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***Mycobacterium paratuberculosis* infection
in sheep: aspects of diagnosis and immunity**

Jacek Michał Andrzej Gwózdź

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...the essential part of scientific research is dedicated to struggling, not against the evils of Nature, but against the evils begotten by our so-called progress...

Henri-Frederic Blanc

Abstract

Paratuberculosis is a chronic, wasting disease of ruminants caused by *Mycobacterium paratuberculosis*. Programmes aimed at controlling paratuberculosis are based on either vaccination or detection and culling of infected animals. Because of its chronic nature, and the lack of suitable tests for early diagnosis, control of the disease using the latter approach is difficult.

Standard procedures for the isolation of *M. paratuberculosis* are time-consuming and some strains are difficult or impossible to grow. Using the published sequence data of IS900, an insertion sequence considered unique for *M. paratuberculosis*, a polymerase chain reaction (PCR) assay was developed. With purified extracts of bacterial DNA the PCR assay was found to be highly sensitive and specific. Among 30 bacterial species tested, the assay showed cross-reactivity only with DNA from *M. scrofulaceum*. The possibility of *M. scrofulaceum* causing false positive results in clinical samples from sheep was considered remote, and the assay was subsequently applied to clinical samples. In a study involving 20 sheep suspected of having clinical paratuberculosis, *M. paratuberculosis* DNA was detected in 90% of liver samples and 66% of blood samples from sheep with advanced clinical paratuberculosis. However, in a longitudinal study involving 14 sheep infected experimentally with *M. paratuberculosis*, the PCR failed to consistently detect the target DNA in liver biopsy specimens and blood samples of subclinically infected and clinically affected sheep with mild or moderate extraintestinal infection. Furthermore, the sensitivity of the PCR on samples of ileum and ileocaecal lymph node was similar to that achieved by histological examination.

An experimental model of ovine paratuberculosis, which was developed primarily to validate the PCR assay, created an opportunity to evaluate the diagnostic performance of three commercially available antibody assays for paratuberculosis: complement fixation test (CFT), agar gel immunodiffusion test (AGID), and enzyme-linked immunosorbent assay (ELISA). Two experimental trials demonstrated a limited value of serology for the control of ovine paratuberculosis, as none of the antibody assays was able to detect all sheep with histologically confirmed paratuberculosis. In comparison, the whole-blood interferon- γ (IFN- γ) assay, which

was assessed only during the second trial, detected significantly more experimentally infected sheep and over shorter period of time than any of the serological tests. Furthermore, in a pilot study involving 19 sheep infected experimentally with *M. paratuberculosis*, 18 of the 19 sheep gave positive reactions in the IFN- γ assay on samples of prescapular lymph node (PLN). The PLN-based IFN- γ assay detected significantly more experimentally infected sheep than the CFT, AGID, ELISA or the blood-based IFN- γ assay. Since the specificities of the blood- and PLN-based IFN- γ assay were similar to that of the serological tests, these data indicate the potential utility of this assay, using blood or samples of peripheral lymph nodes, for the detection of sheep exposed to *M. paratuberculosis*. Interestingly, among the 18 sheep tested positive by PLN-based IFN- γ assay, 13 had no histological evidence of paratuberculosis at the time of collection of the PLN samples. In addition, results obtained in a study involving 14 sheep infected experimentally with *M. paratuberculosis* suggest a positive relationship between the magnitude of antigen-induced IFN- γ response in blood and animal's ability to control the infection. Thus, attempts to use this assay in control programmes that are based on testing and culling of positive reactors could result in the removal of animals that have successfully mounted an immune response to the infection.

Vaccination provides an alternative method to test-and-cull programmes of controlling paratuberculosis. Results of a study involving 28 lambs infected experimentally with *M. paratuberculosis*, 14 of which were vaccinated against paratuberculosis with a live-attenuated vaccine 2 weeks postinfection, indicate that vaccination of lambs already exposed to the organism triggered early and strong humoral and cell-mediated immune responses and led to a reduced mycobacterial burden.

But remember,
nothing is actually happening,
and nothing will occur
till the end.

M. Zablocki

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Table of contents

Abstract.....	I
Acknowledgements.....	III
Table of contents.....	V
List of Tables	XI
List of Figures	XIII

CHAPTER 1

Introduction	1
1.1. Literature review	1
1.1.1 The organism	1
1.1.2. Transmission	3
1.1.3. Clinical manifestation	5
1.1.4. Pathology	5
1.1.5. Pathogenesis.....	7
1.1.6. Resistance	8
1.1.7. Immunology	10
1.1.8. Diagnosis of paratuberculosis	14
1.1.8.1. Methods that identify <i>M. paratuberculosis</i> and provide direct evidence of infection.....	14
1.1.8.2. Tests which provide indirect evidence of infection with <i>Mycobacterium paratuberculosis</i>	16
1.1.9. Control	22
1.1.10. Vaccination	22
1.1.11. Treatment	24
1.1.12. Economic impact	25
1.1.13. Crohn's disease in people and <i>M. paratuberculosis</i>	25
1.2. Aims of the thesis	28

CHAPTER 2

Development of a rapid assay based on polymerase chain reaction for the detection of *Mycobacterium paratuberculosis*.....30

2.1. Introduction.....	30
2.2. Materials and methods.....	30
2.2.1. Oligonucleotide primer selection.....	30
2.2.2. Templates for amplification.....	32
2.2.2.1. DNA extraction from bacteria.....	32
2.2.2.2. DNA extraction from ovine solid tissues.....	35
2.2.3. PCR reaction mixture.....	36
2.2.4. Generation and labelling of 194-bp probe.....	37
2.2.5. Validation of identity of 194-bp PCR product.....	38
2.2.6. PCR product analysis.....	38
2.2.7. Optimisation procedures.....	39
2.2.8. Specificity of the PCR.....	42
2.2.9. Sensitivity of the PCR.....	42
2.3. Results.....	42
2.3.1. Optimisation of magnesium concentration and sensitivity of the PCR.....	42
2.3.2. Specificity of the PCR.....	44
2.3.3. Methods of DNA extraction from mammalian tissues.....	45
2.3.4. Expand Long Template vs. Taq polymerase system.....	46
2.3.5. Validation of identity of 194-bp PCR product.....	46
2.4. Discussion.....	46

CHAPTER 3

Validation of the polymerase chain reaction assay for the detection of *Mycobacterium paratuberculosis* on samples of solid tissue and blood from sheep with clinical paratuberculosis.50

3.1. Introduction.....	50
3.2. Materials and methods.....	51
3.2.1. Animals.....	51
3.2.2. Sample collection.....	51
3.2.3. Processing of samples and DNA extraction from tissues and blood.....	51
3.2.4. Polymerase Chain Reaction.....	52
3.2.5. Histology.....	54

3.2.6. Serology	54
3.3. Results	55
3.3.1. Histology	55
3.3.2. Polymerase Chain Reaction	56
3.3.3. Serology	59
3.4. Discussion.....	59

CHAPTER 4

Development of experimental model of paratuberculosis in sheep..... 63

4.1. Introduction	63
4.2. Materials and methods.....	64
4.2.1. Preparation of inoculum.....	64
4.2.2. Animals and husbandry conditions	64
4.2.3. Necropsy and sample collection for histology	65
4.2.4. Collection and processing of samples for serology and PCR	66
4.2.5. Polymerase Chain Reaction	66
4.2.6. Histology	68
4.2.7. Serological testing.....	68
4.2.8. Microscopic examination of faeces for AFO's.....	69
4.2.9. Statistical analysis	69
4.3. Results	69
4.3.1. Clinical signs and results of microscopic examination of faeces for AFO's	69
4.3.2. Gross pathology	71
4.3.3. Histology	73
4.3.4. Polymerase Chain Reaction	80
4.3.4. Serological testing.....	83
4.4. Discussion.....	84

CHAPTER 5

Vaccination against paratuberculosis of lambs already infected experimentally with *Mycobacterium paratuberculosis*..... 89

5.1. Introduction	89
5.2. Materials and methods.....	90
5.2.1. Animals and husbandry conditions	90
5.2.2. Inoculum	91

5.2.3. Necropsy and histology.....	91
5.2.4. Sample collection and handling	92
5.2.5. Polymerase Chain Reaction	94
5.2.6. Immunological testing.....	94
5.2.7. Microscopical examination of faecal smears for acid-fast organisms	95
5.2.8. Haematology	95
5.2.9. Statistical analysis.....	96
5.3. Results	97
5.3.1. Death rate and gross pathology	97
5.3.2. Histology.....	98
5.3.2.1. Experimentally infected vaccinated and unvaccinated sheep.....	98
5.3.2.2. Control sheep.....	103
5.3.3. Polymerase Chain Reaction	106
5.3.4. Microscopical examination of faecal smears for acid-fast organisms	106
5.3.5. Immunological testing.....	108
5.3.6. Weight gain and faecal egg count.....	110
5.3.7. Haematology	112
5.4. Discussion.....	115

CHAPTER 6

Application of the polymerase chain reaction assay for the detection of <i>Mycobacterium paratuberculosis</i> to blood and liver biopsy specimens from sheep experimentally infected with the organism.....	121
6.1. Introduction	121
6.2. Materials and methods.....	121
6.2.1. Animals and handling	121
6.2.2. Infection status of animals	122
6.2.3. Sample collection and processing.....	122
6.2.4. DNA extraction.....	123
6.2.5. Polymerase Chain Reaction	123
6.2.6. Statistical analysis.....	124
6.3. Results	124
6.3.1. PCR on samples of ileum and ileocaecal lymph node	125
6.3.2. Liver biopsy PCR.....	128
6.3.3. Blood PCR	129
6.4. Discussion.....	130

CHAPTER 7

Comparison of a complement fixation test, gel immunodiffusion test, enzyme-linked immunosorbent assay and interferon- γ assay for the diagnosis of paratuberculosis in sheep infected experimentally with *Mycobacterium paratuberculosis* 134

7.1. Introduction	134
7.2. Materials and methods.....	135
7.2.1. Animals and collection of samples for immunological testing.....	135
7.2.1.1. Trial 1	135
7.2.1.2. Trial 2	136
7.2.2. Animals infection status.....	137
7.2.3. Sample processing and immunological tests.....	137
7.2.3. Statistical analysis.....	138
7.3. Results	138
7.3.1. Trial 1	138
7.3.2. Trial 2.....	140
7.3.3. Validation of specificity of immunological tests	142
7.4. Discussion.....	144

CHAPTER 8

Antigen-induced production of interferon- γ in samples of peripheral lymph nodes from sheep infected experimentally with *Mycobacterium paratuberculosis*..... 148

8.1. Introduction	148
8.2. Materials and methods.....	149
8.2.1. Source and infection status of animals.....	149
8.2.2. Collection of samples for immunological tests.....	149
8.2.3. Processing of samples for immunological tests	150
8.2.4 Immunological testing.....	151
8.2.5. Statistical analysis.....	152
8.3. Results	153
8.3.1. Infection status of animals	153
8.3.2. Comparison of numbers of sheep detected by immunological tests	153
8.3.3. Comparison of production of IFN- γ in samples of blood and prescapular lymph nodes at various times of incubation	155
8.4. Discussion.....	158

CHAPTER 9

General discussion.....	161
9.1. Development and evaluation of a PCR assay for the detection of <i>Mycobacterium paratuberculosis</i> in tissue samples	161
9.2. Comparison of immunological tests for the diagnosis of paratuberculosis in sheep	165
9.3. Vaccination against paratuberculosis of lambs already infected experimentally with <i>Mycobacterium paratuberculosis</i>	169
9.4. Experimental model of paratuberculosis in sheep	170
9.5. Perineural lesions.....	170
9.6. Conclusions	171
Appendix A.1.....	172
Appendix A.2.....	172
Appendix B.1.....	173
Appendix B.2.....	173
Appendix B.3.....	174
Appendix B.4.....	183
Appendix B.5.....	184
Appendix C.1.....	185
Appendix C.2.....	186
Appendix D.....	187
Bibliography	188

List of Tables

Table 1.1. Published specificities and sensitivities of serological tests for the diagnosis of paratuberculosis in cattle.....	17
Table 1.2. Published specificities and sensitivities of serological tests for the diagnosis of paratuberculosis in sheep.....	18
Table 1.3. Published specificities and sensitivities of serological tests for the diagnosis of paratuberculosis in goats.....	19
Table 2.1. Bacterial species and source of isolates or their DNA used in the assessment of the PCR specificity and sensitivity.....	33
Table 2.2. Amplification conditions evaluated during the process of optimisation of the PCR with the 90 and 91 primers.....	41
Table 3.1. Results of single PCR tests on 4, 8 and 12 mg samples of liver, ileocaecal lymph node and ileum, and on 0.5 and 1.0 ml blood samples from 20 sheep suspected of having clinical paratuberculosis and 10 clinically normal control sheep compared with results obtained by histology and serology.....	58
Table 4.1. Results of microscopic examination of faeces for acid-fast organisms in 30 sheep infected experimentally with <i>M. paratuberculosis</i> compared with histology results.....	71
Table 4.2. Necropsy findings compared with results of histological examination in 29 sheep infected experimentally with <i>M. paratuberculosis</i> and in 12 sheep with naturally occurring paratuberculosis.....	72
Table 4.3. Results of histological examination of sections of ileum and mesenteric lymph node from 28 sheep infected experimentally with <i>M. paratuberculosis</i> and 12 sheep with naturally occurring paratuberculosis.....	74

Table 4.4. Results of the PCR on duplicate samples of ileum and ileocaecal lymph node from 30 sheep infected experimentally with <i>M. paratuberculosis</i> compared with results obtained by histology and by 3 different serological tests.....	81
Table 5.1. Scoring system of numbers of microgranulomas and acid-fast organisms.....	92
Table 5.2. Gross pathology findings in 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with <i>M. paratuberculosis</i> , 14 infected but unvaccinated sheep and 11 uninfected and unvaccinated control sheep.....	98
Table 5.3. Results of microscopic examination of faeces for acid-fast organism in 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with <i>M. paratuberculosis</i> , 14 infected but unvaccinated sheep and 13 uninfected and unvaccinated control sheep compared with histology results.	107
Table 6.1. Results of the PCR assay on duplicate samples of hepatic (liver biopsy) DNA, single samples of peripheral blood leukocyte DNA and duplicate samples of ileal and ileocaecal lymph node DNA compared with results obtained by histology. The DNA samples were obtained from 14 sheep infected experimentally with <i>M. paratuberculosis</i> , 14 sheep vaccinated against paratuberculosis 2 weeks after infection and 13 uninfected and unvaccinated controls.....	126
Table 7.1. Specificities of immunological tests at various sampling points.....	143
Table 8.1. Numbers of sheep tested positive by the AGID, CFT, ELISA and the blood-based and prescapular lymph node -based IFN- γ assay.	154
Table 8.2. The effect of lowering of cut-off values on numbers of sheep tested positive by the blood-based and prescapular lymph node -based IFN- γ assay.....	155

List of Figures

<p>Figure 2.1. Diagrammatic representation of the regions of <i>M. paratuberculosis</i> insertion sequence IS900 where primers 90/91, JG1/JG2, TDB3/TDB4 and AM935/AM936 are located.....</p>	31
<p>Figure 2.2. Optimisation of concentrations of magnesium and primers JG1 and JG2 with the Taq DNA polymerase system.</p>	42
<p>Figure 2.3. Optimisation of concentrations of magnesium and primers 90 and 91 with the Taq DNA polymerase system.</p>	43
<p>Figure 2.4.1. Sensitivity of the PCR with the Taq DNA polymerase system in a one-phase PCR (constant 1-minute cycling conditions) at 35 and 40 cycles.</p>	43
<p>Figure 2.4.2. Sensitivity of the PCR in a two-phase, 45-cycle PCR (Taq DNA polymerase system) in which 1-minute times of denaturation, annealing and extension were reduced by half (30 seconds) after the first 5 cycles.</p>	44
<p>Figure 2.4.3. Sensitivity of the PCR on samples of ovine DNA spiked with <i>M. paratuberculosis</i> DNA in a two-phase, 45-cycle PCR (Taq DNA polymerase system) in which 1-minute times of denaturation, annealing and extension were reduced by half after the first 5 cycles.....</p>	44
<p>Figure 2.5. Specificity of the PCR.....</p>	45
<p>Figure 2.6. Comparison of PCR yields achieved in samples of ovine DNA extracted by boiling and by the Proteinase K digestion/CTAB-phenol-chloroform extraction method.</p>	45
<p>Figure 2.7. Comparison of the Taq DNA polymerase system with the Expand Long Template system.</p>	46
<p>Figure 3.1. Results of the PCR on selected samples of hepatic, ileal and ileocaecal lymph node DNA from 20 sheep suspected of having clinical paratuberculosis.</p>	57
<p>Figure 3.2. Results of PCR on selected samples of blood DNA from sheep suspected of having clinical paratuberculosis.</p>	59

Figure 4.1. Death rate of 30 sheep infected orally with <i>M. paratuberculosis</i> as lambs.	70
Figure 4.2.. Small intestine from a sheep with naturally occurring paratuberculosis. The lamina propria shows diffuse infiltration with macrophages	75
Figure 4.3. Small intestine from a sheep infected experimentally with <i>M. paratuberculosis</i> . The lamina propria shows diffuse infiltration with macrophages.	75
Figure 4.4. Small intestine from a sheep infected experimentally with <i>M. paratuberculosis</i> . Aggregates of macrophages in the lamina propria.	76
Figure 4.5. Small intestine from a sheep infected experimentally with <i>M. paratuberculosis</i> . Small aggregates of macrophages in the ileal Peyer's patch.	76
Figure 4.6.a & b. Small intestine from a sheep with naturally occurring paratuberculosis. Accumulation of mononuclear cells around the submucosal nerve.....	78
Figure 4.7.a & b. Small intestine from a sheep with naturally occurring paratuberculosis. Accumulation of mononuclear cells around a nerve in the muscular layer.	79
Figure 4.7.c. Singular acid-fast organisms within some of the mononuclear cells surrounding the nerve in the muscular layer.	79
Figure 4.8. Results of the PCR on selected samples of ileal and ileocaecal lymph node DNA from sheep infected experimentally with <i>M. paratuberculosis</i> as lambs.	82
Figure 4.9. Antibody indices in serum samples of 14 experimental sheep that had infection with <i>M. paratuberculosis</i> confirmed by histology and the PCR and 9 experimental sheep that were clinically normal throughout the duration of the study. The 9 clinically normal sheep showed no evidence infection with <i>M. paratuberculosis</i> , as determined by the PCR and histology at the time of necropsy, 108 weeks after oral inoculation.	83
Figure 5.1. Death rate of 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with <i>M. paratuberculosis</i> , 14 infected but unvaccinated sheep and 13 uninfected-unvaccinated control sheep.	97
Figure 5.2. Score of granulomas in the jejunal and ileal lamina propria, caudal mesenteric lymph node, ileocaecal lymph node and liver of 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with <i>M. paratuberculosis</i> and 14 infected but unvaccinated sheep ...	99

- Figure 5.3.** Small intestine (ileum) from a sheep infected orally with *M. paratuberculosis*. Numerous macrophages in the lamina propria. 100
- Figure 5.4.** Small intestine (ileum) from a sheep infected orally with *M. paratuberculosis*. Aggregates of macrophages predominantly located in the tips of ileal villi. 101
- Figure 5.5.** Small intestine (ileum) from a sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*. A small aggregate of macrophages in the lamina propria. 101
- Figure 5.6.** Mesenteric lymph node from a sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*. Multinucleate giant cells in the cortex. 104
- Figure 5.7.** Prescapular lymph node, draining the vaccination site, from a sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*. Multinucleate giant cells in the cortex. 104
- Figure 5.8.** Small intestine from a sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*. Necrotic focus surrounded by neutrophils, macrophages and fibroblasts in the lamina propria. 105
- Figure 5.9.** Prescapular lymph node, draining the vaccination sites, from a sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*. Necrotic focus surrounded by neutrophils, macrophages and fibroblasts in the cortex. 105
- Figure 5.10.** IFN- γ production in response to Johnin PPD in blood samples and antibody indices in serum samples of 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*, 14 infected but unvaccinated sheep and 13 uninfected-unvaccinated control sheep. 109
- Figure 5.11.** IFN- γ production in response to Johnin PPD in blood samples and antibody indices in serum samples of 14 sheep infected orally with *M. paratuberculosis*: In 7 experimentally infected sheep acid-fast organisms were detected microscopically in sections of tissues examined, while in the remaining 7 sheep AFO's were not detected. 110
- Figure 5.12.** Weight gain and growth rate in 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*, 14 infected but unvaccinated sheep and 13 uninfected-unvaccinated control sheep. 111

- Figure 5.13.** Nematode egg count in faecal samples of 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*, 14 infected but unvaccinated sheep and 13 uninfected-unvaccinated control sheep. **112**
- Figure 5.14.** Erythrocyte count, haemoglobin concentration and total leukocyte count in blood samples of 14 sheep vaccinated against paratuberculosis 2 weeks after oral infection with *M. paratuberculosis*, 14 infected but unvaccinated sheep and 13 uninfected-unvaccinated control sheep. **113**
- Figure 5.15.** Erythrocyte count, haemoglobin concentration and total leukocyte count in blood samples of 14 sheep infected orally with *M. paratuberculosis*. In 7 experimentally infected sheep acid-fast organisms were detected microscopically in sections of tissues examined, while in the remaining 7 sheep AFO's were not detected. **114**
- Figure 6.1.** Results of single PCR tests on selected samples of ileal and ileocaecal lymph node DNA from 14 sheep infected with *M. paratuberculosis*, 14 sheep vaccinated against paratuberculosis 2 weeks after infection and 11 uninfected-unvaccinated control sheep. **127**
- Figure 6.2.** Results of single PCR tests on selected samples of hepatic (liver biopsy) DNA from 13 sheep infected with *M. paratuberculosis*, 14 sheep vaccinated against paratuberculosis 2 weeks after infection and 11 uninfected-unvaccinated control sheep. **128**
- Figure 6.3.** Results of the PCR on selected samples of peripheral blood leukocyte DNA from 14 sheep infected with *M. paratuberculosis*, 14 sheep vaccinated against paratuberculosis 2 weeks after infection, and 11 uninfected-unvaccinated control sheep. **130**
- Figure 7.1.** Results of the CFT, AGID, ELISA 0.1 cut-off and ELISA 0.05 cut-off in samples of serum sequentially collected from 30 sheep inoculated orally with *M. paratuberculosis* in the first month of life (Trial 1). **139**
- Figure 7.2.** Detection curves of the CFT, AGID, ELISA 0.1 cut-off and ELISA 0.05 cut-off in a group of 30 sheep inoculated orally with *M. paratuberculosis* in the first month of life (Trial 1). **140**
- Figure 7.3.** Results of the CFT, AGID, ELISA 0.1 cut-off, ELISA 0.05 cut-off and IFN- γ assay in serum and plasma samples sequentially collected from 14 sheep inoculated orally with *M. paratuberculosis* in the second month of life (Trial 2). **141**

Figure 7.4. Detection curves of the CFT, AGID, ELISA 0.1 cut-off, ELISA 0.05 cut-off and IFN- γ assay in a group of 14 sheep inoculated orally with *M. paratuberculosis* in the second month of life (Trial 2)..... **142**

Figure 7.5. Results of the CFT, AGID, ELISA with 0.1 cut-off, ELISA with 0.05 cut-off and IFN- γ assay in serum and plasma samples sequentially collected from 13 uninfected control sheep..... **143**

Figure 8.1. IFN- γ indices in samples of blood and prescapular lymph node from 19 sheep infected experimentally with *M. paratuberculosis* and 10 uninfected control sheep..... **156**

Figure 8.2. Adjusted OD values of the IFN- γ assay in samples of blood and prescapular lymph node from 19 sheep infected experimentally with *M. paratuberculosis* and 10 uninfected control sheep..... **157**