fourteen weeks of age or older, at which time their level of protection to infection is undetectable by the MAT and their risk of infection increases as pigs within their own pens become infected.
  - v Around 80% of bacon weight pigs from pig herds with endemic *pomona* infection are leptospiruric when sent for slaughter, and exposure to these pigs imposes a high occupational risk to pig farm workers and abattoir workers.

- vi Pigs retained as self replacement gilts can be a continual source of infection for both grower pigs and pigs within the breeding herd.
- vii The mixing of grower pigs, the standards of hygiene, the methods of cleaning pig pens and the design of piggeries are factors which are believed to influence the pattern of disease within a pig herd.



## Chapter Five

**EXPERIMENTAL STUDIES**

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- Serological results of pigs exposed to *pomona*
- Serological results of pigs exposed to *bratislava*
- Titres of heat treated sera
- Cultural results
- Examination of pigs at postmortem
- Histological examination of tissues

*Discussion*

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- Determining the size of droplets in which leptospires survive
  - Creating leptospiral aerosols
  - Measuring droplet size
- Experimental procedure for determining the viability of leptospires in droplets
- Determining the size of droplets created by spray hosing
- Exposure of pigs to *pomona* via the intranasal route
- Exposure of hamsters to *pomona* via the intranasal route
- Exposure of hamsters to a leptospiral aerosol

*Results*

- The viability of leptospires in droplets
- The size of droplets created by spray hosing
- Exposure of pigs to *pomona* via the intranasal route
- Exposure of hamsters to *pomona* via the intranasal route
- Exposure of hamsters to a leptospiral aerosol

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## INTRODUCTION

From serological studies of leptospiral titres in slaughtered pigs [Study 2, Chapter Three]. It was determined that a large proportion of grower pigs had titres to serovar *bratislava*, but in spite of the presence of these titres, *bratislava* had not been isolated from any pigs throughout the entire study. These results led to the hypothesis that these *bratislava* titres were due to serological cross reactions. An experimental investigation of this hypothesis is described in Study 1 of this chapter.

A second hypothesis area requiring experimental investigation was the prospect that infection occurs via either the intranasal or respiratory route, and this would mean that transmission was by means of infective droplets. Results from observational studies in Chapter Four indicated that the transmission of leptospiral infection between pigs usually occurred as a result of direct contact between susceptible pigs and infected pigs or their effluent. However, it was demonstrated in Study 2 of Chapter Four that infection was also spread between pens of pigs in a grower house where pigs were not mixed together and where the pens had solid concrete walls and floors and separate drainage for their effluent. Generally these design features were believed to reduce the chances of direct spread of infection via effluent or by contact. The experimental study undertaken to investigate the possible transmission of leptospires in droplets and infection via the respiratory route are described in Study 2 of this chapter.

## STUDY 1 - ARTIFICIAL INFECTION OF GROWER PIGS WITH SEROVAR *BRATISLAVA* AND *POMONA*

### *Introduction*

In Europe the presence of pigs infected with serovar *bratislava* has emerged as a problem of potential economic significance (Ellis *et al*, 1985). *Bratislava* has been isolated from the kidneys and reproductive tracts of both sows and boars in Ireland and the U.S.A. (Ellis *et al*, 1985; Ellis and Thiermann, 1986; Ellis *et al*, 1986c) where it is believed to be a cause of abortion in sows. In Europe there has also been widespread serological evidence of *bratislava* in pigs. In Sweden 18% of sera from pigs had agglutinins to *bratislava* (Sandstedt and Engvall, 1985) titres have also been recorded in pigs in The Netherlands (Hartmann *et al*, 1975), in England 12% of pigs in a serological survey had titres to *bratislava* (Hathaway *et al*, 1981), and in Australia pigs have also been reported to have titres to *bratislava* (Chung, 1968). As described in Chapter Three, serum samples obtained from New Zealand pigs at the time of slaughter were tested for agglutinins to serovar *bratislava*. Fifty three percent of sera tested had titres to *bratislava*, however the majority of titres were less than 1/160 and there was no cultural evidence that these were due to infection with *bratislava*. It could however be argued that the high prevalence of *pomona* infection in the pigs was masking a concurrent *bratislava* infection. It was therefore decided to determine if these *bratislava* titres were possibly the result of a serological cross reaction associated with pigs infected with *pomona*. This hypothesis was investigated by serological and cultural examination of two groups of pigs which were artificially exposed to either serovar *pomona* or *bratislava*.

### *Materials and Method*

Ten, ten week old Large White pigs from the same litter, and from a farm free of leptospiral infection [Farm A] were selected for the experiment. In order to confirm that the pigs were indeed free of infection, serum from all were tested for titres to *pomona*, *hardjo*, *tarassovi*, *bratislava*, *ballum*, *balcanica*, *copenhageni* and *australis* at a minimum dilution of 1/4, in doubling dilutions to a titre of 1/254. All pigs were found to be free of detectable titres. The pigs were divided into two groups of five pigs, the selection of pigs into either

group was without any specific form. The two groups were housed in pens on opposite sides of the same building in such a way as to remove any opportunity for direct contact between the two groups. Additionally there was no contact between the two groups via their effluent, as the effluent from each pen drained into an isolated central pit. Straw bedding was provided and the pens were cleaned daily by removing the soiled straw, however pens were not washed or hosed out for the duration of the experiment. Gumboots and equipment used during the experiment were disinfected when moving between pens. The pigs were isolated from contact with other animals including birds and rodents.

Because *bratislava* had not been isolated from any species of animal or humans in New Zealand it was necessary to obtain permission from the Ministry of Agriculture and Fisheries [MAF] to perform the experiment. The premises were placed under quarantine by the MAF for the duration of the investigation. All solid effluent was incinerated and liquid effluent was treated with sodium hypochlorite for at least twenty four hours before being discarded. At the conclusion of the experiment the premises were sealed and fumigated with formalin for twenty four hours.

One group [Group 1], contained five female pigs and this group was artificially exposed to serovar *pomona* [strain UBH] which was isolated from the urine of a grower pig at slaughter. Four of five pigs [Pigs 3, 4, 5 and 7] were artificially exposed by the administration of two ml of  $1$  to  $2 \times 10^8$  leptospires/ ml via the intramuscular route. The fifth pig [Pig 2] was exposed to one ml of  $1$  to  $2 \times 10^8$  leptospires/ ml via the intranasal route. This was done by placing a syringe at the edge of each nostril and dispensing 0.5 ml of the liquid into the nasal cavity while holding the pig's head in a horizontal position.

The other group [Group 2], contained four female pigs and one male pig, which were artificially exposed to *bratislava* [strain Jez-bratislava]. Four of five pigs [Pigs 1, 6, 8 and 10] were artificially exposed to two ml of  $1$  to  $2 \times 10^8$  leptospires/ ml via the intramuscular route, and the fifth pig [Pig 9] was artificially exposed via the intranasal route as described in the previous paragraph.

Serovar *bratislava* had been maintained by passage in liquid and semisolid medium at Massey University's Leptospirosis Laboratory since its arrival in 1982 from Brisbane, Australia. The cultures used to artificially inoculate the pigs had been cultured in liquid EMJH media to a concentration of  $1 - 2 \times 10^8$  leptospire/ml.

The day of artificial exposure to infection was regarded as day 0 of the experiment. The experimental exposure of pigs to *pomona* was commenced twenty two days before pigs in Group 2 were exposed to *bratislava*. Therefore at the conclusion of the experiment, pigs in Group 1 had been exposed to *pomona* for sixty four days, and pigs in Group 2 had been exposed to *bratislava* for forty two days.

Samples of blood and urine were collected from all of the experimental pigs every three to four days for the duration of the investigation. Serological examination of the sera was conducted for dilutions of 1/10 to 1/163,840 using *pomona* and *bratislava* antigens, as described in Chapter Two. Blood was cultured from pigs in Group 1, thirty one days after exposure to *pomona*, and from pigs in Group 2, six days after exposure to *bratislava*. Urine was cultured as described in Chapter Two, except that the culture medium used throughout this experiment was semi-solid T80/40 media containing  $300 \mu\text{g}$  5FU/ml. All cultures were examined for the presence of leptospire for a period of twenty seven weeks. Cultures were examined at two weekly intervals for the first six weeks and thereafter they were examined at three weekly intervals until discarded.

Four sera from a pig exposed to *pomona* [Fig 5 of Group 1] and four sera from a pig exposed to *bratislava* [Fig 8 of Group 2] were heated to  $65^\circ\text{C}$  for twenty minutes in order to selectively inactivate the IgM antibody in whole serum (Hellstrom, 1978). These sera were then subjected to the MAT test as previously described.

At the end of the experimental period, all pigs were euthanased by the intravenous administration of pentobarbitone following intramuscular tranquillisation with acepromizine. After death a postmortem examination of

each pig was conducted and the general health and gross pathological signs of each pig were recorded. Samples of the mandibular, renal and lumbar aortic lymph nodes, spleen, kidney, brain, oviduct, uterus, vagina from females, and the seminal vesicles, prostate gland, bulbourethral gland and testes from the male pig were collected for cultural and histopathological examination. A rib and an eye were also collected for cultural examination. The red bone marrow of each rib was cultured by flaming the end of the rib, cutting off its end, and removing some of the red marrow with a sterile scalpel. The marrow was placed in 9 ml of SBM and separately, one drop, and five drops of the suspension were inoculated into culture medium. The vitreous humor from each eye was aseptically removed by rinsing the eye in 95% alcohol and flaming the surface, an 18 gauge needle with an attached syringe was then used to extract the viscous liquid. The vitreous humor was then added to 9 ml of SBM and separately, one drop, and five drops were inoculated into the culture medium. The other tissue samples were processed as described in Chapter Two. Blood was collected for serological examination and urine was obtained from three pigs after death by aspirating urine from each of their bladders. The carcasses of the pigs were incinerated.

## **Results**

### **- Serological results of pigs exposed to *pomona***

Titres to both *pomona* and *bratislava* were detected in all five pigs following artificial exposure to *pomona*. The serological results of pigs exposed to serovar *pomona* are shown in Table 5.1 and 5.2. The GMT of titres to *pomona* and *bratislava* in this group of pigs are shown in Figure 5.1 and Table 5.3. As expected, a serological response to *pomona* was obtained within two weeks of the pigs being exposed to serovar *pomona*. The first titres to *pomona* occurred seven days after pigs had been exposed via the intramuscular route, and ten days after exposure via the intranasal route. Seven days after exposure the titres of four of the five pigs ranged from 1/80 to 1/640 and had a GMT of 1/190. After fourteen days, the titres of all five pigs were 1/2,560 or greater. In general, the homologous titres to *pomona* tended to continue to increase until the experiment was concluded. The homologous titres were of similar magnitude for the duration of the investigation, irrespective of their route of exposure to *pomona*.

Ten days after artificial exposure to *pomona*, all five pigs had detectable titres to serovar *bratislava* with a GMT of 1/30. The GMT's to *bratislava* initially increased to 1/105 fourteen days after exposure, thereafter the *bratislava* GMT's ranged between 1/20 and 1/80, and by the end of the experimental period the GMT was 1/20. The magnitude of the *bratislava* titres was consistently lower than those of the *pomona* titres.

- *Serological results of pigs exposed to bratislava*

The serological results for the pigs exposed to *bratislava* using antigens *bratislava* and *pomona* are shown in Table 5.4 and 5.5. The four pigs exposed by intramuscular inoculation to serovar *bratislava* had titres to *bratislava* antigen three days after exposure and their titres ranged from 1/40 to 1/640 with a GMT of 1/113. Seven days after exposure to *bratislava* via the intranasal route, Pig 9 had a titre to *bratislava* of 1/40. The homologous *bratislava* titres of Pig 9 were consistently lower than those of pigs exposed via the intramuscular route. Fourteen days after exposure, the GMT of *bratislava* titres increased to a high of 1/23,525, thereafter the GMT's generally declined, reaching 1/2,229 at the conclusion of the experiment, forty two days after exposure, as shown in Figure 5.2 and Table 5.6.

Seven days after exposure, all five pigs had heterologous titres to *pomona*, with a GMT of 1/278, three days later only two of the five pigs still had titres to *pomona* which were 1/10 and 1/20. Thereafter, titres to *pomona* were no longer detected. In contrast to the homologous *bratislava* titres, the heterologous *pomona* titres were low and transient [see Figure 5.2].

- *Titres of heat treated sera*

The titres of heat treated sera of pigs #5 and #8 are shown in Table 5.7a and 5.7b. The homologous titre of both pigs were reduced by the greatest amount in the second week after artificial exposure to either of the serovars *pomona* or *bratislava*.

**Pig #5 exposed to *pomona*:** The heterologous *bratislava* titres, of Pig #5 infected with *pomona*, were reduced by two doubling dilutions for the duration of their presence. The heat sensitive portion of the

homologous *pomona* titres and the heat resistant portion of the heterologous *bratislava* titres both decreased with time, as shown in Figure 5.3 and Table 5.7a.

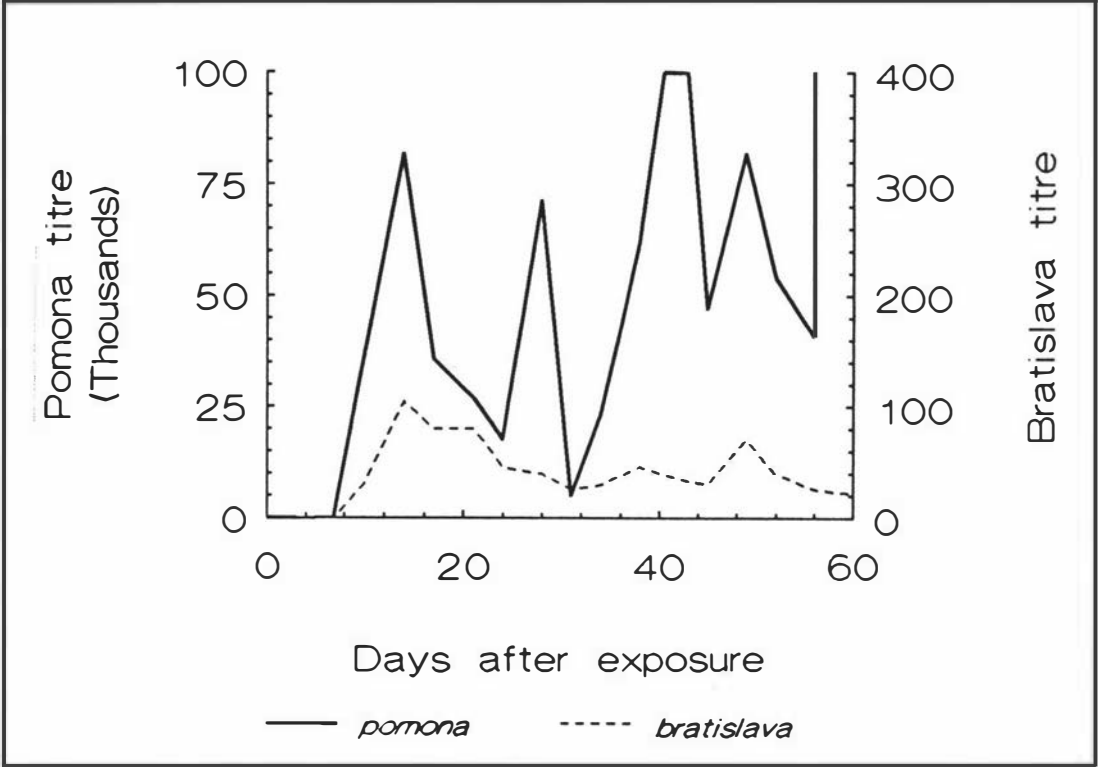
**Pig # 8 exposed to *bratislava*:** The homologous *bratislava* titres were lowered following heat treatment, as was the single heterologous *pomona* titre [see Table 5.7b]. A comparison between the heat sensitive IgM and heat resistant portions of the homologous and heterologous titres was not possible due to the presence of only one detectable *pomona* titre prior to heat treatment, and none following heat treatment.

**Table 5.1** Homologous *pomona* titres of pigs exposed to serovar *pomona* by intramuscular inoculation or via the intranasal route.

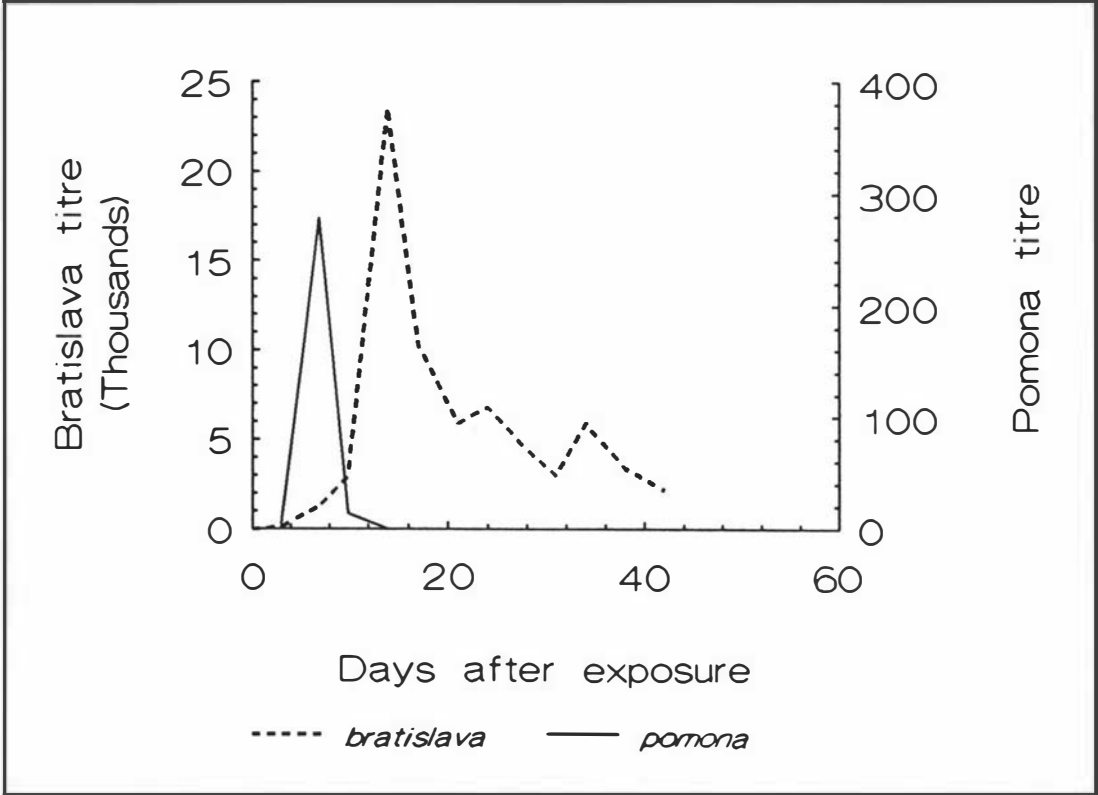
Days post infection	Pig number				
	2	3	4	5	7
0	0	0	0	0	0
3	0	0	0	0	0
7	0	160	80	160	640
10	2,560	2,560	163,840	163,840	327,680
14	20,480	163,840	40,960	81,920	327,680
17	2,560	40,960	81,920	81,920	40,960
21	2,560	81,920	20,480	81,920	40,960
24	1,280	40,960	10,240	81,920	40,960
28	81,920	163,840	40,960	81,920	40,960
31	640	20,480	2,560	2,560	40,960
34	640	163,840	10,240	40,960	163,840
38	1,240	327,680	40,960	163,840	327,680
42	10,240	327,680	81,920	327,680	327,680
45	5,120	327,680	10,240	40,960	327,680
49	81,920	327,680	10,240	40,960	327,680
52	81,920	327,680	5,120	10,240	327,680
56	81,920	327,680	1,280	10,240	327,680
59	327,680	327,680	327,680	327,680	327,680
64	327,680	327,680	327,680	327,680	327,689



**Figure 5.1** *Pomona* and *bratislava* titres of pigs artificially exposed to serovar *pomona*.



**Figure 5.2** *Bratislava* and *pomona* titres of pigs artificially exposed to *bratislava*.



**Table 5.2** Heterologous *bratislava* titres of pigs exposed to serovar *pomona* by intramuscular inoculation or via the intranasal route.

Days post infection	Pig number				
	1	6	8	9	10
0	0	0	0	0	0
3	0	0	0	0	0
7	0	0	0	0	0
10	20	20	80	160	640
14	160	20	40	320	320
17	160	20	20	80	640
21	640	0	0	320	640
24	40	0	20	160	320
28	160	0	0	160	160
31	80	0	0	80	80
34	20	10	10	80	160
38	40	20	20	160	80
42	20	20	20	40	80
45	40	20	20	40	40
49	160	40	20	80	160
52	80	20	20	40	80
56	80	10	10	20	80
59	40	10	10	40	40
64	20	0	10	40	80

**Table 5.3** The geometric mean titres [GMT] of pigs artificially exposed to serovar *pomona*.

Days post infection	GMT of <i>pomona</i> homologous titre	GMT of <i>bratislava</i> heterologous titre
0	0	0
3	0	0
7	190	0
10	35,658	30
14	81,920	105
17	35,658	80
21	27,023	80
24	17,828	46
28	71,315	40
31	5,120	26
34	23,525	30
38	62,084	46
42	124,168	35
45	47,050	30
49	81,920	70
52	54,047	40
56	40,960	26
59	2,292,096	23
64	2,282,096	20

Table 5.4

Homologous *bratislava* titres of pigs exposed to serovar *bratislava* by intramuscular inoculation or via the intranasal route.

Days post infection	Pig number				
	1	6	8	9	10
0	0	0	0	0	0
3	80	80	640	0	40
7	163,840	81,920	327,680	40	1,280
10	640	5,120	81,920	320	2,560
14	81,920	327,680	40,960	320	20,480
17	5,120	81,920	20,480	320	40,960
21	5,120	20,480	10,240	320	20,480
24	2,560	20,480	5,120	640	81,920
28	5,120	10,240	2,560	160	81,920
31	2,560	10,240	1,280	80	81,920
34	20,480	20,480	2,560	80	81,920
38	5,120	10,240	2,560	80	81,920
42	5,120	10,240	2,560	40	10,240

Table 5.5

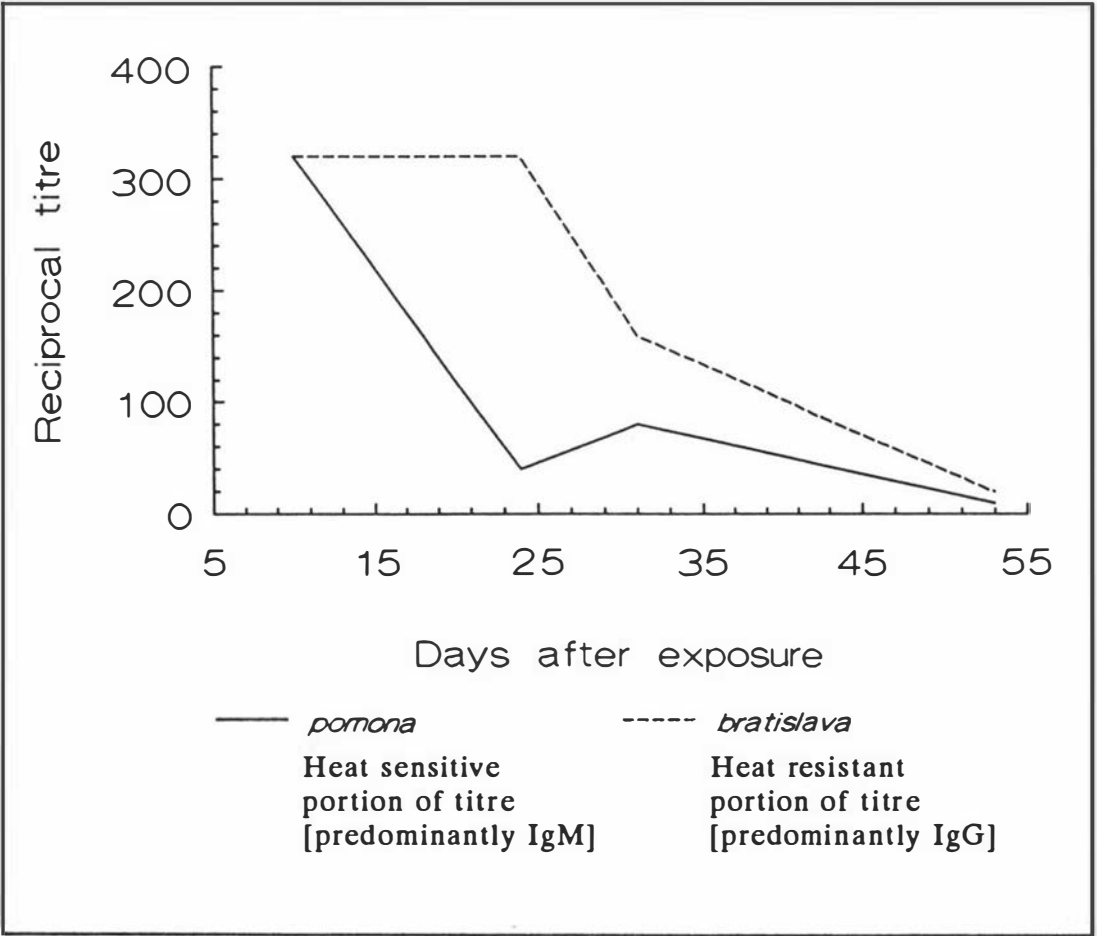
The heterologous *pomona* titres of pigs exposed to serovar *bratislava* by intramuscular inoculation or via the intranasal route.

Days post infection	Pig number				
	1	6	8	9	10
0	0	0	0	0	0
3	0	0	0	0	0
10	640	1,280	40	40	1,280
14	10	0	0	0	20
17	0	0	0	0	0
21	0	0	0	0	0
24	0	0	0	0	0
28	0	0	0	0	0
31	0	0	0	0	0
34	0	0	0	0	0
38	0	0	0	0	0
42	0	0	0	0	0

**Table 5.6**                      **The geometrical mean titres [GMT] of pigs artificially exposed to serovar *bratislava*.**

Days post infection	GMT of <i>bratislava</i> homologous titre	GMT of <i>pomona</i> heterologous titre
0	0	0
3	113	0
7	1,280	278
10	2,941	14
14	23,525	0
17	10,240	0
21	5,881	0
24	6,756	0
28	4,457	0
31	2,941	0
34	5,881	0
38	3,378	0
42	2,229	0

**Figure 5.3**      **Homogenous and heterologous *pomona* and *bratislava* titres of heat treated and untreated sera.**



**Table 5.7** Homogenous and heterologous heat treated and untreated titres of pigs exposed to *pomona* and *bratislava*.

**Table 5.7a**

PIG SERA #5 Days post exposure	Untreated		Heat treated	
	<i>pomona</i>	<i>bratislava</i>	<i>pomona</i>	<i>bratislava</i>
10	1/20480	1/320	1/320	1/80
24	1/5120	1/320	1/1280	1/80
31	> 1/163840	1/160	1/20480	1/40
53	> 1/163840	1/20	1/81920	0/0

**Table 5.7b**

PIG SERA #8 Days post exposure	Untreated		Heat treated	
	<i>bratislava</i>	<i>pomona</i>	<i>bratislava</i>	<i>pomona</i>
10	1/20480	1/40	1/2560	0
24	1/10240	0/0	1/640	0
31	1/1280	0/0	1/320	0
53	1/1280	0/0	1/640	0

*- Culture results*

All pigs artificially exposed to serovar *pomona* became leptospiruric during the investigation. In contrast, leptospires were not isolated from any of the pigs artificially exposed to serovar *bratislava*.

Leptospiuria commenced from the seventh day after exposure to *pomona* and continued until the forty fifth day after artificial exposure, as shown in Table 5.8. The proportion of pigs shedding and the intensity of shedding varied throughout this period. The largest number of pigs which were simultaneously leptospiruric occurred between the twenty first and forty third day after artificial exposure to *pomona*. The highest intensity of leptospiuria, as assessed by the quantitative culture method described in Chapter Two, occurred between the twenty eighth and thirty eighth day after exposure to artificial infection, as shown in Table 5.8. Leptospiuria decreased to undetectable levels within seven days of the highest recorded intensity.

Of those samples from which leptospires were cultured, 88% were detected at

the first examination (2 weeks), 9% at the second examination (4 weeks) and a further 3% at the third examination (6 weeks). All cultures containing leptospires were detected by DFM within one of the first three examinations in the second, fourth or sixth week.

In addition, seventeen days after exposure to artificial infection, leptospires were first seen by direct DFM examination of urine, thus coinciding with increasing intensities of leptospiuria. Leptospires continued to be detectable by direct DFM until forty two days after artificial exposure to *pomona*.

**Table 5.8      The onset, duration and intensity of leptospiuria determine by cultural examination.**

Days post infection	Intensity of leptospiuria				
	2	3	4	5	7
0	o	o	o	o	o
3	o	o	o	o	o
7	o	o	++	o	o
10	o	o	++	o	++
14	p	o	++	o	+++
17	++	o	+++	o	o
21	+++	o	++	o	+++
24	o	+++	+++	+++	+++
28	+++	+++	+++	o	+++
31	+++	o	+++	++	o
34	+++	+++	+++	+++	+++
38	+++	+++	+++	+++	o
42	++	++	+++	o	o
45	++	+	++	o	o
49	o	o	o	o	o
52	o	o	o	o	o
56	o	o	o	o	o
59	o	o	o	o	o
64	o	o	o	o	o

Quantification scale      :

o	=	Leptospires not isolated
+	=	Less than 1000 leptospires/ml
++	=	Between 10 <sup>3</sup> and 10 <sup>6</sup> leptospires/ml
+++	=	At least 10 <sup>6</sup> leptospires/ml

Leptospires were neither isolated from any samples of blood collected in the course of this investigation, nor were they isolated from any of the tissues collected at necropsy.

- *Examination of the pigs at post mortem*

All of the pigs infected with *pomona* had small white spots of approximately one mm in diameter on their kidneys. In contrast, the pigs exposed to *bratislava* had kidneys of normal appearance. No other gross abnormalities or pathological signs were noted in any of the ten experimental pigs at the time of the necropsy.

- *Histological examination of tissues*

Leptospires were visible within renal tubules in the Warthin-Starry silver stained histological sections of pigs exposed to *pomona*, but were not visible in those of pigs exposed to *bratislava*. Leptospires were not detected, nor were any histopathological lesions obvious on examination of any of the other tissue sections.

**Discussion**

The results obtained in this study demonstrated that pigs exposed to serovar *pomona*, irrespective of the route, became infected. This is in contrast to the apparent absence of kidney colonisation by *bratislava*. There was no cultural evidence throughout this study to suggest that pigs exposed to serovar *bratislava*, irrespective of the route, became infected in spite of the high antibody response to this organism.

The strain of *pomona* used to artificially infect pigs in this trial was one that had recently been isolated from the urine of a grower pig slaughtered at a local abattoir. However the *bratislava* isolate used had been passaged through liquid and semisolid medium for a period of five years at Massey University's Leptospirosis Laboratory and repeated passage may have reduced its virulence (Kida, 1969). Due to the restrictions in the use of such a culture in New Zealand, it was not possible to passage the culture through laboratory animals to test or increase its virulence prior to its use in pigs. Leptospires were not isolated from the blood or urine of pigs exposed to *bratislava*. This suggests

that pigs did not become infected with *bratislava* following exposure via the intramuscular or intranasal route. Johnson and Harris (1967a) investigated the response of a host to inoculation with avirulent leptospires. They found that avirulent leptospires could not be isolated from the host 24 to 72 hours after their inoculation by intravenous injection, however antibody-complement system appeared 24 to 72 hours after inoculation. Johnson and Harris (1967) concluded that avirulent leptospires were unable to survive and multiply in the presence of the immune serum and were eliminated from the host, thereby preventing the establishment of infection. It is possible that a similar sequel of events occurred in the pigs exposed to *bratislava*. Leptospirae maintained in the laboratory by passage for long periods, as was the case with the *bratislava* culture used in this study, have been shown to have unaltered antigenicity (Kida, 1969; Ellinghausen, 1973) and thus still induce a strong serological response. In this study, a serological response to *bratislava* was initiated three days after exposure, and maintained in all of the pigs initially exposed to *bratislava*. In the absence of cultural isolation of *bratislava*, and by analogy from the results of the other investigations described previously, the results of this study indicate that pigs had been inoculated with an avirulent leptospiral culture of serovar *bratislava*.

Hathaway *et al* (1981) conducted a serological and bacteriological survey of leptospiral infection in pigs in England and their results showed a seroprevalence to serovar *bratislava* of 5.3% in porker pigs and 18.5% in bacon pigs, however they also failed to isolate *bratislava* from any of these pigs. They considered that it was unlikely that the *bratislava* titres were paradoxical reactions to agglutinins stimulated by infection with other serogroups for several reasons. These were that (1) the *bratislava* titres were frequent in populations infected with different serogroups, (2) that *bratislava* titres were absent or infrequent in some surveys, (3) that the prevalence of *bratislava* titres differed markedly between herds, and (4) that in both field and experimental studies, very high titres occurred in individual animals. It was postulated by these authors that infection with *bratislava* in pigs may be transient, and therefore difficult to isolate. Other workers, reported by Hathaway *et al* (1981) have also had difficulty in isolating *bratislava* from pigs either due to the apparent difficulty of obtaining and maintaining *bratislava* isolates, or the



possibility of dual infection with other serovars which would mask the isolation of *bratislava*. This last possibility was not discounted as one of the reasons for failing to isolate *bratislava* from grower pigs in this study. If this does indeed occur, then one cannot discount the possibility that the *pomona* isolate used in this experiment contained a low percentage of *bratislava* organisms. The UBH culture was tested, to the best of the authors ability, using the simple agglutination test described in Chapter Two and the BRENDA technique, and was on each occasion identified as a pure *pomona* isolate. The following aspects of this discussion assume that it was indeed a pure culture of *pomona* and did not contain contamination with serovar *bratislava*.

It is clear from these results that pigs infected with *pomona* developed detectable heterologous titres to serovar *bratislava*. This finding explains the appearance of *bratislava* titres of less than 1/5,120 in the absence of cultural evidence of infection with *bratislava* in grower pigs at slaughter in New Zealand.

Pigs infected with *pomona* had two distinct phases in which their titres increased, as shown in Figure 5.1. The first increment occurred between the seventh and tenth day, during which the GMT rose from 1/190 to 1/35,658, and a second increment occurred between the fifty sixth and fifty ninth day, in which the GMT rose from 1/40,960 to 1/2,282,096. The initial rise in titre was likely to be due to IgM (Hellstrom, 1978). Hellstrom (1978) determined that the MAT response of cattle naturally infected with *hardjo* was initially due to IgM antibodies and that IgG became predominant within forty two days of infection. The MAT measures both IgG and IgM against leptospira (Hellstrom, 1978; Morris and Hussenini, 1974). Hellstrom (1978) suggested that heat treatment of sera would inactivate IgM and allow the ratio of IgG to IgM of a serum sample to be determined. Therefore the high ratios of IgM:IgG would indicate recent infection while low ratios would indicate longer standing infection. This suggests that the IgM [heat sensitive] portion of the homologous *pomona* titre cross reacts with *bratislava* antigen to form the heterologous (predominantly IgG) *bratislava* titre (see Figure 5.3). These results therefore suggest that heterologous *bratislava* titres are associated with recent infection of pigs with serovar *pomona*. This may also explain the poor

linear association between the magnitude of titres to *pomona* and *bratislava* in slaughtered pigs.

The heterologous *pomona* titres in pigs exposed to *bratislava* were of shorter duration and lower in magnitude than the heterologous titres of pigs infected with *pomona*. However, these titres also occurred soon after exposure to *bratislava*, when the highest IgM levels to *bratislava* were present. The *pomona* titres in pigs exposed to *bratislava* therefore appeared to occur under the same circumstances as the *bratislava* titres in pigs exposed to *pomona*, as previously discussed.

The second rise in *pomona* titres for pigs infected with *pomona* may have been associated with repeated exposure to their own infective urine. In contrast, the *bratislava* titres of pigs exposed to *bratislava* rose to peak levels two to three weeks following exposure, after which they declined. The absence of continued exposure of these pigs to leptospires may explain why their titres declined after the initial rise. Repeated exposure of pigs to virulent leptospires, for example serovar *pomona*, may be important in stimulating the high titres, which have also been found in naturally infected pigs [see Chapters Three and Four].

The onset of a detectable serological response in pigs exposed via the intranasal route was slower than in pigs exposed via the intramuscular route. It is possible that the increased period of time required for leptospires to penetrate the host's first defence system and induce an immune response, and thus the route by which an animal becomes infected, may influence the course of the disease.

The commencement, duration and intensity of detectable leptospiuria in the artificially exposed pigs in this study was similar to findings of other studies in which pigs were experimentally infected with *pomona*. For example, Fennestad and Borg-Petersen (1966) artificially infected two sows with *pomona* and found they were leptospiruric from the twelfth to thirty fourth day, and eleventh to the thirtieth day. Michna and Campbell (1969) reported that leptospiuria was usually evident two to three weeks after exposure and lasted from a few weeks to over two years. Lococo *et al* (1958) observed that artificially infected pigs

shed leptospire consistently for about fifty days. From the results of the above experimental studies and from the prospective cohort studies described in Chapter Four, it can be seen that there is a wide variation in the duration of leptospiruria in pigs infected with *pomona*. The intensity of leptospiruria occurring in the pigs artificially exposed to *pomona* was similar to that seen in pigs of the prospective cohort study described in Chapter Four. Morse *et al* (1958) similarly reported that pigs artificially infected with *pomona* shed their maximum number of leptospire in urine between twenty and thirty days after infection and leptospiruria persisted for up to 122 days in one of their experimentally infected animals. The decline in the intensity of leptospiruria of infected pigs forty five days after exposure to *pomona* suggests that the period of leptospiruria in these pigs was limited to an average duration of twenty nine days and which ranged between nineteen and forty four days. In the observational studies described in Chapter Four, pigs generally remained leptospiruric until they were slaughtered, and many were documented as being leptospiruric for a period exceeding twenty nine days.

The apparent cessation of leptospiruria in the experimentally infected pigs may have resulted from the leakage of leptospiral antibody into renal tubules following the damage caused by the leptospire. Langham *et al* (1958) examined the kidneys of pigs artificially infected with *pomona* and observed that there was wide spread renal damage, with proximal and distal convoluted tubules showing various degrees of degeneration and necrosis. Morse *et al* (1958) measured urinary antibody titres and found that these titres increased to 1/1000 three to four months after infection. They concluded that the presence of urinary antibody was not the result of leakage, but a product of reticuloendothelial cells in the kidney. The basis for this conclusion was that renal damage was found to be greatest two to four weeks after infection and peak urinary titres did not occur until three to four months after infection. Ryan (1978) also measured urinary antibody and found it to be a useful indicator of renal infection in grower pigs. Stalheim (1974) examined the urine of pigs infected with *pomona*, and showed that urine contained large numbers of leptospire and a whitish, flocculent material. It was concluded that the urinary floccule was not a leptospiral product, but was the result of products of the repair process. Therefore it is possible that the intensity of

leptospiuria decreased to undetectable levels as a consequence of renal damage which allowed circulating antibody to enter the renal tubules and result in the agglutination and lysis of leptospires in the renal tubules.

Leptospires were not isolated from the kidneys of any of the pigs in this study. However, the kidneys of the pigs infected with *pomona* had gross pathological signs of leptospiral infection and histopathological examination of the kidneys revealed the presence of leptospires in the renal tubules of infected pigs. It is uncertain why leptospires were not isolated from these kidneys, but it may be related to the use of acepromizine and pentobarbitone when euthanasing these pigs. Either of these drugs may have reduced the viability of leptospires. Leptospires have been isolated from kidneys after the cessation of detectable leptospiuria by Fennestad and Borg-Petersen (1966) and from pigs used in the studies described in Chapter Four.

The repeated use of furosemide diuretic at three and four day intervals may have influenced the detection of leptospiuria. The use of furosemide was reported by MacKintosh (1981) and Nervig and Garrett (1979) to aid in the isolation of leptospires from urine by decreasing contamination rates and altering the pH of urine. However, MacKintosh (1981) also found that administration of furosemide to dogs repeatedly within a 24 hour period reduced the isolation rate of leptospires from the urine samples. Nervig and Garrett (1979) found that the use of furosemide brought physiological changes to the composition of the urine samples. In all cases, furosemide caused the urine samples to be more dilute, lowering the specific gravity and osmolarity, and the pH tended towards neutral from its normal value (Nervig and Garrett, 1979). Furosemide acts on the proximal convoluted tubules of the kidney by actively inhibiting the re-absorption of chlorine ions from the tubular fluid and thus results in increased urinary excretion of water, sodium, potassium and chloride (Booth and McDonald, 1982). This action may have flushed leptospires from the renal tubules increasing the isolation rates in the early stages of leptospiuria but decreasing the isolation rates after repeated use of long duration.

Unfortunately information about the nature of *bratislava* infection in grower pigs could not be determined from this experiment. It did however allow for the presence of *bratislava* titres in grower pigs to be interpreted as serological cross reactions associated with recent infection of pigs with serovar *pomona*. As a result it could be concluded that New Zealand pigs appear to be free of infection with serovar *bratislava*.

## STUDY 2 - THE VIABILITY OF LEPTOSPIRES IN DROPLETS AND TRANSMISSION OF INFECTION VIA THE RESPIRATORY ROUTE

### *Introduction*

The transmission of leptospiral infection via the respiratory route has been reviewed in Chapter One. Infection by this route is recognised as a natural mode for the transmission of infection, however few investigators have addressed the question of the size of a leptospiral aerosol droplet which can be potentially infective to a susceptible host, and whether or not the minimum infective dose via the respiratory route can be delivered by an aerosol. As described in Chapter Four, grower pigs within a piggery became infected without any obvious direct contact between them and infected pigs or their effluent. This led to the hypothesis that leptospires may have been transmitted by aerosol from infected pigs to susceptible pigs of neighbouring pens, and that these aerosols may have been created by the spray hosing which is used to clean the pens.

In Study 1 of this chapter, and in studies reviewed in Chapter One, it was demonstrated that pigs can become artificially infected by exposure to *pomona* via the intranasal route.

The first aspect to be investigated in this part of the study was the size of droplets in which leptospires could remain viable. The next step was to measure the size of droplets, formed by spray hosing, which would be transmitted to a neighbouring pen. Finally, the minimum infective dose for the infection of pigs and hamsters exposed to *pomona* via the intranasal route was determined, and then hamsters were exposed to a leptospiral aerosol to determine if they would become infected.

Throughout this chapter exposure via the intranasal route refers to exposure of the upper respiratory tract of the nasal mucosa to leptospires, while exposure via the respiratory route refers to exposure of the lower respiratory tract of the bronchi, bronchioles and alveoli to leptospires which can be achieved only by exposure to aerosols of less than 50  $\mu\text{m}$  in diameter.

## **Materials and method**

### **- Determining the droplet sizes in which leptospires survive**

#### **- Creating leptospiral aerosols**

Two devices, the spinning disc and a nebuliser, were used to create aerosol droplets of a reasonably uniform size.

#### **Spinning disc**

A spinning disc<sup>1</sup> used for horticultural spraying was initially used to investigate the survival of leptospires in droplets between 50  $\mu\text{m}$  and 200  $\mu\text{m}$  in diameter, classified as a mist to fine spray (Mathew, 1975). The spinning disc apparatus forms droplets of reasonably constant size by delivering a liquid to the centre of the disc while rotating at high speeds. The liquid is then forced to the periphery of the disc where it leaves the disc from fine fins, as shown in Figure 5.4. The spinning disc was surrounded by a plastic curtain, leaving a 3 cm opening for droplets to escape. The apparatus was placed under ultraviolet light for twenty four hours before use. The speed of the rotating disc could be adjusted to alter the droplet size being formed.

#### **Nebuliser**

A hand held nebuliser<sup>2</sup> was used to form droplets of less than 50  $\mu\text{m}$  diameter, and classified as an aerosol by Mathew (1975). The nebuliser was attached to a stand and disinfected with 5% sodium hypochlorite solution. The aerosol created by the nebuliser was directed into a specially constructed cage covered with plastic, see Figure 5.5.

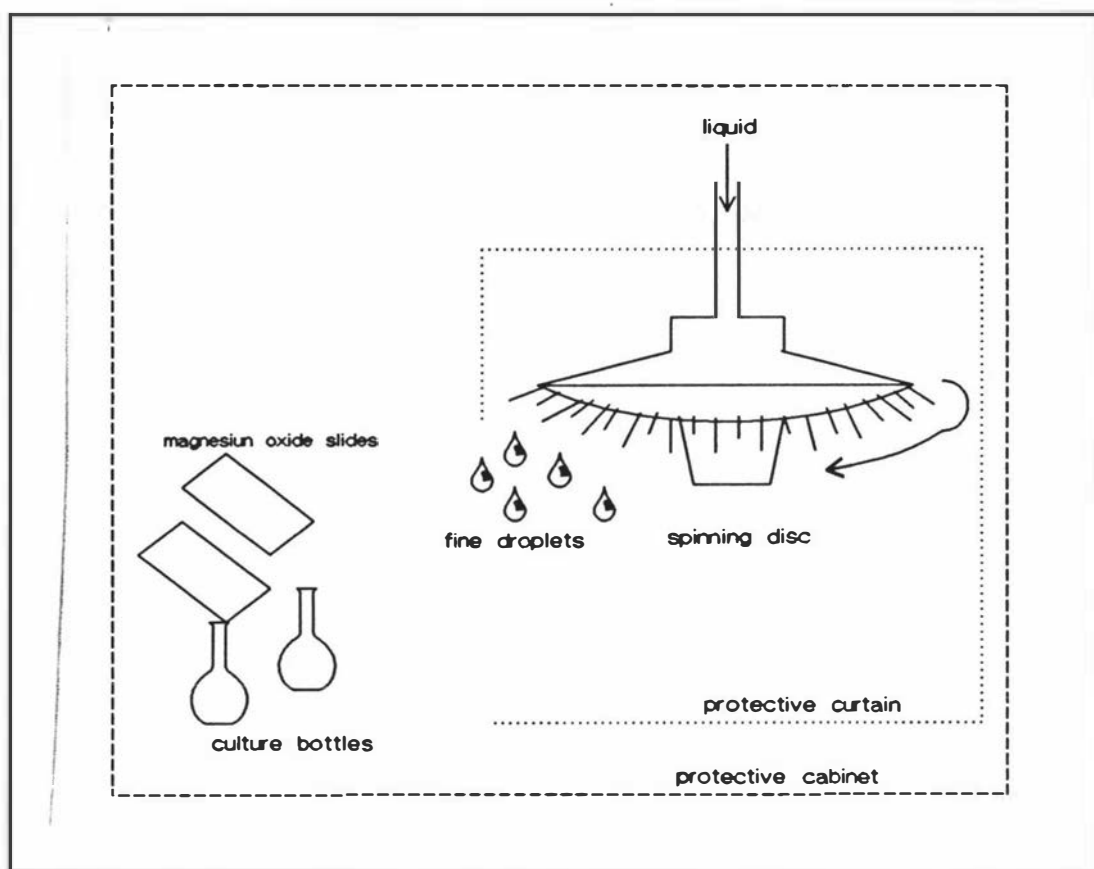
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<sup>1</sup> Spinning Disc, Disc Rotation Micromax 84, Water Aperture Size #4916, 37. Micron Sprayers Ltd, Bromyard, England.

<sup>2</sup> Nebuliser - Hand Held. Aerflo, Cat.No. 1637. Waite and Co. Pty. Ltd. Sydney, Australia.

Figure 5.4

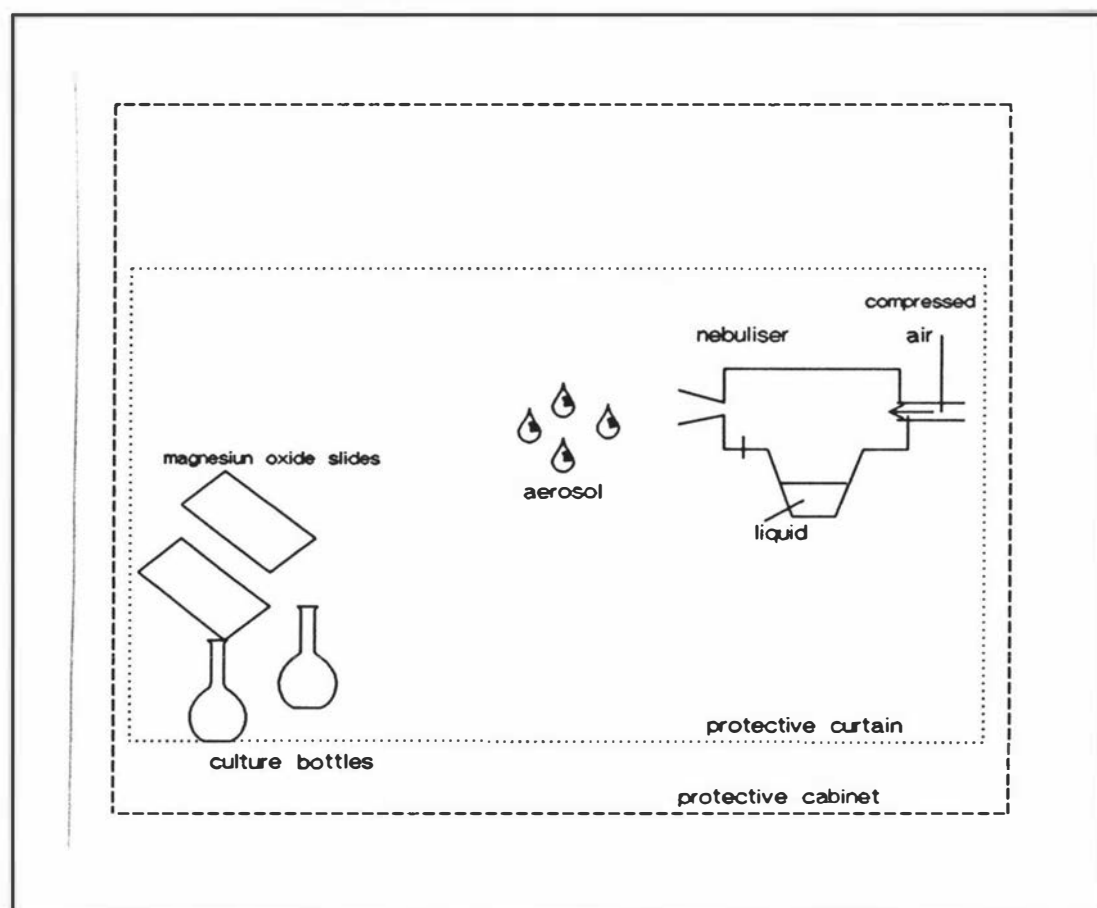
Spinning disc used for the formation of drops greater than  $50\ \mu\text{m}$  in diameter



The cage was exposed to ultraviolet light for twenty four hours before use. Each leptospiral solution was nebulised for 40 seconds using pressurised gas consisting of 5.5% oxygen, 9.75% carbon dioxide and the remainder nitrogen. One ml of leptospiral solution was placed in the nebuliser and after nebulisation the remainder was discarded into disinfectant and replaced with 1 ml of the next leptospiral solution. All droplet formation experiments were conducted within a laminar flow cabinet with a safety glass screen, however the negative pressure was not turned on until after droplet formation had been completed. Protective clothing and breathing apparatus were worn by the author during all of these experiments.



**Figure 5.5** Nebuliser used for the formation of droplets less than 50  $\mu\text{m}$  in diameter.



*- Measuring droplet size*

Droplet size was determined by the "Magnesium Oxide Method" (May, 1950). The method detects and measures the airborne droplets by forming permanent impressions when they strike a layer of magnesium oxide which has been smoked onto a glass slide. The diameter of the impression can be measured microscopically. May (1950) calibrated a wide range of liquids under different impact velocities, and found that the ratio of true drop size to the impression size was constant at 0.86 for drops of any liquid with a diameter of 20  $\mu\text{m}$  or greater. The method is less accurate for drops which are less than 10  $\mu\text{m}$  in diameter due to relative increasing grain size of the magnesium oxide, however droplets down to 5  $\mu\text{m}$  diameter and perhaps less can be detected at sufficiently high impact velocities (May, 1950). May (1950)

recommended the measurement of the diameter of ten droplets from a randomly selected line across each slide, ignoring those droplet impressions which were misshapen, indistinct or had coalesced.

Magnesium oxide slides were prepared by holding a clean glass slide to the smoke of a burning magnesium ribbon. Ten cm of ribbon were burned to smoke each slide.

*- Experimental procedure for determining the viability of leptospires in droplets*

A suspension of serovar *pomona* [strain T A5] was used for all experimental work with droplets. The leptospires were grown in liquid culture medium, which was used as the liquid for the formation of droplets. Three leptospiral suspensions of increasing concentration containing  $6 \times 10^7$ ,  $1.8 \times 10^8$  and  $3.6 \times 10^8$  leptospires per ml of liquid were used in the spinning disc to form droplets between 50  $\mu\text{m}$  and 200  $\mu\text{m}$  in diameter. Two magnesium oxide slides were placed 18 and 30 cm from the edge of the spinning disc, a clean microscope slide was placed 24 cm from the disc, and at 15 cm, 21 cm and 27 cm standard 10 ml culture bottles containing semisolid culture medium were placed with their caps removed, the diameter of the opening of these bottles was 14 mm. After spinning to form a spray, the clean slide was immediately examined for the presence of viable leptospires by DFM. The caps were replaced on the culture bottles and these were incubated at 29°C for eight weeks and examined at two weekly intervals by DFM for the presence of leptospires. The magnesium oxide slides were examined using a light microscope with an eyepiece calibrated to measure 0.1 mm intervals over 1 cm. The diameter of twenty droplets were measured to the nearest 0.05 mm and the diameter adjusted by the microscope correction factor and the droplet diameter calibration factor.

Six leptospiral suspensions of increasing concentration containing  $2 \times 10^7$ ,  $3.3 \times 10^7$ ,  $6.5 \times 10^7$ ,  $1.3 \times 10^8$ ,  $2.6 \times 10^8$  and  $4 \times 10^8$  leptospires per ml of liquid were used in the nebuliser to form droplets of 50  $\mu\text{m}$  or less in diameter. Two

magnesium oxide slides, a clean microscope slide and three culture bottles containing semisolid medium with their caps removed were placed 15 cm from the end of the nebuliser. The clean glass slide, culture bottles and the magnesium oxide slides were processed in the same way as previously described.

*- Determining the size of droplets created by spray hosing*

Two clean concrete pens adjacent to one another were selected for the experiment. A pressure hose, similar to those used on many piggeries was connected to a standard water supply with 150 Kpa of pressure. Three magnesium oxide slides were placed in the pen adjacent to the one being hosed. The slides were placed 28 cm from the floor at distances of 0.5 m, 1.0 m and 1.5 m from the wall separating the two pens. The pen without the magnesium oxide slides, was hosed for one minute and a settle time of three minutes was allowed before removing the slides. The experiment was repeated twice and the second time an additional slide was placed 2.2 m from the separating wall. Twenty five droplet diameters were recorded from five of the seven slides, and only four and five drops were found on the remaining two slides, and the diameter of all of those drops was recorded.

*- Exposure of pigs to pomona via the intranasal route*

Twenty pigs, five months of age, known to be free of leptospiral infection, and without detectable titres to *pomona* were used for the experiment. The twenty pigs were non-selectively divided into four groups of five. Each group of pigs were penned together in solid concrete pens with separate effluent disposal channels, and all pigs were housed within the same building.

Each pig was exposed to *pomona* [strain T A5] via the intranasal route. A multiple dispensing syringe was calibrated to deliver a volume of 0.25 ml of liquid medium containing increasing concentrations of leptospires. All five pigs in each of the four groups were exposed to a total of either  $10^2$ ,  $10^4$ ,  $10^6$ , or  $10^8$  *pomona* organisms. Samples of blood and urine were collected eleven days after exposure. Twenty days after exposure, all of the pigs were slaughtered and blood and kidneys were collected. The serological and cultural examinations were conducted as described in Chapter Two.

- *Exposure of hamsters to pomona via the intranasal route*

Ten weanling syrian hamsters, less than forty two days of age were non selectively divided into two groups of five. The first group was exposed to a total of  $10^4$  *pomona* [strain T A5] by delivering 25  $\mu$ l to each nostril, and the second group was exposed to a total of  $10^6$  *pomona* in the same way. The hamsters were closely monitored for signs of disease, in which case they were anaesthetized, bled and euthanased, and their kidneys cultured as described in Chapter Two. Hamsters which did not appear ill were bled, euthanased and their kidneys cultured twenty one days after exposure to *pomona*.

- *Exposure of hamsters to a leptospiral aerosol*

Five hamsters were individually exposed for one minute to an aerosol formed by nebulising a leptospiral solution containing  $1.8 \times 10^8$  leptospores per ml. A magnesium oxide slide and a culture bottle containing semisolid medium were placed next to each of the hamsters. Twenty droplet diameters were recorded from each slide and the culture bottles were incubated and examined over a period of eight weeks. The hamsters were monitored and samples were collected and processed as previously described.

## **Results**

- *The viability of leptospores in droplets*

The size of droplets formed by the spinning disc ranged between 78  $\mu$ m and 114  $\mu$ m in diameter, as shown in Table 5.9. The magnesium oxide slides closest to the spinning disc, at 18 cm, on average had larger droplets than the magnesium oxide slides placed 30 cm from the disc. Leptospores were isolated from all culture bottles placed in the path of the droplets at 15, 21 and 27 cm from the disc, irrespective of the concentration of leptospores being used to form the droplets [see Table 5.9]. Viable leptospores with normal motile actions were observed in all of the wet slides examined by DFM. Thus, for droplets between 75  $\mu$ m and 115  $\mu$ m in diameter, formed from concentrations of  $6 \times 10^7$  to  $3.6 \times 10^8$  leptospores per ml, leptospores remained viable and capable of multiplying.

**Table 5.9**      **Diameter of droplets formed by spinning disc.**

Concentration of leptospirae/ml	Distance from spinning disc	Droplet diameter ( $\mu\text{m}$ )				Culture result
		mean	min.	max.	S.D.	
$3.6 \times 10^8$	18 cm	114	104	113	4.4	+
	30 cm	93	78	104	6.1	
$1.8 \times 10^8$	18 cm	112	70	121	9.6	+
	30 cm	87	78	104	20.1	
$0.9 \times 10^8$	18 cm	114	113	121	3.5	+
	30 cm	93	87	104	5.2	

+ = Leptospirae isolated from culture medium exposed to droplets, and leptospirae observed by DFM

The size of droplets formed by nebulisation recorded at 15 cm from the nebuliser averaged  $12 \mu\text{m}$  and ranged from the smallest measurable size of  $9 \mu\text{m}$  to  $27 \mu\text{m}$  in diameter, Table 5.10. Leptospirae were isolated from two of twelve culture bottles placed 15 cm from the nebuliser. One culture was obtained from droplets which were formed from a concentration of  $2 \times 10^7$  leptospirae per ml, and the other from  $6.5 \times 10^7$  leptospirae per ml. The average size of droplets formed from the solution containing  $2 \times 10^7$  leptospirae per ml was  $16 \mu\text{m}$  in diameter, and larger than the average size of  $12 \mu\text{m}$  for drops formed from other solutions, see Table 5.10. Leptospirae were observed by DFM in droplets formed from concentrations of  $2 \times 10^7$ ,  $3.25 \times 10^7$  and  $2.6 \times 10^8$  leptospirae per ml, however fewer than ten leptospirae could be counted per drop, and drops readily desiccated. Thus, leptospirae in some droplets ranging in size from  $9 \mu\text{m}$  to  $27 \mu\text{m}$  in diameter were shown to remain viable, and in some cases remain capable of multiplying.

Table 5.10 Diameter of droplets by nebuliser.

Concentration of leptospires/ml	Distance from nebulizer	mean	min.	max.	S.D.	Culture result
Droplet diameter ( $\mu\text{m}$ )						
4.0 x $10^8$	15 cm	12	9	27	4	o
	15 cm	12	9	27	5	o
2.6 x $10^8$	15 cm	12	9	22	5	o
	15 cm	12	9	22	4	o
1.3 x $10^8$	15 cm	12	9	27	4	o
	15 cm	12	9	27	5	o
6.5 x $10^7$	15 cm	12	9	27	5	+
	15 cm	-	-	-	-	-
3.3 x $10^7$	15 cm	12	9	18	4	o
	15 cm	12	9	22	4	o
2.0 x $10^7$	15 cm	16	9	22	4	o
	15 cm	16	9	27	4	+
+ = Leptospires isolated from culture medium exposed to droplets o = Leptospires not cultured - = Not examined						

- *The size of droplets created by spray hosing*

Droplets, formed by spray hosing, which landed in an adjacent pen, decreased in size with increasing distance from the source, as shown in Figure 5.7. The size of drops recorded ranged from 13  $\mu\text{m}$  to 265  $\mu\text{m}$  in diameter. The average diameter of droplets at the specified distances from the separating wall on the two sampling occasions were; 102  $\mu\text{m}$  and 120  $\mu\text{m}$  at 0.5 m; 74  $\mu\text{m}$  and 109  $\mu\text{m}$  at 1.0 m; 31  $\mu\text{m}$  and 84  $\mu\text{m}$  at 1.5 m; and 47  $\mu\text{m}$  at 2.2 m, as shown in Figure 5.7.

- *Exposure of pigs to pomona via the intranasal route*

All pigs exposed to  $10^6$  and  $10^8$  leptospires via the intranasal route had leptospires isolated from their kidneys and had high titres to *pomona* at the time of slaughter. Serological and cultural results are shown in Table 5.11. All five of the pigs exposed to  $10^8$  leptospires and three of the five pigs exposed to  $10^6$  leptospires had detectable titres to *pomona* eleven days after exposure, but only one pig was leptospiruric at that time. Leptospires were not isolated and titres to *pomona* were not detected in any of the two groups of five pigs which were exposed to  $10^4$  or  $10^2$  leptospires via the intranasal route.

Figure 5.6                      The size of droplets landing in pen adjacent to pen which was spray hosed.

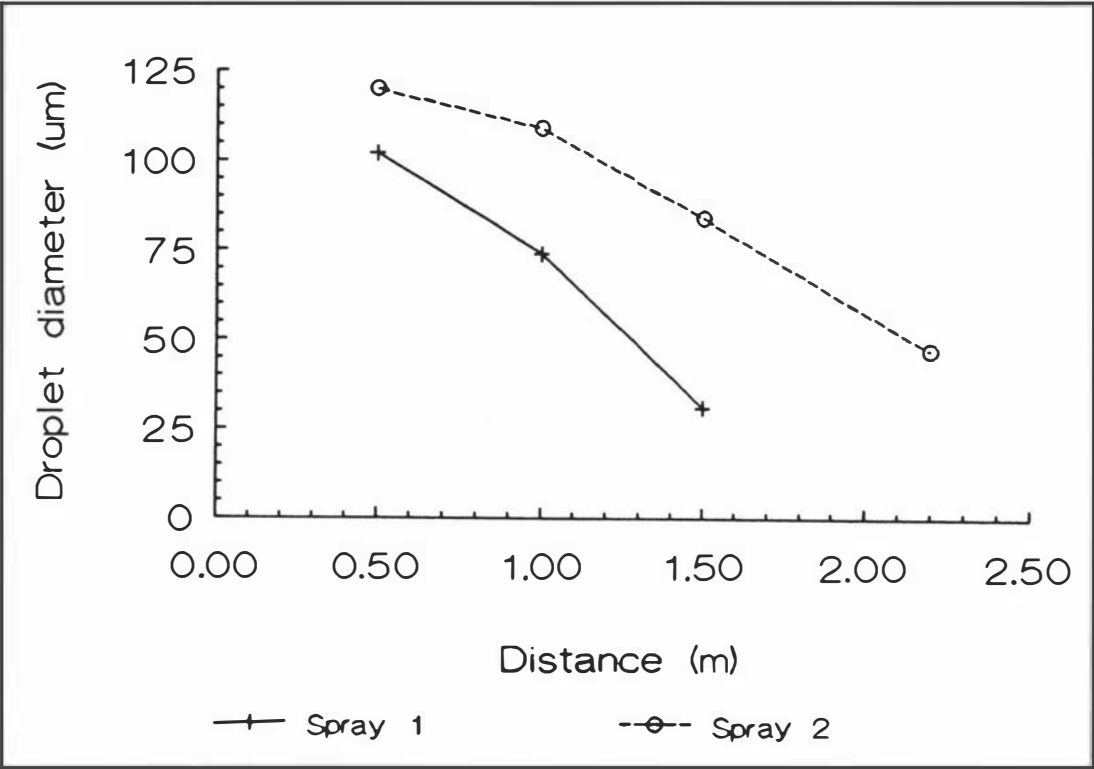


Table 5.11      Minimum infective dose of *po*mona for pigs via the intranasal route.

Infective dose of <i>po</i> mona via the intra-nasal route	Sex	Day 11 <i>po</i> mona titre	Day 11 Urine Culture	Day 21 <i>po</i> mona titre	Day 21 Kidney culture
10 <sup>8</sup>	F	20	o	81,920	+
10 <sup>8</sup>	F	81,920*	+	81,920	+
10 <sup>8</sup>	F	81,920	o	81,920	+
10 <sup>8</sup>	F	81,920	o	81,920	+
10 <sup>8</sup>	F	81,920	o	81,920	+
10 <sup>6</sup>	M	81,920	o	81,920	+
10 <sup>6</sup>	M	0	o	81,920	+
10 <sup>6</sup>	M	0	o	81,920	+
10 <sup>6</sup>	F	81,920	o	81,920	+
10 <sup>6</sup>	F	5,120	o	81,920	+
10 <sup>4</sup>	M	0	o	0	o
10 <sup>4</sup>	M	0	o	0	o
10 <sup>4</sup>	M	0	o	0	o
10 <sup>4</sup>	F	0	o	0	o
10 <sup>4</sup>	F	0	o	0	o
10 <sup>2</sup>	M	0	o	0	o
10 <sup>2</sup>	M	0	o	0	o
10 <sup>2</sup>	F	0	o	0	o
10 <sup>2</sup>	F	0	o	0	o
10 <sup>2</sup>	F	0	o	0	o

+    =    Leptospire

o    =    Leptospire

\*    =    Maximum titre

- *Exposure of hamsters to pomona via the intranasal route*

Leptospire were isolated from the kidneys of four of the five hamsters which were euthanased ten days after exposure to  $10^6$  *pomona*, as shown in Table 5.12. These hamsters were euthanased following the onset of general malaise and conjunctivitis. Titres of 1/10 to *pomona* were detected in only two of the hamsters exposed to  $10^6$  leptospire. Leptospire were also isolated from the kidneys of one of the five hamsters exposed to  $10^4$  leptospire via the intranasal route. This hamster had died twelve days after exposure to *pomona*. All remaining hamsters were euthanased twenty one days after exposure, however leptospire were not isolated from their kidneys, nor did they have detectable titres to *pomona*.

**Table 5.12**    The minimum infective dose of *pomona* for hamsters via the intranasal route.

Number of <i>pomona</i> organisms via the intranasal route	Cause of death and number of days after exposure		Culture of leptospire from kidneys
$10^6$	Euthanased	Day 10	+
$10^6$	Euthanased	Day 10	+
$10^6$	Euthanased	Day 10	+
$10^6$	Euthanased	Day 10	+
$10^6$	Euthanased	Day 10	o
$10^4$	Died	Day 12	+
$10^4$	Euthanased	Day 20	o
$10^4$	Euthanased	Day 20	o
$10^4$	Euthanased	Day 20	o
$10^4$	Euthanased	Day 20	o

---

+    =    leptospire isolated from culture medium exposed to droplets  
 o    =    leptospire not cultured



- *Exposure of hamsters to a leptospiral aerosol*

Leptospire were not isolated and titres to *pomona* were not detected in any of the five hamsters exposed to the leptospiral aerosol. The average size of the aerosol droplets which were measured 8 cm from the nebuliser and that the hamsters were exposed to, was 45  $\mu\text{m}$  in diameter, with a range from 17  $\mu\text{m}$  to 87  $\mu\text{m}$  and a standard deviation of 16  $\mu\text{m}$ , as shown in Table 5.13. Leptospire were isolated from four of six culture bottles which had been exposed to the same leptospiral aerosol.

**Table 5.13** Results of exposing of hamsters to *pomona* via the respiratory route using an aerosol.

Concentration of leptospire/ml	Distance from nebuliser	Droplet diameter ( $\mu\text{m}$ )				S.D.	Culture result	Hamster
		mean	min.	max.				
$1.86 \times 10^8$	8 cm	-	-	-	-	-	+	-
	8 cm	-	-	-	-	-	+	o
	8 cm	38	17	44	5		o	o
	8 cm	45	26	61	10		+	o
	8 cm	43	17	87	20		o	o
	8 cm	53	26	87	18		+	o

+ = Leptospire isolated from culture medium exposed to droplets  
 o = Leptospire not cultured  
 - = Not examined

### Discussion

From the results of these experiments there is little doubt that the intranasal route is potentially a natural route of leptospiral infection for susceptible animals. Burnstein and Baker (1954), also reported that all pigs inoculated via the intranasal route became infected. Will and Diesch (1976) infected hamsters with  $10^5$  *pomona* via the intranasal route, and Blackmore and Schollum (1986 Unpublished) infected rats with  $10^3$  *copenhageni* via the

intranasal route. The minimum infective dose of *poona* [strain T A5] for pigs and hamsters was found to be between  $10^4$  and  $10^6$  leptospires.

Will and Diesch (1976) successfully infected hamsters via the intranasal route, and in this study both pigs and hamsters became infected following exposure to *poona* via the intranasal route. Drops larger than  $100\ \mu\text{m}$  in diameter may carry sufficient numbers of leptospires to permit infection of a susceptible host. Spray hosing of the concrete pen showed that the largest droplets travelled the shortest distance, thus one would expect the risk of transmission of disease to decrease with increasing distance from the source of droplets. Theoretically, transmission can occur via the intranasal route if the product of the number of drops, and the number of leptospires contained within the drops, is equal to, or greater than, the minimum infective dose. Therefore the arrival of droplets containing viable leptospires at the nasal mucosa of a susceptible host, if in sufficient numbers, may result in the infection of that host.

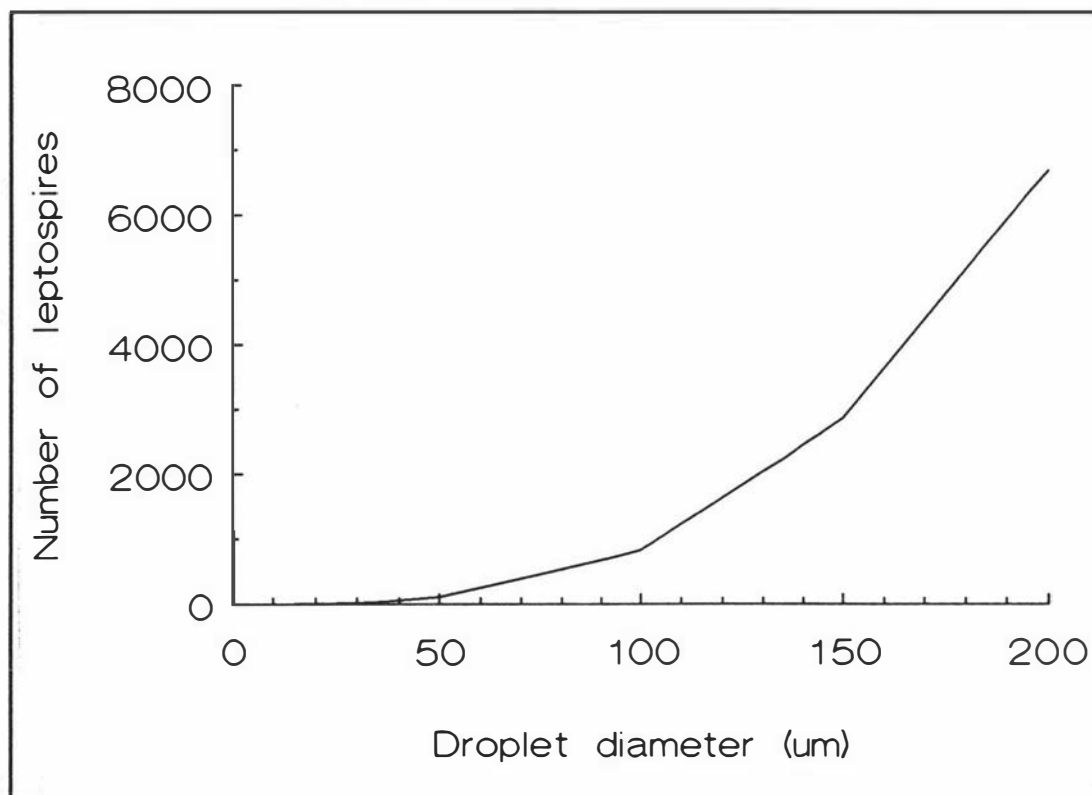
Within a pig herd, transmission of infection by droplets, and infection via the intranasal route may be one of a number of important means by which infection is transmitted between pigs in different pens and between pigs of the same pen. Within a pen of pigs, once one pig is infected and becomes leptospiruric, infection may well occur via the intranasal route by the normal behaviours of rooting and urine sniffing, as shown in Figures 5.9 and 5.10. In piggeries where there appears to be no direct contact of pigs between pens, transmission of infection from one pen to another by droplets may constitute an important means for maintaining infection within the herd.

In these experiments it was demonstrated that leptospires could survive within droplets with a diameter of  $30\ \mu\text{m}$  or greater.

In theory, one leptospira  $0.1\ \mu\text{m}$  in diameter and  $20\ \mu\text{m}$  in length has a volume of  $6.28 \times 10^{-13}$  ml. Therefore, the theoretical maximum number of leptospires which could be contained within a droplet of known diameter can be calculated.

Figure 5.7

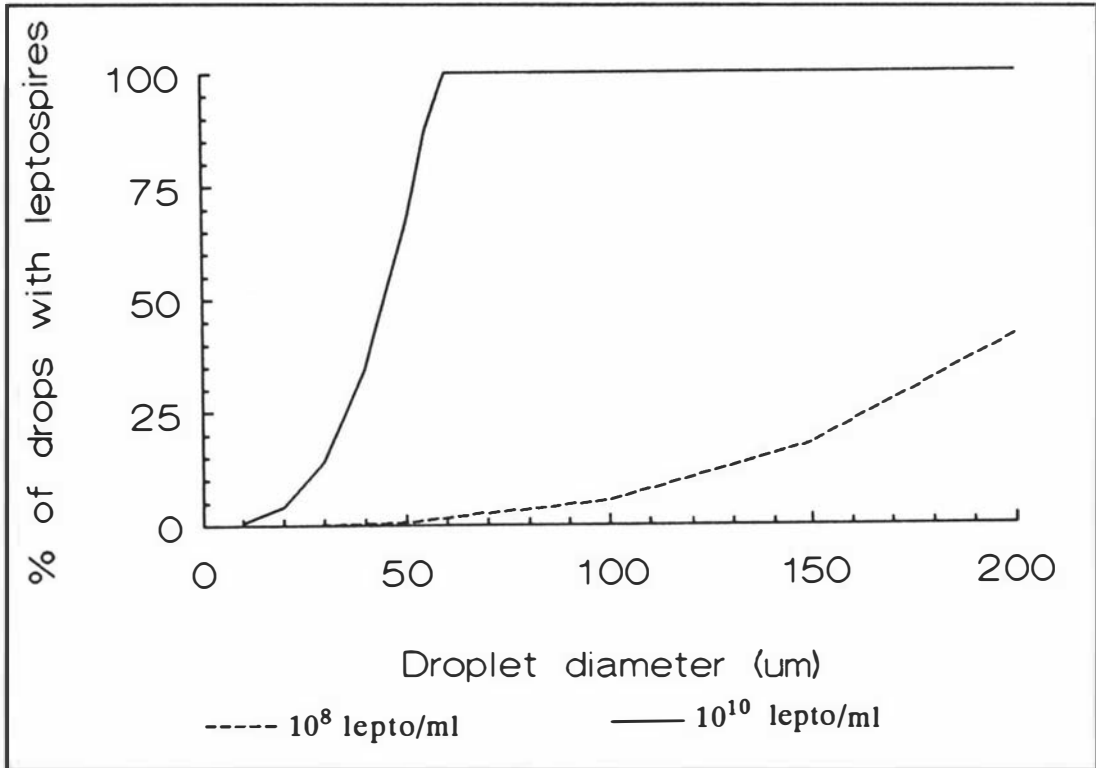
The theoretical maximum number of leptospires in a drop of known diameter.



From the calculations, less than one leptospira [0.82 of a leptospira] would occupy a droplet which was 10  $\mu\text{m}$  in diameter, and therefore it is unlikely that leptospires can be transmitted in droplets of 10  $\mu\text{m}$  or less in diameter. A droplet of 20  $\mu\text{m}$  in diameter, with a volume of  $4.2 \times 10^{-12}$  ml could theoretically contain six leptospires, and thus droplets of increasing size would have greater chances of containing leptospires, as shown in Figure 5.7. These calculations have been based on the assumption that the volume of a leptospira is totally dynamic and that a theoretical concentration of  $1.6 \times 10^{15}$  leptospires per ml was used to form droplets. Cultures of leptospires and urine of leptospiruric pigs normally contain less than  $10^{10}$  leptospires per ml. The number or percentage of drops containing leptospires which are formed from a liquid containing either  $10^8$  and  $10^{10}$  leptospires per ml can also be calculated and is shown in Figure 5.8. Logically, greater numbers of leptospires will be contained in droplets of increasing size. Examination of the calculations suggest that droplets of 100  $\mu\text{m}$  or less in diameter, formed from a concentration of less than  $10^8$  leptospires per ml, are unlikely to contain sufficient leptospires to infect susceptible animals via the respiratory route.

**Figure 5.8**

**The theoretical percentage of drops formed from two leptospiral concentrations which contain at least one leptospira.**



It was demonstrated in this investigation, that hamsters did not become infected following exposure to an aerosol consisting of droplets between 15  $\mu\text{m}$  and 90  $\mu\text{m}$  in diameter and which contained viable leptospires. This indicates that the minimum infective dose was not attained by the aerosol during the one minute period of exposure. Similarly, Will and Diesch (1978) also failed to infect hamsters by exposing them to the air above a model oxidation ditch which contained leptospires, and from which leptospiral aerosols were being formed. The low number of leptospires in droplets less than 50  $\mu\text{m}$  in diameter as indicated by both theoretical calculations and these experimental results does suggest that infection via the respiratory route, by the inhalation of leptospiral aerosols, is an unlikely route of natural infection, even though leptospires have been shown to survive in droplets of less than 50  $\mu\text{m}$  in diameter.

Figure 5.9

Pigs "rooting" amongst infective effluent.



There are many routes for the transmission of leptospiral infection all of which may have varying degrees of importance for the maintenance of infection within a pig herd. In this chapter the hypothesis has been advanced that the intranasal route is a common route of natural infection for pigs within a pig herd, and is likely to occur in conjunction with the many other natural routes of infection described in Chapter One. The control of infection within a pig herd may be most effectively approached by increasing the resistance of pigs to infection, rather than attempting to control the means by which pigs are becoming infected. This is one of the reasons why vaccination was chosen as the means to attempt to control leptospiral infection in a grower herd with endemic *pomona* infection. A vaccination trial, testing four vaccinal regimes was conducted, and is described and discussed in the next chapter.

**Figure 5.10** Pigs "urine sniffing", a behaviour allowing infection via the intranasal route.



## SUMMARY AND CONCLUSIONS

1. An experiment was conducted in an attempt to determine whether *bratislava* titres could be the result of a serological cross reaction in pigs infected with *pomona*, or whether they only occurred following exposure to *bratislava*. Five pigs were artificially exposed to *pomona*, and five to *bratislava*. One pig in each group was artificially exposed to the leptospires via the intra-nasal route, and the remaining four pig in each group were exposed via the intramuscular route.
2. It is believed by the author that the strain of *bratislava* used was avirulent and did not result in infection becoming established in exposed pigs. However a serological response following exposure of the pigs to the avirulent antigen was recorded, as well as a transient heterologous serological response to *pomona*.
3. Pigs exposed to *pomona* became infected. This infection was characterised by leptospiuria and a serological response. Heterologous titres to *bratislava* were recorded in pigs exposed to *pomona*, and were highest during the initial stages of infection.
4. It was concluded that *bratislava* titres in pigs can be the result of a serological cross reaction associated with infection with serovar *pomona*. The magnitude of the *bratislava* titres is generally lower than that of the *pomona* titre, and highest during the initial weeks of infection when the ratio of *pomona* IgM:IgG is greatest. This conclusion has possibly explained the observation of the occurrence of *bratislava* titres of less than 1/5,120 in 50% of pigs at slaughter in the absence of the isolation of *bratislava*, and the poor linear association between the magnitude of the *pomona* and *bratislava* titres in the same pig [Chapter Three].
5. A second experimental study was undertaken in an attempt to determine if leptospiral suspensions could successfully form

infective aerosols, and to determine if infection via the upper and lower respiratory routes are probable routes for natural infection.

6. Droplets of varying size were formed from leptospiral suspensions using either a spinning disc apparatus or a hand held nebuliser. Magnesium oxide coated slides were used to determine the size of droplets formed.
7. Droplets formed using the spinning disc apparatus from solutions containing at least  $6 \times 10^7$  leptospores per ml ranged between  $75 \mu\text{m}$  and  $115 \mu\text{m}$  in diameter and contained viable leptospores capable of multiplying.
8. Droplets formed by nebulisation of solutions containing at least  $2 \times 10^7$  leptospores per ml ranged in size between  $9 \mu\text{m}$  and  $27 \mu\text{m}$  in diameter however only few droplets contained viable leptospores capable of multiplying.
9. Droplets formed by spray hosing a concrete pen which settled in an adjacent pen ranged in size from  $13 \mu\text{m}$  to  $265 \mu\text{m}$ . The size of droplets decreased with increasing distance from the source of the spray hosing.
10. The minimum infective dose of *pomona* via the intra-nasal route in hamsters and pigs appears to be around  $10^4$  leptospores. It was concluded that transmission of infection by aerosol droplets of greater than  $50 \mu\text{m}$  can result in the natural infection of pigs via the intra-nasal route.
11. Hamsters exposed to a leptospiral aerosol formed by nebulisation, failed to become infected. It was concluded that infection via the respiratory route resulting from the inhalation of droplets of less than  $50 \mu\text{m}$  in diameter was an unlikely natural route of infection.



## Chapter Six

**VACCINATION TRIALS**

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## INTRODUCTION

In New Zealand virtually all leptospiral infection due to serovar *pomona* originate either directly or indirectly from pigs. If endemic *pomona* infection of pigs in New Zealand were to be effectively controlled, a major source of leptospiral infection would be eliminated. In this chapter, a description is given of four vaccination regimes which have been tested for their effectiveness in reducing the prevalence of endemic leptospiral infection due to serovar *pomona* in a grower pigs. A review of the literature of previous relevant vaccination trials is also given.

The control of leptospiral infection within a piggery can be approached in at least two ways. One method is to prevent direct or indirect transmission of leptospires between pigs, and another is to increase the resistance of pigs to infection. Prevention of transmission can theoretically be achieved by the segregation of infected pigs from susceptible pigs, the use of chemotherapeutic treatment for infected pigs and by maintaining a high standard of hygiene. In cases where the prevention of transmission is not possible, infection may be prevented by the application of vaccination or by the presence of high levels of maternal antibody.

### *Review of literature*

#### *- History of the production of leptospira bacterin*

One of the early problems associated with the production of a leptospiral bacterin was the difficulty of attaining adequate concentrations of leptospires in culture. This was due to the fastidious growth requirements of leptospires, which include fatty acids, nitrogen, vitamins B<sub>1</sub> and B<sub>12</sub>, iron, calcium, magnesium and zinc to enable them to be grown in culture (Ellinghausen and McCullough, 1965; Johnson and Harris, 1967b). This was initially overcome by the addition of rabbit serum to the culture medium, however attempts to consistently achieve concentrations of at least 10<sup>9</sup> leptospires/ ml in culture were hindered by the variability of the rabbit serum used in the culture medium (Bey and Johnson, 1986). A medium containing albumin was developed in the 1960's, which had the capability of supporting the growth of final concentrations of 1 to 2 x 10<sup>9</sup> leptospires/ ml from initial concentrations of only 1 to 1000 leptospires/ ml (Ellinghausen and McCullough, 1965; Johnson and

Harris, 1967b; Bey and Johnson, 1978). The presence of serum or albumin proteins in a suspension of a bacterin introduces the possibility of adverse reactions to these other proteins, in animals immunized with such a bacterin (Bey and Johnson, 1986). It can therefore be appreciated why albumin protein free medium was subsequently developed (Bey and Johnson, 1978). The immunogenicity of leptospires cultivated in protein free medium was found to be similar to that of leptospires cultivated in medium containing albumin (Bey and Johnson, 1982).

- *History of the use of vaccines*

Bacterin produced from chemically inactivated, whole cultures have been developed and tested for their effectiveness in preventing infection against a variety of serovars in different species of animal (Bey and Johnson, 1986). Formalin inactivation was used by Olitzki *et al* (1953) to develop one of the first *pomona* bacterins. Brown *et al* (1955) incorporated an adjuvant into a formalin inactivated bacterin and found that calves inoculated with this bacterin had significantly higher agglutination titres than calves inoculated with previously produced bacterin. The type and quantity of antigen administered by vaccination influences the type, amount, and duration of agglutinating antibody produced (Bey and Johnson, 1986). Attenuated live vaccines have also been shown to prevent infection in pigs with leptospires not being isolated from the kidneys of vaccinated pigs (Stalheim, 1967; 1968). Using a live avirulent *pomona* vaccine, Fish and Kingscote (1973) apparently immunized pigs, however only low transient agglutination titres were developed. Sera from vaccinated pigs that did not have detectable titres, were capable of passively protecting guinea pigs from challenge. Attenuated live vaccines are however not available for commercial use. This may be due to both the difficulty in maintaining the viability of the vaccine and the possible risk that attenuated organisms in the vaccine may revert to virulent organisms in the inoculated animal (Bey and Johnson, 1986).

- *Vaccination of breeding pigs*

Inoculation of pigs with bacterin has often resulted in undetectable or low levels of agglutinating antibodies (Hanson *et al*, 1972; Dobson and Davos, 1975; Bey and Johnson, 1983). Vaccination of gilts and sows with inactivated vaccines has resulted in the suppression of acute clinical signs of illness and

in the prevention of abortion and perinatal losses, however it has not always prevented kidney colonisation (Cook, 1964; Hanson *et al* 1972). Vaccination of breeding pigs in New Zealand and Australian piggeries is commonly practised in order to prevent abortion and perinatal losses which are primarily due to serovar *pomona* (Cook, 1964; Whyte *et al*, 1982). Unfortunately, vaccination of breeding pigs generally does not prevent endemic leptospiral infection in New Zealand grower pig herds (Study Three, Chapter Three).

- *New Zealand vaccination trials*

In New Zealand, the first attempts to control leptospirosis by vaccination were carried out in sheep (Webster and Reynolds, 1955). Since 1979, vaccination has been, and still is, widely used for the prevention of leptospiral infection in dairy cattle due to serovars *hardjo* and *pomona*. As a result of the widespread use of vaccines, the reported annual incidence of human beings contracting leptospirosis has fallen from above 500 to around 100 over the last ten years (Marshall, 1987). In New Zealand, vaccination as a means of controlling endemic leptospiral infection in pigs has been investigated on three occasions by Hodges *et al* (1976), Hodges (1977), and Hodges *et al* (1985).

Hodges *et al* (1976) studied the efficacy of two commercial vaccines for the prevention of infection with *pomona* in grower pigs. They noted that over 50% of vaccinated pigs became leptospiruric following exposure to natural infection. In their trial, pigs were first inoculated at six to eight weeks of age and given a second inoculation two to three weeks later. Two weeks after the second inoculation the pigs were transferred to a grower house known to contain infected pigs. Agglutination titres to *pomona* were not detected in either vaccinated or unvaccinated pigs before they were exposed to natural infection. Following exposure to infection, titres were detected in 80% of the vaccinated pigs and in all of the unvaccinated pigs. Leptospiruria was detected by DFM in nearly all [23/25] of the unvaccinated pigs and in over half [18/32] of the vaccinated pigs six to eighty days after exposure to natural infection.

Hodges (1977) set out to determine whether or not repeated inoculations of a *pomona* bacterin would prevent infection in pigs which were subsequently exposed to natural infection. A culture of serovar *pomona* organisms was killed by the addition of thiomersal to a final ratio of 1:10,000 and aluminium gel was

added to a final concentration of approximately 1% of the bacterin. Over a period of three weeks, four intramuscular and four subcutaneous inoculations of this bacterin were administered to fourteen of twenty four, six week old piglets. Five days after the last inoculation the twenty four pigs were moved to a grower house containing other pigs known to be naturally infected with *pomona*. Agglutination titres of 1/10 or greater to serovar *pomona* were detected before exposure to natural infection in all of the vaccinated pigs but not in any of the control pigs. Eight weeks after exposure to natural infection, all of the control pigs had developed titres, however the titres of the vaccinated pigs had decreased. Neither leptospiuria nor renal lesions typical of leptospiral infection were detected in any of the vaccinated pigs but were present in all of the control pigs.

Hodges *et al* (1985) reported the results of another vaccination trial which was designed to determine the efficacy of a vaccine for the prevention of leptospiuria in grower pigs exposed to natural *pomona* infection. They used a commercial vaccine which was licensed for use in cattle and contained serovars *hardjo* and *pomona*. Pigs were inoculated with the vaccine on two occasions, initially when they were five weeks of age and then again when they were eight weeks of age. Two weeks after the last inoculation the pigs were transferred to a finisher house containing other pigs known to be naturally infected with *pomona*. Six of nineteen vaccinated pigs were subsequently diagnosed as leptospiuric by DFM. The frequency of the detection of leptospiuria and intensity of leptospiuria in vaccinated pigs was significantly lower [ $P < 0.005$ ] than that of the control pigs. Hodges *et al* (1985) concluded that vaccination of pigs with a cattle vaccine resulted in a significant degree of protection against leptospiuria. In the same trial, agglutination titres to *pomona* were detected in twelve of the nineteen vaccinated pigs prior to exposure. Of sixteen vaccinated pigs exposed to natural infection, the titres of seven pigs increased, five remained the same, and those of four pigs decreased.

There is little doubt that Hodges (1977) and Hodges *et al* (1985) demonstrated that the use of vaccination has the potential to control endemic *pomona* infection in pigs of New Zealand piggeries. The most efficacious of the three trials involved multiple inoculations with a bacterin (Hodges 1977), but as

discussed by Hodges, this method of immunisation would not be economically practical under field conditions. The work did however demonstrate the theoretical ability of a bacterin to produce an immune response capable of protecting pigs from natural infection.

Throughout their trials, Hodges (1977) and Hodges *et al* (1976; 1985) used DFM as the main means of detecting leptospiuria, while cultural examination was a secondary means of detecting leptospiuria and was used less frequently than DFM. Hodges *et al* (1976) used culture of urine only to obtain an isolate which was subsequently used as an antigen for the MAT, and Hodges (1977) cultured urine at least weekly over the twelve week sampling period while Hodges *et al* (1985) used culture for the detection of leptospiuria on only two occasions. Culture is accepted as a more sensitive method for the detection leptospiuria than DFM. DFM is a relatively insensitive method, especially when the intensity of leptospiuria is less than  $10^4$  leptospires/ ml of urine [see Chapter Two]. Therefore, conclusions drawn about the efficacy of a vaccine in reducing the intensity of leptospiuria which are based on the results of DFM only, must be interpreted with caution. Although a reduction in the intensity of leptospiuria of some vaccinated pigs reported by Hodges *et al* (1976; 1985) probably occurred, the use of standardised dilutions of urine in culture as described in Chapter Two would have allowed a more quantitative assessment of the intensity of leptospiuria to have been made.

Exposure of vaccinated pigs to natural infection as reported by Hodges (1977) and Hodges *et al* (1976; 1985) is in this authors opinion the most appropriate method of challenge to test the efficacy of a vaccine. In pig herds with endemic *pomona* infection, exposure to natural infection can be expected to be reasonably consistent as infected pigs continue to contaminate their immediate environment [see Chapter Five]. The continuing risk of infection to susceptible pigs was reflected by the high incidence and prevalence of infection amongst grower pigs (Chapter Five), and by Hodges (1977) and Hodges *et al* (1976; 1985) who repeatedly showed that most of their control pigs became infected. As stated by Hodges (1977) "most studies on the efficacy of leptospiral vaccines have involved experimental infections in which it is impossible to compare the challenge with that encountered under field conditions".

Infection has been eliminated from Massey University's piggery using a combination of quarantine measures for incoming stock, rearing pigs to pork weight, and the vaccination of their breeding pigs (Dr R. Marshall and Mr P. Webber, Pers. Comm<sup>1</sup>). For a period of three years, all incoming pigs to this farm were placed in quarantine on a separate property and treated with a three day course of dihydrostreptomycin [25 mg/ kg]. At the commencement of the vaccination program, permanent management changes were made which included the total segregation of breeding pigs from grower pigs, and rearing the majority of pigs to porker weight, attained at approximately four months of age. Breeding sows on this farm continue to be vaccinated twice a year.

- *Australian vaccination trials*

In Australia, trials to vaccinate against endemic leptospiral infection in pigs have also been conducted.

Gill and Williamson (1978) had the most impressive results from a vaccination regime which resulted in the apparent eradication of endemic leptospiral infection from a piggery. All sows were vaccinated four weeks before farrowing, and for a period of six months, all grower pigs were vaccinated at six weeks of age. The exact inoculation regime used was not specified by these authors. Management procedures used throughout the trial period remained unchanged. These included the weekly dispatch of bacon weight pigs, the presence of an efficient effluent disposal system which resulted in minimal soiling of pens, and the continual systematic movement of pigs along the grower shed until removed for slaughter. Blood, urine and kidneys were collected from slaughtered bacon weight pigs on four occasions. The first occasion was from unvaccinated pigs prior to implementation of vaccination, the second from vaccinated pigs during the six month period of vaccination and the third and fourth were from unvaccinated pigs eight months and fifteen months after the period of vaccination. Kidneys were examined histologically, urine was examined by DFM, and sera were tested by the MAT for agglutinating titres to serovar *pomona*. It was shown in this study that the prevalence of interstitial nephritis in bacon weight pigs was significantly

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<sup>1</sup>. Massey University, Palmerston North, New Zealand. Personal communication about the use of isolation and chemotherapy for incoming boars to the Massey University Piggery.

reduced after the end of the vaccination program. They reported that leptospires were detected by DFM in nine of fifty urine samples collected from the unvaccinated group of bacon weight pigs prior to commencement of the vaccination regime, however subsequently leptospires were not detected by DFM in any of the urine samples. The average *pomona* titres of pigs slaughtered prior to the vaccination program was 1/5,000,000, and after the vaccination program all pigs were seronegative. It was concluded by these authors that endemic *pomona* infection had been eliminated from the piggery. The management procedures used on the farm were thought by the authors to contribute to the success of the eradication scheme by reducing the opportunity for young susceptible pigs to be close to or in contact with older infected pigs.

Gill and Williamson (1978) apparently succeeded in breaking the cycle of the maintenance of *pomona* infection, the ultimate goal in controlling endemic leptospiral infection, leading to the elimination of infection from the grower herd within the piggery. This goal was achieved by increasing resistance to infection by vaccination, and by decreasing exposure of susceptible pigs to infection through management procedures. Unfortunately, there was no discussion by Gill and Williamson about the expected efficacy of the vaccine they used, as a means of preventing leptospiral infection. It is therefore difficult to ascertain the relative importance of both vaccination and management in controlling endemic *pomona* infection in the piggery.

Palit *et al* (1988) reported on the short and long term effectiveness of a killed *pomona* and *tarassovi* vaccine containing an aluminium adjuvant for preventing urinary shedding of leptospires by domestic pigs. Pigs without agglutination titres to serovar *pomona* were selected for the vaccination trial. Pigs twelve to fourteen weeks of age were inoculated on two occasions, four weeks apart, with two ml of vaccine. Four weeks after the second inoculation, three vaccinated pigs and four unvaccinated controls were challenged by exposure to  $8 \times 10^9$  virulent serovar *pomona* leptospires, however the route of challenge was not specified by the authors. Six months after the second inoculation, a second group of eight vaccinated pigs and seven unvaccinated controls were similarly challenged. Urine was collected and examined by DFM, and kidneys were cultured at slaughter for the presence of leptospires. Adequate priming of the immune system appeared to occur in both groups of vaccinated pigs, as shown



by a rapid and marked secondary immune response following vaccination. Six months after vaccination, titres had fallen to low levels. Leptospirosis was not detected in any of the vaccinated pigs, however all control pigs became infected. The authors concluded that "the cycle of serovar *pomona* infection can be broken and the concomitant health risk to man further reduced".

Palit *et al* (1988) stated that "the cycle of *pomona* infection can be broken" but did not specify whether the target population was the grower pig herd, the breeding pig herd or both. However, from the strategy of vaccination at six monthly intervals and commencing at twelve to fourteen weeks of age, it can perhaps be assumed that they were referring to the use of the vaccine for the prevention of leptospiral infection in breeding pigs. The concomitant health risk of which Palit *et al* (1988) speak, appears in this authors opinion to be associated more with *pomona* infection in grower pigs than with infection in breeding pigs (Chapters Three and Four). It is however *pomona* infection in the breeding pigs with which economic loss is associated. Although all pigs in the control group become infected in this investigation, it is unfortunate that the method of challenge was not described by the authors. As a result of this oversight, information about the infective dose and the route of infection were not recorded. The Palit *et al* (1988) trial can not therefore be repeated, nor can a comparison of these results be made with other trials. Only three pigs were used to determine the effectiveness of the vaccine for the prevention of infection four weeks after the second inoculation. With such low numbers it is difficult to accurately assess the outcome. As discussed previously, the use of DFM is less sensitive for the detection of leptospirosis in comparison with culture.

Cargill and Davos (1981) tested two commercial vaccines to determine their ability to prevent renal lesions due to leptospiral infection in grower pigs. The first inoculation was administered to pigs twelve to fourteen weeks of age, followed four weeks later by a second inoculation. Vaccinated pigs were exposed to artificially infected leptospiric pigs, four, seven and twelve months following vaccination. Histological lesions consistent with leptospiral infection were observed in six, seven and eight of ten kidneys respectively, and the renal lesions of vaccinated pigs were reported to be less severe than those of controls. Leptospirae were demonstrated in kidney cultures obtained from

two, three and five of ten vaccinated pigs at the respective intervals of four, seven and twelve months after vaccination. It was concluded that the degree of protection afforded by this vaccination regime was insufficient to prevent kidney colonisation by leptospires.

Many vaccine trials have been undertaken to determine the efficacy of a vaccine administered to animals three, six and twelve months before they were challenged. These intervals between challenge are relevant to the protection of infection in cattle as the maintenance of leptospiral infection in cattle usually has an annual cycle associated with spring calving (Hellstrom, 1978). However they are less relevant to pigs as sows breed all the year round, and the cycle of maintenance of infection amongst grower pigs is continuous due to the constant influx of susceptible pigs into a piggery. Vaccination of breeding pigs is commonly conducted at the time of weaning or mating and therefore most breeding pigs are re-vaccinated every six months. The requirement of a vaccine, in order for it to be efficacious for the prevention of infection in grower pigs, should be that it rapidly induces a protective immune response, and that protection lasts for the duration of the pigs life which may be up to six months of age. Vaccination at intervals greater than six months only offers advantages in flexibility of vaccinal regimes for breeding pigs, however such a prolonged interval has no relevance to the immunisation of grower pigs for the control of endemic leptospiral infection.

Given the present understanding of the epidemiology of leptospira *pomona* infection of pig herds in New Zealand piggeries, and the results of the vaccination trials reviewed in this chapter, it was decided that a vaccination trial should be undertaken to determine the potential effectiveness of vaccination for the control of endemic *pomona* infection in grower pigs. A vaccine already commercially available for use in pigs was selected. Four different regimes of inoculation were tested for their ability to prevent *pomona* infection in grower pigs. The presence of infection being determined by the isolation of leptospires by culture from either urine and kidneys. It was essential that the efficacy of each inoculation regime be measured by the most sensitive means of detecting leptospiral infection available to this author. In conjunction with cultural examination, the serological response of each pig over a period of time, at regular intervals, was also measured to determine if

there was any relationship between protection from infection and measurable agglutinating antibody to serovar *pomona*.

## MATERIALS AND METHOD

### *General experimental design*

Grower pigs of around ten weeks of age, were the subjects of this vaccination trial. The breeds of pigs used for the trial included Large white, Landrace, and their crosses. Four inoculation regimes [Groups 1 to 4] were studied on two farms [Farm A and B]. One farm was free of leptospiral infection [Farm A] and pigs were not exposed to infection at any time throughout the study period. While the other farm had endemic *pomona* infection [Farm B] and all inoculated and unvaccinated pigs on this farm were exposed to natural infection.

### *Leptospiral bacterin*

"Leptovax Bivalent 1007" [batch number 179, expiry date August 1989] produced by Coopers Animal Health N.Z. Limited was used for the vaccination trial. The vaccine is a combined bivalent *pomona* and *hyos [tarassovi]* bacterin, registered in New Zealand for use in pigs. An accompanying instruction sheet recommended the use of the "Leptovax" vaccine at six monthly intervals for the prevention of abortion and premature death of piglets amongst breeding pigs. The recommended regime was two inoculation doses of two ml, administered four to six weeks apart, and thereafter continuing inoculations every six months.

The "Leptovax bivalent" vaccine used throughout this vaccination trial will be referred to as a bacterin, and administration of the bacterin will be termed inoculation. Administration of a series of inoculations, the objective of which is to produce an increased resistance to infection, will be termed vaccination.

The bacterin was inoculated via the subcutaneous route into the anterior neck region of pigs using a multiple inoculation gun with an 18 gauge 1/2 inch [0.65 cm] needle. It was stored at 4°C between use, and transported on ice when used at the farms.

### *Regimes of inoculation*

Four vaccination regimes, shown in Table 6.1, were tested in this vaccination trial. The first regime was a single inoculation of 3 ml, given in the first week of the trial [Group 1]. The second regime was two inoculations of 1.5 ml of bacterin, administered in the first and fifth weeks of the trial [Group 2]. The third group received three inoculations of 1 ml of bacterin, at two weekly intervals, in the first, third and fifth week of the trial [Group 3]. The fourth group received three inoculations of 1 ml of bacterin, at weekly intervals, in the first, second and third week of the trial [Group 4]. Each vaccination regime consisted of a total dose of 3 ml of bacterin.

**Table 6.1** Regimes of inoculation tested in grower pigs.

Inoc. Regime	Number Doses	Each Volume	Total Volume	Week of First	Second Inoculation	Third
1	: one	3.0 ml	3.0 ml	1	-	-
2	: two	1.5 ml	3.0 ml	1	5	-
3	: three	1.0 ml	3.0 ml	1	3	5
4	: three	1.0 ml	3.0 ml	1	2	3

### *Selection and number of pigs*

Pigs aged approximately ten weeks were sequentially allocated, without any specific form of selection, to one of the four inoculation groups on each farm. Each pig was ear tagged for future identification, bled by the jugular route (Chapter Two), and inoculated with bacterin according to the vaccination regime to which it had been allocated.

A total of forty pigs on the farm free of leptospiral infection [Farm A] were selected for the trial. Ten pigs were allocated to each group.

A total of eighty pigs on the farm with endemic *pomona* infection [Farm B] were selected for the vaccination trial, provided they had entered the grower house within one week at the time of their selection. Twenty pigs were allocated to each group. No more than half the pigs in any one pen were subjected to the trial. Pens contained either pigs from one vaccination regime only or a mixture of pigs from two vaccination regimes. The remainder of the pigs in these pens were not inoculated.

#### *Time at which pigs were examined*

Pigs from the farm free of leptospiral infection [Farm A], which received the double and triple inoculations [Groups 2, 3, and 4] were bled every week from the first week of the trial [Week 1], for ten weeks [up to Week 10]. Pigs which received a single inoculation [Group 1] were bled every week for the first seven weeks, but only four of the ten pigs in this group were bled in the eighth week. Eight of the forty pigs were followed through to slaughter at which time blood and kidneys were collected for serological and cultural examination.

On the farm with endemic leptospiral infection [Farm B] between twenty to forty inoculated pigs were bled and samples of urine were collected each week for ten weeks. This resulted in samples of blood and urine being collected from pigs in each inoculation group at approximately three weekly intervals, as shown in Table 6.2. Blood and kidneys were collected at the time of slaughter from both vaccinated and unvaccinated control pigs. Vaccinated pigs ranged from approximately twenty four to forty weeks of age at the time of slaughter. Blood and kidneys of forty one vaccinated, and thirty two unvaccinated control pigs, were collected at the time of slaughter. At the abattoir, pigs from the same farms were penned together and driven through a narrow race to the place where they were electrically stunned and bled. Pigs arrived at the place of slaughter in single file and in no particular order. Each vaccinated pig was identified by the presence of an ear tag, and the first unvaccinated pig of the same gender which followed a vaccinated pig was then selected as a control pig.

**Table 6.2** The number of pigs in each inoculation group which were examined at the farm with endemic *pomona* infection [Farm B] throughout the ten week period of sampling, and at the time of slaughter.

Inoculation group	1	2	3	4
<u>Week</u>	-----			
1	20	20	20	20
2	0	0	0	0
3	0	0	0	20
4	20	10	20	0
5	0	10	0	20
6	0	0	0	0
7	19	0	19	20
8	0	17	0	0
9	0	0	0	0
10	16	18	18	18
Slaughter				
- vaccinated	8	6	14	13
- unvaccinated	3	5	13	11

### ***Challenge***

Only pigs on the farm with endemic *pomona* infection were challenged by exposure to natural infection. Pigs were housed in grower houses known to contain infected pigs. As pigs grew larger and older they were moved from one end of the building to the other and were continually exposed to natural infection (Chapter Four). This movement of the pigs throughout the trial was by normal management procedures, and was not interfered with by the author.

### ***Serological and cultural examinations***

The serological changes of pigs inoculated with bacterin were measured by the MAT, and their infection status was monitored by the culture of urine and kidneys as described in Chapter Two. For the MAT, serum samples were initially tested at dilutions of 1/4 to 1/256. As titres reached 1/256, sera were retested at dilutions ranging from 1/10 to 1/327,680. Thereafter, in the later weeks of the trial, sera were only tested at dilutions ranging from 1/10 to 1/327680.

### *Statistical methods*

Statistical analysis of titres to serovar *pomona* and calculations of geometrical mean titre [GMT] were conducted using coded titre units [CTU] of only seropositive results [see Chapter Two and Appendix II]. Statistical analysis of data was computed using "Statistix" computing package. The Chi squared and Student's T tests were used for statistical analysis.

## **RESULTS**

The results are presented in four sections, each section reports the results of one of the four regimes from both the uninfected [Farm A] and the infected farm [Farm B]. The results from both farms are further divided into initial and final results. Initial results refer to the results of serological and cultural examinations of pigs throughout the ten week period of investigation. Final results refer to the serological and cultural examinations of pigs at the time of slaughter. In describing the serological results, the measures of geometrical mean titre (GMT) and seroprevalence have been used (see Chapter Two, Processing of serological results).

### *Single inoculation with vaccine in the first week [Group 1]*

#### *- Leptospira free farm [Farm A]*

##### *Initial serological results -*

The serological results of this group are given in Table 6.3, and illustrated in Figure 6.1. None of the pigs developed titres greater than 1/4 in the first two weeks. The highest seroprevalence of 60% occurred in the third and fourth weeks, however with the exception of Pig number 9 which had a titre of 1/128 in the third week, the titres of the other seropositive pigs in these weeks did not exceed 1/16. Therefore, although the seroprevalence was high in the third and fourth week, the GMT was low. Thereafter, for the following four weeks, titres of 1/16 to 1/256 occurred in only three pigs [Pig numbers 4, 8 and 9] thus raising the GMT, as shown in Figure 6.1 and Table 6.3.

##### *Final results -*

Serological and cultural results at the time of slaughter were not available from these pigs.

- *Farm with endemic pomona infection [Farm B]*

Initial Serological and cultural results -

As illustrated in Figure 6.2 and shown in Table 6.4, eight of twenty pigs [40%] were seropositive in the first week, and the GMT was 1/21. In the fourth week, only two pigs [10%] [Pig numbers 41 and 49] had titres of 1/40 and 1/320, and in the seventh week none of the pigs had detectable titres. In the tenth week two of sixteen [13%] pigs had titres of 1/40,960 and 1/80, dramatically raising the GMT to 1/1,810.

Leptospire were not isolated from any of the urine samples collected from this group over the ten week period of examination.

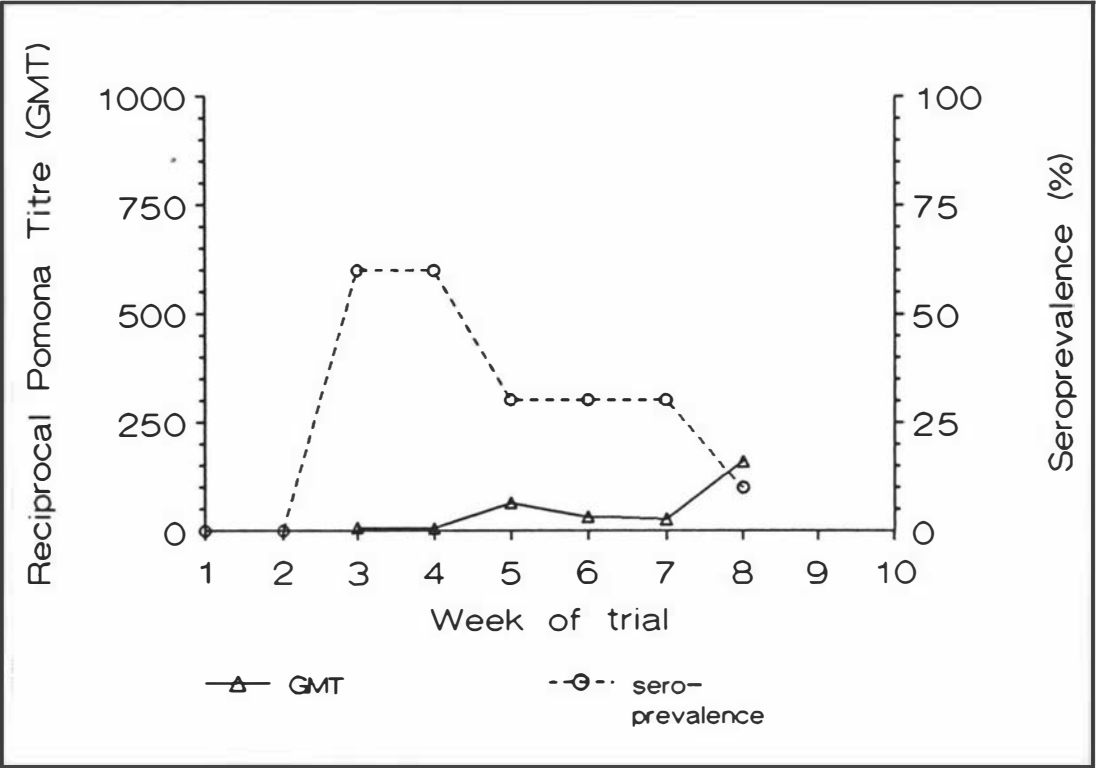
Final serological and cultural results -

Leptospire were cultured from the kidneys of seven of eight vaccinated pigs [88%] and from all five unvaccinated control pigs, as shown in Figure 6.9a and Table 6.4. Three vaccinated pigs [Pig numbers 43, 49 and 78] which had titres in either the fourth or the tenth week of the study subsequently had leptospire isolated from their kidneys. The kidneys from which leptospire were cultured, all came from pigs with high titres which ranged from 1/20480 to 1/327680. Leptospire were not isolated from the kidney of one vaccinated pig [Pig number 44] which had a titre of 1/2,560.

The titres and isolation rates of leptospire from the kidneys of vaccinated and unvaccinated pigs at slaughter did not differ significantly [in both cases  $P > 0.1$ ].



**Figure 6.1** Serological results of pigs raised on the farm free of leptospiral infection [Farm A], which were inoculated with vaccine in the first week only [Group 1].



**Figure 6.2** Serological results of pigs raised on the farm with endemic *pomona* infection [Farm B] which were inoculated with vaccine in the first week only [Group 1].

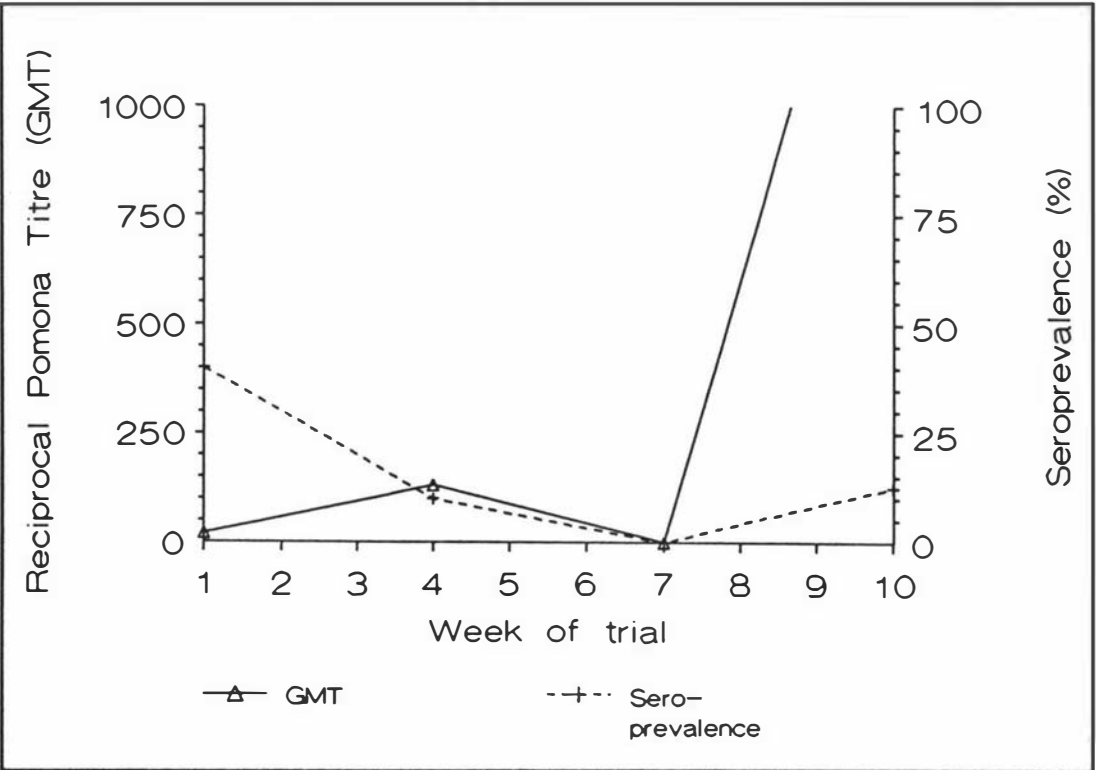


Table 6.3

Reciprocal titres of pigs inoculated in the first week [Group 1]  
which were from the farm free of leptospiral infection [Farm A].

Plg #	Week									
	1	2	3	4	5	6	7	8	9	10
1	0*	0	0	0	0	0	0	0	-**	-
2	0	0	0	0	0	0	0	0	-	-
3	0	0	0	4	0	4	0	0	-	-
4	0	0	0	16	8	128	160	160	-	-
5	0	0	4	0	0	0	0	-	-	-
6	0	0	4	0	0	0	0	-	-	-
7	0	0	4	4	0	0	0	-	-	-
8	0	0	4	4	256	40	16	-	-	-
9	0	0	128	8	128	0	16	-	-	-
10	0	0	4	4	0	0	0	-	-	-
GMT <sup>g</sup>	0	0	7	6	64	32	27	160	-	-
SP <sup>sp</sup>	0%	0%	60%	60%	30%	30%	30%	25%	-	-

\* = titre < 1/4  
\*\* - not examined

<sup>g</sup> = Reciprocal Geometric Mean Titre  
<sup>sp</sup> = Seroprevalence

**Table 6.4** Reciprocal titres and kidney culture results of pigs inoculated in the first week [Group 1] and unvaccinated pigs which were from the farm with endemic *pomona* infection [Farm B]

Pig #	Week					Kidney	Control	
	1	4	7	10	End		Titre	Kidney
41	0*	40	0	0	-.***	-	-	-
42	0	0**	0	0	-	-	-	-
43	0	0	0	40960	327680	+	-	-
44	0	0	0	0	2560	o	-	+
45	0	0	-	-	-	-	-	-
46	0	0	0	0	-	-	-	-
47	4	0	0	0	-	-	-	-
48	16	0	0	-	-	-	-	-
49	0	320	0	0	327680	+	-	-
50	32	0	0	-	-	-	-	-
71	256	0	0	-	-	-	-	-
72	16	0	0	0	-	-	-	-
73	0	0	0	0	-	-	-	-
74	0	0	0	0	20480	+	327680	+
75	0	0	0	0	327680	+	327680	+
76	16	0	0	0	-	-	-	-
77	16	0	0	0	327680	+	-	+
78	0	0	0	80	40960	+	327680	+
79	16	0	0	0	-	-	-	-
80	0	0	0	0	163840	+	-	-
GMT <sup>g</sup>	21	131	0	1810	89003		327680	
SP <sup>sp</sup>	40%	10%	0%	12.5%	100%		100%	

\* = Titre < 1/4  
 \*\* = Titre < 1/10  
 \*\*\* = not examined  
 o = Negative

g = Reciprocal Geometric Mean Titre  
 sp = Seroprevalence  
 + = Positive

***Inoculation with vaccine in the first and fifth week [Group 2]***

***- Leptospira free farm [Farm A]***

**Initial serological results -**

As shown on Table 6.5 and illustrated in Figure 6.3, in the first five weeks the titres of pigs did not rise above 1/16, and the seroprevalence was 30% or less. The titres of these pigs did not rise appreciably until after the second inoculation. In the sixth week, one week after vaccination, the titres of seven of the ten [70%] pigs had risen and the GMT was 1/1,723 and the titres ranged from 1/80 to 1/20,480. In the seventh week, the seroprevalence rose to 90%, and the GMT decreased to 1/806. The seroprevalence declined to 60% in the eighth week, increased to 70% in the ninth week and then decreased again to 50% in the tenth week. Meanwhile the GMT rose steadily from 1/719 in the eighth week to 1/1,114 in the tenth week as shown in Figure 6.3. The post vaccination titres of pigs were variable, and ranged from 1/40 to 1/163840, however after vaccination the titres of individual pigs tended to decrease [see Table 6.5].

**Final serological and cultural results -**

Two vaccinated pigs that were sampled at slaughter had titres of 1/5,120, however leptospires were not isolated from their kidneys. Neither were titres detected, nor leptospires isolated from two unvaccinated pigs at slaughter.

***- Farm with endemic pomona infection [Farm B]***

**Initial serological and cultural results -**

The serological results of this group of pigs are given in Table 6.6, and the seroprevalences and GMTs are illustrated in Figure 6.4. Seven of twenty pigs [35%] in the first week had titres ranging from 1/8 to 1/64, and the GMT was 1/20. The seroprevalence fell to zero in the fourth and fifth week. As on the farm which was free of leptospiral infection, the titres of pigs increased after the second inoculation. In the sixth week, the seroprevalence rose to 70%, the GMT was 1/131, and the titres ranged from 1/40 to 1/640. Thereafter, the seroprevalence decreased to 65% in the eighth week and to 28% in the tenth

week. The GMTs of pigs in the eighth and tenth weeks were lower than in the sixth week, and were 1/21 and 1/62 respectively.

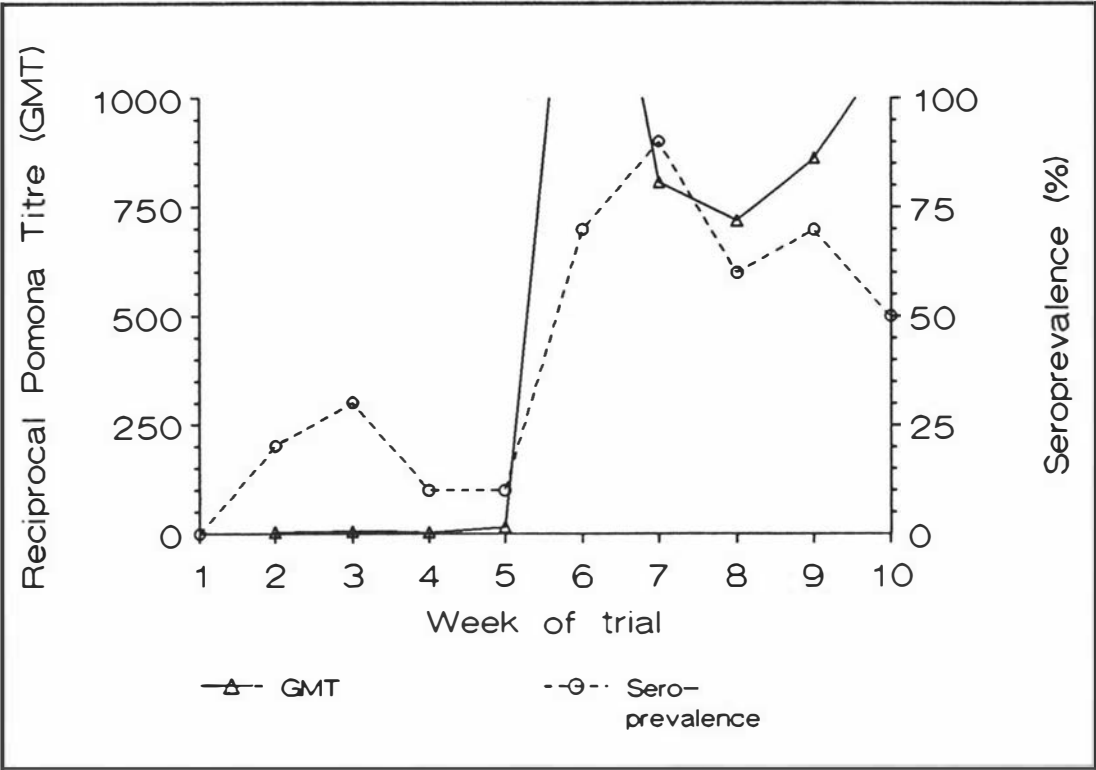
Leptospire were not cultured from the urine of pigs inoculated twice with vaccine [Group 2] over the ten week period of examination.

**Final serological and cultural results -**

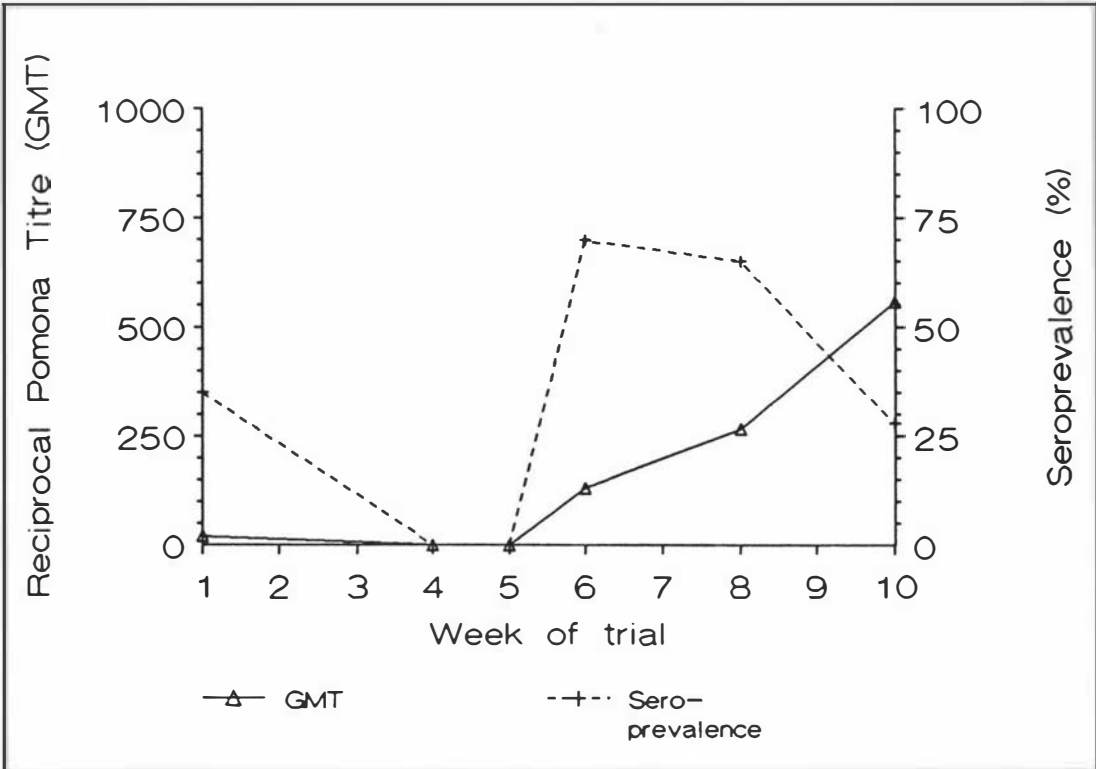
As shown in Table 6.6, the GMTs of the vaccinated and unvaccinated pigs were 1/5,120 and 1/163,840 respectively. The titres of vaccinated pigs and unvaccinated pigs at the time of slaughter ranged from 1/10 to 1/81,920 and from less than 1/10 to 1/327,680 respectively, however the two groups were not significantly different [ $P > 0.13$ ].

Leptospire were cultured from one of six vaccinated pigs' kidneys and from four of five unvaccinated pigs' kidneys, see Figure 6.9b. The isolation rates for the vaccinated and unvaccinated pigs were significantly different [ $P < 0.08$ ].

**Figure 6.3** Serological results of pigs raised on the farm free of leptospiral infection [Farm A] which were inoculated with vaccine in the first and fifth week [Group 2].



**Figure 6.4** Serological results of pigs raised the farm with endemic *pomona* infection [Farm B] which were inoculated with vaccine in the first and fifth week [Group 2].



**Table 6.5**      **Reciprocal titres of pigs inoculated in the first and fifth week [Group 2]**  
**which were from the farm free of leptospiral infection [Farm A].**

Pig #	Week									
	1	2	3	4	5	6	7	8	9	10
11	0*	0	0	0	0	0	40	0	0	0
12	0	0	4	0	0	0	0	0	0	0
13	0	0	0	0	0	0	80	0	0	0
14	0	0	0	4	16	20480	163840	81920	40960	20480
15	0	0	16	0	0	20480	40960	5120	5120	5120
16	0	0	0	0	0	320	40	80	80	0
17	0	0	0	0	0	2560	1280	80	640	320
18	0	4	4	0	0	5120	10240	320	1280	640
19	0	4	0	0	0	80	80	0	40	0
20	0	0	0	0	0	320	160	160	640	80
GMT <sup>g</sup>	0	4	6	4	16	1723	806	719	862	1114
SP <sup>sp</sup>	0%	20%	30%	10%	10%	70%	90%	60%	70%	50%

\* = titre < 1/4  
 \*\* = not examined

<sup>g</sup> = Reciprocal Geometric Mean Titre  
<sup>sp</sup> = Seroprevalence

**Table 6.6 Reciprocal titres and kidney culture results of pigs inoculated in the first and fifth week [Group 2] and unvaccinated pigs which were from the farm with endemic *pomona* infection [Farm B]**

Plg #	Week							Kidney	Control	
	1	4	5	6	8	10	End		Titre	Kidney
61	0*	0	***	320	0	0	81920	o	0	o
62	0	0	-	640	160	0	-	-	-	-
63	0	0	-	80	2560	0	-	-	-	-
64	16	0	-	0**	160	0	-	-	-	-
65	0	0	-	320	-	0	-	-	-	-
66	0	0	-	40	0	0	-	-	-	-
67	0	0	-	40	160	0	-	-	-	-
68	0	0	-	0	-	0	-	-	-	-
69	0	0	-	80	40	0	-	-	-	-
70	16	0	-	0	0	0	-	-	-	-
51	64	-	0	-	0	40960	-	-	-	-
52	0	-	0	-	5120	640	20480	o	-	-
53	0	-	0	-	0	0	-	-	-	-
54	16	-	0	-	160	160	2560	o	32780	+
55	16	-	0	-	320	80	5120	o	32780	+
56	8	-	-	-	-	-	-	-	-	-
57	0	-	0	-	0	0	-	-	-	-
58	0	-	0	-	320	160	81920	+	32780	+
59	32	-	0	-	80	0	-	-	-	-
60	0	-	0	-	160	-	10	o	20480	+
GMT <sup>g</sup>	20	0	0	131	265	557	5120		163840	
SP <sup>sp</sup>	35%	0%	0%	70%	65%	28%	100%		80%	

\* = Titre < 1/4  
 \*\* = Titre < 1/10  
 \*\*\* = not examined  
 + = Positive  
 g = Reciprocal Geometric Mean Titre  
 sp = Seroprevalence  
 o = Negative



***Inoculation with vaccine in the first, third and fifth week [Group 3]***

***- Farm free of leptospiral infection [Farm A]***

**Initial serological results -**

The titres of this group of pigs are shown in Table 6.7 and Figure 6.5 illustrates the seroprevalences and GMTs. For the first three weeks, the titres of these pigs did not rise above 1/4, however the seroprevalence reached 50% in the third week. In the fourth week, one week after the second inoculation, the seroprevalence and GMT rose to 90% and 1/87 respectively. The seroprevalence remained at 90% for the fifth and sixth week, and in the seventh and eighth week it rose to 100%, and then decreased to 80% in the ninth and tenth week. Meanwhile, the GMT of pigs in the sixth week, one week after the third inoculation, increased to 1/338. Thereafter, the GMTs steadily decreased to 1/80 by the tenth week.

**Final serological and cultural results -**

Leptospire were not cultured from either the kidneys of the three vaccinated pigs or from those of the three unvaccinated control pigs. Two of the three vaccinated pigs had titres of 1/80 and 1/40 at slaughter, the remaining pigs did not have detectable titres.

***- Farm with endemic pomona infection [Farm B]***

**Initial serological and cultural results -**

The serological and cultural results of pigs in this groups are shown in Table 6.8, and the serological results are illustrated in Figure 6.6. Nine of twenty pigs [45%] were seropositive in the first week, the GMT was 1/15, and their titres ranged from 1/4 to 1/128. In the fourth week, one week after the second inoculation the seroprevalence had risen to 65% and the GMT had increased to 1/245. The seroprevalence and GMT changed little between the fourth and tenth week, as shown in Figure 6.6. The seroprevalences and GMTs in the seventh week were 58% and 1/206, and in the tenth week were 61% and 1/320 respectively.

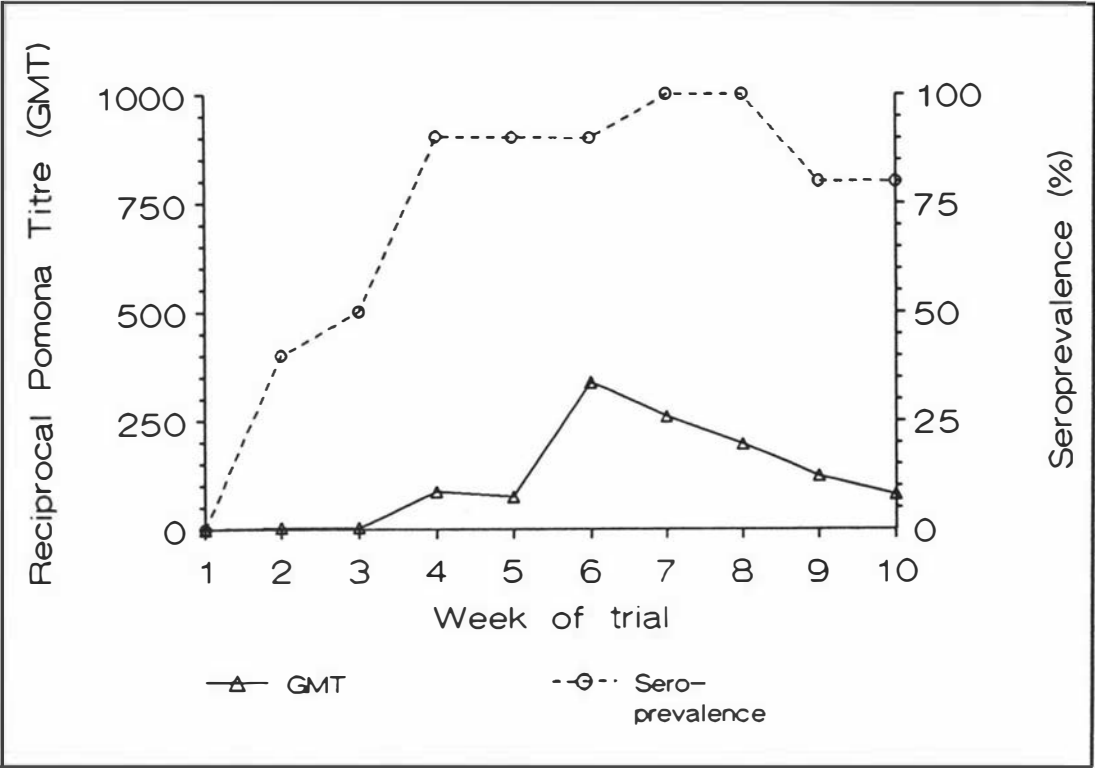
Eight of thirteen pigs which had titres in the third week did not have titres in the first week, while the remaining five of thirteen pigs had titres in both the first and the third week. The GMT of pigs in the third week which did not have titres in the first week was 1/640, and was significantly higher than the GMT of 1/53 of the pigs which had titres in both the first and third weeks [ $P < 0.003$ ]

Throughout the period of examination, leptospires were cultured from the urine of only one vaccinated pig [Pig number 98, see Table 6.8]. This pig was also the only vaccinated pig to develop and maintain a titre as high as 1/327,680.

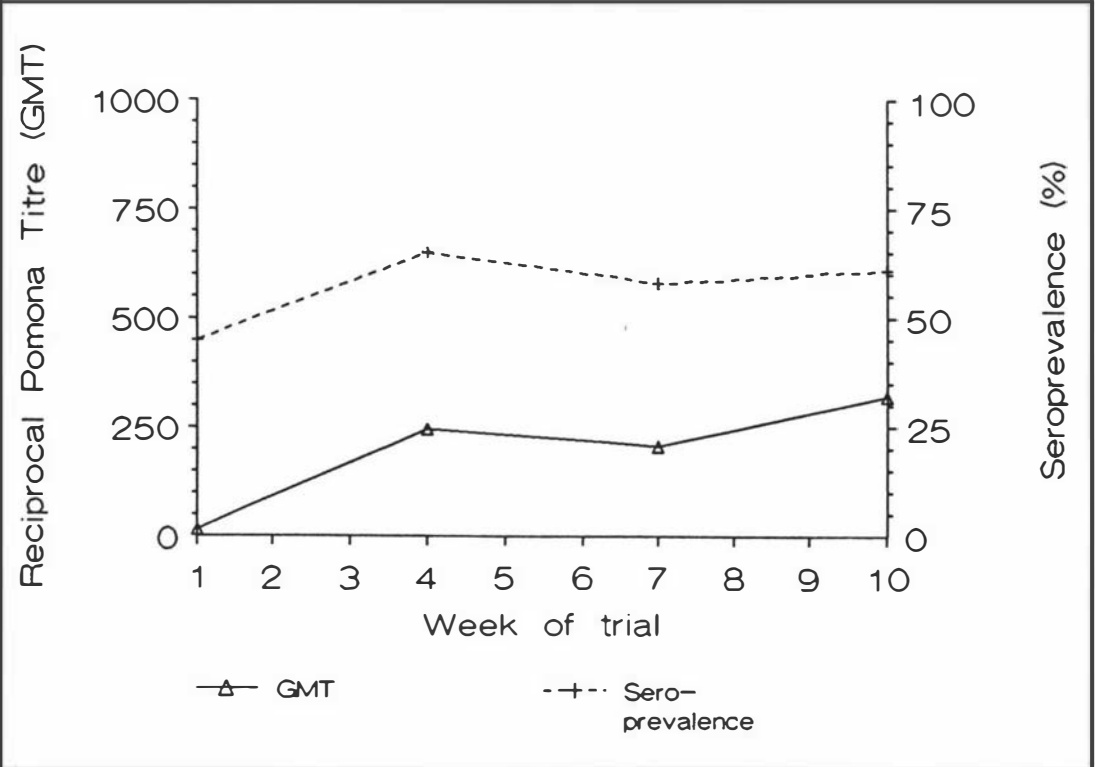
#### Final serological and cultural results -

Leptospires were cultured from the kidneys of three of fourteen [21%] vaccinated pigs, and from ten of thirteen [77%] unvaccinated pigs, as shown in Figure 6.9c and Table 6.8. Vaccinated pigs had a significantly lower prevalence of infection than unvaccinated control pigs [ $P < 0.003$ ]. At the time of slaughter, the GMT of vaccinated pigs was 1/3,978 and less than the GMT of unvaccinated pigs which was 1/68,886 [ $P < 0.02$ ]. The GMT of vaccinated pigs which were not apparently infected was 1/1,396 [ $n = 8$ ] and less than the GMT of 1/65,170 [ $n = 3$ ] of vaccinated pigs which were infected [ $P < 0.07$ ].

**Figure 6.5** Serological results of pigs raised on the farm free of leptospiral infection [Farm A] which were inoculated with vaccine in the first, third and fifth week [Group 3].



**Figure 6.6** Serological results of pigs raised on the farm with endemic *pomona* infection [Farm B] which were inoculated with vaccine in the first, third and fifth week [Group 2].



**Table 6.7**      **Reciprocal titres of plgs inoculated in the first, third and fifth week [Group 3]  
which were from the farm free of leptospiral infection [Farm A].**

Plg #	Week									
	1	2	3	4	5	6	7	8	9	10
21	0*	0	0	64	32	64	40	40	0	0
22	0	0	4	16	32	256	1280	2560	640	160
23	0	0	4	0	4	256	160	160	160	40
24	0	0	4	256	256	256	320	160	160	40
25	0	0	4	256	256	2560	1280	320	160	160
26	0	4	0	256	256	40	40	40	0	0
27	0	4	4	256	64	640	320	160	40	80
28	0	0	0	64	64	2560	2560	320	40	40
29	0	4	0	4	0	0	40	160	40	40
30	0	4	0	256	256	320	320	320	320	320
GMT <sup>g</sup>	0	4	4	87	75	338	260	197	123	80
SP <sup>sp</sup>	0%	40%	50%	90%	90%	9%	100%	100%	80%	80%

\* = titre < 1/4  
 \*\* = not examined

<sup>g</sup> = Reciprocal Geometric Mean Titre  
<sup>sp</sup> = Seroprevalence

**Table 6.8 Reciprocal titres and kidney culture results of pig inoculated in the first, third and fifth week [Group 3] and unvaccinated pigs which were from the farm with endemic *poona* infection [Farm B]**

Pig #	Week					Kidney	Control	
	1	4	7	10	End		Titre	Kidney
81	4	40	160	0	0	o	80	o
82	0*	0**	0	0	0	o	327680	+
83	0	320	0	0	2560	+	1280	o
84	0	1280	12780	320	-	-	-	-
85	4	40	40	40	0	o	0	o
86	0	2560	40	40	5120	o	327680	+
87	0	5120	0	0	5120	o	327680	+
88	0	80	0	-	-	-	-	-
89	4	80	0	0	-	-	-	-
90	0	640	40	0	80	o	2560	+
91	64	0	***	-	-	-	-	-
92	4	80	80	320	320	o	327680	+
93	0	0	320	80	-	-	-	-
94	32	0	0	0	327680	+	327680	+
95	0	1280	5120	1280	320	o	327680	+
96	0	80	160	10240	20480	o	327680	+
97	16	40	40	40	18920	o	327680	+
98	32	0	2560	327680	327680	+ <sup>LU</sup>	-	-
99	0	0	0	40	-	-	-	-
100	128	0	0	40	40	o	327680	+
<hr/>								
GMT <sup>g</sup>	15	245	206	206	320	3978	68886	
SP <sup>sp</sup>	45%	65%	58%	58%	61%	78%	92%	

*	=	Titre < 1/4	g	=	Reciprocal Geometric Mean Titre
**	=	Titre < 1/10	sp	=	Seroprevalence
***	=	not examined	LU	=	Leptospirosis: Week 10
+	=	positive	o	=	negative

***Inoculation with vaccine in the first, second and third week [Group 4]***

***- Farm free of leptospiral infection [Farm A]***

**Initial serological results -**

The titres of the pigs in this group are given in Table 6.9, and illustrated in Figure 6.7. Nine of the ten pigs did not have detectable titres for the first two weeks. In the third week, one week after the second inoculation, eight of the ten pigs had titres, with a GMT of 1/15. In the fourth week, one week after all inoculations, the seroprevalence reached 100% and the GMT was 1/208, and there was little variation between the titres of pigs. All pigs remained seropositive in the fifth week, however the GMT decreased to 1/91 and the range of titres increased. Thereafter, the seroprevalence steadily declined to 50% by the eighth week, and the GMTs remained reasonably constant at around 1/110 after a low of 1/46 in the sixth week, as shown in Figure 6.7.

**Final serological and cultural results -**

Leptospire were not isolated from any of the kidneys nor were agglutinating antibodies detected at a minimum dilution of 1/10 from three vaccinated pigs and three unvaccinated control pigs at the time of slaughter.

***- Farm with endemic pomona infection [Farm B]***

**Initial serological and cultural results -**

The serological and cultural results of pigs from this group are shown in Table 6.10, and the seroprevalences and GMTs are illustrated in Figure 6.8. In the first week, seven of the twenty pigs [35%] had titres which ranged from 1/4 to 1/128, and the GMT was 1/15. The seroprevalence increased to 55% in the third week, one week after the second inoculation and thereafter remained around this level, except in the tenth week when it rose to 72%, as shown in Figure 6.8. Meanwhile, the GMT of the third week increased to 1/530 and thereafter, until the tenth week the GMT fluctuated between 1/530 and around 1/350.

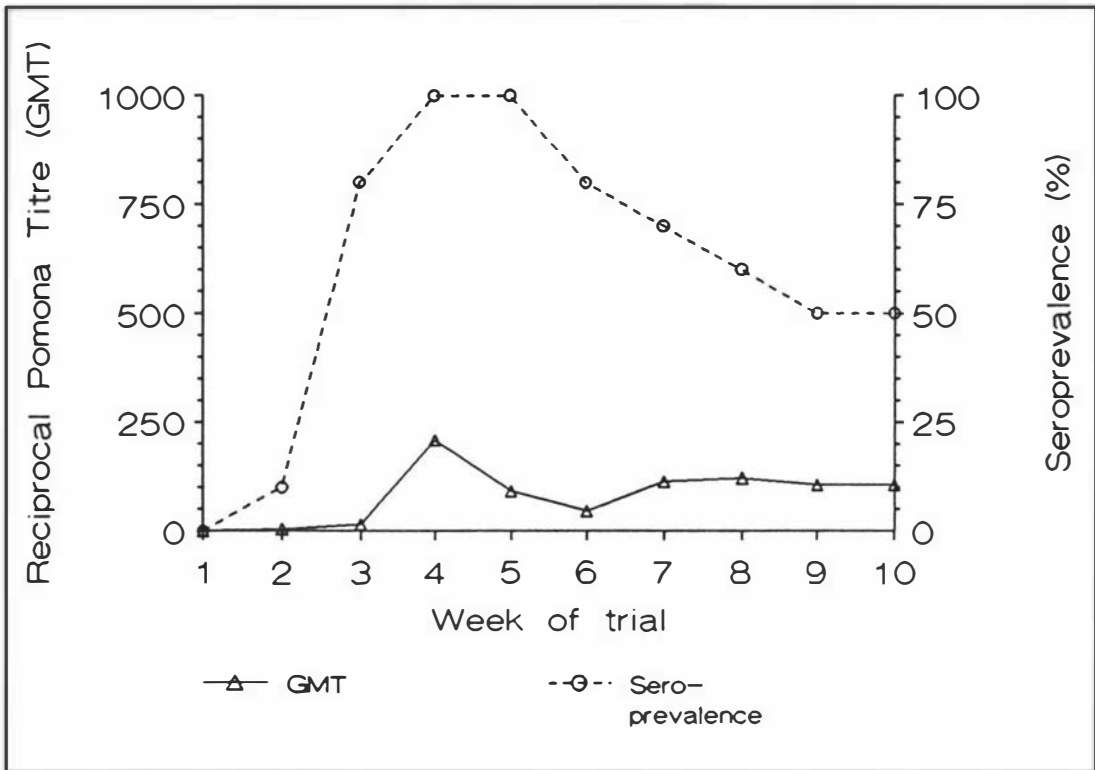
There was no significant difference [ $P > 0.3$ ] between the GMT of pigs in the third week of examination which had either detectable or undetectable titres in the first week of examination. The GMT of pigs in the third week, which had undetectable titres in the first week was  $1/862$  [ $n = 7$ ] and the GMT of pigs which were seropositive in the first and third week was  $1/243$  [ $n = 5$ ].

Leptospire were isolated from six samples of urine originating from three pigs taken at different times throughout the ten week period of sampling [see Table 6.10]. Leptospire were isolated from Pig number 114 in the fifth and seventh week, its maximum titre of  $1/327,680$  was recorded in the tenth week and again at the time of slaughter. Pig number 117 was leptospiruric in the tenth week, and its' highest titre was  $1/20\ 480$  which occurred in the seventh week. Pig number 119 was leptospiruric in the seventh and tenth week and had a titre of only  $1/640$  and  $1/320$  in the seventh and tenth week.

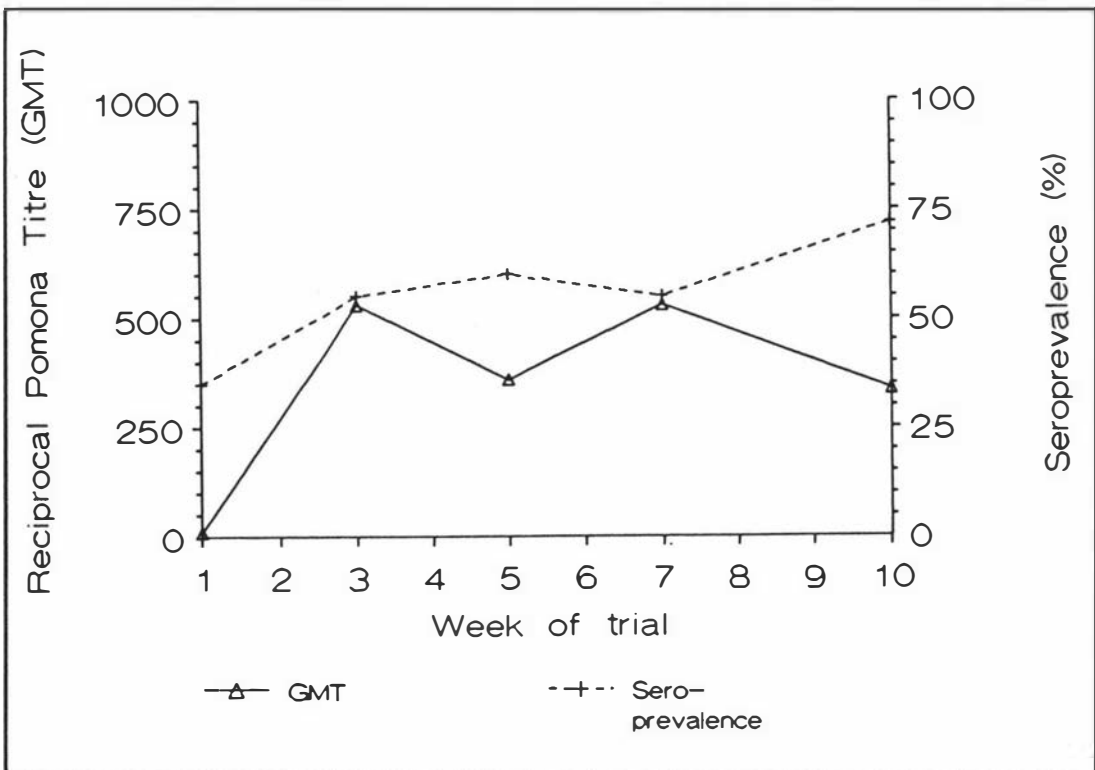
#### Final serological and cultural results -

As shown in Table 6.10 and Figure 6.9d leptospire were isolated from the kidneys of three of thirteen [23%] vaccinated pigs and from ten of eleven [91%] unvaccinated control pigs. The prevalence of infection in vaccinated pigs is significantly lower [ $P < 0.001$ ] than in unvaccinated pigs. The seroprevalence of vaccinated pigs at slaughter was 85%. The GMT of vaccinated pigs at slaughter was  $1/6,174$  and lower [ $P < 0.003$ ] than GMT of  $1/307,669$  from the eleven unvaccinated control pigs. Leptospire were not isolated from the kidneys of two vaccinated pigs [Pig numbers 110 and 115] both of which had undetectable titres throughout the period of examination at the farm, and at the time of slaughter.

**Figure 6.7** Serological results of pigs raised on the farm free of leptospiral infection [Farm A] which were inoculated with vaccine in the first, second and third week [Group 4].



**Figure 6.8** Serological results of pigs raised on the farm with endemic *pomona* infection [Farm B] which were inoculated with vaccine in the first, second and third week [Group 4].





**Table 6.9**      **Reciprocal titres of plgs inoculated in the first, third and fifth week [Group 3]  
which were from the farm free of leptospiral infection [Farm A].**

Plg #	Week									
	1	2	3	4	5	6	7	8	9	10
31	0*	0	4	256	256	0	40	80	80	40
32	0	0	4	256	64	0	0**	0	0	0
33	0	0	0	256	128	4	0	0	0	0
34	0	0	4	256	256	128	80	320	320	160
35	0	4	16	256	64	40	80	80	80	160
36	0	0	16	256	128	40	80	0	0	0
37	0	0	32	256	256	320	640	320	160	160
38	0	0	128	256	32	40	0	0	0	0
39	0	0	0	128	8	0	0	0	0	0
40	16	0	32	64	128	40	160	40	40	80
GMT <sup>g</sup>	0	4	15	208	91	46	113	121	106	106
SP <sup>sp</sup>	0%	10%	80%	100%	100%	70%	60%	50%	50%	50%

\* = titre < 1/4  
 \*\* = not examined

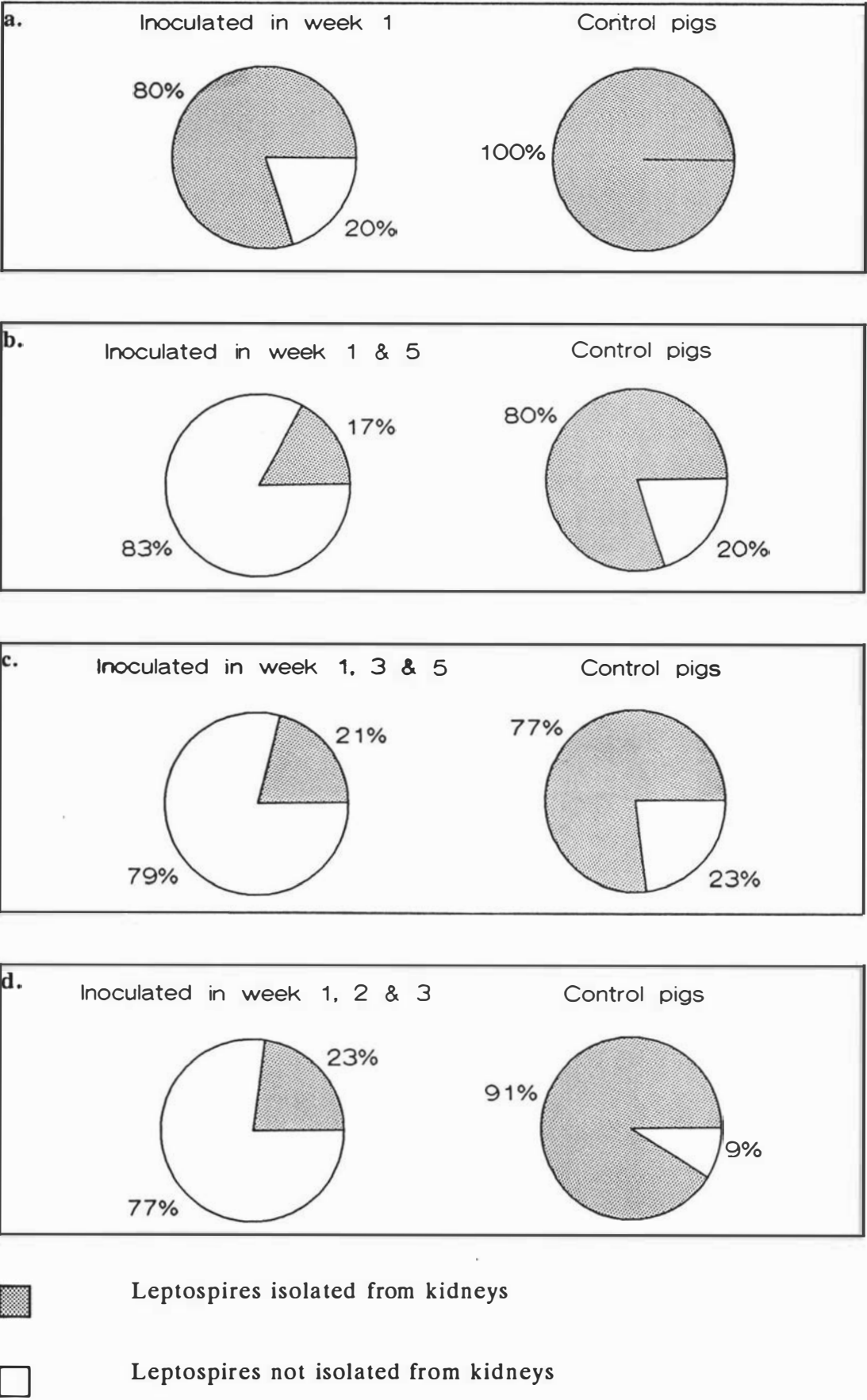
<sup>g</sup> = Reciprocal Geometric Mean Titre  
<sup>sp</sup> = Seroprevalence

**Table 6.10 Reciprocal titres and kidney culture results of pigs inoculated in the first, second and third week [Group 4] and unvaccinated pigs which were from the farm with endemic *pomona* infection [Farm B]**

Plg#	week					End	Kidney	Titre	Kidney
	1	3	5	7	10				
101	4	40	0	0	0	10240	o	327680	+
102	0*	320	0	80	160	320	o	163840	o
103	4	1280	10240	5120	2560	-	-	-	-
104	4	40	40	80	160	-	-	-	-
105	8	40	0	0	****	320	o	327680	+
106	32	10240	160	0	0	-	-	-	-
107	4	0**	0	0	160	160	o	327680	+
108	0	0	80	0	-	-	-	-	-
109	0	160	640	40	80	-	-	-	-
110	0	0	0	0	0	0	o	327680	+
111	0	0	160	80	80	160	-	-	-
112	128	0	0	0	0	327680	o	-	+
113	0	320	80	40	40	327680	o	327680	+
114	0	2560	2560	5120	32768	327680	+LU1	327680	+
115	0	0	0	0	0	0	o	327680	+
116	0	80	160	0	40	40	o	327680	+
117	0	0	0	20480	5120	40960	+LU2	327680	+
118	0	0	40	10240	320	-	-	-	-
119	0	10240	2560	320	40	10240	+LU3	327680	+
120	0	10240	2560	640	320	5120	o	-	-
GMT <sup>g</sup>	10	530	359	530	338	6174		307669	
SP <sup>sp</sup>	35%	55%	60%	55%	72%	85%		100%	

*	=	Titre < 1/4	g	=	Reciprocal Geometric Mean Titre
**	=	Titre < 1/10	sp	=	Seroprevalence
***	=	not examined	LU1	=	Leptospirosis: Weeks 5 and 7
LU2	=	Leptospirosis: Week 10	LU3	=	Leptospirosis: Weeks 7 and 10
+	=	Positive	o	=	Negative

**Figure 6.9a - d**                      **The proportion of kidneys from vaccinated and unvaccinated pigs from which leptospires were isolated.**



*Other observations*

It was observed that it took more time for leptospires to be demonstrated in cultures of urine and kidneys from vaccinated pigs than from the cultures of unvaccinated pigs. Miscellaneous urine samples were collected from unvaccinated pigs raised on the farm with endemic *poona* infection throughout the period of sampling. Leptospires were not isolated from these pigs in the first weeks of sampling, however leptospires were cultured from these pigs' urine in the later weeks of the trial.

## DISCUSSION

It has been demonstrated from the results of this study that grower pigs which received at least two inoculations of a commercial vaccine containing a *pomona/tarassovi* bacterin, resulted in increased levels of agglutinating antibodies to serovar *pomona* and gave protection from natural infection due to serovar *pomona*.

The use of urine culture as a method of determining the time at which vaccinated pigs were becoming infected on the farm with endemic *pomona* infection was of limited use as the majority of pigs did not appear to become infected until after the selected ten week period of examination. The period over which the collection of urine took place may therefore have been more informative had it occurred in the ten weeks prior to slaughter, rather than the ten weeks following commencement of the trial. In spite of the fact that vaccinated and unvaccinated pigs were penned together, the majority of vaccinated pigs resisted infection even when there was both direct and indirect exposure to leptospires. At the commencement of this trial it was anticipated that the pigs would be sent for slaughter between 22 and 24 weeks of age. However, the majority of the pigs were not sent for slaughter until they were between 26 and 30 weeks of age. This resulted in the prolonged period between collection of the last urine samples and collection of kidneys at slaughter. Leptospirae were isolated from the kidneys of all vaccinated pigs which had been leptospiruric during the ten week period of examination. It would therefore appear that the duration of leptospiruria in vaccinated animals was not shortened. It was observed that it took longer for leptospirae to grow in the cultures prepared from both kidney tissue and urine of vaccinated pigs compared with those of unvaccinated pigs. This may indicate as Hodges *et al* (1985) suggested, that the intensity of leptospiruria and severity of renal colonisation are reduced in vaccinated pigs.

At slaughter, the cultural examination of kidneys was the most definitive means of determining the infection status of pigs. The prevalence of infection at slaughter was significantly reduced in pigs which had been inoculated either two or three times. The degree of significance by which the cultural prevalence

had been reduced varied between those three groups. The double vaccinated group [Group 2] had the lowest prevalence of infected pigs at slaughter [one out of six]. This group had the smallest sample size and as a consequence the results were less significant [ $P < 0.08$ ] than the results of pigs in Groups 3 and 4 [ $P < 0.003$ ] even though the prevalence of infection in pigs of these two groups was higher than that of pigs in Group 2. As the prevalence of infection in vaccinated pigs was significantly reduced, it is reasonable to assume that it was indeed the effect of vaccination which brought about the reduction in prevalence. Knowledge of the prevalence of infection in both control and vaccinated groups allows the apparent efficacy of each vaccinal regime to be calculated by firstly determining the relative risk of infection for the vaccinated pigs in each group. A risk factor of less than one indicates that the vaccination regime reduces the risk of infection. The relative risk of infection for vaccinated pigs in this trial has been calculated relative to that for unvaccinated control pigs where the overall prevalence at slaughter was 85% [29/34]. The relative risk for pigs inoculated only once is 1.0 [87/85] indicating that a single inoculation had no effect on the prevalence of infection in these pigs. The relative risk for the remaining three groups are 0.20 for pigs inoculated twice [Group 2], 0.25 for pigs inoculated three times at two weekly intervals [Group 3], and 0.27 for pigs inoculated three times at weekly intervals [Group 4]. Perhaps it would be more meaningful to measure the efficacy of vaccination as the relative risk of infection for unvaccinated animals compared with vaccinated animals. In this case the efficacy of the vaccination regimes 2, 3, and 4 are 5.0, 4.0, and 3.7 respectively. These results therefore suggest that the most efficacious is the regime consisting of two inoculations, four weeks apart, thus indicating that unvaccinated pigs are five times more likely to become infected than pigs vaccinated by that regime. However, administration of either of the triple inoculation regimes also effectively reduce the risk of infection by around four times. In calculating the relative risk, no account of sample size is incorporated into the final result. Therefore, when judging the most appropriate vaccination regime, one must also consider the results of statistical analyses, relative risk or vaccinal efficacy, and the farm management procedures where vaccination is to be practised.

A detailed profile of the serological response of pigs to the various regimes of inoculation, in the absence of both maternal antibody titres and exposure to infection was obtained by the weekly serological examination of forty pigs which originated from the farm free of endemic *porcine* infection [Farm A]. Thirty nine of the forty pigs from the farm free of leptospiral infection did not have detectable titres in the first week, and one pig [Pig number 40] had a titre of 1/16. The titres of pigs on the farm with endemic leptospiral infection [Farm B] were initially higher than the titres of pigs from the farm free of leptospiral infection [Farm A]. It has been assumed that the titres detected in pigs in the first week of the trial, prior to any inoculations, were titres of maternally derived antibody. The reasons for this assumption are as follows. Maternal antibody has been estimated to have a half life of approximately sixteen days [see Chapter Four]. In a population of pigs with endemic leptospiral infection, it was estimated [see Figure 6.10] that 35% of ten week old pigs would have a persistent maternal antibody titre of 1/10 or greater. In the first week of the trial, the four groups of pigs from the farm with endemic leptospiral infection [Farm B] had seroprevalences of 40%, 35%, 45% and 35% and GMT's of 1/21, 1/20, 1/15 and 1/10 respectively. The seroprevalence of pigs given a single inoculation [Group 1] fell to zero by the seventh week, and the seroprevalence of pigs inoculated twice [Group 2] also fell to zero by the fifth week. The declining titres of pigs before they received a second inoculation, also therefore supports the hypothesis that titres detected in the first week were residual maternal antibody titres.

It was noted that some pigs which had maternal antibody titres in the first week subsequently had lower post inoculation titres than pigs which did not have maternal antibody titres in the first week. This phenomenon was statistically significant [ $P < 0.003$ ] in pigs which had received two of three inoculations at two weekly intervals [Group 3]. This statistical association possibly represents a direct cause and effect relationship, in which the presence of maternal antibody in some way neutralises the injected bacterin, reducing its effectiveness for initiating antigenic stimulation. Hodges (1977), Hodges *et al* (1976; 1985) and Palit *et al* (1988) only used grower pigs which at the commencement of their vaccination trials had undetectable titres at a minimum dilution of 1/10, thereby eliminating the possible influence of maternal antibody

from their results. The influence of maternal antibody on vaccine efficacy has been investigated with respect to the vaccination of puppies and kittens for the prevention of infectious viral diseases. The presence of maternal antibody has been found to interfere with the efficacy of inactivated vaccines for the prevention of canine adenovirus and canine parvovirus infection in puppies, and feline panleukopenia infection in kittens (Carmichael *et al*, 1983; Pollock and Carmichael, 1982; Scott *et al*, 1970). Scott *et al* (1970) reported that the presence of maternally derived antibody in kittens prevented the development of an immune response following the administration of inactivated [and live] vaccine. Subsequent exposure of the kittens to the panleukopenia virus resulted in their infection. Scott *et al* (1970) concluded that any amount of passive immunity was sufficient to interfere with the response to vaccination.

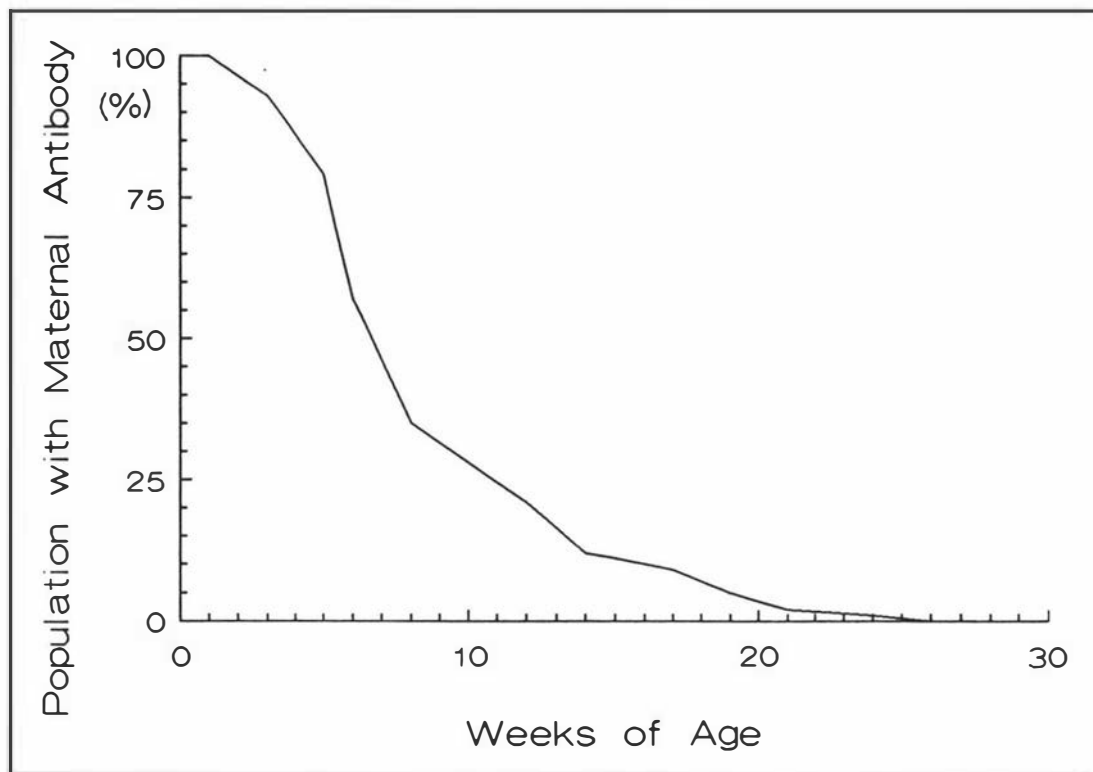
While Pollock and Carmichael (1982) concluded that vaccination could not effectively commence until after puppies were susceptible to canine parvovirus infection, unless multiple vaccinations were given beyond the duration of maternal antibody. For optimal results vaccination should be delayed until the titre of maternal antibody has declined to near zero, however this results in a "window of susceptibility" especially under conditions of crowding and in a highly contaminated environment (Fenner *et al*, 1986). Interference with the efficacy of bacterial vaccines is not well documented but has been implicated as a possible reason for the poor performance of vaccination regimes in which young calves are vaccinated.

The distribution of declining maternal antibody titres in a population of grower pigs from a pig herd with endemic leptospiral infection, based on the distribution of titres in breeding pigs (Ryan 1978) is shown in Figure 6.10. The age at which a pig can first be vaccinated should be based on its' dams titre at the time of farrowing [see Figure 6.11]. Therefore the optimum age at which pigs are first inoculated will be a compromise between the level of waned maternal antibody, the age of exposure to infection, and farming practices.

Consequently, on a farm with endemic leptospiral infection, vaccination has only a few weeks in which its effect can be maximised, as shown Figure 6.12. This is in contrast to semi-intensive farming of dairy cattle where the cycle of production is annual, and the time between waned maternal antibody and exposure to infection is usually a period of months.



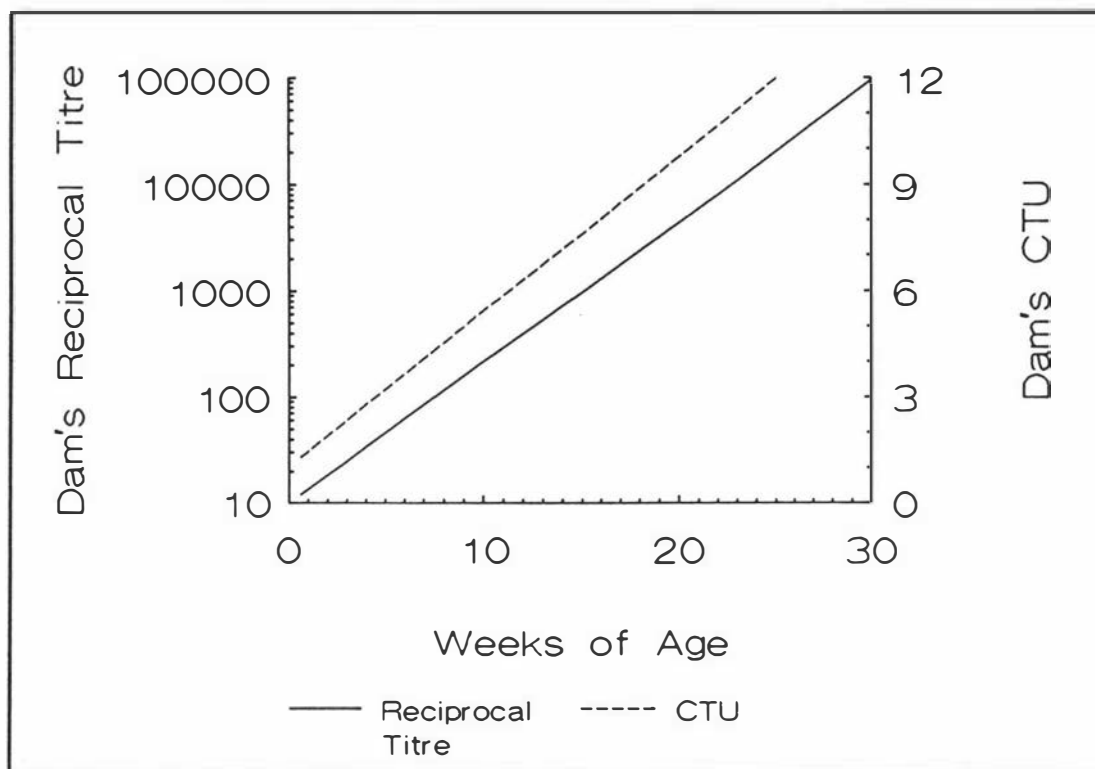
Figure 6.10 The percentage of pigs in a population with residual maternal antibody.



Titres of low intensity and duration occurred in some of the pigs which had received only a single inoculation of bacterin. A similar finding has been reported following the administration of a single inoculum of bacterin to cattle, in which most post vaccination titres of 1/24 diminished in less than four weeks, and a few titres of 1/48 and 1/96 persisted for longer (Hanson *et al*, 1964). The titres of pigs in this trial, which diminished to undetectable levels in less than seven weeks after the inoculation, did not protect these pigs from natural infection. Pigs which received a single inoculum, and which did not develop detectable titres also became infected. Hodges *et al* (1976) also reported that pigs which did not have detectable titres following vaccination and prior to exposure to natural infection, subsequently became infected.

Dobson and Davos (1975) reported that the agglutinin titres of vaccinated pigs increased one to two weeks after vaccination. Similarly, pigs in this investigation which received two or three inoculations showed increased titres one to two weeks after a booster inoculation. Post vaccination titres were present in the majority of pigs which had received at least two inoculations of the bacterin, and generally, these pigs were protected from natural infection.

**Figure 6.11** Nomograph indicating optimum times for the commencement of vaccination of pigs with a *porona* vaccine, based on the antibody titre of the dam and a half life of sixteen days.

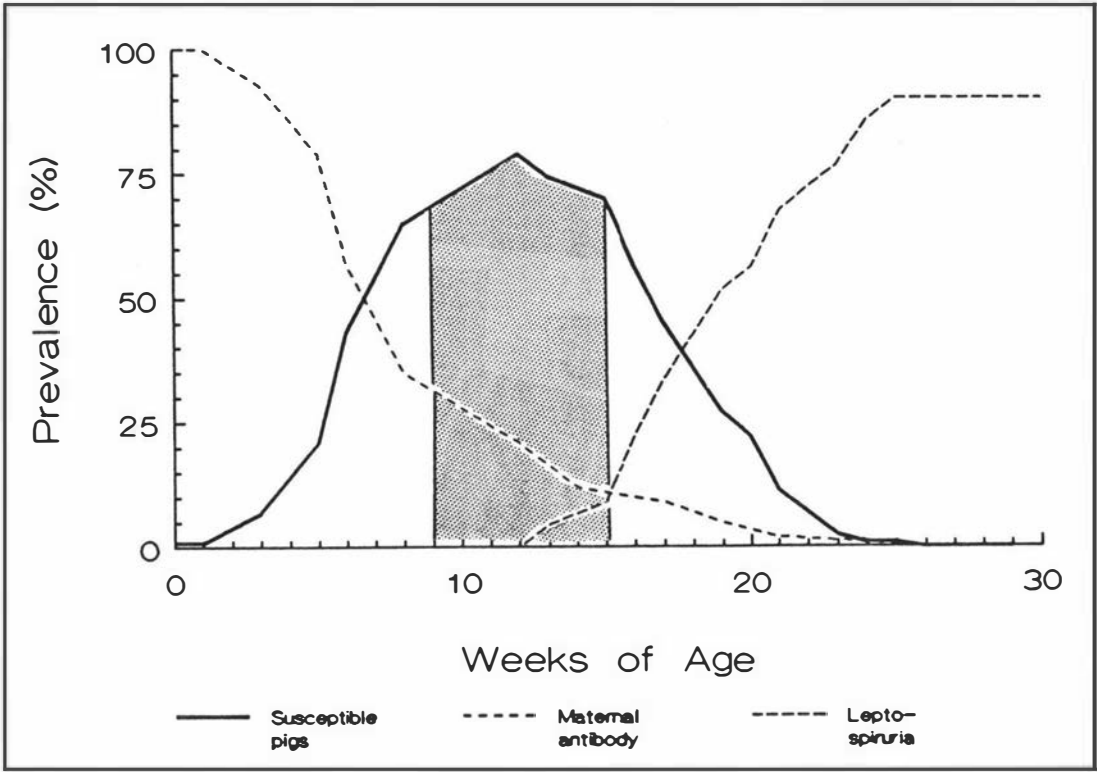


The results of this trial suggest that the agglutinating antibody titres following a second or third inoculation with bacterin are indicative of the presence of protective immunity. Hodges (1977) also reported that all pigs which developed detectable titres after vaccination, and before exposure to natural infection, were protected from infection. It was suggested by Hodges (1977) that the development of agglutination titres following the inoculation of a bacterin, and prior to exposure, were indicators of adequate antigenic stimulation.

The triple inoculation regime given at weekly intervals [Group 4] resulted in the most rapid development of post vaccination titres. These titres appeared in pigs from the third week of vaccination onwards, thus when pigs were thirteen weeks of age or older. Post vaccination titres in pigs inoculated three times but given at two weekly intervals [Group 3] clearly showed an increase following the second and third inoculations. This suggested that a double

inoculation alone, at two a week interval was sufficient to stimulate the production of agglutinating antibody, indicating antigenic stimulation and a level of resistance to infection, but a third inoculation clearly demonstrated a further serological response. It is possible that a third inoculation might increase the level of resistance to infection, above and beyond that which occurs following only two inoculations, this especially may be the case when maternal antibody was present at the first inoculation.

**Figure 6.12** The "windows of susceptibility" for a population of pigs, indicating the optimum age range for the commencement of vaccination.



Generally the titres of individual vaccinated pigs varied. However, it does appear that an absence of detectable titres in vaccinated pigs is an indication that such a pig is susceptible to infection. Vaccinated pigs which subsequently became infected also developed titres and these were similar in magnitude to those of unvaccinated pigs which became infected. In spite of these two observations, the use of the MAT as the sole determinant of the infection status of individual vaccinated pigs is not recommended by this author.

At slaughter, the distribution of titres in pigs which had received a single inoculation was similar to that of unvaccinated pigs [see Figure 6.13]. Titres of the unvaccinated control pigs fell into three areas of distribution. The majority of unvaccinated pigs from the farm with endemic *pomona* infection [Farm B] had high titres and these were associated with infection. Less than 10% had undetectable titres and these were associated with the absence of infection. The remainder [less than 20%] had intermediate titres which could not be specifically related to either the presence or absence of infection. The titres of pigs which received two or three inoculations were more even in their distribution when compared with the distribution of titres of unvaccinated pigs [see Figures 6.14 to 6.16]. The final titres of pigs which had received three inoculations were significantly [ $P < 0.02$ ] lower than those of their controls. Therefore a comparison of the distribution and GMT's of titres of groups of vaccinated and unvaccinated pigs at slaughter could be cautiously used as a guide for distinguishing farms which are effectively vaccinating their grower pigs.

It is important to select the appropriate age for the commencement of inoculation, as well as a regime which is practical and effective. The fact that the three different regimes of inoculation all reduce the prevalence of infection in pigs exposed to natural infection by increasing their resistance to infection is evidence that inoculation regimes can be varied to some degree to suit individual farming practices.

Figure 6.13 The distribution of *pomona* titres of pigs inoculated once and of unvaccinated pigs at slaughter.

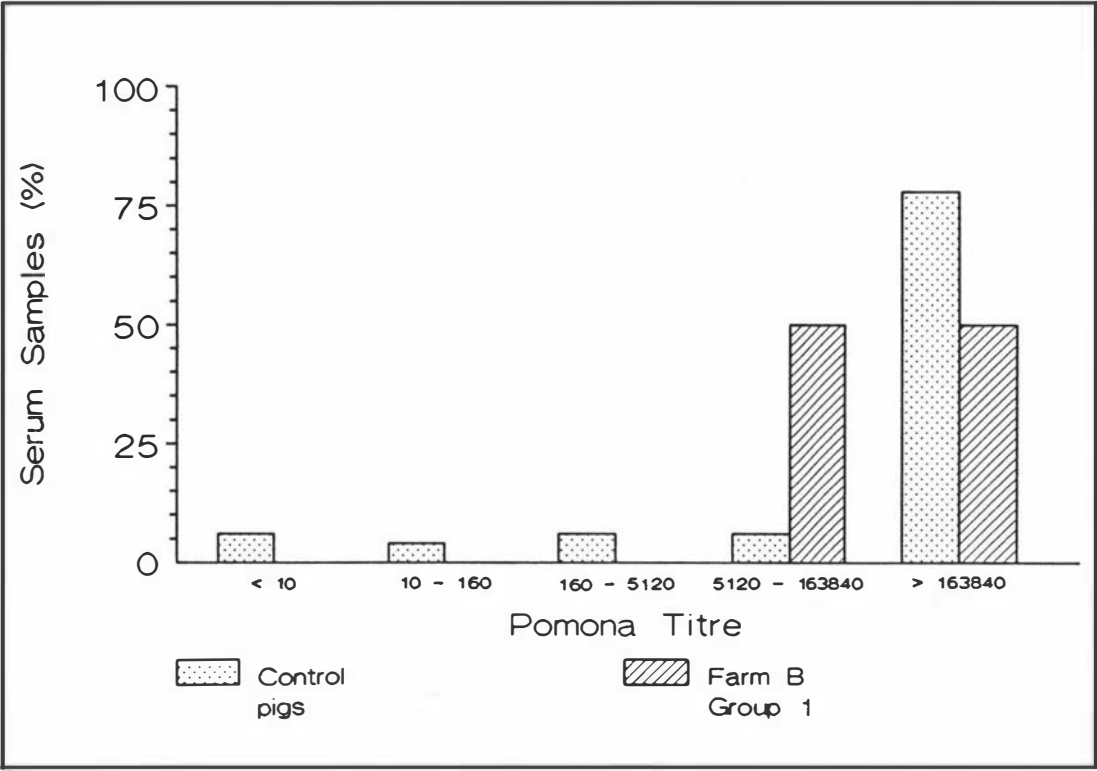


Figure 6.14 The distribution of *pomona* titres of pigs inoculated twice, and of unvaccinated pigs at slaughter.

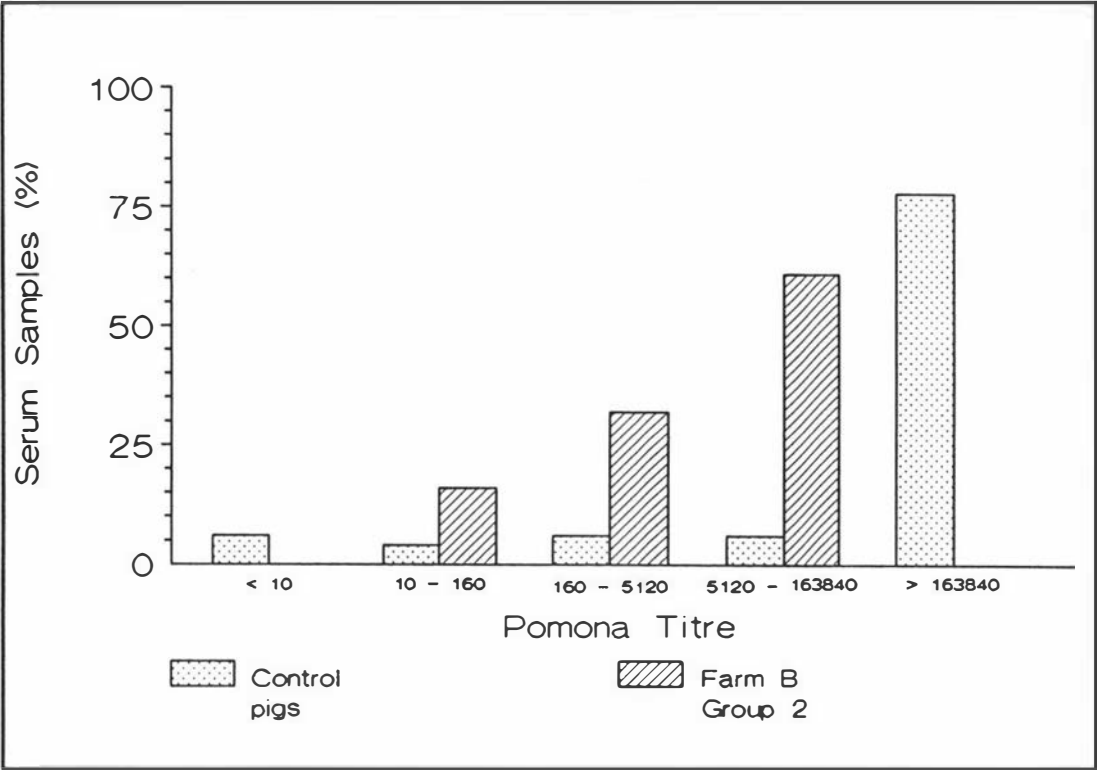


Figure 6.15 The distribution of *pomona* titres of pigs inoculated three times at two weekly intervals, and of unvaccinated pigs at slaughter.

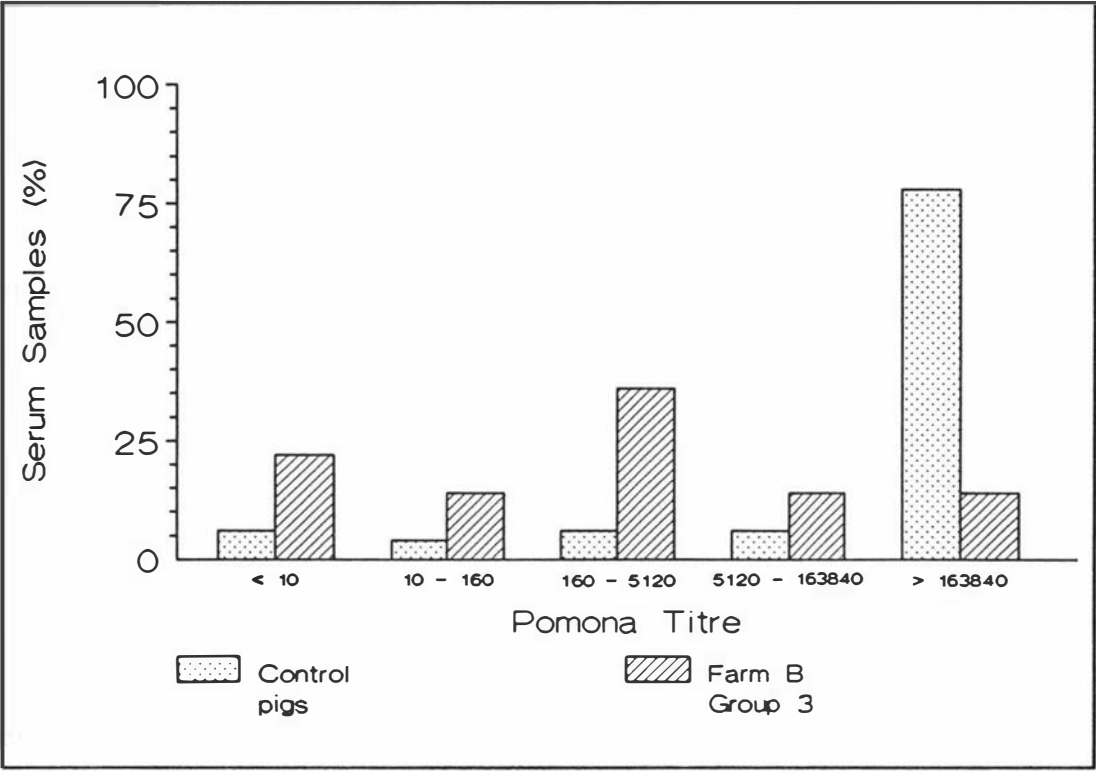
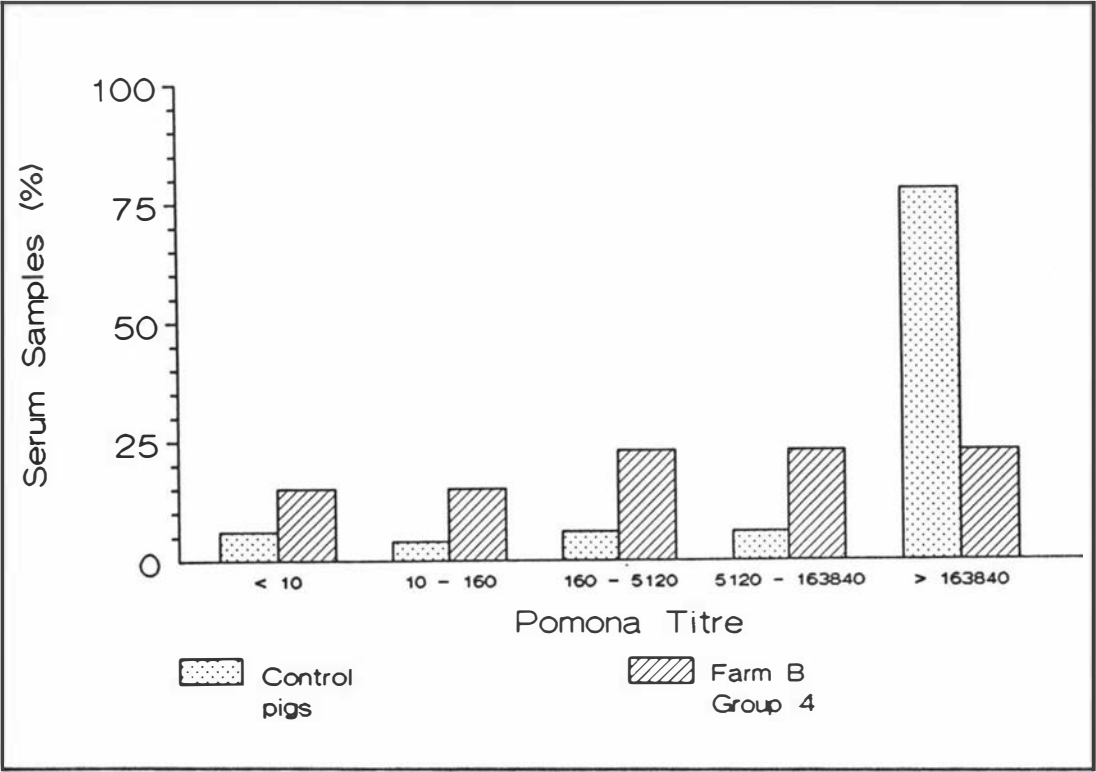


Figure 6.16 The distribution of *pomona* titres of pigs inoculated three times at weekly intervals, and of unvaccinated pigs at slaughter.



The double inoculation of 1.5 ml of vaccine [Group 2], was the regime which was closest to the manufactures recommendations for use of the vaccine in breeding pigs. The advantage of a double inoculation, is that it involves the least amount of labour to administer. The disadvantage of the double inoculation, at a four week interval, is the long period of time between the inoculations, a period during which they remain susceptible to infection. Theoretically, the larger the "window of susceptibility" in the face of exposure to natural infection, the greater the chance that these pigs will become infected. The increased chance of becoming infected during this period was not apparent in this trial. Pigs given a single inoculation [Group 1] did not become infected during the initial ten week period of examination, however they had become infected by the time they were slaughtered. This absence of infection may have been because the pigs were not exposed to infection during the initial period of examination which includes their period of susceptibility. The level of exposure to infection increases as pigs become older and they are surrounded by infected leptospiruric cohorts. The majority of pigs become infected after sixteen weeks of age (Chapter Four).

The administration of both triple inoculation regimes is more labour intensive to administer, however in this investigation both significantly reduced the prevalence of infection in grower pigs. The regime of three weekly inoculations, has the advantage that it fits neatly into most systems of piggery management. Every week pigs are moved into finisher houses, where they can be initially inoculated and boosters given in the succeeding two weeks. This would require minimal tracking of individual pigs, as whole pens of pigs could be vaccinated every week. Logistically, three inoculations at a two weekly intervals would require a more sophisticated means for keeping track of pigs, such as ear tagging, and the associated additional expenses, because the inoculation regime would span over a period of six weeks. Another possibly important advantage of using three inoculations is that if pigs did miss one inoculation, they will still have received two inoculations.

It would be an impractical and an endlessly factorial task to test various regimes of inoculation, commencing inoculation at different ages, and using different volumes of bacterin. However, by altering a few variables, the four

vaccination trials that were undertaken revealed many aspects of the complexities of effective vaccination in the field. It was interesting to note for example, that although the total volume of only 3 ml of vaccine was used for all of the regimes, which is 1 ml below the total dose [2 x 2 ml] recommended by the manufactures for use in breeding pigs. The triple regimes of inoculation used 1 ml per inoculation dose, which was half of the recommended volume for a single dose. These reduced volumes of vaccine, however, still resulted in antigenic stimulation and resistance to infection.

It makes one wonder to what extent commercial companies which produce these vaccines, trial them under experimental and field situations. It is this authors opinion that the majority of these companies do the minimal amount of investigation, and often only sufficient to allow registration of the product for use.

The age range over which vaccination can most effectively take place will broaden as the prevalence of leptospiral infection within the piggery declines. This is due to two variables which may influence the prevalence of infection within a piggery. One is resistance to infection due to the presence of maternal antibody and the other is the risk of infection. As fewer gilts and sows in the piggery become infected, overall, the titres of pigs in the breeding pig herd will decline. As a result, the period of protection that piglets derive from maternal antibody will be reduced, and this would allow the first inoculation to commence in pigs at a younger age. Vaccination of all grower pigs will result in an increased resistance to infection, and a concomitant reduction in the prevalence of leptospiruria which together, will continually reduce the risk of cohorts becoming infected. Vaccination of successive generations of grower pigs will progressively reduce the prevalence of leptospiral infection within the piggery, and thus bring about control of infection. In theory, a continual decline in the prevalence of infection, coupled with continued resistance to infection by vaccination, will result in failure of endemic leptospiral infection to be maintained within a grower herd and thus the eventual eradication of infection from the piggery.



In order to determine the effect of a vaccination regime, pigs should be monitored six months after the commencement of a vaccination program. This should be done by cultural and serological examination of slaughtered grower pigs. The greatest reduction in the prevalence of infected pigs can be expected to occur in the first six months following the commencement of vaccination. Thereafter, assuming the prevalence is reduced to between 20% and 30%, abattoir monitoring can be less frequent, however larger sample sizes will be required to detect the lower prevalence of infection amongst the grower pigs.

From the discussion of these vaccination trials, the following strategies for implementing vaccination of grower pigs are proposed:

1. Determine whether endemic *poimona* infection exists amongst grower pigs of the farm. This can be achieved by blood sampling at least ten pigs at the time of their slaughter.
2. Inform the farmer about the epidemiology of endemic leptospiral infection on his/her farm, and the risk of infection to human beings and other domestic animals.
3. Determine what farming practices are used in respect to the following factors:
  - The source of grower pigs [purchased or bred at farm].
  - Age of weaning pigs.
  - The degree of separation of pigs from each other.
  - Age at which pigs are moved to the grower house[s].
  - Number of grower houses pigs used to accommodated pigs until they are sent for slaughter.
  - Housing design, especially effluent systems.
  - Hygiene standards and means of cleaning pens.
  - Degree to which stock of similar or varying ages are mixed.
  - Determine from where the breeding stock is obtained, specifically determine if self replacement is practised.

- Determine if breeding stock are vaccinated against leptospiral infection and if there is a history of poor reproductive performance which may be related to leptospiral infection.
  - The age at which pigs are sent for slaughter [pork or bacon].
  - General health status of the breeding and grower pigs.
4. Plan a vaccination regime for the pigs, based on the factors discussed in this chapter. Include suggestions to the farmer of practices which, when altered, may contribute the control of endemic leptospiral infection by reducing exposure to infection.
5. Ensure that the vaccination regime is strictly adhered to.
6. Monitor vaccination progress, preferably by kidney culture six months after the commencement of vaccination. Continue to monitor on a regular basis until the herd is considered to be free of infection. If kidney culturing is not possible then serology can be used, but it is less accurate and must be interpreted in relation to serological results from the same farm prior to vaccination.

## SUMMARY AND CONCLUSIONS

1. Four vaccination regimes using a commercially available *pomona/tarassovi* vaccine were tested for their ability to induce a serological response and to prevent leptospiral infection in grower pigs. Trials were conducted on a farm free of leptospiral infection [Farm A] and on a farm known to have endemic leptospiral infection [Farm B].
2. The first vaccination regime consisted of one inoculation of 3 ml of bacterin [Group 1]. The second regime was of two inoculations each of 1.5 ml of bacterin four weeks apart [Group 2]. The third and fourth regimes were triple inoculations each of 1 ml of bacterin two weeks apart [Group 3], and at weekly intervals [Group 4]. Inoculation of pigs commenced when they were approximately ten weeks of age. Pigs were retained within their respective piggeries and handled by normal management procedures.
3. Blood and urine samples were regularly collected from inoculated pigs in the ten weeks following the first inoculation. Blood and kidneys were collected from selected trial pigs and unvaccinated control pigs at the time of slaughter.
4. The three regimes administering two or more inoculations [Groups 2, 3 and 4] resulted in significantly reduced cultural prevalence of infection. The average for unvaccinated control pigs was 85% and for pigs from the farm with endemic leptospiral infection in Groups 2, 3 and 4 respectively the cultural prevalence was 17%, 21% and 23%.
5. Results of pigs given only one inoculation [Group 1], produced insufficient evidence to suggest that pigs responded differently from unvaccinated control pigs. The other three regimes [Groups 2, 3 and 4] resulted in significantly increased titres to

serovar *pomona* following the booster inoculations. The triple inoculation regimes [Groups 3 and 4] resulted in significantly lower agglutinin titres to serovar *pomona* than control pigs at the time of slaughter.

6. It was concluded that vaccination can be used as a means of controlling endemic *pomona* infection in pig herds, however vaccination regimes must consist of at least two inoculations. Vaccination regimes can be tailored to suit individual farms. The epidemiology of endemic *pomona* infection within a herd must be comprehensively understood so that the effect of vaccination can be effectively monitored and regimes altered to fit changing patterns of infection. Flexibility in planning vaccination programs may facilitate the control of endemic *pomona* infection in New Zealand piggeries.

Chapter Seven

**A COMPUTER SIMULATION MODEL OF ENDEMIC  
LEPTOSPIRAL INFECTION IN A PIG HERD**

**Simulated Leptospiral Infection within a Pig herd [SLIP89]**

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## INTRODUCTION

This chapter describes a computer simulation model of endemic *pomona* infection within a pig herd. Epidemiological studies of *pomona* infection in pigs and pig herds which have been described in this thesis and in the scientific literature, have identified several important factors which influence the epidemiological behaviour of this disease.

This computer simulation model was developed with four main purposes in mind:

- 1) To increase understanding of the dynamics and multifactorial nature of the epidemiology of *pomona* infection within a pig herd.
- 2) To confirm previously identified and new factors which appear to influence the epidemiological behaviour of *pomona* infection within a pig herd.
- 3) To observe by simulation, the extent of variation in the patterns of leptospiral infection within a pig herd which are due to chance.
- 4) To identify those variables in the model which may affect the epidemiological behaviour of the disease within the pig herd. The model may provide an initial assessment of a variety of control measures in the absence of field trials.

A computer model should define and represent key features of a system, so that an evaluation can be made of the behaviour of the system under various hypothesised conditions (Marsh, 1986). The process of simulation has been defined by Smith and LaDue (1974) as a dynamic representation of a system, achieved by building a model and moving it through time. Throughout this thesis the combined processes of simulation and modelling is referred to as computer simulation modelling.

This computer simulation model is a representation of the physical process which occurs when a pig herd is infected with *pomona*. The physical process can be divided into a series of logical steps, each of which can be considered as an event. The simulation uses probabilities to determine the outcome of

predetermined events, which is termed stochastic modelling. Computer model simulations can therefore simulate a multitude of dependent and independent events, mimicking biological systems in which there is dynamic interaction between the host, agent and environment.

Traditionally, computer models which were developed to represent biological systems were formulated using complex mathematical techniques. These models are difficult to develop and understand. More recently, a method known as Monte Carlo simulation has been used successfully; for example by Morris (1976), Roe (1977), Marsh (1986) in computer simulation models of bovine mastitis, bovine brucellosis and animal production systems respectively. Many important aspects of the behaviour of a biological system can be divided into a logical series of choices of one of two possible events, known as binary events (Roe, 1977). Outcomes of individual binary events are determined in the model by random sampling on a Binomial distribution. The probability of each event occurring is assigned a value based on information available from epidemiological studies. The probability that an event will occur can be either fixed or variable over time. For example, there is a fixed probability that a sow will become pregnant following mating, while there is a variable probability for the transmission of *pomona* infection to a pig as it becomes older. During the computer simulation the outcome of each event is determined by a randomly generated number between zero and one. If the number falls equal to or below the value of the probability of an event happening then the event is said to occur, otherwise the event does not occur. This is sampling based on a Binomial distribution. The same principle can be applied to other distributions such as the Normal, Lognormal or Poisson (Marsh, 1986). The random numbers generated by the computer are based on a mathematical formula and is therefore not a strictly true random process but a "pseudo-random" process which is suitable for models of this nature. Different sequences of random numbers are initiated from different "seed" numbers which can either be entered from the keyboard, or drawn from the computer clock, as is the case in SLIP89. These numbers fall in an extremely long sequence which eventually repeats itself. The Monte Carlo method considers one event at a time and therefore

allows a departure from the previously mathematically complex simulations (Hammersley and Handscomb, 1979). The use of a computer allows a large number of events to be rapidly simulated.

This computer simulation model should only be used as an aid to understanding the dynamics of leptospiral infection in a pig herd. The accuracy of the model is limited by the assumptions made, the interpretation of quantitative epidemiological results obtained from observational and experimental studies, and their representation within the model. Willeberg (1979) described models to be "valuable tools in the analysis of particular disease situations" which "do not take the place of epidemiological analyses".

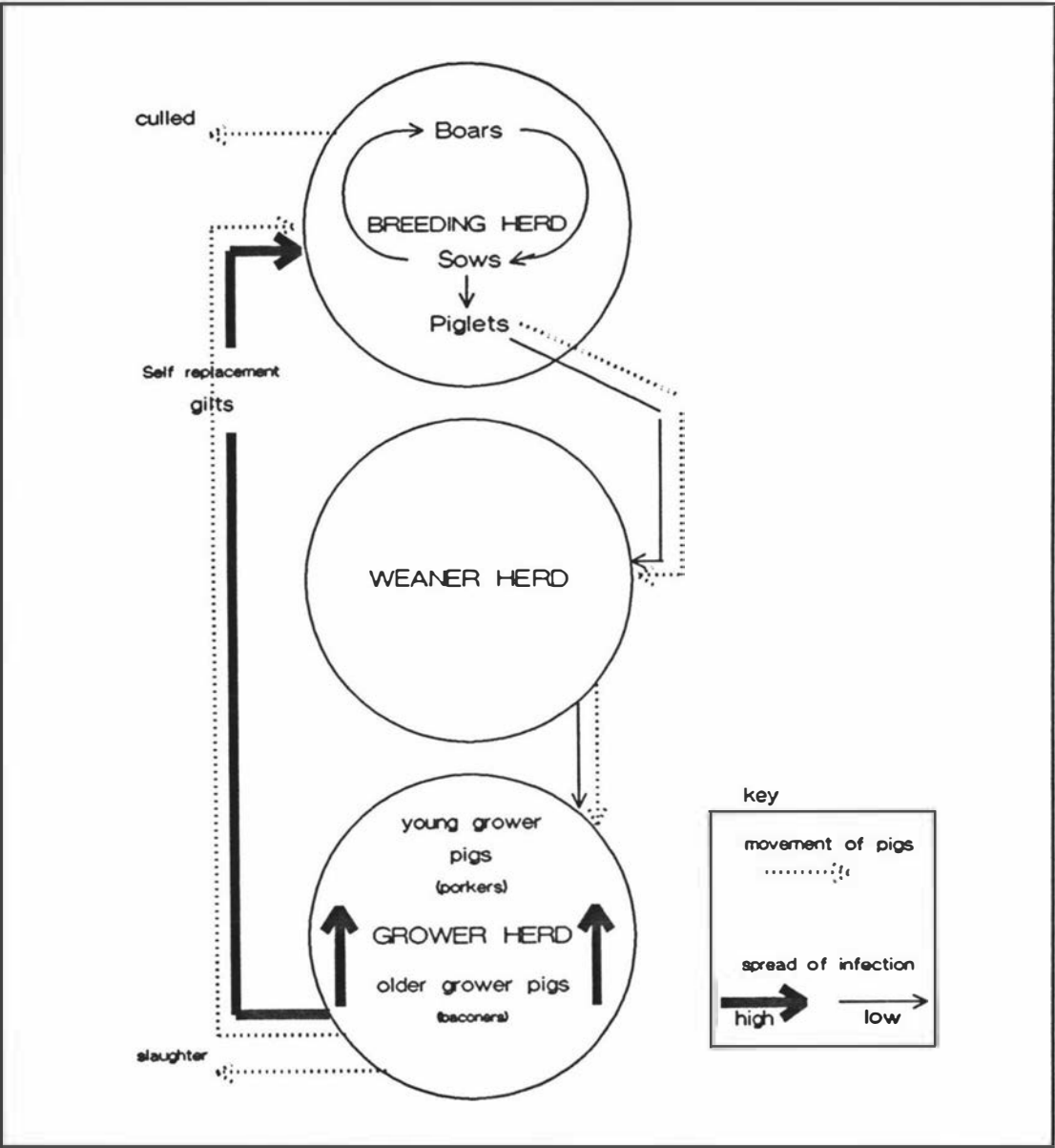
### DEVELOPMENT OF THE SLIP89 MODEL

#### *The epidemiological behaviour of pomona within a pig herd*

Endemic *pomona* infection is maintained within pig herds throughout New Zealand. Figure 7.1 summarizes the movement of pigs and the transmission of infection that occurs within most piggeries (Chapters Three and Four). A high prevalence of infection was common and often exceeded 80% in bacon weight pigs. Only between 2% and 10% of breeding sows, and up to 30% of gilts were leptospiruric. Suckling piglets were not found to be infected, and were protected from infection by passive immunity acquired soon after birth from their dams' colostrum. Piglets were usually weaned from their dams at five weeks and held in weaner houses until they were about ten weeks old. During this period of time they were physically separated from the breeding herd and older grower pigs. Meanwhile, the piglets' level of passive protection had declined due to its half-life of only sixteen days. Upon being moved to grower houses, these pigs became exposed to leptospires shed from infected older pigs. Without resistance to infection, and with continual exposure to infection, these susceptible pigs became infected. These pigs then become the source of infection for other young grower pigs; completing the cycle of maintenance of infection within the pig herd.



**Figure 7.1** Pathways for the transmission of infection between pigs in SLIP89.



### ***The computer simulation model of SLIP89***

SLIP89 simulates the cycle of maintenance of *pomona* infection within a pig herd. The simulation is achieved by repeatedly simulating the events which occur in a piggery over the period of a week, the unit time interval used in the model. In previous epidemiological studies, infection within pig herds was monitored using serological and cultural techniques. Therefore titres and leptospiruria have been used within the simulation model to represent the immune and infection status of individual pigs in any one week. During each simulated week the age, infection and immune status, reproductive status, and the location of each pig within the herd is updated. A flow diagram representing the sequences of events which takes place in the model is shown in Figure 7.2. Development of the computer simulation model started with modelling the population dynamics of a pig herd. Aspects of the epidemiology of *pomona* infection within a pig herd were added to the basic structure of the program in a stepwise manner. Therefore over a period of time the model represented an increasingly complex, although logical series of events.

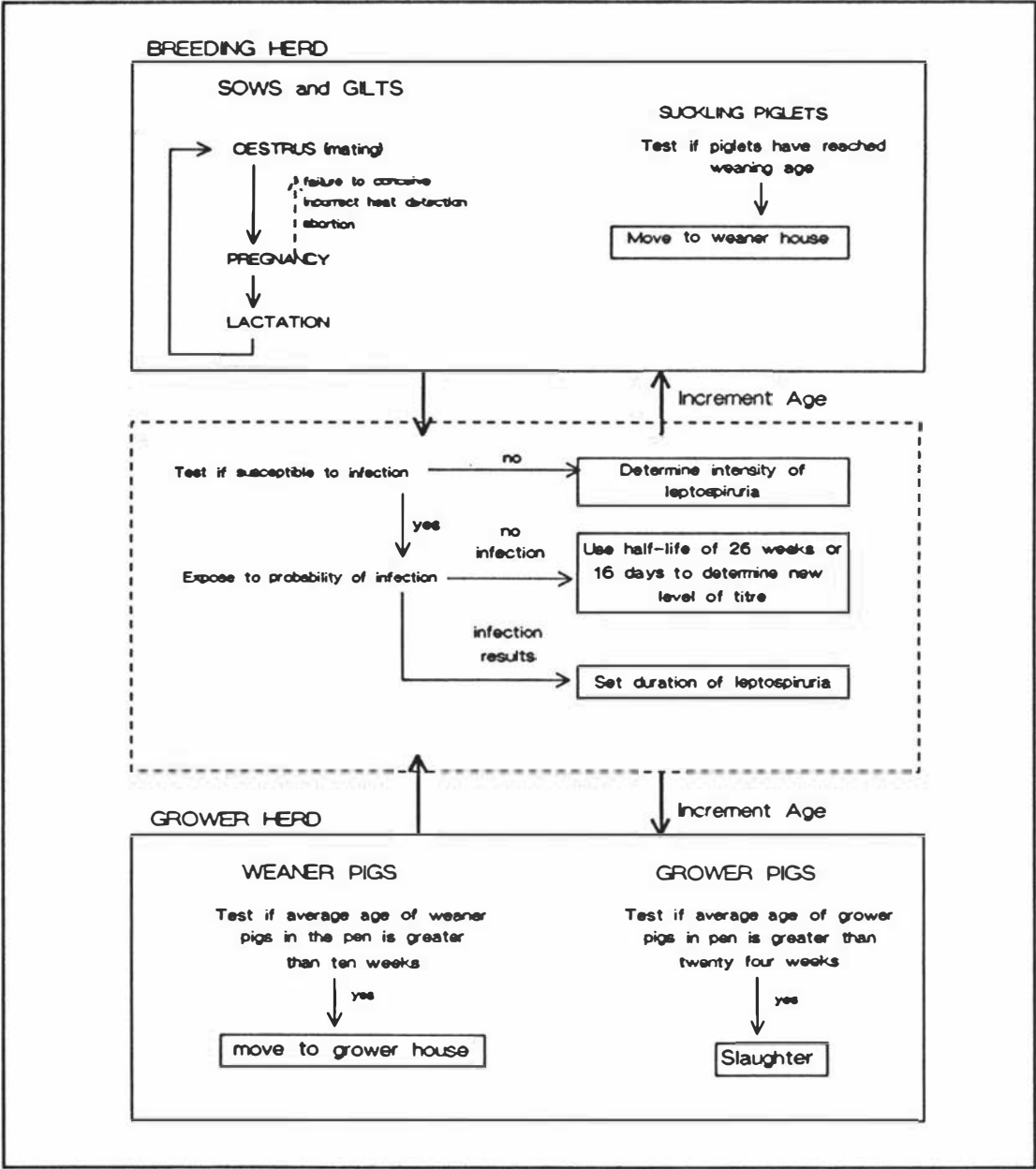
The program for the model was written using Turbo Pascal 4.0 and is shown in Appendix VI. A herd size of 75 breeding sows allows the model to simulate a maximum of 250 weeks. The maximum number of grower pigs in a herd which this model will allow is 2000, this equates to around 200 breeding pigs. Larger herd sizes reduce the number of weeks which can be simulated and further increases the execution time for the program.

### ***Representation of the population of pigs in SLIP89***

The first step in constructing the model was defining the logical sequence of demographic events which occur within a typical New Zealand pig herd (see Chapter One). The following events are simulated weekly:

- 1) Sows and gilts are mated with one or two boars.
- 2) Successful pregnancies result in the birth of piglets to the sow, and piglets suckle from their own dam until the time of weaning.

Figure 7.2     Flow diagram of SLIP89.



- 3) Following weaning, several litters of piglets are held together in pens within a weaner house. When pigs reach approximately ten weeks of age they are transferred to a grower house.
- 4) Pigs remain in the grower house until they are either sent for slaughter or transferred into the breeding herd as self-replacement gilts. All grower pigs are bred on the farm and none are purchased from other farms.
- 5) Breeding pigs are removed from the herd and replaced with either self-replacement gilts or gilts which have been purchased from another farm.
- 6) Boars are removed and replaced only with boars from other farms.

These events, repeated over time, represent the cyclical population dynamics within a piggery.

The information retained for individual sows and gilts, boars, and grower pigs within the program are recorded as shown in Figures 7.3a and 7.3b.

*- The breeding herd*

The breeding herd consists of boars, sows and gilts. The reproductive cycle of the female breeding pig is based on that described by Moller (Massey University Press). Sows make up most of the breeding herd, and include all female breeding pigs which are equal to or older than fifty two weeks of age. Gilts are classified as the female breeding pigs which are younger than fifty two weeks of age (the classification has not been based on parity) and make up around 30% of the breeding herd. Each sow and gilt is assigned to one of three phases of the reproductive cycle which are; oestrus (which includes the time from weaning to conception), pregnancy, and lactation. Gilts which have recently entered the breeding herd are assigned to the oestrus phase of the cycle.

**Figure 7.3a SLIP89 Data records for sows and boars.**

DATA RECORDS FOR GILTS AND SOWS
Sow identity number Parity number Production index Reproductive phase: oestrus pregnancy lactation Data record: age (weeks) duration of infection time to peak titre post infection sex titre (Coded Titre Unit) intensity of leptospiruria (natural logarithm) weeks post infection infectious status previous infection Litter reference and identity of all piglets from previous litters
DATA RECORD FOR BOARS
Boar identity number Number of matings per week and identity number of sows mated Data record: age (weeks) duration of infection time to peak titre post infection sex titre (Coded Titre Unit) intensity of leptospiruria (natural logarithm) weeks post infection infectious status previous infection

The reproductive phase of a sow or gilt only alters after the positive occurrence of one of three events: mating, farrowing and weaning. In the event of mating, sows or gilts are mated by two different boars. There is a fixed probability of [p = 0.85] that mating will result in pregnancy, in which case the reproductive status changes from oestrus to pregnancy. Of the fifteen percent of pigs which fail to become pregnant, the reproductive status remains in oestrus and the next date for mating is specified according to the reason for the failure to become pregnant. Three percent of these failures are due to incorrect oestrus detection and these pigs are mated within three weeks of the last mating. Ten percent are due to failure to conceive and are mated again in three weeks time, and two percent experience abortion and these pigs are mated again, on average, in ninety days with a standard deviation of ten days. At the end of pregnancy, after a gestation period of 113 to 115 days, the pigs farrow, and their reproductive status changes from pregnant to lactating. The number of piglets born is based on a Normal distribution, and the gender of each piglet randomly assigned. Each breeding pig remains in the lactation phase until its piglets are weaned. Thereafter, the reproductive status of the breeding pig returns to oestrus, thus completing a full cycle, which takes about twenty three weeks.

Boars make up around five percent of the breeding herd. Young boars are not capable of as many matings as older experienced boars, as shown in Table 7.1. If there are insufficient boars for the weekly mating requirements, a new boar is added to the boar herd.

**Table 7.1                                      Age related mating frequency for boars.**

Age of Boar (weeks):					
	<32	32 - 45	46 - 65	66 - 80	>80
Maximum number of matings per week:					
	0	1	2	3	4

Pigs within the breeding herd are culled on the basis of their age, reproductive performance and for miscellaneous causes. In the case of a pig being culled it

is removed from the herd. Breeding herd numbers are maintained by the addition of new gilts or boars to the herd.

*- The grower herd*

The grower herd makes up the largest proportion of pigs within the whole herd and accounts for around ninety percent of the total population when the herd is fully established and has a zero population growth. The grower herd is divided into three categories: suckling piglets, weaner pigs and grower pigs.

**Figure 7.3b SLIP89 Data records for grower pigs.**

DATA RECORD OF GROWER PIGS	
Grower identity number	
Data record:	
	age (weeks)
	duration of infection
	time to peak titre post infection
	sex
	titre (Coded Titre Unit)
	intensity of leptospiruria (natural logarithm)
	weeks post infection
	infectious status
	previous infection
Pen number	
Pen record:	
	number of pigs in pen (maximum thirty)
	number of infected pigs
	number of seropositive pigs
	average age of pigs in pen
	geometrical mean titre (GMT)
	environmental contamination (average per pig)
	probability of infection for pigs in pen

Suckling piglets.

These are piglets, which prior to weaning, are with their dam. Separate litters of piglets are not mixed together, and they piglets only have effective contact with their own dam.

Weaner pigs.

Following weaning, pigs are transferred to pens within a "weaner house", each pen can hold up to thirty pigs. The design of the weaner pens in the model consist of two adjacent rows of five pens, as shown in Figure 7.4. Litters of pigs are only mixed together when pens are being filled. When mixing occurs, it is only with pigs which are no more than two weeks older than themselves. The weaner house is considered to be a separate building which is isolated from contact with breeding pigs or older grower pigs.

**Figure 7.4    Floor plan of weaner and grower houses in SLIP89.**

WEANER HOUSE					GROWER HOUSE					
PEN #					PEN #					
1	3	5	7	9	11	13	-	-	47	49
2	4	6	8	10	12	14	-	-	48	50

Grower pigs.

When the average age of pigs in a weaner pen reaches ten weeks, the pigs are transferred to pens within the "grower house". Grower pigs are kept in the same pen for the duration of their time within the grower shed. The pens within the grower shed consist of two adjacent rows of twenty pens, as shown in Figure 7.4. When the average age of pigs in a grower pen reaches twenty four weeks, they are removed for slaughter. Female pigs which have reached twenty two weeks of age may be selected as breeding stock replacements.

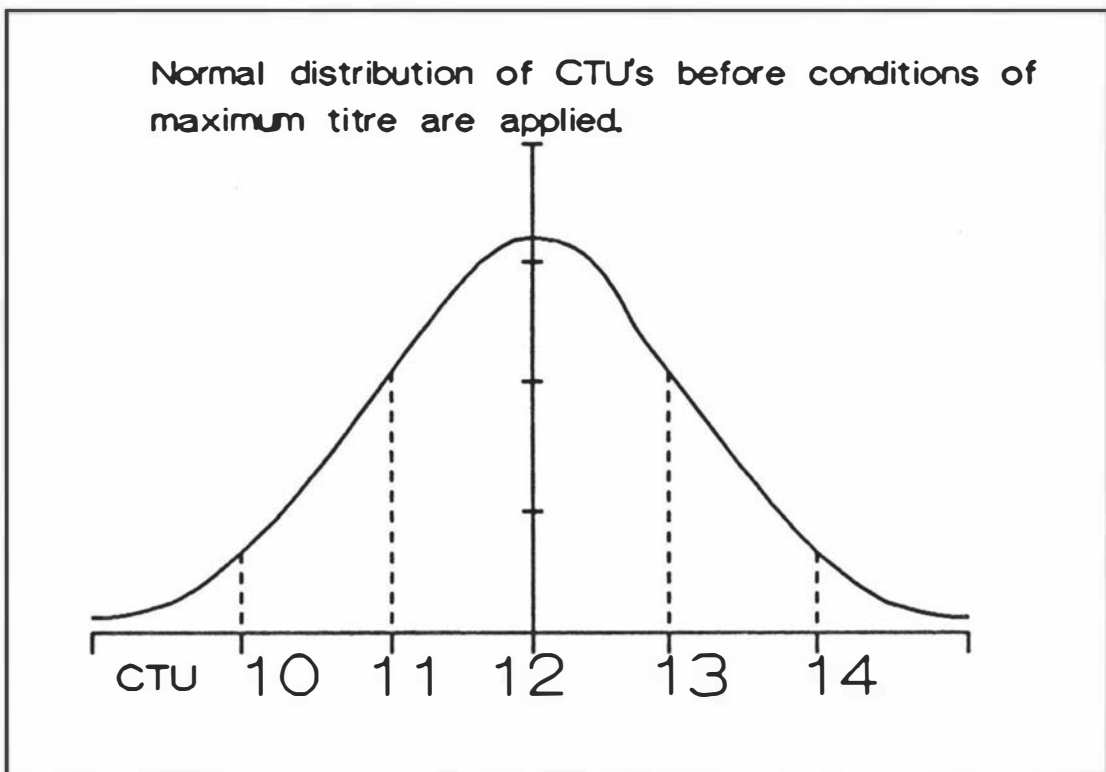


***Selection and representation of epidemiological variables in SLIP89***

***- Immune status***

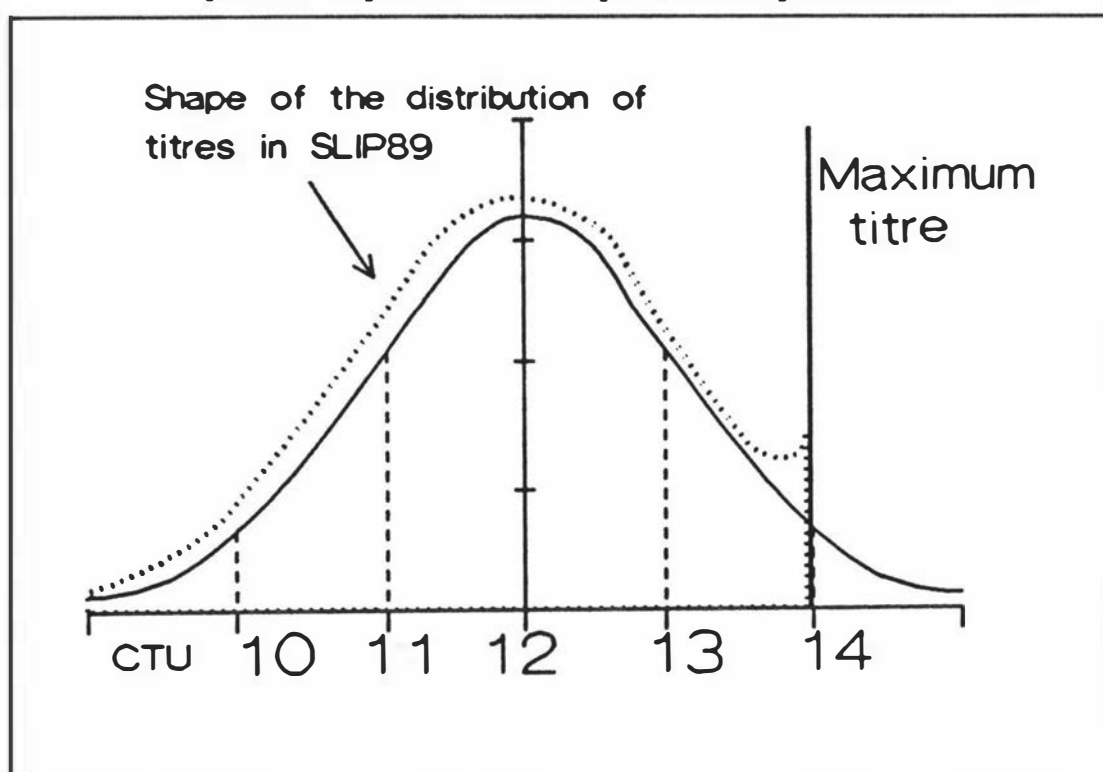
There are two variables which hold information about a pig's immune status. The first variable [TITRE] represents the coded titre unit [CTU] of a MAT titre between 1/10 and 1/327,680 which a seropositive pig may have at any one time. Seronegative pigs are represented by having a CTU of zero. A titre of less than 1/10 indicates that a pig does not have titre detectable by the MAT. All titres are expressed as CTU in the program and are converted to titres using the equation given in Chapter Two, Figure 2.5.

Figure 7.5a Normal distribution of post-infection titres.



In the model, when a pig becomes infected its maximum titre is determined from a normal distribution, Figure 7.5a. The distribution of post infection titres are bound by the specified minimum titre in the presence of infection [MINSENS] and the maximum allowable titre of 1/327,680 [MAXTITRE].

**Figure 7.5b** The distribution of post-infection titres after applying minimum [MINSPEC] and maximum [MAXTITRE] titre restrictions.

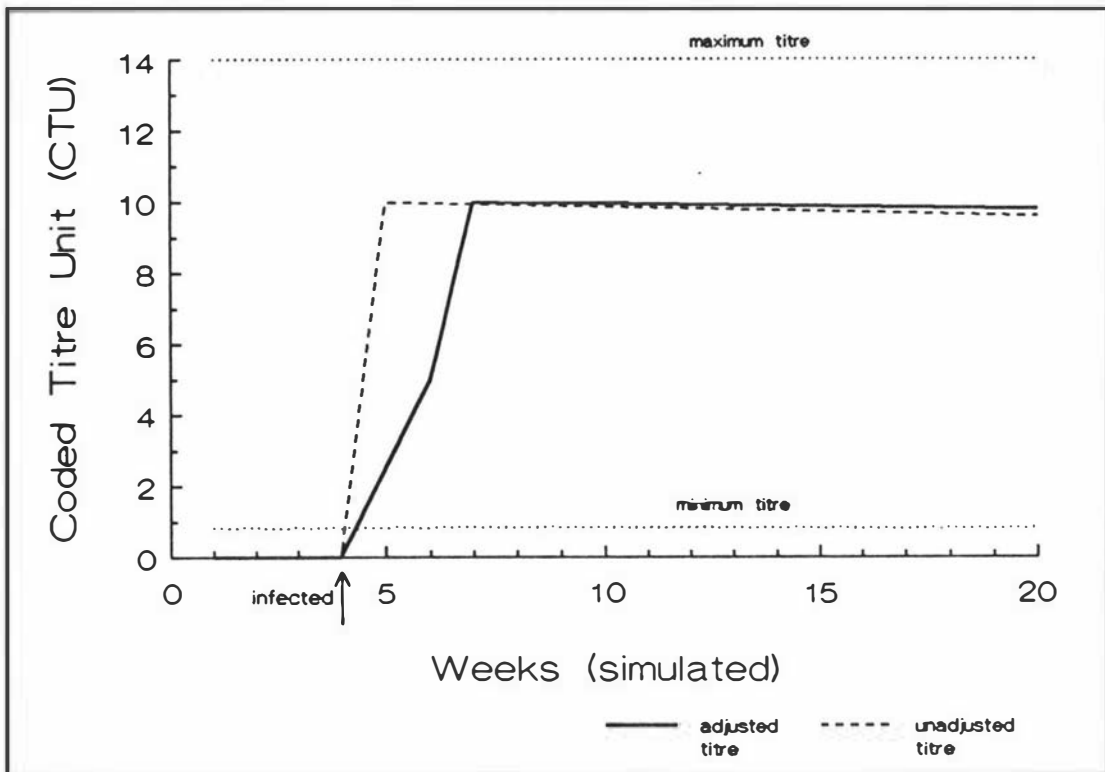


Any titre selected from the normal distribution which falls below the minimum allowable titre [MINSPEC] is discarded and a new titre is selected until it is equal to or above the minimum allowable titre. This ensures that newly infected pigs do not initially have a titre which is less than MINSPEC. Any titre which falls above the maximum titre of  $1/327,680$  [MAXTITRE] it is given the maximum value of  $1/327,680$ , as this is the highest titre the model will accept. As a result, the distribution of simulated pigs' titres will be skewed to the right, as shown in Figure 7.5b. Field studies in Chapter Four and Study 1 of Chapter Five demonstrated that pigs do not attain their maximum titre for at least two weeks after the time at which they become infected. A second variable [PEAK] was therefore incorporated to delay the maximum titre from occurring at the time of infection. This has been achieved by halving the predetermined CTU at the time of infection for the number of weeks to peak titre. The time to peak titre has been fixed at two weeks post infection. Thus,

for the first two weeks following infection, the CTU is doubled until the peak titre is reached, Figure 7.6. In the weeks following infection and subsequent to the peak titre, the titre of an infected pig will decline according to a half life of twenty six weeks [ADULTHL26], although Ryan (1978) has suggested that the half life of MAT titres for infected pigs may be as long as one year.

**Figure 7.6**

**The simulated increase of pig's titres following infection in SLIP89.**



Piglets acquire passive immunity from their dam soon after birth. Generally piglets initially have the same titre as that of their dam. The titres of piglets and other pigs which have not been previously infected are reduced every week in accordance with their titre having a half life of sixteen days [PIGLETHL16].

Simulated pigs remain passively protected while they have a titre which is equal to or greater than the specified maximum titre for susceptibility to infection [MAXSPEC], this value is preset to 0.8, thus representing a titre of just less than 1/10.

*- Infection status*

The infection status of each pig in the model is represented by five variables.

- 1) Variable [INF] records whether or not the pig is currently infected.
- 2) Variable [WKPI] records the number of weeks since the pig became infected.
- 3) Variable [PREVIN] records whether the pig has been previously infected or not.
- 4) Variable [DURINF] is a measure of the duration for which the infected pig will remain leptospiruric. The duration of leptospiruria is determined from a normal distribution, the average and standard deviation of which can be specified by the user. The default values are on average a duration of 56 days, with a standard deviation of fourteen days. These estimated values have been selected as a result of the studies described in Chapters Four and Five, as well as from accounts in the literature about the duration of leptospiruria in naturally infected pigs.
- 5) Variable [SHED] stores the intensity of leptospiruria, measured as the number of leptospires per ml of urine. A pig does not shed leptospires in its urine until two weeks after it becomes infected. Thereafter, for the duration of infection [DURINF] a new intensity of leptospiruria is selected each week. The average and standard deviation for the intensity of leptospiruria are determined by the user. A wide range of concentrations of leptospiruria have been demonstrated to occur in naturally infected pigs [Study 2 of Chapter Four]. Natural logarithm of the concentration of leptospiruria is used for determining the intensity of leptospiruria from a Normal distribution. The result from the Normal distribution is then converted back to a the number of leptospires per ml of urine that pig will shed for that simulated week.

*- Exposure to infection*

Suckling piglets are only exposed to infection if either their dam or their litter mates are leptospiruric.

Weaned pigs, held in the weaner house are only exposed to infection when either pigs within the same pen or within other pens of the same building are leptospiruric.

Grower pigs housed within the grower house are exposed to infection from leptospiruric pigs within the same pen and from infective pens within the grower shed.

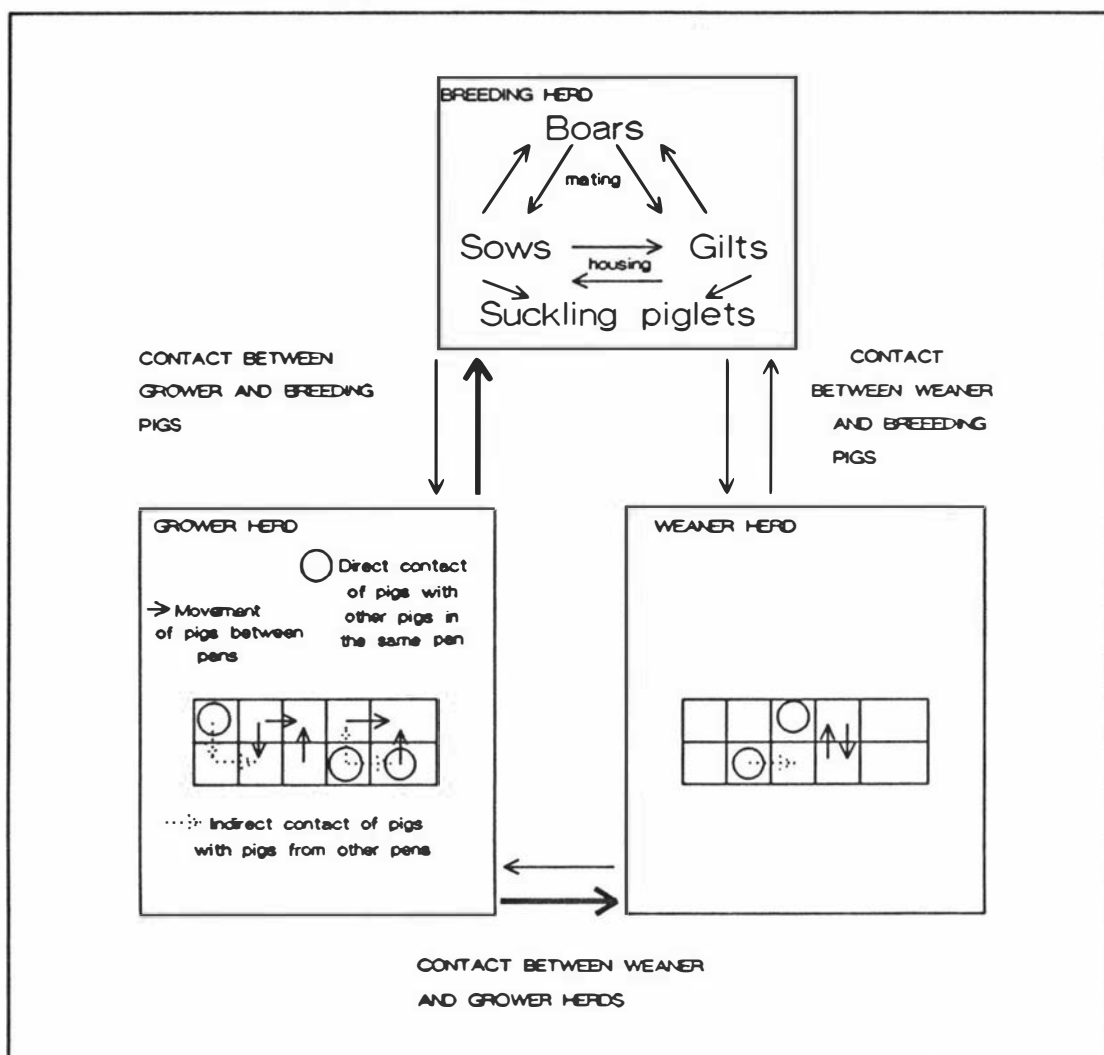
The amount of contact between the weaner, grower and breeding pig herds can vary between no contact and continuous contact, see Figure 7.7. Contact between either of these herds is another means by which infection may spread to susceptible pigs. The degree of contact is represented as a percentage, 0% being no contact, and 100% being continuous contact. This is a very subjective measure, and a simplified means of representing contact between different sectors of the pig population. However it is the intention that the user to be able to represent different systems of management in which various population sectors can be directly or indirectly mixed together. Occasional contact between two population sectors is given a value of 10%.

The degree of mixing of pigs within the same population sector is also represented with percentages.

*- Determining the probability of infection*

The probability of infection for a susceptible pig is dependent upon the level of exposure to leptospires experienced by that pig. Infection may result from either direct contact with leptospires following "urine sniffing", coitus or intrauterine transfer of leptospires, or by indirect contact, which is most likely to occur following "rooting", "sniffing", or contact of abraded or broken skin with contaminated effluent. Generally, both direct and indirect transmission of

**Figure 7.7** Pathways for direct and indirect transmission of infection between pigs in SLIP89.



infection can be considered to occur between pigs within the same pen, whilst only indirect transmission can be considered to occur between pigs from different pens.

The probabilities for direct and indirect transmission are added to give the final probability for the transmission of infection for each susceptible pig in the course of a simulated week. The calculation comprises four parts [Part A, B, C, and D], each of which represents an independent probability of infection for a susceptible pig. The four independent probabilities are then added together to form a final probability of infection for a susceptible pig, the result of which

is binomial and determines whether the event of infection will occur or not. A schematic representation for calculating the probability of infection for a susceptible pig is shown.

$$\begin{array}{c}
 \text{Transmission from pigs within the same pen.} \\
 + \\
 \text{Transmission from pigs in the same herd} \\
 \text{due to the movement of pigs between pens.} \\
 + \\
 \text{Transmission from pigs in the same herd,} \\
 \text{in other pens.} \\
 + \\
 \text{Transmission from pigs in other herds.} \\
 = \\
 \text{Final probability of infection for a susceptible pig,} \\
 \text{over the period of one simulated week.}
 \end{array}$$

A new probability of infection for every susceptible pig is calculated. Each susceptible pig is then "exposed" to its' probability of becoming infected using the Monte Carlo method. If the pig fails to become infected or the pig has passive protection from infection then its titre will decrease by an amount which equates to a half life of sixteen days, however if the pig has been previously infected its titre is reduced by an amount which equates to a half life of twenty six weeks.

*- Calculating the probability of infection for a susceptible pig*

**Direct transmission.**

The probability of infection resulting from direct transmission is related to the probability of a susceptible pig within a pen having direct contact with an infected pig within the same pen, and is represented by Part A of the

equation. The likelihood of this event occurring is dependent on the number of susceptible pigs within the pen, the number of leptospiruric pigs within the pen, and the intensity of their leptospiuria. The ratio of infected to susceptible pigs within the pen is used in calculating the probability of a susceptible pig becoming infected from an infected pig within the same pen. The probability of an infected pig being moved into the pen is also calculated [Part B].

**PART A:** This segment of the calculation determines the probability of a susceptible pig becoming infected from both direct and indirect contact with infected pigs from the same pen. Provided there are leptospiruric pigs within the same pen as the pig, the average amount being shed by the pigs of that pen is multiplied by the number of leptospiruric pigs. This value is then divided by the number of susceptible pigs, minus one (which represents the pig for which the calculation is being done). This calculation is used to logically represent the effect of dilution of infective effluent with non-infective effluent from non-infected pigs, as well as represent the ratio of direct contact between the susceptible pigs and infected pigs within the pen. The "pen contamination division factor" [PCDF] and natural logarithms have been used to transform the final value to one which can represent a probability, that is a value between zero and one. The PCDF and natural logarithms can therefore be regarded as constants within the equation. This is perhaps a crude representation of a potentially complex mathematical problem. However in an attempt to keep the program operational at a logical level, this representation has been found to be adequate. The probability of infection for pigs within the grower herd generally varies between zero and 0.3, which is in keeping with an incidence finding of infection in field trials of around 10% per week.



**PART B:**

This calculation represents the probability of infection resulting from mixing pigs of the same population sector, which are held within the same building. The probability that an infected pig is transferred to a pen containing susceptible pigs is dependent on the movement of pigs between pens (within the same population sector), and the proportion of infected pigs in the other pens within the same building. This part of the equation has been divided into three steps:

- 1) The proportion of infected pigs in all pens of the same building, except the pen containing the pig for which the probability of infection is currently being calculated, is determined.
- 2) The resultant fraction is multiplied by the probability of contact occurring between pigs in different pens. This value represents the likelihood that an infected pig is mixed with pigs in the pen containing the pig for which the probability of infection is being calculated.
- 3) This probability is then tested using the Monte Carlo method. Should the outcome indicate that an infected pig has been transferred to the pen containing the simulated pig of concern than the probability of a pig within the pen becoming infected is related to the intensity of leptospirosis of the introduced pig. In such an event, the average intensity of leptospirosis of pigs in other pens is calculated and the introduced pig represents an "average" infected pig. From the intensity of leptospirosis, the probability that the susceptible pig will become infected from the introduced pig, is calculated. If an infected pig is not introduced into the pen, then obviously the probability that direct transmission will result from pigs of other pens is zero.

### **Indirect transmission.**

Indirect transmission of infection can occur from leptospiruric pigs contained within the same building, or from pigs in other buildings only if contact between more than one group of pigs occurs, for example; contact between breeding and grower pigs. The probability that a susceptible pig becomes infected from an indirect source, is calculated as described in Part C. Sources of infection include; effluent drainage from adjacent pens, via fomites, or spray hosing of infected pens as suggested in Chapter Five. It is assumed in this calculation that the probability of infection from adjacent pens is higher than from more distant pens. Therefore the amount of environmental contamination in adjacent pens is given greater weight than from more distant pens when calculating the probability of indirect transmission of infection is calculated.

**PART C:** This part of the equation calculates the probability of indirect transmission of infection arising from pigs of the same population segment which are housed in other pens within the building. If there are pigs within the same building which are leptospiruric, than the "total environmental contamination" is divided by the number of pigs in that population segment, for example the total number of weaner, grower or breeding pigs. The natural logarithm of that value is then divided by the "environmental contamination factor" [ECDF], and the resulting value is used to represent the probability of infection from the contaminated environment. The level of contamination of adjacent pens is calculated. The natural logarithm of the level of contamination in adjacent pens is divided by the ECDF and added to the previously calculated probability of infection from the contaminated environment. In this way, the level of contamination contributed by pens adjacent to the pen containing the susceptible pig is given more weighting than contamination arising from more distant pens. The design of a piggery will influence this calculation,

and in this program the design of weaner and grower houses has been assumed to be two rows of adjacent pens, as shown in Figure 7.4. Therefore, pens at the end of the house have only three adjacent pens, while all other pens have five adjacent pens. This difference has been taken into account when calculating the likelihood of contamination arising from adjacent pens.

Environmental contamination from other population segments, which are housed in other buildings may also contribute to the final probability of indirect transmission of infection. These sources of contamination have to be taken into account when calculating the final probability of indirect transmission.

#### PART D

This is the final section of the equation in which the probability of infection between breeding pigs, weaners, and growers is calculated. If contact between the breeding herd and another herd has been specified then the probability of infection spreading between the breeding herd and the other herd is calculated by multiplying the probability of infection from boars by five percent, as they normally constitute five percent of the breeding herd, and the probability of infection from sows and gilts is multiplied by ninety five percent, these are then added together to obtain the probability of infection within the breeding herd. This value is then multiplied by the probability of contact between the breeding herd and the other herd [grower or weaner]. Similarly, the probability of contact between growers or weaners is multiplied by the respective probabilities for the transmission of infection between those two population segments. The resulting probability values for indirect transmission of infection from each population segment are added together to give the total probability of infection from other population segments.

## SLIP89 SIMULATION RESULTS

### *Verification and validation of SLIP89*

Development of a computer simulation model should be followed by a procedure for testing the adequacy of the model (Roe, 1977). A model is a theoretical representation of a series of events, and therefore all testing is subjective, and the usefulness of the model will depend on its ability to meet the objectives for which it was developed. The two activities in testing the adequacy of the model are verification and validation.

#### *- Verification*

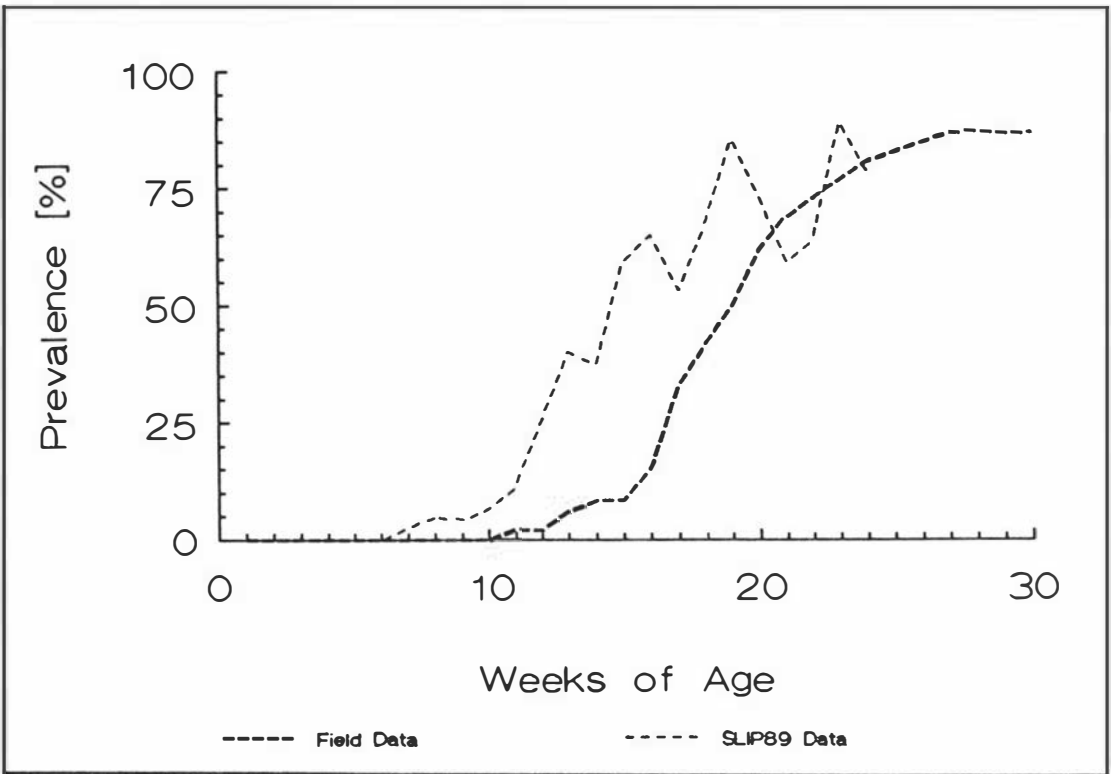
Verification involves checking the logical sequence of events which are claimed to be represented in the model. These steps must be correctly represented in the program language being used. This was tested by scrutinising each segment of the program and testing it, independently of the total program, for its ability to correctly perform the intended function. As part of this process multiple simulation runs were performed and the behaviour of variables were traced throughout the period of simulation. Unexpected behaviour of a variable then led to a detailed investigation into either the logic of the process it was used to simulate, or the possibility of a programming error. These processes are too lengthy to report in full, however all necessary steps were repeated until the correct functioning of model had been verified.

Several structural changes were made to the program over the period of its development and through the process of verification. As the program increased in size and included more interactions and events, the structure had to be altered to better accommodate the latest developments. As the model was developed in a step wise fashion, the programming is by no means the most efficient possible, as the final logic structure was not known until the program had been completed.

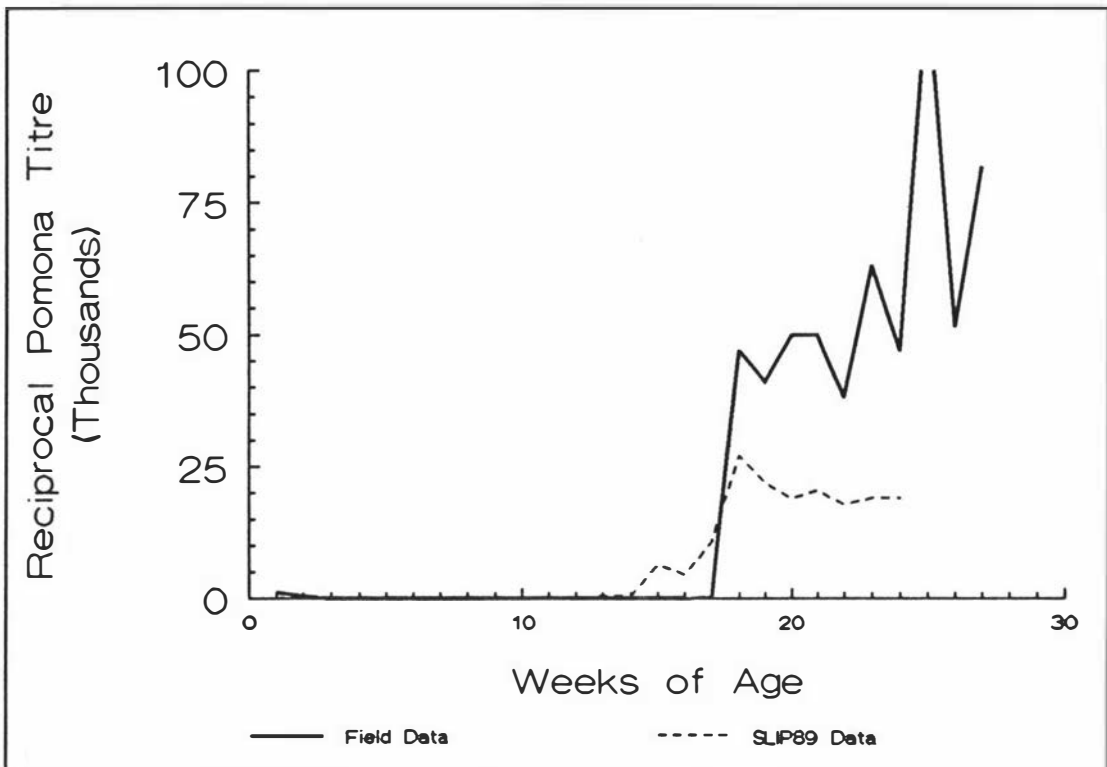
- Validation

For validation of the model, results of field studies described in Chapters Three and Four were used to compare with the results generated by SLIP89. It was found on repeated occasions that the patterns of infection displayed by the model were similar to those found to occur in naturally infected pig herds. Figures 7.8, 7.9 and 7.10 show that the prevalence of infection in grower pigs, the prevalence of leptospirosis in the suckling, weaner, grower and breeding herds, and the titres of grower pigs from results of field studies (Chapter Four) were similar to the average results of five simulations [see Appendix V]. Although totally independent field data was not available with which to validate the results of SLIP89, it is evident that the simulation results closely approximate that of the field data available.

**Figure 7.8** The prevalence of infection for grower pigs for SLIP89 data and field data (Chapter Four).



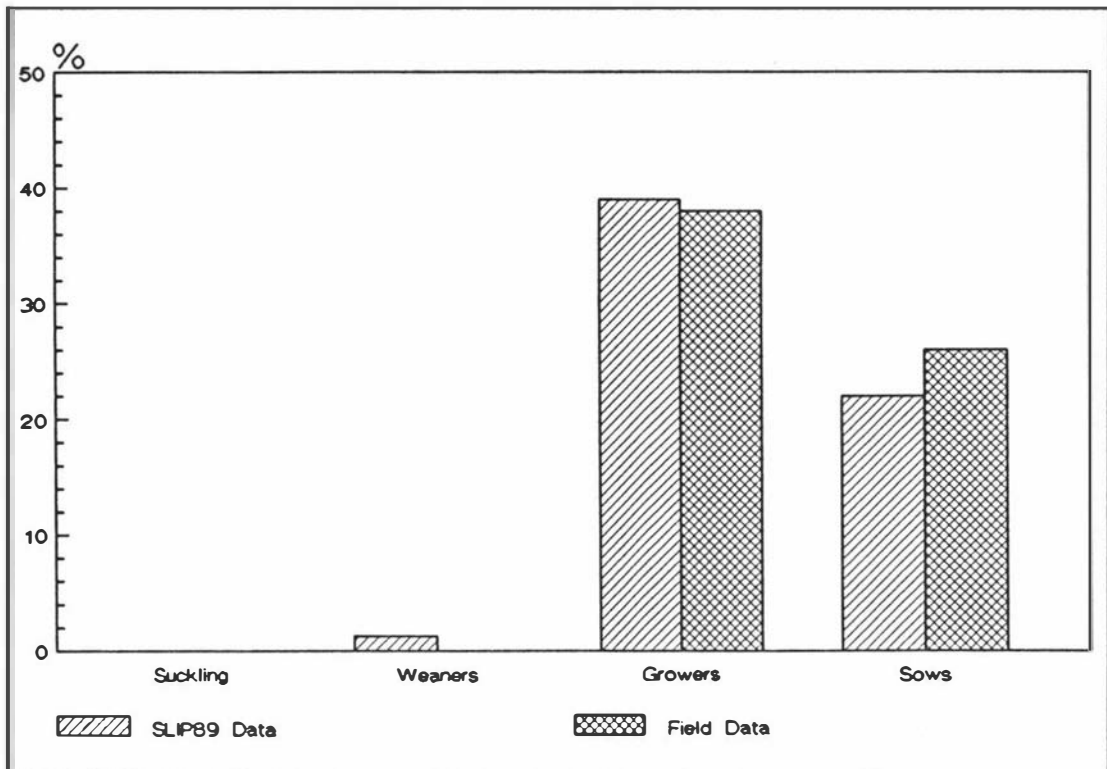
**Figure 7.9** The *pomona* titres of grower pigs for SLIP89 data and field data (Chapter Four).



A further part of validation comprises sensitivity testing. This involves altering the value of each variable and observing the change in the patterns of disease within the population which occur apparently as a result. Sensitivity tests cannot be considered proof that the model is not faulty or free from error, however they do help one gain confidence in the credibility of the model (Morris, 1972). It is difficult to objectively assess the influence of a single parameter on the outcome of a simulation as its alteration may influence several other variables. Given the large number of parameters the model has, the sensitivity testing was restricted to alteration of parameters in each selected variable to a single upper and lower limit. Only the variables considered to be the most important to the behaviour of the model were selected for sensitivity testing. Comparative results of sensitivity tests for key variables of weaning age, duration of leptospiruria, intensity of leptospiruria, virulence of leptospirures [ECDF], and the degree of contact between pig populations for five simulations

over a simulation period of fifty weeks and using fifty sows, are shown in Tables 7.2 to 7.7 and can be viewed in more detail by referencing Appendix V. The average prevalence of infection for grower pigs over the last twenty weeks of each simulation was selected for comparison between simulations, as shown in Tables 7.2 to 7.6. It must be kept in mind that the average prevalence of infection for grower pigs is derived from the infection status of all pigs in the grower house for that simulated week. Other output measures can be used for the comparisons by examining the files stored from the sensitivity test simulations, see Appendix V.

**Figure 7.10** The prevalence of leptospirosis for populations of pigs for SLIP89 data and field data (Chapter Four).



#### Weaning age

Alteration of the weaning age to an average of twenty one days and fifty six days [default is thirty five days] had little effect in changing the prevalence of infection amongst grower pigs. The lower test level [Table 7.3] reduced the

prevalence slightly. Lowering the weaning age will have reduced the likelihood of piglets becoming infected from their dams, and thus lower the potential for infection to occur amongst pigs in the weaner house. Conversely, by increasing the age of weaning to eight weeks, one would expect to see an increased prevalence of infection in grower pigs, however this was not the case [Table 7.3] as the prevalence of infection for grower pigs was similar to that observed for simulations using the default values [Table 7.2]. The age of weaning does not affect the rate of decline of passive immunity. Therefore, the rate of infection of pigs in the weaner house is not altered and it is unlikely that more than five percent of weaners could become infected before ten weeks of age. As a result the prevalence of infection in the grower shed is not noticeably altered by increasing the weaning age to eight weeks.

**Table 7.2      The average prevalence of leptospiral infection for grower pigs in SLIP89, using the default settings.**

Table 7.2      The average prevalence of infection in grower pigs in SLIP89 using all default settings.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	26*	38	39	21	22
32	30	32	36	30	26
33	35	24	42	31	26
34	35	34	44	34	29
35	41	41	42	36	32
36	43	45	38	38	35
37	46	46	44	41	37
38	52	50	44	31	40
39	56	53	48	46	43
40	60	55	50	50	49
41	60	59	48	53	54
42	62	59	53	54	58
43	65	56	53	57	62
44	60	47	49	53	64
45	56	47	50	45	55
46	55	49	54	50	55
47	64	43	54	60	45
48	62	48	61	61	46
49	58	50	62	65	53
50	49	54	65	75	56

\* = Percent rounded to nearest whole number.



**Table 7.3** SLIP89 results of the average prevalence of leptospiral infection in grower pigs, when validating for weaning age.

**Table 7.3a** Age of weaning set to twenty one days.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	41 <sup>*</sup>	29	54	29	45
32	33	27	42	38	43
33	35	27	41	42	44
34	33	32	43	51	40
35	36	39	49	48	44
36	44	47	54	48	43
37	53	48	54	48	51
38	54	53	57	52	54
39	55	54	55	56	54
40	58	62	54	60	56
41	62	62	56	64	56
42	65	60	57	65	61
43	65	60	56	65	62
44	59	60	54	57	50
45	57	50	54	45	52
46	64	49	53	45	53
47	64	52	49	48	53
48	71	51	49	53	55
49	69	50	55	48	52
50	54	49	56	44	39

**Table 7.3b.** Age of weaning set to fifty six days.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	37 <sup>*</sup>	48	39	42	46
32	43	49	41	41	47
33	42	55	49	49	47
34	42	57	54	42	47
35	43	55	60	41	48
36	42	57	59	40	49
37	45	56	56	39	53
38	41	49	50	42	53
39	44	45	54	48	56
40	49	47	56	49	65
41	50	50	60	53	68
42	56	54	60	62	71
43	58	52	62	64	68
44	55	47	60	66	69
45	57	48	55	62	64
46	61	54	51	67	57
47	69	57	59	74	63
48	72	62	64	66	67
49	71	66	68	72	68
50	75	67	58	70	67

\* = Percent rounded to the nearest whole number.

### **Duration of leptospirosis**

An average duration of leptospirosis of twenty one days resulted in a reduced prevalence of infection amongst grower pigs, Table 7.4. However infection was still maintained by the grower pig population which had a reduced average duration of leptospirosis. This result was expected as pigs remain leptospirotic for a short duration only, thus reducing the opportunity for the spread of infection between them. An increase in the duration of leptospirosis on average to 140 days [twenty weeks] slightly increased the prevalence of leptospirosis compared to the default value of fifty six days [eight weeks]. It is likely that other factors limit the spread of infection between grower pigs when the duration of leptospirosis is increased above the preset value of fifty six days.

### **Intensity of leptospirosis**

Decreasing the intensity of leptospirosis for infected pigs to a setting of 500, decreased the prevalence of infection for grower pigs, see Table 7.5. This is an expected result, as fewer leptospires are shed into the environment, therefore, decreasing the likelihood of direct or indirect transmission of infection. Increasing the intensity of leptospirosis to 500,000 (default value is 50,000) did not greatly change the prevalence of infection within the grower herd. SLIP89 simulation is less sensitive to this increase as the preset value is set at a high level, above which there is little effect when altered alone.

### **Environmental and pen contamination division factors**

Both the ECDF and the PCDF are denominators in the equation for calculating the probability of infection for a susceptible pig. Therefore, lowered values will result in an increase in the probability of infection, while high values will decrease the probability of infection, see Table 7.6. These variables have also been termed virulence factors as they influence the ease with which infection is spread between pigs. Decreasing both the ECDF and the PCDF [i.e. increasing the virulence] resulted in a marked increase in the prevalence of infection of grower pigs. This is expected as the probability of infection for each susceptible pig will have been increased, resulting in infection of grower

**Table 7.4** SLIP89 results of the average prevalence of leptospiral infection in grower pigs when validating for the of duration of leptospiruria.

**Table 7.4a** Duration of leptospiruria set to a mean of twenty one days and a standard deviation of seven days.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	37*	32	29	32	31
32	39	28	27	32	36
33	36	33	26	34	36
34	40	34	30	32	35
35	34	34	33	37	34
36	32	35	41	40	42
37	34	37	40	43	37
38	30	44	41	44	42
39	32	48	42	46	43
40	32	49	46	44	48
41	31	53	48	45	49
42	35	56	49	48	50
43	38	57	53	53	50
44	35	53	46	49	45
45	31	52	44	44	41
46	37	48	38	42	43
47	38	53	36	46	45
48	43	54	43	47	45
49	44	59	39	47	50
50	53	61	43	47	52

**Table 7.4b.** Duration of leptospiruria set to a mean of one hundred and forty days with a standard deviation of seven days.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	21*	25	37	49	37
32	29	29	38	55	40
33	37	33	36	57	45
34	42	36	42	63	47
35	45	39	45	65	53
36	50	44	50	70	55
37	55	44	54	75	555
38	57	49	53	76	57
39	62	51	53	79	57
40	62	54	52	78	57
41	66	61	57	77	61
42	69	61	58	79	63
43	69	65	60	83	63
44	63	56	58	82	60
45	59	55	49	76	58
46	56	48	48	81	60
47	59	46	43	80	58
48	58	53	51	82	57
49	51	47	51	79	59
50	46	38	52	80	54

\* = Percent rounded to the nearest whole number.

**Table 7.5** SLIP89 results of the average prevalence of infection in grower pigs when validating the of intensity of leptospirosis.

**Table 7.5a** Intensity of leptospirosis set to a mean of five hundred and a standard deviation of two.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	27*	30	44	28	22
32	25	33	45	27	24
33	27	32	41	29	22
34	21	32	42	28	27
35	25	29	41	31	30
36	27	29	40	30	28
37	34	34	36	32	31
38	37	34	37	32	35
39	38	37	35	36	36
40	39	42	37	41	35
41	42	45	40	39	35
42	46	49	37	41	35
43	51	49	37	47	39
44	50	44	33	45	35
45	52	48	29	44	34
46	52	47	27	46	34
47	57	51	28	49	34
48	65	56	32	49	37
49	68	57	33	51	40
50	70	60	38	50	39

**Table 7.5b.** Intensity of leptospirosis set to a mean of 500,000 and a standard deviation of two.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	47*	40	31	25	36
32	50	36	40	37	32
33	49	33	40	45	36
34	50	40	39	44	38
35	47	39	44	48	39
36	52	41	45	44	44
37	44	46	47	44	46
38	50	49	54	51	48
39	58	54	55	54	55
40	53	57	60	55	57
41	56	60	60	57	65
42	55	62	65	64	63
43	62	67	66	68	65
44	61	67	62	66	61
45	54	59	54	56	59
46	57	55	57	59	53
47	54	54	59	60	50
48	57	53	65	64	60
49	65	54	68	66	62
50	66	58	68	58	68

\* = Percent rounded to the nearest whole number.

pigs soon after their passive immunity wanes. A noticeable decrease in the prevalence of infection was noted when the ECDF and the PCDF were increased, [virulence decreased]. Thus the reverse of the above was occurring; as the probability of infection for susceptible pigs was lowered, there was a longer period of time before pigs susceptible to infection become infected. Therefore the prevalence of infection amongst grower pigs was on average lowered. SLIP89 was moderately sensitive to alteration of the ECDF and PCDF parameters.

### **Contact between pigs**

Decreasing the direct contact between groups of pigs to 0% did not decrease the prevalence of infection for grower pigs, Table 7.7. At first this may appear incorrect, however, there are several reasons why the prevalence did not decrease. Infection is initially seeded into the grower house thus enabling endemic leptospiral infection within the grower herd to be simulated, this occurs when the "Endemic" option is selected, as was the case for all sensitivity tests. Once infection is established within the grower house, infection can spread indirectly via fomites and by aerosol [see Chapter Five]. Therefore endemic infection can persist in the absence of direct contact of pigs. The absence of contact between pigs did however reduce the spread of infection between population sectors. This was reflected by the lowered prevalence of infection of pigs in the breeding herd and in the weaner house. Maximum contact of 100% between pigs increased the prevalence of infection for all population sectors, and is likely to be the result of mixing infected and susceptible pigs, thus allowing great opportunity for the direct transmission of infection. These results suggest that within the model the grower herd is a reservoir of infection for other pigs.

For the maintenance of endemic *poona* infection within a pig herd, the probability of infection is related to the duration and intensity of leptospiuria, and the virulence of the organism for the host species. This has been reflected in the sensitivity studies of SLIP89.

**Table 7.6** SLIP89 results of the average prevalence of infection in grower pigs when validating the contamination division factors.

**Table 7.6a** Environmental and Pen Contamination Division Factors both set to fifty.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	41*	40	77	56	71
32	46	48	74	65	90
33	51	57	79	72	95
34	53	65	78	71	91
35	65	71	86	79	86
36	69	69	92	80	93
37	78	79	95	79	95
38	86	81	96	76	97
39	84	84	97	76	97
40	82	83	98	81	96
41	79	77	99	81	97
42	87	88	98	84	99
43	86	93	97	86	99
44	79	92	98	80	96
45	87	80	97	77	100
46	92	88	96	91	100
47	91	85	97	84	99
48	91	95	97	82	99
49	93	84	99	80	100
50	81	78	94	93	100

**Table 7.6b** Environmental and Pen Contamination Division Factors both set to two hundred and fifty.

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	21*	25	25	25	21
32	24	28	33	23	28
33	17	30	33	24	27
34	18	28	38	23	27
35	23	28	31	24	28
36	27	30	30	31	31
37	32	28	30	36	34
38	38	31	25	39	40
39	39	28	28	38	42
40	40	32	29	37	45
41	40	35	30	42	40
42	44	36	32	40	47
43	48	39	38	45	54
44	42	33	38	41	51
45	42	36	36	33	46
46	42	36	36	37	51
47	44	41	42	33	48
48	44	40	46	28	51
49	44	42	47	26	41
50	49	45	44	32	40

\* = Percent rounded to the nearest whole number.

**Table 7.7**      **Simulation results of the average prevalence of infection for the validation of contact between pigs.**

**Table 7.7a**      **Degree of contact set to zero for all settings.**

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	37*	31	50	31	32
32	46	38	56	36	40
33	46	39	61	47	30
34	52	45	60	50	38
35	45	50	65	56	48
36	50	52	64	54	48
37	54	58	52	62	51
38	60	59	55	63	55
39	58	67	57	66	58
40	60	72	63	71	57
41	63	72	61	75	66
42	66	73	64	79	68
43	70	75	69	78	73
44	65	70	63	67	73
45	58	61	58	61	69
46	61	61	60	60	76
47	59	63	60	71	74
48	64	62	66	63	71
49	61	61	68	67	73
50	59	54	69	68	72

**Table 7.7b**      **Degree of contact set to one hundred percent for all settings.**

Simular Week	SLIP89 Simulation number				
	1	2	3	4	5
31	66*	56	67	73	71
32	75	64	75	81	77
33	81	71	80	80	84
34	71	77	84	84	79
35	74	87	88	79	79
36	78	86	89	80	85
37	82	86	93	79	82
38	89	89	95	79	81
39	89	86	91	82	82
40	90	89	92	88	84
41	89	90	94	86	82
42	91	90	95	91	82
43	92	93	96	93	80
44	94	90	96	94	78
45	83	86	86	92	80
46	85	89	87	91	85
47	79	86	89	85	86
48	76	89	90	85	84
49	73	91	90	81	85
50	73	83	88	88	92

\* = Percent rounded to the nearest whole number

It is part of the function of the model to allow the operator to alter the parameters of one or more variables and to observe its effect. Experience suggests that a minimum of five simulation runs should be conducted to adequately represent natural variation in the outcome (Roe, 1977). This can serve as an important means of understanding the multivariate nature of the disease, and of exploring potential methods for the control of infection within the simulated population. It is important that the user of any computer simulation model understands the series of events being simulated since the outcome of the simulation will reflect personal theories and understanding of the disease being simulated. In this case, the epidemiology of *pomona* infection within a pig herd, as described in this thesis has been used as the basis for the structure of the SLIP89 computer simulation model.

#### ***Examination of SLIP89 results***

The Output Menu provides the means of examining the data generated by computer simulations of SLIP89. There are four options for exploring this data. They are the examination of:

- 1) Cohort Data.
- 2) Time Series Graphs.
- 3) Age Group Data.
- 4) Pen Data.

The variables examined in each of these options has been detailed in Appendix IV on "Procedure for using SLIP89". A set of results from a simulation using only the preset [default] values for reproductive and epidemiological variables, a sow herd size of fifty and a simulation period [runtime] of fifty weeks are summarized in Appendix IV, and can be accessed using a computer by following the instructions described in Appendix IV. Appendix V contains a reference list of data files from other simulations which have been previously run and recorded on disk and these can be similarly examined if one has access to a suitable computer.



Changes in the behaviour of epidemiological variables can be examined by viewing one of a number of graphs. Selecting the Cohort Data option enables the prevalence of infection in grower pigs aged between ten and twenty four weeks to be examined. Generally the average simulated prevalence of infection in these pigs is near 50%, see Figure A4.6. The age groups of grower pigs which are infected, and the period over which they become infected, can be examined by selecting the Age Group Data option, see Figure A4.8. This option displays the average of the results of epidemiological variables for grower pig aged one to twenty four weeks. These results indicate that the majority of pigs become infected following entry into the grower house at ten weeks of age. The titres of young pigs can be seen to decline as their age increases and usually these pigs remain free of infection while in the weaner house, see Figures A4.7 and A4.8. When infection of young pigs occurs, it does so in a cyclical pattern, however the prevalence of infection amongst these pigs is usually less than 5%. The combination of intermittent infection with a low prevalence is a likely reasons why infection was not detected in pigs of less than ten weeks of age in the field studies described in Chapter Four. Further detail of the patterns of infection within one of the first four pens in either the weaner or grower house can be examined by selecting the Pen Data Records option, see Appendix IV. By examining the trends of the epidemiological variables for pigs within a pen, each pen can be seen to be an environment within which an outbreak of infection occurs, as shown in Figure A4.9.

The model simulates a recognisable pattern of infection for grower pigs. Infection generally commences in pigs equal to or older than twelve weeks, and the prevalence of infection peaks before or close to the time of slaughter. Occasionally a whole pen of pigs may escape infection, while at the other extreme, some pigs may become infected while in the weaner house and in these cases the prevalence of infection within a grower house pen may reach a peak within a few weeks of these pigs being housed in it. These patterns of infection, or "pen epidemics", are similar to the findings of the observational studies described and discussed in Chapter Four.

The Time Series Display option enables one to examine trends of variables and different population sectors [suckling, weaner, grower, gilt, sows, and boars], over a number of simulated weeks. The display is a series of graphs, one for each summary interval, which are sequentially displayed (Figure A4.7). The display can give a "moving picture" of the trend of a variable over time. For example, Figure A4.7 shows the prevalence of infection in grower pigs, gilts and sows to remained relatively constant, while a low prevalence was recorded in the weaner pigs for one of the summary intervals, however the temporary infection amongst weaner pigs did not appear to significantly affect the prevalence in grower pigs in the following summary intervals. Examination of the Time Series graphs is useful for comparing the values of a variable between the six groups of pigs.

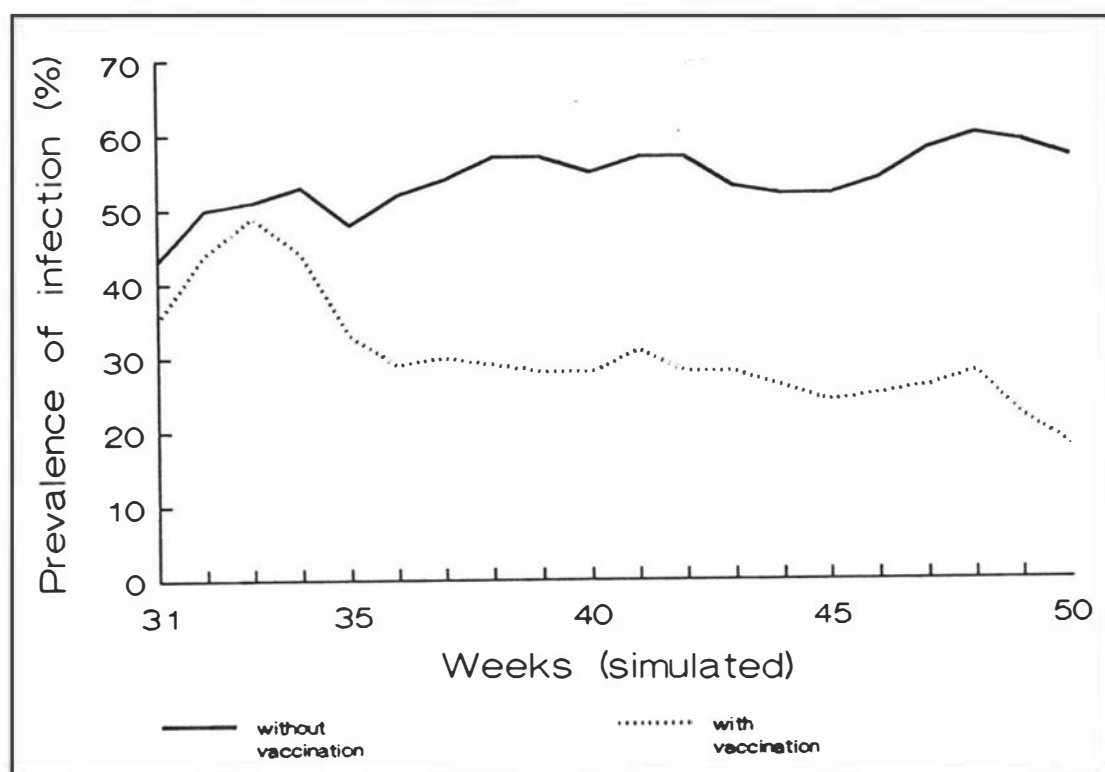
Vaccination of grower pigs has been simulated by SLIP89 and data files are provided (see Appendix V). Initially the prevalence decreased in the grower pigs to around 20%, however towards the end of the 150 week period of simulation, see Figure 7.11. In longer simulations of 150 weeks, the prevalence of infection in the grower pigs initially decreased to around 20% but later increased to around 65%. The most likely reason that this occurred is due to the change of infectious and immunological status of the breeding herd over this period of time. As fewer gilts became infected, the GMT of pigs in the breeding herd fell and piglets became susceptible to infection at an earlier age. The focus for the maintenance of infection was then moved to pigs of a younger age. The consequence of vaccination where self-replacement of gilts is practised is also discussed in Chapter Six.

## DISCUSSION

In the development of a computer simulation model it is firstly important to keep sight of, and achieve the objectives set for the development of the model.

Otherwise development of the model could continue *ad infinitum*, resulting in a more detailed final product, but failing to achieve the initial objectives. The

**Figure 7.11** Prevalence of *pomona* infection in grower pigs following simulated vaccination.



primary objective of this model was to simulate endemic *pomona* infection within a pig herd. Varying degrees of detail were used in the development of SLIP89. Considerable detail was given to the means by which the final probability of infection for susceptible pigs was obtained, and this was based on investigations of the mechanisms for the transmission of infection between pigs, described in Chapters Four and Five. As a result, the means for calculating the probability of infection became increasingly detailed as the options for transmission of infection were explored. In contrast, the means for simulating the effect of vaccination has been simplified and is achieved using a measure of the vaccinal efficacy, which is converted to a measure of relative risk of infection for vaccinated pigs. In this way, a final probability of infection for a vaccinated pig is obtained. The duration of infection and intensity of leptospiruria have not been altered in the model when simulating the effect of vaccination. Therefore when simulating vaccination, these variables will not be accurately predicted. As a result the duration for eradication or control of infection to an acceptable level will be an over estimate because the intensity

and duration of leptospiuria have not been reduced as appeared to occur in field trials (Chapter Six). However the prevalence of infection is affected when simulating vaccination and this affects the infectious status of the simulated population from generation to generation. For example, when vaccination efficacy was simulated to be 80%, and the age of last vaccination at twelve weeks; the results of the simulation were an initial decrease in the prevalence of infection amongst grower pigs, see Figure 7.11. One would expect more promising results if the age of last vaccination could be lowered to ten weeks after an initial period of vaccination at twelve weeks. In the interpretation of the results one must bear in mind that SLIP89 does not account for the specific effects of the vaccination on a pig's immune status. For example, the interaction between residual maternal antibody and bacterin has not been taken into account in SLIP89. These limitations should not prevent one from "experimenting" with vaccination at different ages, so long as they are kept in mind when interpreting the results. For increased accuracy in simulating the effect of vaccination or other control measures within a pig herd, a new sequence of events and therefore further development of the current computer simulation model will have to be made.

The greatest shortcoming in the testing of SLIP89 model has been the lack of opportunity to compare its results with independent results from pig herds with endemic *pomona* infection. Therefore the model can not be said to have been truly validated. Validation is in its self a subjective assessment, and the results obtained from any given pig farm are themselves representative of a series of interactions between the hosts, agent and environment at a particular point in time. The results obtained from the SLIP89 simulation are similar to the epidemiological characteristics of the populations of grower pigs studied on Farms B and C (described in Chapter Four), however the logic of the model was based on the understanding of the epidemiology of infection within these pig herds. Therefore, if the simulation model is a fair representation of the epidemiology of leptospiral infection on those farms, then one would expect the SLIP89 simulation results and the farm results to be similar. True validation in the form of acceptance of the model will come with increasing exposure of the model to veterinarians, epidemiologists and other interested persons. It is

hoped that publication of the model and its applied use in undergraduate and post-graduate teaching will stimulate discussion about the epidemiology of leptospiral infection in pig herds.

Throughout the development of this computer simulation model the process of defining observational and experimental findings into a logical series of events was found to be an interesting and stimulating exercise. The Monte Carlo method was readily applied, as well as providing a suitable means for simulating complex biological interactions in the absence of mathematical formulas. As a result of this detailed thought process some aspects of the research were directed towards the collection of data which appeared to be important in the epidemiology of *pomona* in pig herds, but for which little information was available from current literature, especially with reference to naturally infected pigs. As a consequence it was gratifying to be able to use information obtained in the studies described in this thesis in the development of SLIP89. It is now intended that this model will be used by students [undergraduate and post graduate] to further their understanding of the epidemiological cycle of endemic *pomona* infection within a pig herd, and the use of computer simulation programs.

Future developments of SLIP89 that could readily be undertaken may include the alteration of pigs' records following vaccination, incorporation of the specific effect of current or hypothetical control measures, the inclusion of economic factors, and increasing the efficiency of the program to deal with a larger pig herd and for a longer simulation period.

In conclusion, the development of SLIP89 computer simulation model formed an interesting and educational adjunct to the traditional means of investigating disease within a population. In the author's opinion, this model provides a satisfactory approximation of the epidemiological behaviour of *pomona* within a pig herd. If the model serves to stimulate discussion about both the epidemiology of leptospiral infection in pig herds, and the advantages and disadvantages of computer simulation models, then the model has served a further useful purpose.

## APPENDIX I

*i) Preparation of Stuart's Basal Medium [SBM]*

Prepare 1000 ml of distilled water.

Add 5 g of glycerol

3.4 g Stuart's medium base dehydrated powder<sup>1</sup>

Mix until dissolved.

Dispense into 4.5 ml, 10 ml, or 100 ml bottles.

Autoclave for 20 minutes at 121°C.

*ii) Preparation of Bovine Serum Albumin Diluent [BSAD]*

Dissolve 10.0 g of Fraction V Bovine Albumin<sup>2</sup> [BSA] in 100 ml double distilled water.

Sterilise by filtration through a 0.22  $\mu$ m membrane filter.

Keep refrigerated until required.

Dilute 1/10 with double distilled water to form final concentration of 1% BSA.

Add 1 ml of 5FU [see below] for every 100 ml final volume.

Dispense in 4.5 ml aliquot into sterile glass bottles.

*iii) Preparation of 5 Fluorouracil [5FU]*

Add 1.0 g of 5FU<sup>3</sup> to 50 ml of double distilled water.

Dissolve at 60°C in water bath.

Adjust pH to 7.6 using 1N HCl.

Make up to a total volume of 100 ml using distilled water.

Sterilise by filtration through a 0.22  $\mu$ m membrane filter.

Store in fridge at 4°C until required.

*iv) Preparation of Phosphate Buffer Solution [PBS]*

Dissolve in two litres of distilled water:

15.3 g Sodium Chloride

1.448 g Disodium hydrogen orthophosphate

0.42 g Potassium dihydrogen phosphate

Adjust the pH to 7.4.

Dispense into glass bottles and autoclave at 121°C for 15 minutes.

Store at room temperature until required.

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1 Stuart's Medium Base. Difco Laboratories. Detroit, Michigan, U.S.A.

2 Fraction V Bovine Serum Albumin. Pentax Division. Miles Laboratories. Victoria, Australia.

3 5 Fluorouracil. Sigma. St.Louis, Missouri, U.S.A.

v) Preparation of Tween-albumin serum culture medium [EMJH]

Prepare stock solutions of the following chemicals in 100 ml of deionised water:

1) Ammonium chloride ( $\text{Na}_4\text{Cl}$ )	[25.0 g] [B.H.D.] <sup>4</sup>
2) Zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ )	[ 0.4 g] [M&B] <sup>5</sup>
3) Magnesium chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ )	[ 1.5 g] [Analar] <sup>6</sup>
4) Calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ )	[ 1.5 g] [Analar]
5) Ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ )	[ 0.5 g] [Analar]
6) Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ )	[ 0.3 g] [Analar]
7) Sodium pyruvate	[10.0 g] [B.H.D.]
8) Glycerol	[10.0 g] [B.H.D.]
9) Tween 80	[10.0 g] [Sigma] <sup>7</sup>
10) Thiamine (Vitamin $\text{B}_1$ )	[ 0.5 g] [Sigma]
11) Cyanocobalamin (Vitamin $\text{B}_{12}$ )	[ 0.02 g] [Sigma]

The base medium was prepared by dissolving the following chemicals in 996 ml of deionised water:

1) Disodium hydrogen orthophosphate ( $\text{Na}_2\text{HPO}_4$ )	[ 1.0 g] [Analar]
2) Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	[ 0.3 g] [Analar]
3) Sodium chloride ( $\text{NaCl}$ )	[ 1.0 g] [Analar]

The following stock solutions were then added and then the pH adjusted to 7.4 using either hydrochloric acid (HCl) or sodium hydroxide (NaOH).

1) Ammonium chloride	[ 1.0 ml]
2) Thiamine	[ 1.0 ml]
3) Sodium pyruvate	[ 1.0 ml]
4) Glycerol	[ 1.0 ml]

The base medium was then sterilized by autoclaving at 121°C for 20 minutes.

The albumin supplement was prepared by the dissolving 20 g of Fraction V Bovine Albumin to 100 ml of deionised water by continual stirring, thereafter the following stock solutions were added:

1) Calcium chloride	[ 2.0 ml]
2) Magnesium chloride	[ 2.0 ml]
3) Zinc sulphate	[ 2.0 ml]
4) Copper sulphate	[ 0.2 ml]
5) Ferrous sulphate	[20.0 ml]

<sup>4</sup> B.H.D. British Drug House, Poole, England, U.K.

<sup>5</sup> May and Baker Ltd, Dagenham, England, U.K.

<sup>6</sup> B.D.H. Chemicals Ltd, Poole, England, U.K.

<sup>7</sup> Sigma Chemicals Co., P.O.Box 14508, St Louis, Missouri 63178, U.S.A.

- |                   |           |
|-------------------|-----------|
| 6) Cyanocobalamin | [ 2.0 ml] |
| 7) Tween 80       | [25.0 ml] |

The pH was then adjusted to 7.4 using either hydrochloric acid or sodium hydroxide, and the total volume adjusted to 200 ml using deionised water. This supplement was sterilized by filtration through a 0.22 $\mu$ m membrane filter.

Liquid EMJH medium was prepared by adding 30 ml of the albumin supplement to 270 ml of the base medium and 0.33 ml of Neomycin [2 mg/ml]. Volumes of 5 ml of the medium were dispensed into sterile screw top bottles within a laminar flow cabinet.

Semisolid EMJH medium was prepared by the addition of 0.75 g of agar [Difco, bacteriological grade] to 450 ml of base medium. This was then autoclaved at 121°C for 20 minutes and allowed to cool to approximately 40°C before the addition of 50 ml of albumin supplement. Either 1 ml or 2 ml of 5FU [see Appendix I, iii] were added to the final medium to form a concentration of either 200  $\mu$ g or 400  $\mu$ g of 5FU per ml of medium. The medium was then dispensed into sterile screw cap bottles within a laminar flow cabinet.

New batches of media were incubated at 37°C for three days, then checked for evidence of contamination and stored at room temperature.

vi) Preparation of T80/40LH medium for bratislava

Prepare the following stock solutions by dissolving the following chemicals in 100 ml of distilled water.

- |                            |                   |
|----------------------------|-------------------|
| 1) Zinc sulphate           | [ 0.4 g]          |
| 2) Calcium chloride        | [ 1.0 g]          |
| 3) Magnesium chloride      | [ 1.0 g]          |
| 4) Glycerol                | [10.0 ml]         |
| 5) Vitamin B <sub>12</sub> | [ 0.02 g]         |
| 6) Thiamine chloride       | [ 0.5 g]          |
| 7) Manganese sulphate      | [ 0.3 g] [B.H.D.] |
| 8) Ferrous sulphate        | [ 0.5 g]          |
| 9) 5 Fluorouracil          | [ 1.0 g]          |
| 10) Nalidixic acid         | [ 0.1 g]          |

Prepare the following stock solutions in 180 ml of distilled water:

- |              |           |
|--------------|-----------|
| 11) Tween 80 | [20.0 ml] |
| 12) Tween 40 | [20.0 ml] |

Prepare the albumin supplement by slowly stirring the following chemicals in 50 ml of sterilized distilled water:

- |                                    |          |
|------------------------------------|----------|
| 1) Fraction V bovine serum albumin | [10.0 g] |
|------------------------------------|----------|



- |                            |                               |
|----------------------------|-------------------------------|
| 2) Lactalbumin hydrolysate | [ 1.0 g] [Difco] <sup>8</sup> |
| 3) Superoxide dismutase    | [ 0.1 g]                      |
| 4) Sodium pyruvate         | [ 0.04 g]                     |

When fully dissolved the following stock solutions were added:

- |                            |           |
|----------------------------|-----------|
| 1) Thiamine chloride       | [ 1.0 ml] |
| 2) Calcium chloride        | [ 1.0 ml] |
| 3) Magnesium chloride      | [ 1.0 ml] |
| 4) Zinc sulphate           | [ 1.0 ml] |
| 5) Manganese sulphate      | [ 1.0 ml] |
| 6) Ferrous sulphate        | [10.0 ml] |
| 7) Vitamin B <sub>12</sub> | [ 1.0 ml] |
| 8) Tween 80                | [ 9.0 ml] |
| 9) Tween 40                | [ 3.5 ml] |

This solution was then stirred for one hour to ensure that all ingredients were dissolved, and the pH adjusted to 7.4 using either hydrogen chloride or sodium hydroxide. The volume was then adjusted to 96 ml using sterilized distilled water and 4 ml of rabbit sera, previously heated to 56°C, was added. The addition of 20 ml of both nalidixic acid and 5FU followed. The final solution was then sterilized by filtration through a filter with a pore size of 0.22  $\mu$ m.

The semisolid T80/40LH medium was then prepared by dissolving the following chemicals in 998 ml of distilled water:

- |                                     |          |
|-------------------------------------|----------|
| 1) Disodium hydrogen orthophosphate | [1.0 g]  |
| 2) Potassium dihydrogen phosphate   | [0.3 g]  |
| 3) Sodium chloride                  | [1.0 g]  |
| 4) Ammonium chloride stock solution | [1.0 ml] |
| 5) Glycerol stock solution          | [1.0 ml] |

The pH was adjusted to 7.4 using sodium hydroxide. To 860 ml of this solution 1.5 g of agar [Difco, bacteriological grade] was added and autoclaved at 121°C for 20 minutes and allowed to cool to approximately 50°C before adding 140 ml of supplement. The final solution was then mixed and dispensed in 5 ml volumes into sterile screw top bottles within a laminar flow cabinet.

APPENDIX II

*Conversion table for reciprocal titre to Coded Titre Unit*

Reciprocal titre	Coded titre unit
0	∞
1	-2.323
4	-0.32
8	0.68
10	1
16	1.68
20	2
32	2.68
40	3
64	3.68
80	4
128	4.68
160	5
256	5.68
320	6
640	7
1,280	8
2,560	9
5,120	10
10,240	11
20,480	12
40,960	13
81,920	14
163,840	15
327,680	16

---

Reciprocal titre =  $10 \times 2^{(\text{CTU} - 1)}$

$$\text{CTU} = \frac{\ln(\text{reciprocal titre})}{0.6931} - 2.303 + 1$$

## APPENDIX III

PIG FARMER INFORMATION SHEET

## ADDRESS

## PRODUCTION

Sow Breeding Herd Size (integer) .....

Number of Boars (integer) .....

Number of Growers (integer) .....

Replacement Rate (%) .....

Pigs/Sow/Annum (real) .....

## MANAGEMENT

Farming System (intensive/semi-intensive/extensive) .....

Are Sow Records kept (y/n) .....

Can litter be traced from sow records (y/n) .....

Are sows kept separate from grower pigs (y/n) .....

Degree of contact (continuous/occasional) .....

When are grower pigs slaughtered (pork/bacon) .....

At which abbatoir are pigs slaughtered (sows) .....

(boars) .....

(growers) .....

Are litters mixed (y) .....

Age of mixing (preweaning) .....

(weaning) .....

(postweaning) .....

Effluent system (open/closed) .....

Comments:

## DISEASE STATUS

Are stock vaccinated (y/n) .....  
 Type of vaccine (leptovax/leptovoid) .....

Sows frequency (time interval) .....  
 Boars frequency (time interval) .....  
 Grower frequency (time interval) .....

General health status (A/B/C/D) .....

Have there been any recent abortions (y/n) .....  
 How many (integer) .....  
 Over what time period (dates) .....  
 Sow and Boar ID (Sow ID) .....  
 (Boar ID) .....  
 .....  
 .....

Have any persons on this farm previously

Had leptospirosis (y/n) .....  
 Name .....  
 Date of illness .....  
 Suspected serovar .....

.....  
 .....  
 .....

## APPENDIX IV

**PROCEDURE FOR USING SLIP89**

Simulated Leptospiral Infection [*pomona*] within a Pig herd is called SLIP89. The program can be executed using any IBM compatible computer, and simulation is faster when using an AT computer. A diskette containing the files necessary for running SLIP89 is located on the inside of the back cover of this thesis. A printer is a useful adjunct when running SLIP89. SLIP89 has been programmed using Turbo Pascal 4.0 on a PCXT, IBM compatible computer. A selection of programming segments are shown in Appendix VI. This section will explain in detail how to execute SLIP89, and how to store and retrieve the data created by SLIP89.

***To start***

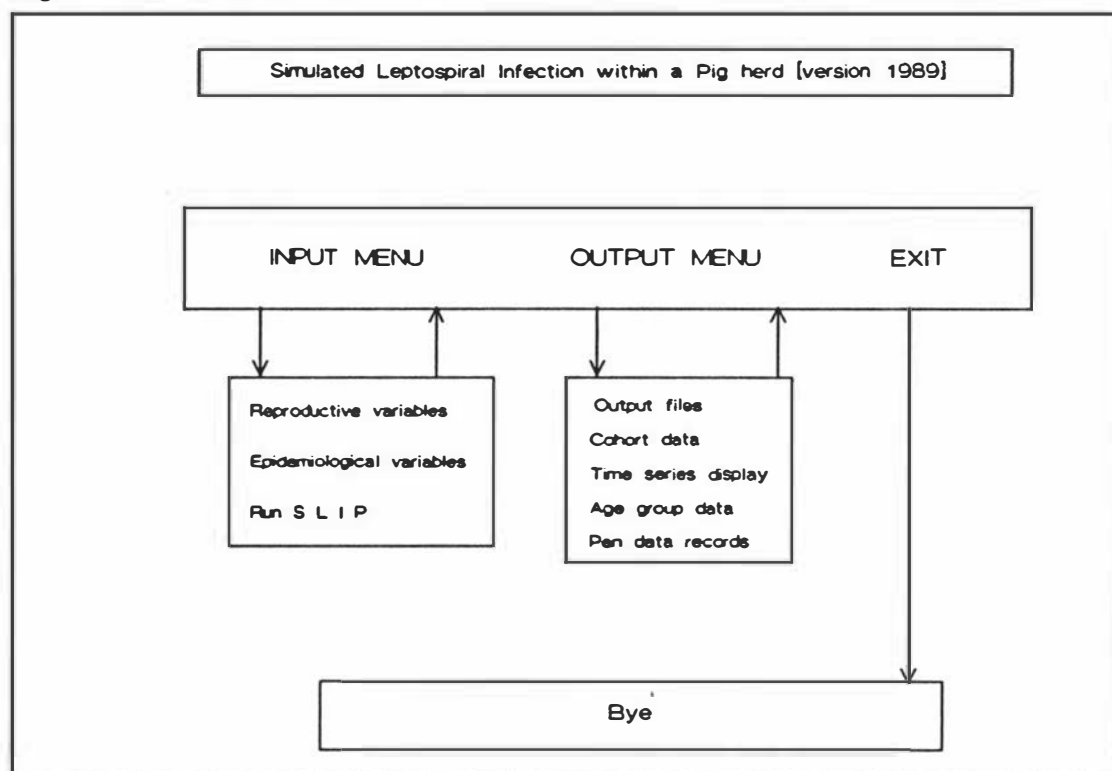
1. Insert the diskette labelled SLIP89 into the disk drive.
2. Type SLIP89 and press return [Enter SLIP89].
3. The title page will appear, press return to continue.

Note: a) Instructions are given at the bottom of the screen.

- b) The ESC key can be used to move back through the menu system.
- c) The X key can be used to return to the last menu setting from a selected menu item.
- d) If a printer is available, check that paper is loaded, and that it is switched ON; otherwise the program will fail if you attempt to use the printer.

***The menu system***

The main menu features three options, these are Input menu, Output menu, and Exit. A summary of the menu is shown in Figure A4.1. The cursor [arrow] keys can be used to move between the menu items, when the appropriate menu is highlighted, press return and the selected menu option will be executed. Alternatively, press the highlighted letter [I, O, or E] of the required menu option and it will be directly executed.

**Figure A4.1 Main menu and sub-menus of SLIP89.**

#### *- Input Menu*

This menu has three sub menus, see Figure A4.1.

- 1) **Reproductive variables**
- 2) **Epidemiological variables**
- 3) **Run SLIP89**

#### 1) **Reproductive variables**

The preset or default values of reproductive variables, given in parenthesis, include those which can potentially influence the population dynamics of the pig herd, see Figure A4.2. To designate a new value to a selected variable, type in the new value and press return, or just press return if the default value is to be used. Once all of the variables have been specified, press return to revert to the Input menu.

**ANNUAL REPLACEMENT RATE:** This refers to the proportion of breeding female pigs which are replaced by gilts in the course of a year. The default value is 30%.

Figure A4.2 Reproductive variables submenu option of SLIP89.

Input Menu - reproductive variables.		
	default	curr
Annual replacement rate (%).....	( 30.0)	30.0
Weaning age, average (days).....	( 35.0)	35.0
Weaning age, standard deviation (days).....	( 0.5)	0.5
Litter size, average.....	( 10.0)	10.0
Litter size, standard deviation.....	( 1.0)	1.0
Mortality to weaning per litter, average.....	( 2.0)	2.0
Mortality to weaning per litter, standard deviation....	( 1.0)	1.0
Press <Enter> to return to Input Menu		

**WEANING AGE:** Refers to the average and standard deviation of the age at which pigs are weaned. The duration is specified in days, and the respective default values are 35 days and 0.5 days.

**LITTER SIZE:** This is the average and standard deviation of the number of live piglets born to breeding females within the simular population. The default values are set to an average of ten live piglets, and a standard deviation of one.

**MORTALITY TO WEANING:** Refers to the expected average and standard deviation of the number of piglets per litter, which would be expected to die before they are weaned. These respective default values are two and one piglets per litter.

## 2) Epidemiological variables

These variables influence the occurrence of disease within the simular population. Default values have been specified, and can be altered in the same way as has been previously described for the reproductive variables, see Figure A4.3. SLIP89 can

be run using the default values for the reproductive and epidemiological variables or after new variables have been entered.

**Figure A4.3 Epidemiological variables sub-menu option of SLIP89.**

```

Input Menu - epidemiological variables.

Titre post infection                                default  current
  average (coded).....( 14.0)    14.0
  standard deviation (coded).....( 1.0)    1.0
Duration of leptospiuria
  average (days).....( 56.0)    56.0
  standard deviation (days).....( 14.0)    14.0
Intensity of leptospiuria
  (ln) average (lepto/ml).....(50000.0) 50000.0
  (ln) standard deviation (lepto/ml).....( 10.0)    10.0
Minimum titre in the presence of infection .....( 6.0)    6.0
Maximum titre for susceptibility to infection ....( 0.9)    0.9
Environmental Contamination Division factor .....( 150.0)   150.0
Pen Contamination Division Factor .....( 150.0)   150.0
Probability (%) of contact between :
  (a) Breeding and Weaner populations .....( 0.0)    0.0
  (b) Weaner and Grower populations .....( 0.0)    0.0
  (c) Grower and Breeding populations .....( 10.0)   10.0
  (d) Breeding stock .....( 10.0)   10.0
  (e) Grower pens .....( 50.0)   50.0
  (f) Weaner pens .....( 10.0)   10.0

Press <Enter> to return to Input Menu

```

**MAXIMUM POST INFECTION TITRE:** This is the average and standard deviation of the maximum MAT *pomona* titre which a pig will acquire after infection. The titres are given in coded titre units [CTU] described in Chapter Two, and conversion tables are shown in Appendix II. The default values are 12 and 1 respectively.

**DURATION OF LEPTOSPIRURIA:** The average and standard deviation of the duration of leptospiuria in infected pigs is measured in days. The average and standard deviation default values have been set at 56 days and 14 days respectively, however a mean of 84 days has also been regularly used by the author.

**INTENSITY OF LEPTOSPIRURIA:** Average and standard deviation of the quantitative measure of the intensity of leptospiuria, the default values are 50,000 and 10



leptospire per ml of urine respectively. Using the default values of 50,000 and 10 leptospire per ml as an example, these values are converted by the natural logarithmic function, to values of 10.8 and 2.3 respectively. Using the normal distribution the 95% confidence interval for these converted concentrations is between 3.9 and 17.7 [ $10.3 \pm 6.9$ ] which when converted back represents concentrations of between 50 leptospire and  $4.8 \times 10^7$  leptospire per ml of urine.

**MINIMUM TITRE IN THE PRESENCE OF INFECTION:** This CTU represents the minimum titre which a newly infected pig can obtain as its maximum titre after infection. Therefore CTU values selected from the normal distribution of "Maximum Post Infection Titre" which fall below the minimum CTU are discarded and a new value selected until it is equal to or above the minimum specified CTU. The default value is set at 6.0 CTU [1/320].

**MINIMUM TITRE FOR PROTECTION FROM INFECTION:** This is the minimum CTU which protects a pig from infection. Therefore when a pig's CTU falls below this specified value, the pig is considered to be susceptible to infection. The default value is set at 0.8 CTU [1/9].

**ENVIRONMENTAL AND PEN CONTAMINATION FACTORS:** These values represent a constant component in the equation used to calculate the probability of infection for a susceptible pig, as has been previously described. Increasing or decreasing this value, serves respectively to decrease or increase the probability for the transmission of disease. For a noticeable effect this value should be altered in steps of around 50. The default value is set at 150.

**THE PROBABILITY OF CONTACT BETWEEN PIGS:** These percentage values reflect the degree of direct or indirect contact between two of the three population sectors; weaners, growers, and breeding pigs, of the similar pig population. The upper level of 100% means that there is contact between each pig in a population sector and pigs of the other population sector each similar week. This would only be considered to occur if there was free mixing of pigs or their effluent between two

population segments. Usually a limited degree of contact is represented by a value of around 10%. The absence of contact between two groups of pigs is assigned a value of 0%. The default values of 0% between all sectors would indicate that population sectors are not mixed together, and that effluent does not flow between the pens. Likewise, direct or indirect contact between pigs within the same population sector is represented on a scale of 0% to 100%. The default values are 10% for breeding stock, indicating a 10% chance that a pig within the breeding herd will have direct contact another breeding pig within the course of a similar week. The default value of 50% for grower pigs indicates that there is frequent contact between pigs from different pens in the course of a week. A value of 0% indicates there is no contact between weaner pigs from different pens.

### 3) **SLIP 89 Simulation**

Enter **S** or press return when Run SLIP is highlighted. Various operating criteria are required before the simulation occurs. These include the name of files for data storage, and the periods for which data is to be stored during the period of the computer simulation, see Figure A4.4.

ENTER TIME FOR SLIP TO RUN: This is the number of simulated weeks for which SLIP89 is to be run. The maximum number of weeks is 250, representing almost five years, or ten generation periods. A 250 week simulation has been found to be more than adequate for examining the maintenance of *pomona* within a pig herd, and to observe changes in the patterns of infection which may occur due to alterations to the preset reproductive or epidemiological values. As a minimum of five separate simulations using the same variables should be examined, it is often convenient to select a simulation period of less than 250 weeks [i.e. 50 weeks]. A simulation period of 50 weeks has a much shorter execution time than 250 weeks and adequately simulates the maintenance of leptospiral infection within a pig herd. The first twenty six simulated weeks an epidemic of infection occurs if the Endemic option is selected [see below]. Also during this initial period of the simulation the population continues to increase in size until it reaches a state of equilibrium. The minimum number of simulated weeks is ten.

**Figure A4.4 SLIP89 sub-menu option for executing SLIP89.**

```

View and change settings.

Input Menu - operating criteria for S L I P 8 9

Enter time for SLIP to run (weeks)..... 50
Sow herd average size..... 50
Simulate endemic leptospirosis ..... y
Vaccination Efficacy..... 0
Last vaccination age..... 0

File storage information:
Enter number of weeks for age group data summary..... 20
Enter number of weeks for cohort summary..... 20

Enter file name for weekly cohort data : a:\xxx.dat
Enter file name for weekly age group data : a:\xxxx.dat
Enter file name for weekly weaner pen data : a:\xxxxx.dat
Enter file name for weekly grower pen data : a:\xxxxxx.dat

Press <Enter> to continue

```

**sow HERD SIZE:** Represents the number of breeding female pigs [sows and gilts] within the pig herd. The size of this population segment influences the size of the weaner and grower herds. Fifty sows has been found to be a good number of sows for the SLIP89 model, which can cope with a maximum of 250 sows. Increasing the sow herd size results in an increase in the execution time. Overloading of the computer's memory and failure of the program may result if a large sow herd size and a high number of simulation weeks have been specified. If too many sows are specified and for too many similar weeks, termination of the program may result.

**SIMULATE ENDEMIC LEPTOSPIROSIS:** Answer with <Y> for yes or <N> for no. The <Y> option elevates the probability of infection for pigs aged twelve weeks and older by 0.1 for the first twenty six weeks. Alternatively with the <N> option, only sows with previous records of infection can act as a reservoir of infection for pigs within the pig herd. Under most circumstances the <Y> option for endemic infection should be selected.

**VACCINATION EFFICACY:** This is a percent value which represents the expected percentage of vaccinated grower pigs which are expected to be free of leptospiral infection at the time of slaughter. A vaccination efficacy value of 0% indicates that vaccination has not occurred. In the absence of vaccination, around 90% of grower pigs are infected at the time of slaughter. The risk of infection for a vaccinated pig is calculated using the vaccination efficacy value and the normally expected prevalence value of 90%. For example, if a vaccination efficacy of 80% is specified, the relative risk is calculated by dividing the percentage of vaccinated pigs which would be expected to become infected  $[100 - 80]$  by the expected percentage of infected pigs in the absence of vaccination  $[90]$ , thus the relative risk of infection for a vaccinated pig would be  $0.22 [(100 - 80)/90]$ . The probability of infection for a vaccinated pig is then calculated by multiplying the relative risk by the probability of infection for that pig, were it a susceptible unvaccinated pig. For example, the probability of infection for a susceptible, unvaccinated pig may be 0.15 in a particular simulated week, this value is multiplied by 0.22 to arrive at a probability of infection of 0.03 for the vaccinated pig.

**LAST VACCINATION AGE:** The simulated age of grower pigs at which the last inoculation of a vaccination regime is given. This variable need only be entered when vaccination efficacy of greater than zero has been used. Pigs are considered to be protected from infection two weeks after their final inoculation.

**FILE STORAGE INFORMATION:** This section specifies the number of simulation weeks for which information generated by the SLIP89 is to be stored on disk, which enables it to be retrieved at a later time for examination. Two information summaries can be stored.

- 1) The "Age Group Data" which records the weekly prevalence, number of infected pigs, number of susceptible pigs, the geometrical mean titre [GMT], and average intensity of leptospiruria of pigs aged one to twenty four weeks.

A maximum of twenty weekly records of "Age Group Data" from the last twenty weeks of the simulation period can be stored on disk. Enter a value

from 1 to 20 to specify the number of weeks for which records are to be retained.

- 2) The "Cohort Data" which is a summary of GMT, average age, average environmental contamination, prevalence of infection, incidence of infection, and the probability of infection for the suckling, weaner, grower, gilt, sow and boar population segments.

"Cohort Data" records can be stored for all weeks of the simulation. Increasing the number of weeks for which Cohort Data is to be stored, increases the execution time of SLIP89. Fifty weeks of data records has been found to be adequate for long simulation periods of 150 weeks or more, while twenty weeks has been most frequently used by the author when simulating for a period of fifty weeks. The data is stored on disk for the last weeks of the simulation period. For example if the period of simulation is 50 weeks and the number of summary weeks for the "Age Group Data" and the "Cohort Data" are both specified as 20, then the records will be stored from week 31 to 50.

Four file names are required for the storage of data. All file names must be accurately defined and must be different from each other. If the name of a previously defined data file is reused then the contents of that data file will be erased. The first file stores the summary information of the "Cohort Data", and the second file stores data from the "Age Group Data" summary. The third and fourth files store summary information about the first four pens within the weaner and the grower houses. If a mistake is made when entering a file names, enter X and reselect the Run SLIP option.

Following the specifications page, an estimate of the execution time for the simulation is given, at this stage there is the opportunity to discontinue. The time estimate will depend on the type of computer being used and may not be very accurate. Press any key to continue, or X to exit.

As SLIP89 commences a summary will appear on the computer screen and it is a summary of some results being obtained during the simulation. The program emits a sound [beep] each time a gilt or sow within the breeding herd aborts due to leptospiral infection. At the end of the simulation, the program will return to the main menu.

#### *- Output Menu*

The output menu has five submenus, see Figure A4.1.

- 1) **Output Files**
- 2) **Cohort Data**
- 3) **Time Series Display**
- 4) **Age Group Data**
- 5) **Pen Data Records**

Except for the first submenu of Output Files, the remaining four submenu options provide various means of examining data stored from previous SLIP89 simulations.

#### 1) **Output Files**

Previously stored data files can only be accessed after their file names and information about the summary intervals has been entered in Output Files, see Figure A4.5.

Five file names need to be entered. Four of these five file names have been previously specified when storing the data from a SLIP89 simulation. The corresponding file names for Cohort Data, Age Group Data, Weaner Pen Data, and Grower Pen Data must be specified to access the data stored in them. For example, if the cohort file name previously specified was A:COH1.DAT then the output file name to examine the Cohort Data must also be A:COH1.DAT. This enables one to conduct several simulations numbering the files consecutively, for example A:COH2.DAT. The results of different simulations can then be examined, using the Output option, by specifying the specific file names. The fifth file name is that of a summary file, and the preset name for this file is SUMMARY.DAT. The summary file is created every time the Output option is selected and contains no new information. Therefore the preset file name can be re-used each time the

Output option is selected.

**Figure A4.5 Output Files submenu of SLIP89.**

```

Output Menu - file names and data summaries.

Enter filename of weekly Cohort Data:      a:\cohort.dat
Enter filename of Age Group Data:          a:\ages.dat
Enter filename of weekly Weaner Pen Data:  a:\wean.dat
Enter filename of weekly Grower Pen Data:  a:\grow.dat
Enter filename for Summary Data:           summary.dat

Data content for Output Files:

<Enter> the first week of summary:         1
<Enter> summary interval (weeks):         1

Start Week of Summary = 1 at Intervals of 1 week(s)
Number of Summary Intervals is 20

Press <Enter> to return to Output Menu

```

The summary interval specifies the number of weeks over which data in the files is summarised and stored in the summary file. The week from which the summary commences must be initially specified. The first week of the summary can be any week for which data is stored. The summary interval must be specified next. This is the number of weeks which are summarised together, to form one summary result. Successive weeks will be sequentially summarised over the specified interval. For example, if data was stored for the last twenty weeks of a simulation, then data could be summarised from the first week of the stored data, in blocks summarising four week at a time, thus resulting in five summary intervals. If the results of every week are to be examined then the summary interval should be set at one.

## 2) Cohort Data

A graphic representation of the average results for one of six cohort groups and one of six epidemiological variables over a specified period of time is shown, see Figure

A4.6. Firstly, Enter a number from 1 to 6 to select one of the six cohort groups for which the data is to be examined.

- 1) suckling piglets
- 2) weaner pigs
- 3) grower pigs
- 4) gilts
- 5) sows
- 6) boars

Secondly, Enter a number from 1 to 6 to select one of the six variables for the Y axis of the graph.

- 1) age
- 2) titre [CTU]
- 3) level of environmental contamination
- 4) prevalence of infection
- 5) incidence of infection
- 6) the probability of infection

The X axis of the graph is formed by the summary results. A graph will be displayed, as shown in Figure A4.6. Press Return in order to return to the Output Menu.

### 3) Time Series Display

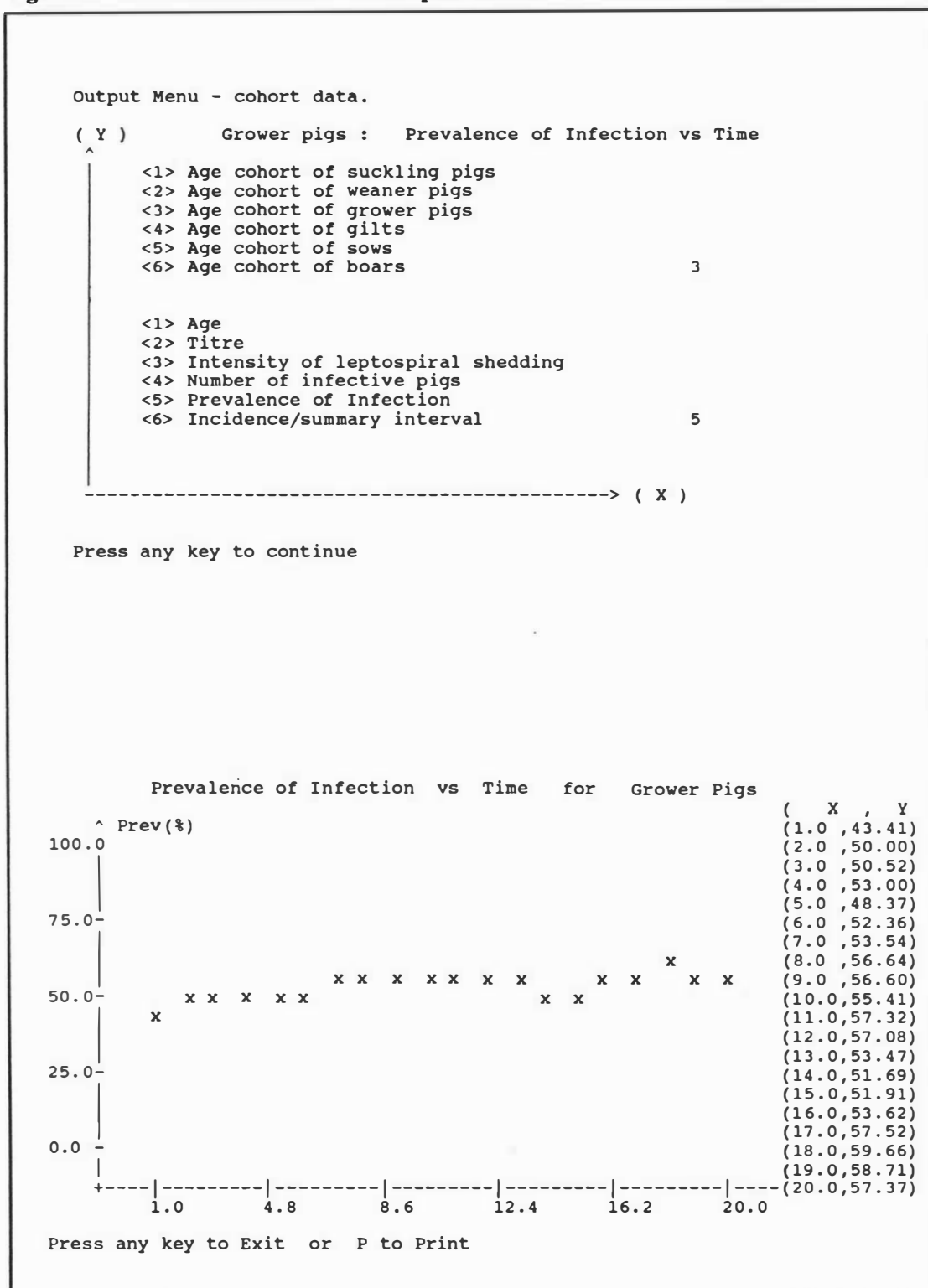
Displays a consecutive series of graphs over a number of specified summary intervals. Each graph shows all six cohort groups [X axis] and the selected variable [Y axis], see Figure A4.7. Firstly, Enter a number from 1 to 6 to select one of the six variables.

- 1) age
- 2) titre
- 3) intensity of leptospiral shedding
- 4) number of infective pigs
- 5) prevalence
- 6) incidence of infection per interval period.

The screen will display the number of weeks that data are stored for, the first summary week, the summary interval, and the number of summary intervals. The



**Figure A4.6 Cohort Data submenu option of SLIP89.**



user must then select which of the summary intervals are to be examined. For example, if there are five summary intervals then the user can examine a full time series by Entering 1 and 5 consecutively, however if only the last two summary intervals need to be examined then one can Enter 4 and then 5. Press return for the next graph or X to exit.

#### 4) **Age Group Data**

Tables of summarised age group data are displayed for the last twenty simulated weeks. The summarised data of pigs aged six to twenty four weeks of age is displayed on the screen, see Figure A4.8. In order to examine the data of pigs aged one to twenty four weeks, press P to print the data [first ensure the printer is turned on]. The epidemiological variables displayed include the number of pigs in each age group, the number infected, the number susceptible to infection, the prevalence of infection, the average intensity of infected leptospiruric pigs in each age group, and the GMT expressed as both a CTU and a MAT titre. Each new table, representing successive weeks is displayed by pressing return, or one can press X to return to the Output Menu.

#### 5) **Pen Data Records**

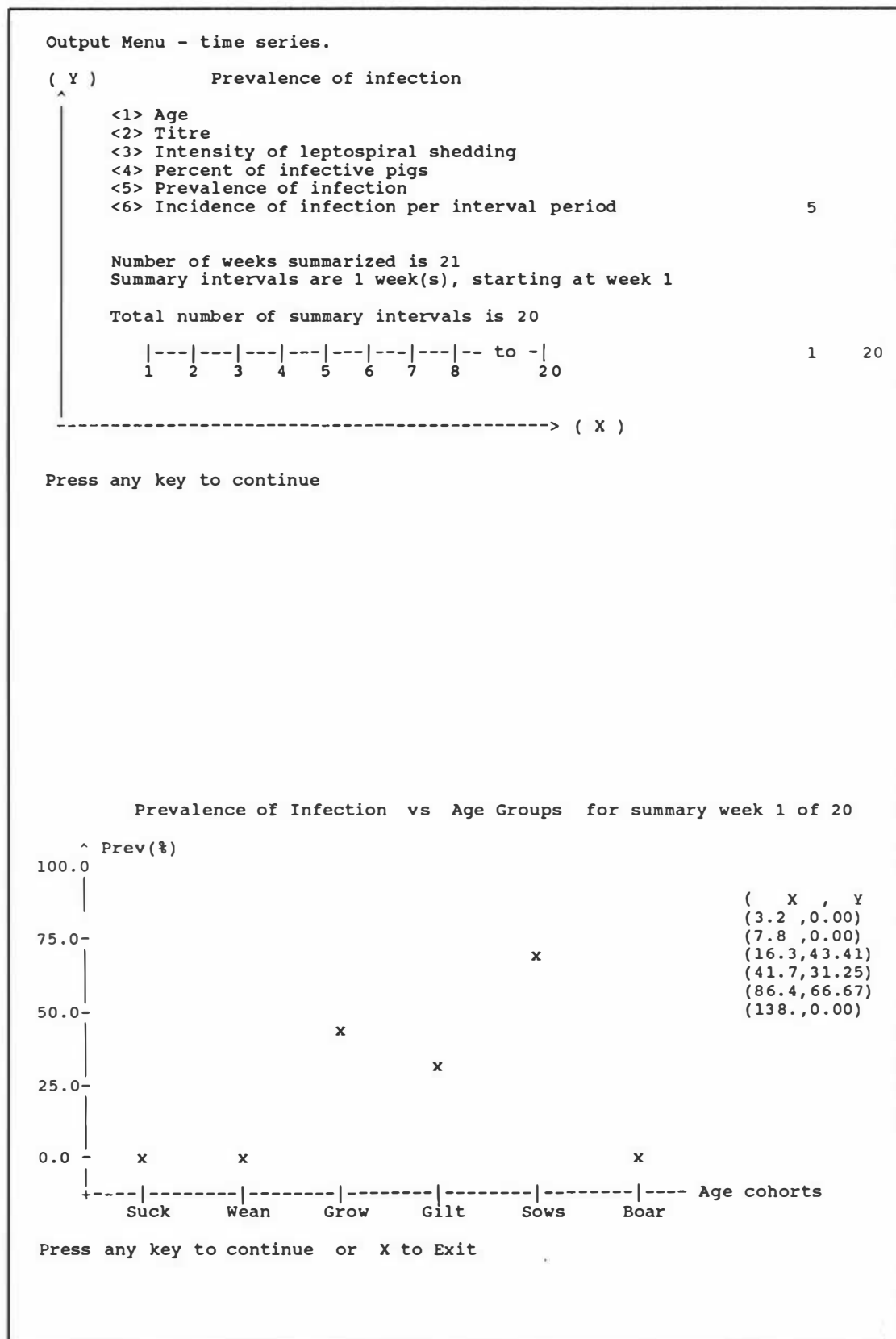
Data from four pens within both the weaner and the grower house can be examined in the form of either a table or graph of a selected variable, as shown in Figure A4.9. Enter either the letter W [weaners] or G [growers] to select which group of pigs is to be examined. Then Enter either the letter A [all data] to select the table option, or P [plot of a selected variable] to obtain a graph.

The table option [A] will display six variables.

- 1) Number of pigs in the pen.
- 2) Percentage of pigs with a high titre [above 1/1280 or CTU of 8].
- 3) Percentage of pigs infective or number with leptospiuria.
- 4) The degree of pen contamination.
- 5) The probability of infection between cohorts of the same pen.
- 6) The probability of infection for pigs within a pen from an indirect source.

Press return to display a new table. Successive tables display the data for

Figure A4.7 Time Series Display submenu option of SLIP89.



**Figure A4.8    Age Data submenu option of SLIP89.**

Age group data - week 1							
Age(wk)	Number	# Inf	# Susc	Prev	L-uria	GMT	Titre
6	18	0	10	0	0	2.4	1/27
7	32	0	15	0	0	1.4	1/14
8	8	0	0	0	0	1.1	1/11
9	18	0	1	0	0	1.0	1/10
10	16	0	16	0	0	0.0	0/0
11	10	0	10	0	0	0.0	0/0
12	38	1	37	3	0	3.5	1/57
13	25	7	18	28	86	6.9	1/580
14	16	8	8	50	241	9.2	1/3044
15	0	-	-	-	-	-	-
16	13	9	4	69	6653	6.1	1/335
17	8	6	2	75	27963	9.6	1/3914
18	10	3	6	40	74210	12.3	1/25428
19	21	8	11	48	98471	11.1	1/11197
20	0	-	-	-	-	-	-
21	17	9	5	71	290194	11.1	1/11008
22	0	-	-	-	-	-	-
23	8	6	0	100	422320	11.5	1/14318
24	16	5	2	88	90672	12.1	1/22157
Press any key to continue    or    P to Print    or    X to Exit							

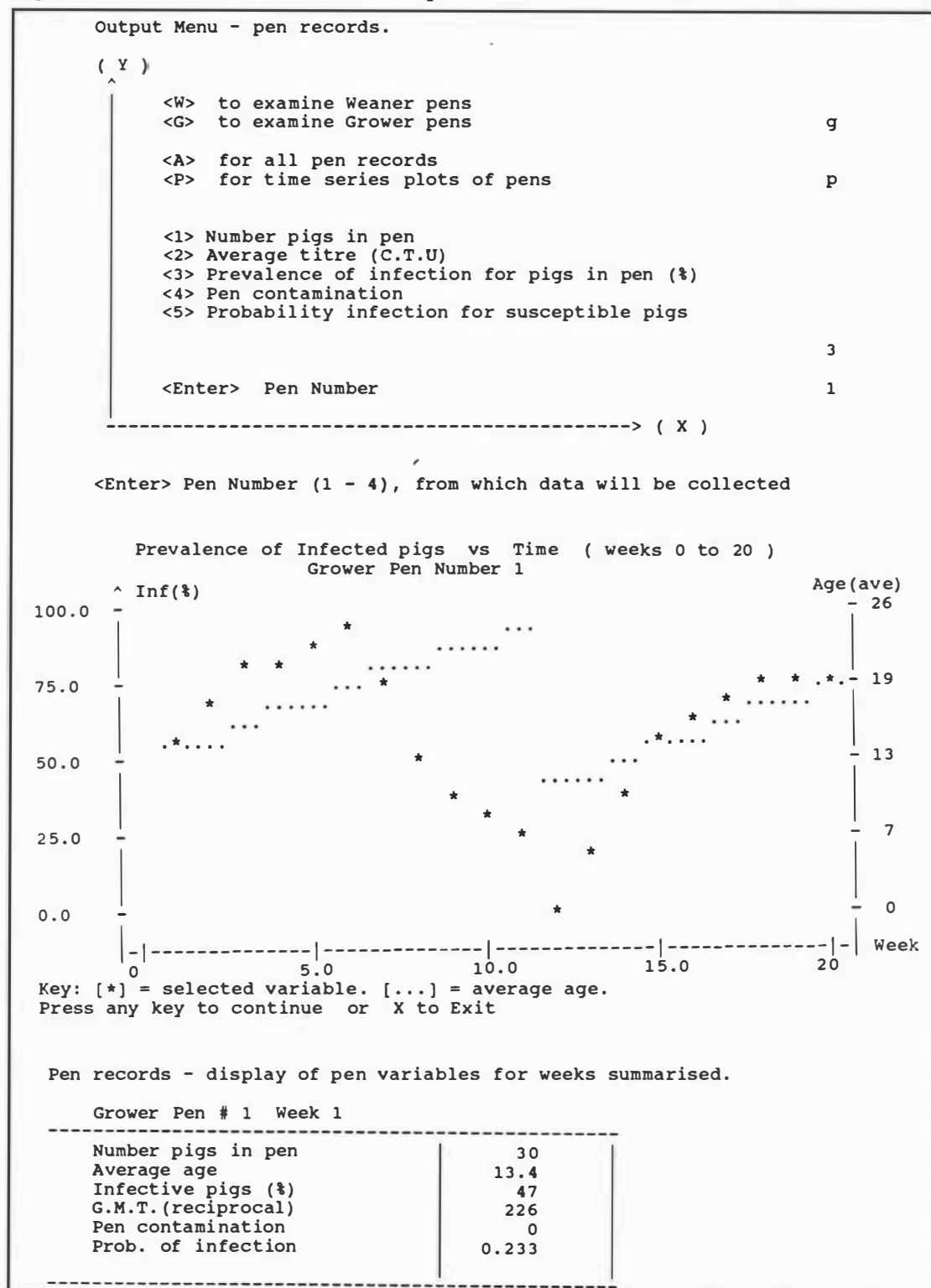
consecutive summary weeks, at the end of the summary period, the table will display the data for the next pen. Press X to return to the Output menu.

The graph option [P] displays one of seven variables over the summary period for a specified pen number. Enter a number from 1 to 7 to select one of the following variables;

- 1) number of pig in the pen.
- 2) prevalence of infection in the pen.
- 3) percentage of pigs with a high titre.
- 4) percentage of infective pigs.
- 5) pen contamination.
- 6) probability of infection from cohorts.
- 7) probability of infection from cross contamination.

Then Enter a number from 1 to 4 to select the pen for which the data is to be displayed. The stars on the graph are the values for the selected variable, while the three dots represent the average age of pigs within the pen. Press any key to select variables for another graph, or press X to return to the Output Menu.

Figure A4.9 Pen Records submenu option of SLIP89.



### *- Exit*

Execution of the SLIP89 can be terminated by entering E for Exit from the Main Menu, or by using the cursor keys to highlight Exit in the Main Menu and then pressing return. The word "bye" will appear on the screen, if you do not want to terminate the use of SLIP89 at this stage, then press the ESC, otherwise press return and the program will conclude.

### *Program Failure*

The execution of SLIP89 may fail when too many sows are specified for too many weeks of simulation. SLIP89 will also terminate if the disk used to store the data files is full, or the Ctrl and C [Ctrl-C] buttons are simultaneously pressed. Safeguard mechanisms have been built into the program to prevent most faulty entries from causing the program to fail. If particularly nonsensical values are entered, these may also cause program failure. SLIP89 is readily recommenced by Entering SLIP89, although the reason for initial failure should be ascertained before recommencing its execution.

### *Alteration of Preset Epidemiological Default Values*

An additional program for altering the preset or default values of the epidemiological variables has been supplied [CHANGE\CHANGEPI.EXE] on the disk, see Appendix V. The program should only be used when repeated simulations are being made, using the same epidemiological variables which differ from the original default values. This saves one from having to continually alter the value of these variables. It is advisable to backup or copy the current default values before commencing with any changes [A:COPY EPIVAR.DAT A:EPIVAR.BAK]. The program can then be executed by typing CHANGEPI and pressing return. It will request that the variable name be given, followed by the new default value. Press return for the first part of each numbered question, thus leaving the variable name option blank. Then Enter the new default value, if the value is not being changed then still Enter the old default value. Enter the values in the order they appear in the epidemiological variable page of the Input Menu. Execute SLIP89, and select Epidemiological data at the Input Menu to check the new default values are correct.

## APPENDIX V

Listing of SLIP89 data files which can be used to examine data generated by SLIP89.

FILE NAMES	CONDITIONS	DIRECTORY
COHORT.DAT	Number of sows = 50	
AGES.DAT	Run Time = 50 weeks	
WEAN.DAT	Default settings only.	
GROW.DAT		
C*	Default settings only.	
A*	* = 1 to 5	DATA
W*	[e.g. C1; A1; W1; G1	
G*	and C2; A2; W2; G2 etc.]	
COH150*.150	Number of sows = 150.	
AGE150*.150	Run Time = 150 weeks.	DATA
WEA150*.150	Default settings only.	
GRO150*.150	* = A to C.	
VC*	Number of sows = 50.	
VA*	Run Time = 50 weeks.	VACDATA
VW*	Vaccination efficacy = 80%	
VG*	Last age of vaccination is 12 weeks. * = 1 to 3.	
VC150*.150	Vaccination efficacy = 80%	
VA150*.150	Last age of vaccination	
VW150*.150	is 12 weeks.	VACDATA
VG150*.150	* = A to C.	
VC8010	Vaccination efficacy = 80%	
VA8010	Last age of vaccination	VACDATA
VW8010	is 10 weeks.	
VG8010		

## APPENDIX VI

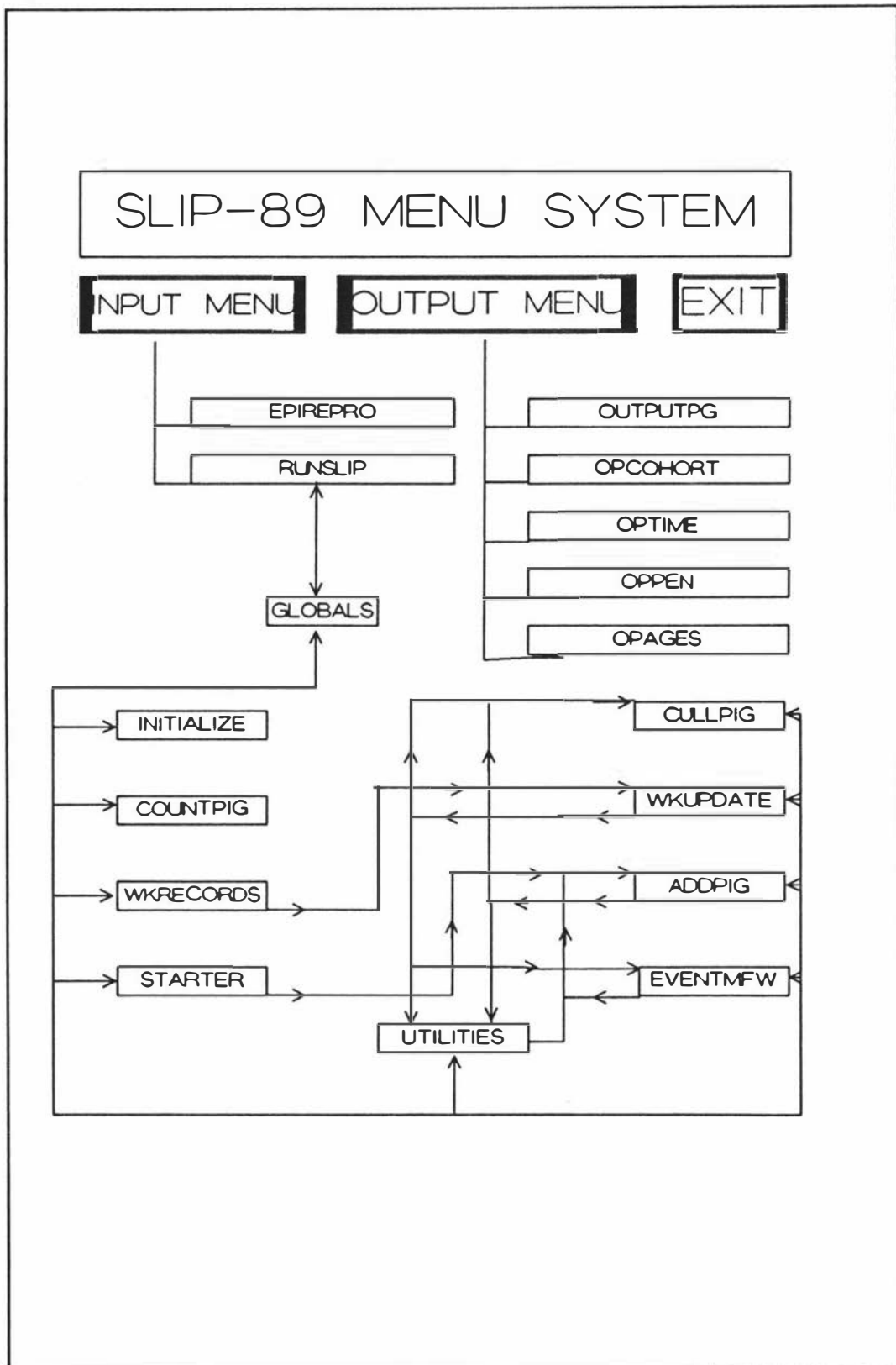
This is a listing of the program units required by the SLIP89 model to simulate endemic leptospiral *pomona* infection within a pig herd. Units which are important for the display of simulated data have been excluded. Figure A6.1 indicates the interactions between program units. The main program units involved in the model are RUNSLIP [page 293] and WKUPDATE [page 308] which are summarized in Figure A6.2 [page 307].

The following program units are listed:

ADDPIG	Adds a pig to the relevant population sector.
COUNTPIG	Tallies the number of pigs in each population sector.
CULLPIG	Removes a pig from the herd.
EVENTMWF	Changes the status of sows from mating to farrowing and weaning, also adds piglets to herd at the time of farrowing.
GLOBALS	Contains the codes for variables used in the program.
INITIAL	Initializes all variables to their correct starting values.
RUNSLIP	Sequence of weekly events as they are programmed to occur in the simulated pig herd.
UTILITIE	Contains standard procedures frequently used by other units.
WKRECORD	Writes data generated by the simulation to data files.
WKUPDATE	Simulates the weekly occurrence of events within the piggery. This includes the movement of pigs and the exposure of pigs to infection.



Figure A6.1 Flow chart of interaction between SLIP89 program units.



```

UNIT ADDPIG;
interface

uses GLOBALS,crt,
    UTILITIES,
    CULLPIG;

procedure ADDSOW ( NOSOWS : INTEGER;
    var BASE : NEXTSOW;
    var Growers : Gpigs);
procedure ADDBOAR (BTYPE : BOARTYPE;
    var BASE : NEXTBOAR);
procedure ADDGROWER(NEXTS : NEXTSOW; var pig : growptr);

implementation
function MAKEID (X,A : INTEGER; PC : CHAR):ID;
    const IDMAX = 9999;
    var IDNUM : STRING[5];
    TEMPSTRING : STRING[10];
    PS, AROUND: CHAR;
    RAND : REAL;
begin { MAKEID }
    case PC of
        'S': begin
            PS:= 'F';
            AROUND:=CHR(A);
            STR(X : 1, IDNUM);
            TEMPSTRING:=(PC + PS + IDNUM + AROUND);
            MAKEID:=TEMPSTRING;
        end;
        'B': begin
            PS:= 'M';
            AROUND:=CHR(A);
            STR(X : 1, IDNUM);
            TEMPSTRING:=( PC + PS + IDNUM + AROUND);
            MAKEID:=TEMPSTRING;
        end;
        'G': begin
            RAND := RANDOM;
            if RAND < 0.5 then PS:= 'F'
            else PS:= 'M';
            AROUND:=CHR(A);
            STR(GX : 1, IDNUM);
            TEMPSTRING:=( PC + PS + IDNUM + AROUND);
            MAKEID:=TEMPSTRING;
        end;
    end; { case }
end; { MAKEID }

procedure ADDSOW;
    var NOGILTS, RAND, N : INTEGER;
    RANDINF, RANDTITRE : REAL;
    NEXTS : NEXTSOW;
    Pig: growptr;

begin { ADDSOW }
    NOGILTS:= 0;
    i:=1;
    while (i <= 2000) and (nogilts < nosows) do
        begin
            if (Growers[i] <> nil) and
                (Growers[i]^ .sex = 'F') and
                (Growers[i]^ .age >= 22) then
                begin
                    nogilts:= nogilts + 1;

```

```

Pig:= Growers[i];
NEW(NEXTS);
with NEXTS ^ do
begin
  sdata.age:= pig^.age;
  sdata.durinf:= pig^.durinf;
  sdata.peak:= pig^.peak;
  sdata.wkpi:= pig^.wkpi;
  sdata.sex:='F';
  sdata.titre:=pig^.titre;
  sdata.shed:=pig^.shed;
                                if sdata.inf = pos then
                                pig^.inf:=plus;
  if sdata.previnf then
    SX:= SX+1;
    SID:= MAKEID(SX,A,'S');
    if SX = 9999 then begin SX:= 0;
                                A :=
                                A +
                                1;
                                end;
  REFLIT:= NIL;
  PSA:= 1;
  NEWPSA:=0;
  PARITY:=0;
  EVENT:= OESTRUS;
  MATEDATE:= TIME + 2 + RANDOM(4);
  NOGILTS:= NOGILTS + 1;
end; { with }
NEXTS^.SLINK:= SBASE;
SBASE:= NEXTS;
Dispose(pig);
Growers[i]:= nil;
end; { if }
i:= i+1;
end; { if }
while NOGILTS < NOSOWS do
begin
  NEW(NEXTS);
  with NEXTS ^ do
  begin
    SX:= SX + 1;
    SID:= MAKEID(SX, A, 'S');
    if SX = 9999 then begin SX:=0; A:=A +1;
                      end; {if}
    PSA:=1;
    NEWPSA:=0;
    if TIME = 1 then
    begin
      N:= WEANAGE + 18;
      RAND:= RANDOM(N) + 1;
      case RAND of
        1..2 : begin
                  EVENT:= OESTRUS;
                  MATEDATE:= (RANDOM(2)+TIME);
                                SDATA.AGE:=
                                25+RANDOM(47)+MATEDATE;
                end;
        3..20 : begin
                  EVENT:= PREGNANCY;
                  FARROWDATE:=
                                RANDOM(17)+TIME;
                  SDATA.AGE:= 25 + 17 +
                                RANDOM(47) +
FARROWDATE;
                end
      else if (RAND > 20) and (RAND <= N) then
      begin
        EVENT:= LACTATION;
        WEANDATE:=
RANDOM(ROUND(WEANAVE/7))+TIME;
      end
    end
  end
end

```

```

        SDATA.AGE:= 25+ 17+
RANDOM(47)+WEANDATE;
    end;
    end; { case }
end {if}
else
    begin
        RAND:= RANDOM(3)+1;
        EVENT:= OESTRUS;
        MATEDATE:= RAND + TIME;
        SDATA.AGE:= 24 + RANDOM(10);
    end;
    PARITY:= TRUNC(SDATA.AGE/25)-1;
    if PARITY <0 then PARITY:=0;
    with SDATA do
    begin
        SEX:= 'F';
        INF:= NEG;
        PREVINF:= FALSE;
        DURINF:= 0;
        WKPI:= -1;
        TITRE:= RANDOM;
        {in comming gilt/sows are susceptible pigs }
        {with undetectable titres}
        SHED:=0;
        PEAK:= 0;
    end; {with}
    REFLIT:=NIL;
    SLINK:= BASE;
    BASE:= NEXTS;
end; { with }
NOGILTS:= NOGILTS + 1;
end; { while }
end; { ADDSOW }

procedure ADDBOAR;
var N : INTEGER;
    NEXTB : NEXTBOAR;
procedure FILLBOARRECS(BTYPE:BOARTYPE;TARGET:ID; var BBASE:NEXTBOAR);
const YGBOARMINAGE = 27;
    YGBOARMAXAGE = 79;
    MATBOARMINAGE = 80;
    MATBOARMAXAGE = 150;
var RANDAGE, N : INTEGER;
    RANDINF, RANDTITRE : REAL;
begin { FILLBOARRECS }
    NEXTB:= BBASE;
    while NEXTB <> NIL do
    begin
        if NEXTB^.BID = TARGET then
        begin
            with NEXTB^.BDATA do
            begin
                if BTYPE = YOUNG then
                begin { if }
                    RANDAGE:=0;
                    repeat RANDAGE :=RANDOM(YGBOARMAXAGE + 1)
                        until RANDAGE
>= YGBOARMINAGE;
                    AGE := RANDAGE;
                    SEX := 'M';
                end { if }
                else
                begin
                    RANDAGE:=0;
                    repeat RANDAGE:=RANDOM(MATBOARMAXAGE + 1);
                        until RANDAGE
>= MATBOARMINAGE;
                    AGE := RANDAGE;

```

```

        SEX := 'M';
    end; { else }

    INF := NEG;
    PREVINP:= FALSE;
    DURINF:=0;
    PEAK:=0;
    WKPI:= -1;
    TITRE:= RANDOM;
    SHED:= 0;
    end; {with}
    while NEXTB <> NIL do NEXTB:= NEXTB^.BLINK;
    end
    else NEXTB:= NEXTB^.BLINK;
    end; {while}
end; { FILLBOARRECS }

begin { ADDBOAR }
    NEW(NEXTB);
    with NEXTB^ do
    begin
        BX:= BX+1;
        BID:= MAKEID( BX, A, 'B');
        if BX = 9999 then
            begin
                BX:= 0;
                A:= A+1;
            end; { if }
        SERVPERWK:= 0;
        BLINK:= BASE;
        BASE:= NEXTB;
    end; { with NEXTB^ }
    FILLBOARRECS(BTYPE, NEXTB^.BID, BBASE);
end; { ADDBOAR }

procedure ADDGROWER;
    const IMMTRANSFER = 100;
        COLIMMSD = 0.5;
    var N : INTEGER;
        RAND, COLTRANSFER : REAL;

    function SowIDtoPigPen(sowid:id):integer;
    var  sownum, error : integer;
        sn: string[5];

    begin
        sn:=copy(sowid,3,length(sowid)-3);
        val(sn, sownum, error);
        SowIDtoPigPen:=10000+sownum;
    end;

begin { ADDGROWER }
    NEW(pig);
    with pig^ do
    begin
        GX:= GX + 1;
        GID:= MAKEID(GX, A, 'G');
        if GX = 9999 then
            begin
                GX:= 0;
                A:= A + 1;
            end; { if }
        AGE:= 0;
        COLTRANSFER:=(NEXTS^.SDATA.TITRE *
                                                                IMMTRANSFER)/100;
        TITRE:= NORMAL(COLTRANSFER, COLIMMSD);
        if TITRE < 0 then TITRE:=0;
        if TITRE > COLTRANSFER then TITRE := COLTRANSFER;
    end;
end;

```

```

    INF:= min;
    PREVINF:= FALSE;
    DURINF:=0;
    WKPI:= -1;
    SHED:=0;
    PEAK:=0;
    RAND:= RANDOM;
    if RAND >= 0.5 then SEX:= 'M'
    else SEX:= 'F';
    Penno:= SowIDtoPigPen(NEXTS^.SID);
end; { with }
i:=1;
while ((i<=2000) and (Growers[i] <> nil)) do i:=i+1;
  Growers[i]:=pig;
end; { ADDGROWER }

end.

```

```

UNIT COUNTPIGS;

interface

uses GLOBALS;

procedure BOARCOUNT ( BASE : NEXTBOAR;
  var YGBOARS, MATBOARS, TOTBOARS : INTEGER);

function SOWCOUNT ( BASE : NEXTSOW): INTEGER;

implementation
procedure BOARCOUNT;
var NEXTB : NEXTBOAR;
begin { BOARCOUNT }
  if BASE = NIL then
    begin
      TOTBOARS:=0;
      MATBOARS:=0;
      YGBOARS:=0;
    end { if }
  else
    begin
      TOTBOARS:=0;
      MATBOARS:=0;
      YGBOARS:=0;
      NEXTB:= BASE;
      while NEXTB <> NIL do
        begin
          if NEXTB^.BDATA.AGE < 80 then YGBOARS:=YGBOARS+1
          else MATBOARS:=
MATBOARS + 1;
          NEXTB:= NEXTB^.BLINK;
        end; { while }
        TOTBOARS:= YGBOARS + MATBOARS;
      end; { else }
    end; { BOARCOUNT }

function SOWCOUNT : INTEGER;
var N : INTEGER;
    NEXTS : NEXTSOW;
begin { SOWCOUNT }
  if BASE = NIL then SOWCOUNT:=0
  else
    begin
      N:=0;
      NEXTS:= BASE;
      while NEXTS <> NIL do
        begin
          N:=N+1;
          NEXTS:= NEXTS^.SLINK;
        end; { while }
        SOWCOUNT := N;
      end; { else }
    end; { SOWCOUNT }

end.

```

```
UNIT EVENTMFW;
interface
```

```
uses crt, GLOBALS,
  ADDPIG,
  UTILITIES;
```

```
procedure MATESOW ( var NEXT : NEXTSOW; var TOTBOAR : INTEGER;
  NEXTBOAR );
```

```
var BASE :
```

```
procedure FARROW ( var NEXT : NEXTSOW; var Growers:Gpigs);
  var N, LITNO, DEAD, LIVE : INTEGER;
```

```
procedure WEAN( var NEXT : NEXTSOW);
```

```
function MATINGS : INTEGER;
```

```
implementation
```

```
procedure MATESOW;
  var STOP, MATING, RANDM, COUNTER : INTEGER;
  RANDI : REAL;
  NEXTB : NEXTBOAR;
```

```
begin { MATESOW }
  MATING:=0;
  STOP:=0;
  if TOTBOAR = 0 then
  begin
    ADDBOAR(MATURE,BBASE);
    TOTBOAR:=TOTBOAR + 1;
  end;
  while MATING < 2 do
  begin
    RANDM:= RANDOM(TOTBOAR)+1;
    NEXTB:= BBASE;
    if RANDM = 1 then NEXTB:=BBASE
    else for COUNTER:= 1 to (RANDM - 1) do
      NEXTB:= NEXTB^.BLINK;
    if ((NEXTB^.SERVPERWK < 5) and (NEXTB^.BDATE.AGE >
  MATINGFREQ[NEXTB^.SERVPERWK])) then
      begin
        NEXTB^.SERVPERWK:= NEXTB^.SERVPERWK + 1;
        MATING:= MATING + 1;
      end; { if }
    STOP:= STOP + 1;
    if ((STOP > (TOTBOAR*10)) and
      (STOP > (TOTBOAR*TOTBOAR)))
    then begin
      STOP:=0;
      TOTBOAR:=TOTBOAR + 1;
      ADDBOAR(MATURE, BBASE);
    end;
  end; { while }
  RANDI:=0;
  RANDI:= RANDOM;
  if RANDI > FF then
  begin
    NEXT^.EVENT:= PREGNANCY;
    NEXT^.FARROWDATE:= DATE(BIRTH);
  end { if }
  else
  begin
    if RANDI <= 0.10 then NEXT^.MATEDATE:= TIME + 3
    else if ((RANDI <= 0.13) and (RANDI > 0.10)) then
      NEXT^.MATEDATE:= TIME + RANDOM(3) + 1
    else if ((RANDI <=FF) and (RANDI >0.13)) then
      begin
```



```

        NEXT^.MATEDATE:= DATE(SLIP);
        if (NEXT^.MATEDATE - TIME) > 17 then
            NEXT^.MATEDATE:= TIME + 17;
        end;
    end; { else }
end; { MATESOW }

procedure FARROW;
begin { FARROW }
    LITNO:=0;
    DEAD:=0;
    LIVE:=0;
    N:= 1;
    LITNO:= NORMAL(LITAVE, LITSD);
    DEAD:= NORMAL(DEADAVE, DEADSD);
    LIVE:= LITNO - DEAD;
    for N:= 1 to LIVE do ADDGROWER(next,pig);
    if (TIME - NEXT^.NEWPSA) > 0 then NEXT^.PSA:=
        LIVE/(TIME - NEXT^.NEWPSA)
    else NEXT^.PSA:=0;
    NEXT^.NEWPSA:= TIME;
    NEXT^.PARITY:= NEXT^.PARITY +1;
    NEXT^.EVENT:= LACTATION;
    NEXT^.WEANDATE:= DATE(ENDLACT);
end; { FARROW }

procedure WEAN;
    var LIVENO : INTEGER;
begin { WEAN }
    NEXT^.EVENT:= OESTRUS;
    NEXT^.MATEDATE:= DATE(SERV);
end; { WEAN }

function MATINGS;
    const YEAR = 52;
    var CTIME : WEEKS;
        LPS, TBMW : REAL;
        WEANAGE : INTEGER;
begin { MATINGS }
    WEANAGE:= ROUND(WEANAVE/7);
    CTIME:= (16 + WEANAGE + 1);
    LPS:= YEAR/CTIME;
    TBMW:= ((LPS*TEMPSOWMAX)*(100/85)*2)/52;
    MATINGS:= ROUND(TBMW);
end; { MATINGS }
end.

```

UNIT GLOBALS;

interface

```

const FF = 0.15;
  MAXTIME = 520;
  IDMAX = 10;
  MAXAGE = 450;
  SERVAGE0 = 31;
  SERVAGE1 = 45;
  SERVAGE2 = 67;
  SERVAGE3 = 80;
  SERVAGE4 = 200;
  MAXTITRE = 16;
  NUMWPENS = 4; NUMGPENS = 4;
  PIGLETHL2 = 0.3465735;
  PIGLETHL16 = 0.3032518;
  ADULTHL26 = 0.0266595;

type Z = file of REAL;
  ZNORM = array [1..35] of REAL;
  FARRAY = array [1..16] of REAL;
  MATINGARRAY = array[0..4] of INTEGER;
  ZNORMAL = ^ZNORM;
  WEEKS = 0..MAXTIME;
  PRODINDEX = REAL;
  CYCLE = (OESTRUS, PREGNANCY, LACTATION);
  ID = STRING[IDMAX];
  MAT = REAL;
  CHOICE = (SERV, BIRTH, ENDLACT, SLIP, DURATINF);
  BOARTYPE = (YOUNG, MATURE);
  STRING_8 = STRING[8];
  FNSTRING = STRING[30];

  PENPTR = ^PENRECORD;
  PENRECORD = record
    NUMINPEN, NOINFECTED, SEROPOS : INTEGER;
    AVEAGE, AVETITRE, AVESHED, PROBINF : REAL;
  end;

  PENARRAY = array[1..50] of PENPTR;

  PENDATARECORD = record
    POP, INFECTED : array[1..NUMWPENS] of INTEGER;
    SEROLOGY, AGEAVER, PREV, CONTAMIN, INFCOHORT :
      array[1..NUMWPENS] of REAL;
  end;

  DATARECORD = record {for GROWERREC, BOARREC and SOWREC}
    AGE, DURINF, PEAK : INTEGER;
    SEX : CHAR;
    TITRE : MAT;
    SHED : REAL;
    WKPI : INTEGER;
    INF : (POS, NEG);
    PREVINF : BOOLEAN;
  end; { DATARECORD }

  NEXTBOAR = ^BOARREC;
  BOARREC = record
    BID : ID;
    BDATA : DATARECORD;
    SERVPERWK : INTEGER;
    BLINK : NEXTBOAR;
  end; { BOARREC }

```

```

GrowPtr = ^Growerpig;
Growerpig = record
    gid:string[10];
    age, durinf,peak,wkpi:integer;
    sex:char;
    titre, shed:real;
    previnf:boolean;
    inf:(plus,min);
    penno:integer;
end;
Gpigs = array[1..2000] of GrowPtr;

LITTRACE = ^IDLITTER;
IDLITTER = record      { for SOWREC }
    GID : ID;
    NEXTMATE : LITTRACE;
end; { IDLITTER }

REFLIT = LITTRACE;
NEXTSOW = ^SOWREC;
SOWREC = record
    SID : ID;
    SDATA : DATARECORD;
    PSA,NEWPSA : PRODINDEX;
    PARITY : INTEGER;
    SLINK : NEXTSOW;
    REFLIT : LITTRACE;
    case EVENT : CYCLE of
        OESTRUS : (MATEDATE : WEEKS);
        PREGNANCY : (FARROWDATE : WEEKS);
        LACTATION : (WEANDATE : WEEKS);
    end; { SOWREC }

var ZTABLE : Z;
ZTABLES : ZNORMAL;
PPINFECTION, WEANAVE, LITAVE, WEANSO, LITSD, DEADAVE,      DEADSD, MINSO,
MAXSPEC, SOWHERDAVE, SOWHERSD,      TITREAVE, TITRESO, HALFLIFE, LUDURAVE,
LUDURSD, LUINTAVE,      LUINTSD,
PCBW, PCWG, PCGB,
{Prob.Contact between Breeders and Weaners etc}
TWEC, TGEC, TSEC, TBEC,
{Total Weaner Environmental Contamination}
ECDF, PCDF,
{Environmental/Pen Contamination Division Factor}
PCSSB, PWPWP, PGPGP,
{Probability Weaner Pen to Weaner Pen etc}
VRR, VA : REAL;
{Vaccination Relative Risk, Vaccination age}
REPRO, EPI : FARRAY;
MINPSA : PRODINDEX;
MATINGFREQ : MATINGARRAY;
BBASE : NEXTBOAR;
SBASE : NEXTSOW;
PIGLETS : LITTRACE;
GROWERS:GPIGS;
PIG:GROWPTR;
HOUSE:PENARRAY;
TEMPBOAR, EXMATBOARS, EXYGBOARS, EXTOTBOARS, YGBOARS,      MATBOARS,
TOTBOARS,DIFFYGBOARS, DIFFMATBOARS, DIFFTOTBOARS, NN,      TEMPSOWMAX,
WEANAGE, COUNT, SX, BX, GX, X, A, RANDSOW, SPACE,      COUNTER, CULL,
SOWNO, MAXSOWAGE, Y,X1,X2,Y1,Y2, SUMINT,NNX,NSW,
NUMWEANPIGS, NUMGROWPIGS, IDNO, LIVE, DEAD, LITNO,i,
STARTWK, INTERVAL: INTEGER;
BID, SID,GID: ID;
MATINGFLAG, FINISH, ENDEMIC : BOOLEAN;
RUNTIME,TIME,SERVAGE : WEEKS;
BTYPE : BOARTYPE;

```

```
CH, CH1, CH2 : CHAR;  
WEANFILENAME, GROWFILENAME, COHORTFILENAME,  
SUMFILENAME, AGESFILENAME, FILENAME : FNSTRING;  
PENDATA : PENDATARECORD;
```

```
implementation  
begin  
end.
```

UNIT INITIAL;

interface

uses tpcrt, Globals, SlipGtbl;

procedure StartSettings;

implementation

procedure StartSettings;

var pen : penrecord;

begin

ASSIGN( ZTABLE, 'NORMAL.DIS');

RESET(ZTABLE);

SEEK(ZTABLE, 1);

NEW(ZTABLES);

for NN := 1 to 35 do

begin

READ(ZTABLE,P);

ZTABLES^[NN]:= P;

end;

CLOSE(ZTABLE);

SX:=0;

BX:=0;

GX:=0;

X:=0;

A:=65;

EXYGBOARS:=0; EXMATBOARS:=0; EXTOTBOARS:=0;

TEMPSOWMAX:=0;

MATINGFREQ[0]:= SERVAGE0;

MATINGFREQ[1]:= SERVAGE1;

MATINGFREQ[2]:= SERVAGE2;

MATINGFREQ[3]:= SERVAGE3;

MATINGFREQ[4]:= SERVAGE4;

SOWHERDS:=RepArray[1]/52;

WEANAVE:=RepArray[2]; WEANS:=RepArray[3];

LITAVE:=RepArray[4]; LITSD:=RepArray[5];

DEADAVE:=RepArray[6]; DEADSD:=RepArray[7];

TITREAVE:=EpiArray[1]; TITRES:=EpiArray[2];

LUDURAVE:=EpiArray[3]; LUDURSD:=EpiArray[4];

LUINTAVE:=ln(EpiArray[5]); LUINTSD:=ln(EpiArray[6]);

MINSENS:=EpiArray[7]; MAXSPEC:=EpiArray[8];

ECDF:=EpiArray[9]; PCDF:=EpiArray[10];

if ECDF < 1 then ECDF:=1;

if PCDF < 1 then PCDF:=1;

PCBW:=EpiArray[11]/100; PCWG:=EpiArray[12]/100;

PCSSB:=EpiArray[14]/100; PGPGP:=EpiArray[15]/100;

TSEC:=0; TBEC:=0; TGEC:=0; TWEC:=0;

PINFECTION:=0;

TIME:= 1;

NEW(BBASE);

BBASE:= NIL;

NEW(SBASE);

SBASE:= NIL;

for i:=1 to 50 do

house[i]:= nil;

for i:= 1 to 2000 do

growers[i]:= nil;

MINPSA:= 0.25;

WEANAGE:= ROUND(WEANAVE/7);

MAXSOWAGE:= 180;

RANDOMIZE;

end; { initialize }

end.

PCGB:=EpiArray[13]/100;

PWPWP:=EpiArray[16]/100;

*UNIT RUNSLIP;*

*Interface*

*Uses*

*TpCrt,  
Globals,  
SlipGlbl,  
Initial,  
Addpig,  
Cullpig,  
Utilities,  
Countpigs,  
Eventmfw,  
Wkrecords,  
WkUpDate,  
Starter;*

*var NEXTS : NEXTSOW;  
NEXTB : NEXTBOAR;  
CohortFileName, AgesFileName : FNString;*

*Procedure SlipBody;*

*Implementation*

*Procedure SlipBody;*

*const PrevConst = 90;*

*var YN, CH: Char;*

*Minutes, Error, IOCode: Integer;*

*VE: Real;*

*R: FNString;*

*F: Text;*

*Procedure Errormsg(min, max : integer);*

*begin*

*SOUND(440); DELAY(50); NOSOUND;*

*GOTOXY(1,25); clreol;*

*WRITE('Input error, <Enter> value ',min,' to ',max);*

*end;*

*Function ErrorCheck(xx,yy:integer;*

*MinVal, MaxVal:Real;*

*var StrVal:FNString):boolean;*

*var Value: Real;*

*Error:integer;*

*EscChar: Char;*

*begin*

*ErrorCheck:= True;*

*Value:=0;*

*gotoxy(xx,yy);ClrEol; Readln(StrVal);*

*EscChar:= StrVal[1];*

*if upcase(EscChar) = 'X' then Exit;*

*gotoXY(1,24); ClrEol;*

*Val(StrVal,Value,ERROR);*

*if (ERROR <> 0) then ErrorCheck:= false;*

*if ((Value < MinVal) or (Value > MaxVal)) then*

*end;*

*ErrorCheck:= false;*

*begin*

*textmode(bw80);*

*window(1,1,80,25);*

*ClrScr;*

*RUNTIME:=0;*

*for i:= 1 to 2000 do Growers[i]:= nil;*

*GOTOXY(1,1); WRITE('Input Menu*

*- operating criteria for S L I P 8 9');*

```

textColor(8);
GOTOXY(5,3); WRITE('Enter time for SLIP to run      (weeks)..... ');
GOTOXY(5,4); WRITE('Sow herd average              size..... ');
GOTOXY(5,5); WRITE('Simulate endemic leptospirosis   ');
GOTOXY(5,6); WRITE('Vaccination                    Efficacy..... ');
GOTOXY(5,7); WRITE('Last vaccination              age..... ');
GotoXY(1,9); write('File storage information:');
GOTOXY(5,10); WRITE('Enter number of weeks for age group      data summary..... ');
GOTOXY(5,11); WRITE('Enter number of weeks for cohort      summary..... ');
GotoXY(5,13); write('Enter file name for weekly cohort    data : ');
GotoXY(5,15); write('Enter file name for weekly age      group data : ');
GotoXY(5,17); write('Enter file name for weekly weaner    pen data : ');
GotoXY(5,19); write('Enter file name for weekly grower   pen data : ');
X1:=65;
for i:= 1 to 7 do
begin
  GotoXY(1,25);clrEol;
  case i of
    1: write('<Enter> number of simular weeks from      20 to 250 ');
    2: write('<Enter> number of simular sows from      10 to 150 ');
    3: write('<Enter> Y/N');
    4: write('<Enter> 0 for NO vaccination, or a      value 1 to 100');
    5: if VE > 0 then
        write('<Enter> last age (weeks) of      vaccination from 4 to 36')
        else begin GotoXY(X1,I+2); Write('0');end;
    6: write('<Enter> a value from 1 to 20');
    7: write('<Enter> a value from 10 to a maximum      of
',runtime-1);

end;
Y1:= I+2;
if (i = 6) or (i = 7) then Y1:= Y1+2;
GOTOXY(X1,Y1);
TextColor(15);
case i of
  1: begin
    while not ErrorCheck(X1,Y1,20,250,R) do
      Errormsg(20, 250);
      YN:= R[1]; if upcase(YN) = 'X' then      Exit;
      Val(R,Runtime,Error);
    end;
  2: begin
    while not Errorcheck(X1,Y1,10,150,R) do
      ErrorMessage(10, 150);
      YN:= R[1]; if upcase(YN) = 'X' then      Exit;
      Val(R,SowHerdAve,Error);
    end;
  3: begin
      YN:= R[1];
      if upcase(YN) = 'X' then exit;
      while ((upcase(YN) <> 'Y')and
        (upcase(YN) <> 'N')) do
        begin
          gotoXY(X1,Y1); ClrEol;
          Readln(R);
          YN:= R[1];
        end;
      if upcase(YN) = 'Y' then Endemic:= True
      else Endemic:= False;
    end;
  4: begin
    while not ErrorCheck(X1,Y1,0,100,R) do
      YN:= R[1]; if upcase(YN) = 'X' then      ErrorMessage(0, 100);
      val(R,VE,Error);      Exit;
    end;
  5: begin
    if VE = 0 then VA:=0

```

```

else
  begin
    while not ErrorCheck(X1,Y1,4,36,R)
      YN:= R[1]; if upcase(YN) = 'X' then
        Val(R,VA,Error);
    end;
  end;
6: begin
  while not ErrorCheck(X1,Y1,1,20,R)
    YN:= R[1];
    if (upcase(YN) = 'X') then Exit;
    Val(R,NNX,Error);
  end;
7: begin
  while not ErrorCheck(X1,Y1,1,RunTime,R)
    YN:= R[1];
    if (upcase(YN) = 'X') then Exit;
    Val(R,NSW,Error);
  end;
end;
end;
gotoXY(1,24); ClrEol; write('Type new file name or');
GotoXY(1,25); ClrEol;
write('Press <Enter> for default = cohort.dat');

{$I-}
repeat
  GotoXY(50,13); ClrEol;
  Readln(R);
  YN:= R[1];
  if upcase(YN) = 'X' then Exit;
  if length(R) = 0 then R:= 'cohort.dat';
  Assign(F, R);
  Rewrite(F);
  Close(F);
  IOCode:= IOResult;
  if IOCode <> 0 then
    begin
      SOUND(440); DELAY(50); NOSOUND;
      GOTOXY(1,25);clrEol;
      WRITE('Input error, <Enter> path name for file
        e.g. a:xxx.dat');
    end;
  until IOCode = 0;
{$I+}
CohortFilename:= R;
gotoXY(50,13); ClrEol; write(CohortFileName);

GotoXY(1,25); ClrEol;
write('Press <Enter> for default = ages.dat');
{$I-}
repeat
  GotoXY(50,15); ClrEol;
  Readln(R);
  YN:= R[1];
  if upcase(YN) = 'X' then Exit;
  if length(R) = 0 then R:= 'ages.dat';
  Assign(F, R);
  Rewrite(F);
  Close(F);
  IOCode:= IOResult;
  if IOCode <> 0 then
    begin
      SOUND(440); DELAY(50); NOSOUND;
      GOTOXY(1,25);clrEol;
      WRITE('Input error, <Enter> path name for file
        e.g. a:xxx.dat');
    end;

```



```

until IOCode = 0;
{$I+}
AgesFileName:= R;
gotoXY(50,15); ClrEol; write(AgesFileName);

GotoXY(1,25); ClrEol;
write('Press <Enter> for default = wean.dat');
{$I-}
repeat
  GotoXY(50,17); ClrEol;
  Readln(R);
  YN:= R[1];
  if upcase(YN) = 'X' then Exit;
  if length(R) = 0 then R:= 'wean.dat';
  Assign(F, R);
  Rewrite(F);
  Close(F);
  IOCode:= IOResult;
  if IOCode <> 0 then
    begin
      SOUND(440); DELAY(50); NOSOUND;
      GOTOXY(1,25); clreol;
      WRITE('Input error, <Enter> path name for file   e.g. a:xxx.dat');
    end;
until IOCode = 0;
{$I+}
WeanFileName:= R;
gotoXY(50,17); ClrEol; write(WeanFileName);

GotoXY(1,25); ClrEol;
write('Press <Enter> for default = grow.dat');
{$I-}
repeat
  GotoXY(50,19); ClrEol;
  Readln(R);
  YN:= R[1];
  if upcase(YN) = 'X' then Exit;
  if length(R) = 0 then R:= 'grow.dat';
  Assign(F, R);
  Rewrite(F);
  Close(F);
  IOCode:= IOResult;
  if IOCode <> 0 then
    begin
      SOUND(440); DELAY(50); NOSOUND;
      GOTOXY(1,25); clreol;
      WRITE('Input error, <Enter> path name for file   e.g. a:xxx.dat');
    end;
until IOCode = 0;
{$I+}
GrowFileName:= R;
gotoXY(50,19); ClrEol; write(GrowFileName);
gotoXY(1,24); ClrEol;
gotoXY(1,25); ClrEol; write('Press <Enter> to continue');
gotoxy(79,25); Readln(R);
ClrScr;

gotoXY(1,1); write('Simulating Leptospiral Infection   within a Pig herd');
if RunTime <= 25 then Minutes:= 5
else minutes:= round((sowherdave/50) * (Runtime/3));
gotoXY(1,5);
write('Simulation estimated to take 'minutes,'
      minutes - do you want to continue ?');
gotoXY(1,25);
write('Press <Enter> to continue   or   X to Exit');
gotoXY(79,25);
CH:= ReadKey;

```

```

ClrScr;
if upcase(Ch) = 'X' then Exit;
gotoXY(5,5); ClrEol;

if VE > 0 then VA:= VA+2;
{Vaccination effective two weeks after last booster}
if VE > (100-PREVCONST) then
  VRR {Vaccination Relative Risk} := (100 - VE)/PREVCONST
else VRR:= 1;
STARTSETTINGS;
ClrScr;
READSOWS(ROUND(SOWHERDAVE),SBASE);
GotoXY(1,1);
Write('Run Time Summary S L I P 8 9      Week: ');
gotoXY(40,3); write('^ Age Groups');
for i:= 4 to 19 do
begin
  gotoXY(40,i); write('| ');
  case i of
    5,6,9,10,13,14,17,18:
      begin GotoXY(39,i); write('.'); end;
  end;
  case i of
    5,9,13,17:
      begin gotoXY(35,i); write('Inf'); end;
    6,10,14,18:
      begin gotoXY(35,i); write('LU'); end;
  end;
end;
for i:= 4 to 20 do
gotoXY(1,4); write('Key -----+');
gotoXY(1,5); write('| Inf = Past or Present |');
gotoXY(1,6); write('| Infection (%) |');
gotoXY(1,7); write('| LU = Leptospiruric (%) |');
gotoXY(1,8); write('+-----+');
gotoXY(31,4); write('Weaners');
gotoXY(31,8); write('Growers');
gotoXY(31,12); write('Sows');
gotoXY(31,16); write('Boars');
for i:= 40 to 80 do
begin
  gotoXY(i,20); write(' ');
  case i of
    40,50,60,70,80: begin
      gotoXY(i,21); write('| ');
      gotoXY(i-1,22);
      write((i-40)*2);
    end;
  end;
end;
gotoXY(75,22); write('(% )');
gotoXY(1,10); write('+-----+');
gotoXY(1,11); write('| Susceptible |');
gotoXY(1,12); write('| Pigs Result | Age Titre |');
gotoXY(1,13); write('| - - - - - + - - - - |');
gotoXY(1,14); write('| Not Infected |');
gotoXY(1,15); write('| Infected |');
gotoXY(1,16); write('+-----+');
gotoXY(1,17); write('| ');
gotoXY(1,18); write('| Abortions this week = 0 |');
gotoXY(1,19); write('| ');
gotoXY(1,20); write('+-----+');
gotoXY(50,25); write('(Ctrl-C to terminate program)');

while (TIME <= RUNTIME) do
begin { while TIME }

```

```

gotoXY(46,1); ClrEol; write(time);
TEMPSOWMAX:= SOWCOUNT(SBASE);
SOWNO:= NORMAL(SOWHERDAVE, SOWHERDSD);
{*} if time = 1 then readsows(sowno, sbase);
if SOWNO <> TEMPSOWMAX then
begin
if SOWNO > TEMPSOWMAX then
begin
NN:= SOWNO - TEMPSOWMAX;
ADDSOW(NN, SBASE, Growers);
end { if }
else
begin
NN:= TEMPSOWMAX - SOWNO;
for CULL := 1 to NN do
begin
CULLSOW(SBASE);
TEMPSOWMAX:= TEMPSOWMAX - 1;
end;
end; { else }
end; { if }
TEMPSOWMAX:= SOWNO;
BOARCOUNT(BBASE, YGBOARS, MATBOARS, TOTBOARS);
BOARAGES(EXYGBOARS, EXMATBOARS, EXTOTBOARS);
DIFFTOTBOARS:= EXTOTBOARS - TOTBOARS;
DIFFMATBOARS:= EXMATBOARS - MATBOARS;
DIFFYGBOARS:= EXYGBOARS - YGBOARS;
if DIFFTOTBOARS > 0 then
begin
for NN:=1 to DIFFMATBOARS
do ADDBOAR(MATURE, BBASE);
for NN:=1 to DIFFYGBOARS
do ADDBOAR(YOUNG, BBASE);
end
else
if DIFFTOTBOARS < -1 then
begin
for NN:= 1 to ABS(DIFFTOTBOARS) do
CULLBOAR(TOTBOARS, BBASE);
end; { else }
UPDATEBOARS(BBASE, TBEC);
gotoXY(43,1);
BOARCOUNT(BBASE, YGBOARS, MATBOARS, TOTBOARS);
UPDATEGROWERS(House, Growers, TWEC, TGEC);
gotoXY(43,1);
UPDATESOWS(SBASE, TSEC);
if Time >= RunTime-NSW then WEEKOUT(Growers, SBASE, BBASE, house, NSW,
CohortFileName, WeanFileName, GrowFileName); if Time >= RunTime-NNX then
AGEGRPSUMMARY(Growers,NNX, AgesFileName);
TIME:=TIME+1;
if Keypressed then if readkey = #3 then Exit;
end; { while TIME }
WRITEHERD;
CLRSCR;
end;

End.

```

UNIT UTILITIES;

interface

uses GLOBALS, {\*} printer;

function NORMAL( MEAN, SD : REAL ): INTEGER;

function DATE( OPTION : CHOICE ): WEEKS;

procedure BOARAGES (var EXYGBOARS, EXMATBOARS,  
EXTOTBOARS {input/output}: INTEGER);

implementation

function NORMAL;

var N : INTEGER;

TEMPSD, RAND, sdX : REAL;

begin { NORMAL }

N:=1;

sdX:= SD;

RAND:= RANDOM;

if RAND < > 0.5 then

begin

if RAND > 0.5 then RAND:= 1.0 - RAND

else sdX:= -SD;

while RAND >= ZTABLES^[N] do N:= N + 1;

TEMPSD:= ((35 - N)/10)\*sdX;

end { if }

else TEMPSD:= 0;

NORMAL:= ROUND(MEAN + TEMPSD);

end; { NORMAL }

function DATE;

const GESTAVE = 115; GESTSD = 0.5;

MATEAVE = 10; MATESD = 1;

ABORTAVE = 90; ABORTSD = 10;

var MEAN, SD : REAL;

N : INTEGER;

begin

case OPTION of

SERV : begin MEAN:= MATEAVE; SD:= MATESD end;

BIRTH : begin MEAN:= GESTAVE; SD:= GESTSD end;

ENDLACT : begin MEAN:= WEANAVE; SD:= WEANS D end;

SLIP : begin MEAN:= ABORTAVE; SD:= ABORTSD end;

DURATINF : begin MEAN:= LUDURAVE; SD:=LUDURSD; end;

end; { case }

N:= ROUND(NORMAL(MEAN, SD)/7) + TIME;

if N < TIME then N:= TIME;

DATE:= N;

end; { DATE }

function MATINGS: INTEGER;

const YEAR = 52;

var CTIME : WEEKS;

LPS, TBMW : REAL;

WEANAGE : INTEGER;

begin { MATINGS }

WEANAGE:= ROUND(WEANAVE/7);

CTIME:= (16 + WEANAGE + 1);

LPS:= YEAR/CTIME;

TBMW:= ((LPS\*TEMPSOWMAX)\*(100/85)\*2)/52;

MATINGS:= ROUND(TBMW);

end; { MATINGS }

procedure BOARAGES;

begin { BOARAGES }

```
EXTOTBOARS:= ROUND(MATINGS/3);  
EXYGBOARS:= TRUNC(EXTOTBOARS/3);  
EXMATBOARS:= (EXTOTBOARS - EXYGBOARS);  
end; { BOARAGES }  
  
end.
```

UNIT WKRECORDS;

Interface

Uses Globals,  
WkUpdate,  
Crt;

Procedure WEEKOUT(Growers:Gpigs;  
SBASE:NEXTSOW;  
BBASE:NEXTBOAR;  
House:PenArray;  
NumSumWks:Integer;  
CohortFileName, Wean1, Grow1: FNString);

Procedure AGEGRPSUMMARY(Growers:Gpigs;  
NUMWEEKS:INTEGER;  
AgesFileName: FnString);

Implementation

```

procedure AGEGRPSUMMARY;
  type AGEDATAINT = array[1..24] of integer;
  AGEDATAREAL = array[1..24] of real;
  AGEGRPREC = record
    NUMBER, NUMINFECTED, NUMSUSC, NUMSEROPOS,
    PREVALENCE : AGEDATAINT;
    LEPTOSPIRURIA, AVERAGECT : AGEDATAREAL;
  end;
  AGESFILE = file of AGEGRPREC;
  TEMPAGR = AGEGRPREC;
var AGESDATA : TEMPAGR;
  FILEAGESDATA : AGESFILE;
  NM : INTEGER;
  Pig:growptr;

  procedure AGECLASSSUMMARY(AGECLASS:INTEGER; pig:growptr;
    var AGESDATA:TEMPAGR);
  begin
    if ((AGECLASS > 0) and (AGECLASS < 25)) then
      with AGESDATA do
        begin
          NUMBER[AGECLASS]:= NUMBER[AGECLASS] + 1;
          if pig^.INF = plus then
            NUMINFECTED[AGECLASS]:= NUMINFECTED[AGECLASS] + 1
          else if pig^.TITRE <= MAXSPEC then
            NUMSUSC[AGECLASS]:= NUMSUSC[AGECLASS] + 1;
          if pig^.TITRE >= 1 then
            begin
              NUMSEROPOS[AGECLASS]:= NUMSEROPOS[AGECLASS] + 1;
              AVERAGECT[AGECLASS]:=
                AVERAGECT[AGECLASS] + pig^.TITRE;
            end;
          if pig^.PREVINP then
            PREVALENCE[AGECLASS]:= PREVALENCE[AGECLASS] + 1;
          LEPTOSPIRURIA[AGECLASS]:=
            LEPTOSPIRURIA[AGECLASS] + pig^.SHED;
        end;
      end;
    end;
  end;

  begin
    if TIME = RunTime-NumWeeks then
      begin
        assign(FILEAGESDATA, AGESFILENAME);
        rewrite(FILEAGESDATA);
        close(FILEAGESDATA);
      end;
    for NM:= 1 to 24 do

```

```

begin
  with AGESDATA do
    begin
      NUMBER[NM]:=0;
      NUMINFECTED[NM]:=0;
      NUMSUSC[NM]:=0;
      NUMSEROPOS[NM]:=0;
      PREVALENCE[NM]:=0;
      LEPTOSPIRURIA[NM]:=0;
      AVERAGECT[NM]:=0;
    end;
  end;
  for i:= 1 to 2000 do
    if Growers[i] <> nil then
      begin
        pig:= growers[i];
        AGE SUMMARY(Pig^.AGE, pig, AGESDATA);
      end;
      with AGESDATA do
        begin
          for NM:= 1 to 24 do
            begin
              if NUMINFECTED[NM] > 0 then
                LEPTOSPIRURIA[NM]:=
                  LEPTOSPIRURIA[NM]/NUMINFECTED[NM];
              if NUMSEROPOS[NM] > 0 then
                AVERAGECT[NM]:= AVERAGECT[NM]/NUMSEROPOS[NM];
            end;
          end;
        end;
      assign(FILEAGESDATA, AGESFILENAME);
      reset(FILEAGESDATA);
      seek(FILEAGESDATA, (TIME-(RUNTIME-NUMWEEKS)));
      write(FILEAGESDATA, AGESDATA);
      close(FILEAGESDATA);
    end;
  end;

procedure WEEKOUT;
  type COHORTRECORD = record
    AGE, TITRE, SHED, INF, PREV, INCIDENCE, NOINFLASTWK: REAL;
  end;
  COHORTARRAY = array [1..6] of COHORTRECORD;
  FILEOF COHORTS = file of COHORTARRAY;
  PENFILE = file of PENDATARECORD;
  FILETITLE = STRING[30];
  var COHORT : COHORTARRAY;
  COHORTFILE : file of cohorts;
  FILEOF WPENS, FILEOF GPENS : PENFILE;
  II, I, RT26, WEEK, TOTSUCK, TOTWEAN, TOTGROW, TOTGILT, TOTSOW,
  IDNO, TOTBOAR, SuckSP, WeanSP, GrowSP, GiltSP, SowsSP, BoarSP: INTEGER;
  V: REAL;
  PENFOUND : BOOLEAN;
  pig: growptr;
  NEXTS : NEXTSOW;
  NEXTB : NEXTBOAR;

procedure GROWERVALUES(I : INTEGER; pig: growptr;
  var COHORT : COHORTARRAY);
begin
  with pig^ do
    begin
      COHORT[I].AGE:=COHORT[I].AGE + AGE;
      if TITRE >= 1 then
        COHORT[I].TITRE:=COHORT[I].TITRE + TITRE;
        COHORT[I].SHED:=COHORT[I].SHED + SHED;
      if INF = plus then
        COHORT[I].INF:=COHORT[I].INF + 1;
      if PREVINF then

```

```

        COHORT[I].PREV:=COHORT[I].prev + 1;
    end;
end;

procedure SOWVALUES(I : INTEGER; NEXTS : NEXTSOW;
                    var COHORT : COHORTARRAY);
begin
    with NEXTS^.SDATA do
    begin
        COHORT[I].AGE:=COHORT[I].AGE + AGE;
        if Titre >= 1 then
            COHORT[I].TITRE:=COHORT[I].TITRE + TITRE;
            COHORT[I].SHED:=COHORT[I].SHED + SHED;
            if INF = POS then
                COHORT[I].INF:=COHORT[I].INF + 1;
            if PREVINF then
                COHORT[I].PREV:=COHORT[I].PREV + 1;
            end;
        end;
    end;

procedure BOARVALUES(I : INTEGER; NEXTB : NEXTBOAR;
                    var COHORT : COHORTARRAY);
begin
    with NEXTB^.BDATA do
    begin
        COHORT[I].AGE:=COHORT[I].AGE + AGE;
        if Titre >= 1 then
            COHORT[I].TITRE:=COHORT[I].TITRE + TITRE;
            COHORT[I].SHED:=COHORT[I].SHED + SHED;
            if INF = POS then
                COHORT[I].INF:=COHORT[I].INF + 1;
            if PREVINF then COHORT[I].PREV:=
                COHORT[I].PREV + 1;
            end;
        end;

procedure RECORDVALUES(I, TOT, TotTitre : INTEGER;
                    var COHORT : COHORTARRAY);
begin
    if TOT <> 0 then
    begin
        COHORT[I].INCIDENCE:=
            COHORT[I].INF - COHORT[I].NOINFLASTWK;
        COHORT[I].NOINFLASTWK:= COHORT[I].INF;
        if COHORT[I].INCIDENCE < 0 then
            COHORT[I].INCIDENCE:=0;
        COHORT[I].AGE:= COHORT[I].AGE/TOT;
        if TotTitre > 0 then COHORT[I].TITRE:=
            COHORT[I].TITRE/TOTTITRE
        else Cohort[I].Titre:= 0;
        COHORT[I].SHED:= COHORT[I].SHED/TOT;
        COHORT[I].INF:= COHORT[I].INF/TOT;
        COHORT[I].PREV:= COHORT[I].PREV/TOT;
    end
    else
    begin
        COHORT[I].AGE:=0.0;
        COHORT[I].TITRE:=0.0;
        COHORT[I].SHED:=0.0;
        COHORT[I].INF:=0.0;
        COHORT[I].PREV:=0.0;
        COHORT[I].INCIDENCE:=0.0;
    end;
end;
end;

```





```

with house[idno+10] ^ do
begin
  PENDATA.POP[IDNO]:= NUMINPEN;
  if numinpen > 0 then
    PENDATA.PREV[IDNO]:= (NoInfected/NumInPen)*100
  else
    PenData.Prev[idno]:=0;
    PENDATA.INFECTED[IDNO]:= NOINFECTED;
    PENDATA.SEROLOGY[IDNO]:= AVETITRE;
    PENDATA.AGEAVER[IDNO]:= AVEAGE;
    PENDATA.CONTAMIN[IDNO]:= AVESHED;
    PENDATA.INFCOHORT[IDNO]:= PROBINF;
  end;
end;
assign(FILEOFGPENS, GROW1);
reset(FILEOFGPENS);
seek(FILEOFGPENS, NumSumWks-RT26);
write(FILEOFGPENS, PENDATA);
close(FILEOFGPENS);
ASSIGN(COHORTFILE, COHortFILENAME);
RESET(COHORTFILE);
if RT26 = NumSumWks then
for I:= 1 to 6 do COHORT[I].NOINFLASTWK:=0
else
begin
  SEEK(COHORTFILE, NumSumWks-RT26-1);
  READ(COHORTFILE, COHORT);
  FOR I:= 1 TO 6 DO
    COHORT[I].NOINFLASTWK:= COHORT[I].NOINFLASTWK;
end;
SEEK(COHORTFILE, NumSumWks-RT26);
for I:= 1 to 6 do
begin
  COHORT[I].AGE:=0.0;
  COHORT[I].TITRE:=0.0;
  COHORT[I].SHED:=0.0;
  COHORT[I].INF:=0.0;
  COHORT[I].PREV:=0.0;
  COHORT[I].INCIDENCE:=0.0;
end;
TOTSUCK:=0; TOTWEAN:=0; TOTGROW:=0;
TOTSOW:=0; TOTGILT:=0; TOTBOAR:=0;
SuckSP:=0; WeanSP:=0; GrowSP:=0;
GiltSP:=0; SowsSP:=0; BoarSP:=0;
i:=1;
while i<= 2000 do
begin
  Pig:= Growers[i];
  if (pig^.AGE >=1) and (pig^.AGE <=WEANAGE) then
  begin
    TOTSUCK:=TOTSUCK + 1;
    if pig^.titre >= 1 then SuckSP:= SuckSP + 1;

    GROWERVALUES(1, pig, COHORT);
  end;
  if (pig^.AGE > WEANAGE) and (pig^.AGE <=10) then
  begin
    TOTWEAN:=TOTWEAN + 1;
    if pig^.titre >= 1 then WeanSP:= WeanSP + 1;
    GROWERVALUES(2, pig, COHORT);
  end;
  if (pig^.age > 10) and (pig^.AGE <= 24) then
  begin
    TOTGROW:=TOTGROW + 1;
    if pig^.titre >= 1 then GrowSP:= GrowSP + 1;
    GROWERVALUES(3, pig, COHORT);
  end;
end;

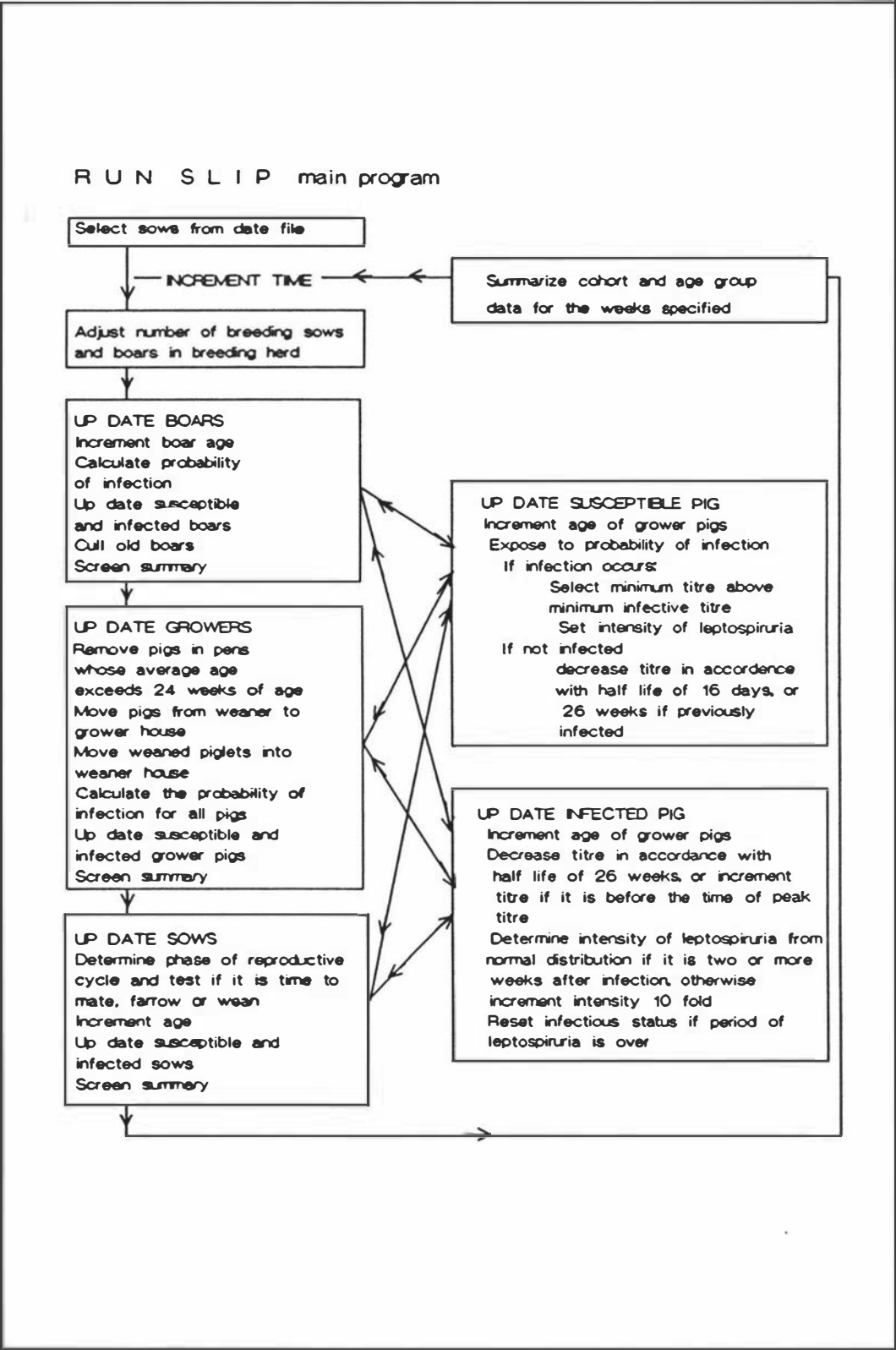
```

```

i:= i+1;
end;
RECORDVALUES(1, TOTSUCK, SuckSP, COHORT);
RECORDVALUES(2, TOTWEAN, WeanSP, COHORT);
RECORDVALUES(3, TOTGROW, GrowSP, COHORT);
NEXTS:=SBASE;
while NEXTS <> NIL do
begin
  if NEXTS^.SDATA.AGE < 52 then
  begin
    TOTGILT:=TOTGILT+1;
    if NextS^.SData.Titre >= 1 then
      GiltSP:= GiltSP + 1;
    SOWVALUES(4, NEXTS, COHORT);
  end
  else
  begin
    TOTSOW:=TOTSOW +1;
    if NextS^.SData.Titre >= 1 then
      SowsSP:= SowsSP + 1;
    SOWVALUES(5, NEXTS, COHORT);
  end;
  NEXTS:=NEXTS^.SLINK;
end;
RECORDVALUES(4, TOTGILT, GiltSP, COHORT);
RECORDVALUES(5, TOTSOW, SowsSP, COHORT);
NEXTB:=BBASE;
while NEXTB <> NIL do
begin
  TOTBOAR:= TOTBOAR + 1;
  if NextB^.BData.Titre >= 1 then BoarSP:= BoarSP + 1;
  BOARVALUES(6, NEXTB, COHORT);
  NEXTB:= NEXTB^.BLINK;
end;
RECORDVALUES(6, TOTBOARS, BoarSP, COHORT);
WRITE(COHORTFILE, COHORT);
CLOSE(COHORTFILE);
end; { WEEKOUT }
end.

```

Figure A6.2 Summary of the interaction of program procedures within the WKUPDATE and RUNSLIP units.



UNIT WKUPDATE;

interface

uses crt,turbo3,printer,  
 GLOBALS,  
 UTILITIES,  
 EVENTMFW,  
 CULLPIG;

procedure UPDATEBOARS(var BASE : NEXTBOAR; var TBEC : REAL);

procedure UPDATESOWS(var BASE : NEXTSOW; var TSEC : REAL);

procedure UPDATEGROWERS(var house: PenArray;  
 var Growers : Gpigs; var TWEC, TGEC : REAL);

implementation

procedure UPDATEBOARS;

const MAXCULLAGE = 176;  
 PEAKTITRE = 2;  
 var NN, num,i,ii,TINF,  
 NoBoar, NoInfBoar, NoLUBoar : INTEGER;  
 RANDI : REAL;  
 NEXTB : NEXTBOAR;

begin { UPDATEBOARS }

NoBoar:= 0; NoInfBoar:= 0; NoLUBoar:= 0;

NEXTB:= BBASE;

TBEC:= 0; {TotalBoarEnvironmentalContamination}

TINF:=0; NUM:=0;

while NEXTB <> NIL do

begin

NUM:= NUM+1;

TBEC:= NEXTB^.BDATA.SHED + TBEC;

if NEXTB^.BDATA.INF = POS then TINF:= TINF + 1;

NEXTB:= NEXTB^.BLINK;

end;

if TBEC > 0 then TBEC:= ln(TBEC/NUM)/ECDF;

{In the case of environmental contamination,  
 where contraction of leptospirosis is likely via an  
 indirect path, dilution proportion to number  
 shedding and total number of pigs}

{shedding is important vs direct contact as in pens}

NEXTB:= BBASE;

while NEXTB <> NIL do

begin

NEXTB^.BDATA.AGE:= NEXTB^.BDATA.AGE + 1;

NEXTB^.SERVPERWK:= 0;

with NEXTB^.BDATA do

if WKPI > -1 then

begin

WKPI:= WKPI + 1;

if TIME <= PEAK then

begin

TITRE:= TITRE \* 2;

if TIME = PEAK then SHED:=

exp(normal(LuIntAve, LuIntSd)) /100

else SHED:= 0;

if SHED < 0 then SHED:=0;

end

else if TIME <= DURINF then

begin

SHED:= exp(NORMAL(LUINTAVE, LUINTSD));

if TITRE > 0 then

```

        TITRE:= exp(ln(TITRE) - ADULTHL26);
    end;
    if SHED < 0 then SHED:= 0;
    if TIME = DURINF then
        begin
            SHED:= 0;
            INF:= NEG;
            WKPI:= -1;
            PEAK:= 0;
            DURINF:= 0;
        end;
    end
    else
        if ((INF = NEG) and (TITRE <= MAXSPEC)) then
            begin
                RANDI:= RANDOM;
                PINFECTION:= VRR*((PCSSB*(0.95*TSEC+0.05*TBEC))
                    + (PCGB*TGEC) + (PCBW*TWEC));
                if RANDI <= PINFECTION
            then
                begin
                    PREVINF:= TRUE;
                    INF:= POS;
                    DURINF:= DATE(DURATINF);
                    WKPI:= 0;
                    repeat TITRE:= NORMAL(TITREAVE, TITRES D);
                    until TITRE >= MINSENS;
                    if TITRE > MAXTITRE then TITRE := MAXTITRE;
                    {squewered distribution of titre toward high titre}
                    for NN:= 1 to 2 do TITRE:= TITRE
                * 0.5;
                    PEAK:= PEAKTITRE + TIME;
                    SHED:= 0;
                end
                else if TITRE > 0 then
                    TITRE:= exp(ln(TITRE) - ADULTHL26);
                end;
                if NEXTB^.BDATA.AGE > MAXCULLAGE then
                    DELETEBOAR(NEXTB^.BID, BASE);
                NoBoar:= NoBoar + 1;
                if NextB^.BData.Previnf = True then
                    NoInfBoar:= NoInfBoar + 1;
                if NextB^.BData.Shed > 0 then
                    NoLUBoar:= NoLUBoar + 1;
                NEXTB:= NEXTB^.BLINK;
            end; { while }
            GotoXY(41,17); ClrEol;
            GotoXY(41,18); ClrEol;
            if NoBoar > 0 then
                begin
                    ii := 41 + round((NoInfBoar/NoBoar)*100/2);
                    if ii > 80 then ii:= 80;
                    if ii > 41 then for i:= 41 to ii do
                        begin GotoXY(i,17); write('X'); end;
                    ii := 41 + round((NoLuBoar/NoBoar)*100/2);
                    if ii > 80 then ii:= 80;
                    if ii > 41 then for i:= 41 to ii do
                        begin GotoXY(i,18); write('X'); end;
                    end;
                end;
            end; { UPDATEBOARS }

procedure UPDATEGROWERS;
const CULLAGE = 24;
MOVEAGE = 10;
PEAKTITRE = 2;
var SETTOBASE : BOOLEAN;
Pig: Growptr;
NEXTS : NEXTSOW;
PEN : penptr;

```

```

(*)      ii,iii, lower, transfer,totweaners, totweanersinf,
          totgrowers, totgrowersinf, totpopulation,
          totinfectd, NoGrow, NoWean, NoInfGrow, NoInfWean,
          NoLUGrow, NoLUWean : integer;
          ProbMixPigs, ProbCrossInf, AdjPenShed,
          OtherPenShed : Real;

Function PigPentoSowID(penno:integer):ID;
var sowid : string[10];
    sn : string[5];
    penn : integer;

begin
    penn:= penno-10000;
    str(penn:1, sn);
    sowid:=concat('SF'+sn+'A');
    PigPentoSowID:= sowid;
end;

procedure UPDATEINFPIG;
begin
    with Growers[i]^ do
    begin
        age:= age+1;
        if WKPI > -1 then
            begin
                WKPI:=WKPI + 1;
                if TIME <= PEAK then
                    begin
                        TITRE:= TITRE * 2;
                        if TITRE > MAXTITRE then TITRE:= MAXTITRE;
                        if TIME = PEAK then
                            SHED:= exp(normal(LuIntAve, LuIntSd)) /100
                        else SHED:= 0;
                        if SHED < 0 then SHED:=0;
                    end
                else
                    if TIME <= DURINF then
                        begin
                            SHED:= exp(NORMAL(LUINTAVE, LUINTSD));
                            if TITRE > 0 then
                                TITRE:= exp( ln(TITRE) - ADULTHL26 );
                            end;
                            if SHED < 0 then SHED:= 0;
                            if TIME = DURINF then
                                begin
                                    SHED:= 0;
                                    INF:= min;
                                    WKPI:= -1;
                                    PEAK:= 0;
                                    DURINF:= 0;
                                end;
                            end;
                        end;
                    end;
                end; {UpDateInfPig}

procedure UPDATESUSCPIG(CHANCEINF : REAL);
const PEAKTITRE = 2;
var RANDI : REAL;
    NN : INTEGER;
begin
    with Growers[i]^ do
    begin
        Age:= Age + 1;
        if TITRE <= MAXSPEC then
            begin
                RANDI:= RANDOM; {for prob.inf }

```

```

if Time < 26 then
  begin
    if ((Endemic) and (Age > 12)) then
      CHANCEINF:= CHANCEINF + 0.1
    end
  else
    if Age >= VA then CHANCEINF:= VRR * CHANCEINF;
    if RANDI <= CHANCEINF then
      begin
        PREVINF:= TRUE;
        INF:= plus;
        DURINF:= DATE(DURATINF);
        WKPI:= 0;
        repeat TITRE:= NORMAL(TITREAVE, TITRESD);
        until TITRE >= MINSSENS;
        if TITRE > MAXTITRE then TITRE := MAXTITRE;
        for NN:= 1 to PEAKTITRE
          do TITRE:= TITRE * 0.5;
        PEAK:= PEAKTITRE + TIME;
        SHED:= 0;
      end;
    end
  else
    if (PREVINF and (TITRE > 0)) then
      TITRE:= exp(ln(TITRE) - ADULTHL26)
    else if ((AGE > 1) and (TITRE > 0)) then
      TITRE:= exp(ln(titre) - PIGLETHL16);
    {derived from t1/2 life equation, with y = logtitre, x = time}
    end;
  end; {UpDateSuscPig}

```

Function PenSurrounding(a:integer; house:PenArray):real;

Var AdjShed : Real;

b: integer;

begin

AdjShed:=0;

case a of

1,2: for b:= 1 to 4 do

if b <> a then

if house[b] <> nil then

AdjShed:= AdjShed + house[b]^a.avedshed;

9,10: for b:= 7 to 10 do

if b <> a then

if house[b] <> nil then

AdjShed:= AdjShed + house[b]^a.avedshed;

11,12: for b:= 11 to 14 do

if b <> a then

if house[b] <> nil then

AdjShed:= AdjShed + house[b]^a.avedshed;

49,50: for b:= 47 to 50 do

if b <> a then

if house[b] <> nil then

AdjShed:= AdjShed + house[b]^a.avedshed;

else

if odd(a) then

begin

for b:= a-2 to a+3 do

if (b <> a) and (house[b] <> nil) then

AdjShed:= AdjShed + house[b]^a.avedshed;

end

else

begin

for b:= a-3 to a+2 do

if (b <> a) and (house[b] <> nil) then

AdjShed:= AdjShed + house[b]^a.avedshed;

end;

end;



```

PenSurrounding:= AdjShed;
end;

```

```

begin { UPDATEGROWERS }

```

```

{pen pigs... pigs which are now 5 weeks will be penned into a
pen numbered 1..10. Before penning 5 week old pigs remove
oldest pigs ready for slaughter, then move up weaner pigs
aged 10 weeks, then move in suckling pigs }

```

```

{ remove pigs of cull age }

```

```

for i:= 11 to 50 do

```

```

if house[i] <> nil then

```

```

begin

```

```

if house[i]^aveage >= cullage then

```

```

begin

```

```

for ii:=1 to 2000 do

```

```

if Growers[ii]^penno = i then

```

```

deletegrower(Growers[ii]^gid);

```

```

pen:= house[i];

```

```

dispose(pen);

```

```

house[i]:= nil;

```

```

end;

```

```

end;

```

```

{ transfer pigs from weaner to grower house }

```

```

for i:= 1 to 10 do

```

```

if ((house[i] <> nil) and (house[i]^aveage >= 10)) then

```

```

begin

```

```

{ first fill incompletely filled pens }

```

```

while house[i]^numinpen > 0 do

```

```

begin

```

```

ii:=10;

```

```

repeat ii:= ii+1;

```

```

until (((house[ii] <> nil) and

```

```

(house[ii]^numinpen < 30) and

```

```

(house[ii]^aveage <= 12)) or

```

```

(ii = 50));

```

```

if house[ii] <> nil then

```

```

begin

```

```

iii:=0;

```

```

repeat

```

```

iii:= iii+1;

```

```

if ((Growers[iii] <> nil) and

```

```

(Growers[iii]^penno = i)) then

```

```

begin

```

```

house[i]^numinpen:=house[i]^numinpen -1;

```

```
house[ii]^numinpen:=house[ii]^numinpen +1;

```

```
Growers[iii]^penno:= ii;

```

```

end;

```

```

until ((house[i]^numinpen = 0) or

```

```

(house[ii]^numinpen = 30)

```

```

or (iii = 2000));

```

```

end;

```

```

{ fill unoccupied pens }

```

```

ii:=10;

```

```

repeat ii:= ii+1;

```

```

until ((house[ii] = nil) or (ii = 50));

```

```

if house[ii] = nil then

```

```

begin

```

```

new(pen);

```

```

pen^.numinpen:=0;

```

```

pen^.noinfected:=0;

```

```

pen^.seropos:=0;

```

```

pen^.aveage:=0;

```

```

pen^.avetitre:=0;

```

```

pen^.probinf:=0;

```

```

    iii:=0;
    repeat
        iii:= iii + 1;
        if ((Growers[iii] <> nil) and (Growers[iii]^penno = i)) then
            begin
                house[i]^numinpen:= house[i]^numinpen -1;
                pen^numinpen:=
pen^numinpen +1;
                Growers[iii]^penno:= ii;
            end;
        until ((house[i]^numinpen = 0) or
            (iii = 2000));
        house[ii]:= pen;
    end;
end;
pen:=house[i];
dispose(pen);
house[i]:= nil;
end;

{ transfer pigs from dams to weaner house }
transfer:=0;
for i:=1 to 2000 do if growers[i] <> nil then
    if Growers[i]^age = weanage
        then transfer:= transfer +1;
if transfer > 0 then
{ first fill incompletely filled pens }

for ii:= 1 to 10 do
if house[ii] <> nil then
begin
    if (house[ii]^numinpen < 30) and
        (house[ii]^aveage <= weanage +1) then
        begin
            i:=0;
            repeat
                i:= i+1;
                if ((Growers[i] <> nil) and
                    (Growers[i]^age = weanage) and
                    (Growers[i]^penno >50)) then
                    begin
                        house[ii]^numinpen:= house[ii]^numinpen +1;
                        Growers[i]^penno:= ii;
                        Transfer:= transfer -1;
                    end;
                until ((transfer = 0) or (i = 2000) or
                    (house[ii]^numinpen = 30));
                if transfer = 0 then ii:= 10;
            end;
        end;
    end;

{ fill new empty pen }
ii:=0;
while transfer > 0 do
begin
    repeat ii:= ii + 1;
    until ((house[ii] = nil) or (ii = 10));
    if house[ii] = nil then
        begin
            new(pen);
            pen^numinpen:=0;
            pen^noinfected:=0;
            pen^seropos:=0;
            pen^aveage:=0;
            pen^avetiire:=0;
            pen^aveshed:=0;
            pen^probinf:=0;
            i:=0;

```

```

repeat
  i:= i + 1;
  if ((growers[i] <> nil) and
      (Growers[i]^age = weanage) and
      (Growers[i]^penno > 50)) then
  begin
    Growers[i]^penno:= ii;
    pen^.numinpen:= pen^.numinpen + 1;
    transfer:= transfer -1;
  end;
  until ((pen^.numinpen = 30) or (transfer = 0) or
        (i = 2000));
  house[ii]:=pen;
end;
end;

```

{Complete pen records}

```

NoGrow:= 0; NoWean:= 0;
NoInfGrow:= 0; NoInfWean:= 0;
NoLUGrow:= 0; NoLUWean:= 0;
for i:= 1 to 50 do
begin
  if house[i] <> nil then
  with house[i]^ do
  begin
    aveage:=0;
    avetitre:=0;
    aveshed:=0;
    noinfected:=0;
    numinpen:=0;
    seropos:= 0;
    probinf:=0;
  end;
end;
for i:= 1 to 2000 do
begin
  if Growers[i] <> nil then
  if Growers[i]^penno <=50 then
  with house[growers[i]^penno]^ do
  begin
    numinpen:= numinpen + 1;
    aveage:= aveage + Growers[i]^age;
    if Growers[i]^titre >=1
    then seropos:=seropos+1;
    avetitre:= avetitre + Growers[i]^titre;
    aveshed:= aveshed + Growers[i]^shed;
    if Growers[i]^inf = plus
    then noinfected:= noinfected + 1;
  end;
end;
twec:=0; tgec:=0;
totweaners:=0; totweanersinf:=0;
totgrowers:=0; totgrowersinf:=0;

for i:= 1 to 50 do
if house[i] <> nil then
begin
  with house[i]^ do
  begin
    if numinpen <> 0 then aveage:= aveage/numinpen
    else aveage:= 0;
    if seropos > 0 then avetitre:= avetitre/seropos
    else avetitre:= 0;
    if i < 11 then
    begin

```

```

    twec:= aveshed + twec;
    totweaners:= totweaners + numinpen;
    totweanersinf:= totweanersinf + noinfected;
end
else
begin
    tgec:= aveshed + tgec;
    totgrowers:= totgrowers + numinpen;
    totgrowersinf:= totgrowersinf + noinfected;
end;
aveshed:= aveshed/numinpen;
end;
end;

{Calculate probability of transmission of infection}
for i:= 1 to 50 do
    if house[i] <> nil then
        with house[i]^ do
            begin
                if i < 11 then
                    begin
                        ProbMixPigs:=0; ProbInf:=0;
                        AdjPenShed:=0; OtherPenShed:=0;

                        if (TotWeaners-NumInPen) > 0 then
                            ProbMixPigs:=(TotWeanersInf-NoInfected)
                                /(TotWeaners-NumInPen)
                                {Proportion of infected pigs outside pen}
                        else ProbMixPigs:=0;

                        if Random(1) < (PwPwP * ProbMixPigs) then
                            begin
                                {infected pig transfered to pen - theoretically}
                                if ( ((twec - (aveshed*numinpen)) > 0) and
                                    ((totweanersinf-noinfected) > 0) ) then
                                    probinf:=
                                        abs(ln((twec - (aveshed*numinpen))
                                            /(totweanersinf-noinfected)))/PCDF);
                                {direct transmission of inf from pig mixed into current pen}
                                else probinf:= 0;

                                {direct transmission between pigs in pen}
                                {total probability of direct transmission}
                                if ( ((aveshed*Numinpen) > 0) and
                                    ((numinpen -1 -noinfected) > 0) ) then
                                    probinf:=
                                        abs(ln((aveshed*numinpen)
                                            /(numinpen -1 -noinfected)))/pcdf)
                                        + probinf
                                    else ProbInf:= probinf;

                                {indirect transmission from adjacent and other pens}
                                if (totweaners*TWEC > 0)
                                    then TWEC:= abs(ln(TWEC/totweaners))/ECDF)
                                    else TWEC:= 0;
                                {now TWEC represents prob of inf from average env contam}
                                PenSurrounding(i, house);
                                if AdjPenShed > 0
                                    then ProbCrossInf:=
                                        TWEC + abs(ln(AdjPenShed))/ECDF)
                                    else ProbCrossInf:= TWEC;

                                {all probabilities of transmission of
                                infection added together}

                                probinf:= probinf + ProbCrossInf +

```

{twec = tot num lepto in env}

end

AdjPenShed:=

```

        (PCBW*(TBEC*0.05+TSEC*0.95));
    if TGEc*TotGrowers > 0 then
        ProbInf:=
            ProbInf+abs(PCWG*(ln(TGEc/totgrowers)/ECDF))
    else ProbInf:= ProbInf;
    end
else {grower pens}
begin
    ProbMixPigs:=0; ProbInf:=0;
    AdjPenShed:=0; OtherPenShed:=0;

    if (TotGrowers-NumInPen) > 0 then
        ProbMixPigs:=
            (TotGrowersInf-NoInfected)
            /(TotGrowers-NumInPen)
    else ProbMixPigs:=0;

    if Random(1) < (PgPgP * ProbMixPigs) then
        begin
            {infected pig transfered to pen - theoretically}
            {tgec = tot num lepto in env}
            if ( ((tgec - (aveshed*numinpen)) >0) and
                ((totgrowersinf-noinfected) >0) ) then
                probinf:=
                    abs(ln( (tgec - (aveshed*numinpen))
                        /(totgrowersinf-noinfected) ))/PCDF)
            else TGEc:= 0;
        end
    {direct transmission of inf from pig mixed into current pen}
    end
    else ProbInf:= 0;

    {direct transmission between pigs in pen}
    {total probability of direct transmission}
    if ( ((aveshed*Numinpen) >0) and
        ((numinpen -1 -noinfected) >0) ) then
        probinf:=
            abs(ln((aveshed*numinpen)
                /(numinpen -1 -noinfected)))/pcdf)
            + probinf
    else ProbInf:= probinf;

    {indirect transmission from adjacent and other pens}
    if (TGEc*totgrowers) > 0 then
        TGEc:= abs(ln(TGEc/totgrowers)/ECDF)
    else TGEc:= 0;
    {now TGEc represents prob of inf from average env contam}
    AdjPenShed:= PenSurrounding(i, house);
    if AdjPenShed > 0 then
        ProbCrossInf:=
            TGEc + abs(ln(AdjPenShed)/ECDF)
    else ProbCrossInf:= TGEc;

    {all probabilities of transmission of infection
    added together}
    probinf:= probinf + ProbCrossInf +
        (PCBW*(TBEC*0.05+TSEC*0.95))+(PCWG*TWEC);
    end;
end;

for i:= 1 to 2000 do
begin
    if Growers[i] <> nil then
    begin
        if Growers[i]^wkpi = -1 then
        begin
            if Growers[i]^penno < 51 then
                pinfection:= house[growers[i]^penno]^probinf
            else
                begin

```

```

    Nexts:= sbase;
    repeat NEXTS:= NEXTS^.SLINK
    until (NEXTS = NIL) or
        (NEXTS^.SID =
            PigPentoSowID(Growers[i]^.penno));
    if nexts^.sdata.shed > 1 then
        pinfection:= (ln(nexts^.sdata.shed)/pcdf)
    else pinfection:= 0;
    end;
    updatesusc pig(pinfection);
end
else updateinfpig;

if Growers[i]^.previnf then
begin
    gotoXY(17,15); write('      | ');
    gotoXY(18,15); write(growers[i]^.age);
    gotoXY(22,15);
    write(exp((growers[i]^.titre-1)*0.6931 + 2.303):6:0);      end
else
begin
    gotoXY(17,14); write('      | ');
    gotoXY(18,14); write(growers[i]^.age);
    gotoXY(22,14);
    write(exp((growers[i]^.titre-1)*0.6931 + 2.303):6:0)      end;
end;
end;
{End of pen records and updating grower pigs}

{Run Time SLIP Summary}
for i:= 1 to 2000 do
if Growers[i] <> nil then
if ( (Growers[i]^.penno > 10) and (Growers[i]^.penno < 51) ) then
begin
    NoGrow:= NoGrow + 1;
    if Growers[i]^.previnf = true
    then NoInfGrow:= NoInfGrow + 1;
    if Growers[i]^.Shed > 0
    then NoLUGrow:= NoLUGrow + 1;
end
else
begin
    NoWean:= NoWean + 1;
    if Growers[i]^.previnf = true
    then NoInfWean:= NoInfWean + 1;
    if Growers[i]^.Shed > 0
    then NoLUWean:= NoLUWean + 1;
end;

GotoXY(41,5); ClrEol;
GotoXY(41,6); ClrEol;
if NoWean > 0 then
begin
    ii := 41 + round((NoInfWean/NoWean)*100/2);
    if ii > 80 then ii:= 80;
    if ii > 41 then for i:= 41 to ii do
    begin GotoXY(i,5); Write('X'); end;
    ii := 41 + round((NoLuWean/NoWean)*100/2);
    if ii > 80 then ii:= 80;
    if ii > 41 then for i:= 41 to ii do
    begin GotoXY(i,6); Write('X'); end;
end;

GotoXY(41,9); ClrEol;
GotoXY(41,10); ClrEol;
if NoGrow > 0 then
begin

```

```

ii := 41 + round((NoInfGrow/NoGrow)*100/2);
if ii > 80 then ii:= 80;
if ii > 41 then for i:= 41 to ii do
begin GotoXY(i,9); Write('X'); end;
ii := 41 + round((NoLUGrow/NoGrow)*100/2);
if ii > 80 then ii:= 80;
if ii > 41 then for i:= 41 to ii do
begin GotoXY(i,10); Write('X'); end;
end;
end; { UPDATEGROWER }

procedure UPDATESOWS;
const MAXCULLAGE = 180;
PEAKTITRE = 2;
var RANDI : REAL;
NN, TINF, num, numfarrow,
NoSows, NoInfSows, NoLUSows,i,ii : INTEGER;
NEXTS : NEXTSOW;
nextb : nextboar;
abortions : integer;

begin { UPDATESOWS }
NoSows:= 0; NoInfSows:= 0;
NoLUSows:= 0;
Abortions:= 0;
gotoXY(25,18); write(' ');
gotoXY(25,18); write(Abortions);
NEXTS:= SBASE;
TSEC:= 0; {TotalGrowerEnvironmentalContamination}
NUM:=0; TINF:=0;
while NEXTS <> NIL do
begin
NUM:= NUM+1;
TSEC:= NEXTS^.SDATA.SHED + TSEC;
if NEXTS^.SDATA.INF = POS then TINF:= TINF+1;
NEXTS:= NEXTS^.SLINK;
end;
if TSEC > 0 then TSEC:= ln(TSEC/NUM)/ECDF;
NEXTS:= BASE;
while NEXTS <> NIL do
begin
case NEXTS^.EVENT of
OESTRUS : begin
if NEXTS^.MATEDATE = TIME
then
MATESOW(NEXTS,TOTBOARS,BBASE);
end; { oestrus
}

PREGNANCY : begin
if NEXTS^.FARROWDATE = TIME
then
FARROW(NEXTS, Growers);
if (NEXTS^.FARROWDATE - TIME)
<=5
then
if ((NEXTS^.FARROWDATE - TIME)
>0) and
(NEXTS^.SDATA.WKPI = 1)
then
begin
SOUND(500); DELAY(20);
NOSOUND;
NEXTS^.EVENT:= OESTRUS;
NEXTS^.MATEDATE:=
DATE(SERV);
Abortions:= abortions + 1;
gotoXY(25,18); write('
');
```

```

        gotoXY(25,18);
        write(Abortions);
        end;
    end;
    LACTATION : if NEXTS^.WEANDATE = TIME then
        WEAN(NEXTS);
    end; { case }
    NEXTS:=NEXTS^.SLINK;
end; { while }
NEXTS:= BASE;
while NEXTS <> NIL do
begin
    with NEXTS^.SDATA do
    begin
        if WKPI > -1 then
        begin
            WKPI:=WKPI + 1;
            if TIME <= PEAK then
            begin
                TITRE:= TITRE * 2;
                if TIME = PEAK then
                    SHED:= exp(normal(LuIntAve, LuIntSd)) /100
                else SHED:= 0;
                if SHED < 0 then SHED:=0;
            end
        else if TIME <= DURINF then
            begin
                SHED:=exp(NORMAL(LUINTAVE, LUINTSD));
                if TITRE > 0 then
                    TITRE:= exp(ln(TITRE) - ADULTHL26);
                end;
            if SHED < 0 then SHED:= 0;
            if TIME = DURINF then
            begin
                SHED:= 0;
                INF:= NEG;
                WKPI:= -1;
                PEAK:= 0;
                DURINF:= 0;
            end;
        end
    else
        if ((INF = NEG) and (TITRE <= MAXSPEC)) then
        begin
            RANDI:= RANDOM;
            PINFECTION:= VRR* ((PCSSB*(0.95*TSEC+0.05*TBEC))
                +(PCGB*TGEC)+(PCBW*TWEC));
            if RANDI <= PINFECTION then
            begin
                PREVINF:= TRUE;
                INF:= POS;
                DURINF:= DATE(DURATINF);
                if Time = 1 then WKPI:= Random(DurInf) +1
                else WkPI:= 0;
                repeat TITRE:= NORMAL(TITREAVE, TITRESD);
                until TITRE >= MINSENS;
                if TITRE > MAXTITRE then TITRE := MAXTITRE;
                if Time = 1 then
                begin
                    Peak:= 0;
                    Shed:= exp(Normal(LUIntAve, LUIntSD));
                end
            else
            begin
                for NN:= 1 to PEAKTITRE
                do TITRE:= TITRE * 0.5;
                PEAK:= PEAKTITRE + TIME;
            end
        end
    end
end

```



```

        SHED:= 0;
    end;
end
else if TITRE > 0 then
    TITRE:= exp( ln(TITRE) - ADULTHL26 );
end;
AGE:= AGE + 1;
if AGE > MAXCULLAGE then
    if ((NEXTS^.EVENT = OESTRUS)
    or (NEXTS^.EVENT = PREGNANCY)) then
        CULLSOW(SBASE);
    end;
    NOSOWS:= NOSOWS + 1;
    if NEXTS^.SDATA.PREVINF = TRUE then
        NOINFSOWS:= NOINFSOWS + 1;
        if NEXTS^.SDATA.SHED > 0 then NOLUSOWS:= NOLUSOWS+ 1;
NEXTS^.SLINK;
    end; { while }

    GotoXY(41,13); ClrEol;
    GotoXY(41,14); ClrEol;
    if NoSows > 0 then
        begin
            ii := 41 + round((NoInfSows/NoSows)*100/2);
            if ii > 80 then ii:= 80;
            if ii > 41 then for i:= 41 to ii do
                begin GotoXY(i,13); write('X'); end;
            ii := 41 + round((NoLUSows/NoSows)*100/2);
            if ii > 80 then ii:= 80;
            if ii > 41 then for i:= 41 to ii do
                begin GotoXY(i,14); write('X'); end;
            end;

        end; { UPDATESOWS }

end.

```

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