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Final report

project

The identification of constraints and possible remedies to livestock production by zoonotic diseases in the South Pacific

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1 Acknowledgments

We wish to thank the National Agriculture Quarantine and Inspection Authority (NAQIA) and its veterinary field staff in Lae, the management of Trukai Industries, Ramu Beef, Rumion piggery, Numundo Beef, Pelgen piggery and smallholder cattle farmers and villagers in the Markham Valley. We also thank the residents of Naduri and Burebusaga villages in Fiji and the Department of Health, Fiji for their cooperation

2 Executive summary

The need to address the zoonotic disease implications of increasing numbers of livestock being raised in close proximity to human populations was identified at several Pacific Island regional forums during the 1990's. The 2001 Permanent Heads of Agriculture and Livestock Production Services (PHALPS) in the South Pacific Region Conference identified the need to develop strategies to reduce the impact of zoonotic diseases on both animal production and human health. The zoonotic infections of leptospirosis, trichinellosis and angiostrongylosis were identified as the priority diseases for this project based on preliminary data from surveys completed by the SPC Animal Health Service. Leptospirosis is a significant cause of infertility in cattle, which has a direct impact on farm income, especially in PNG, and the main impact of trichinellosis is through trade restrictions. The overall aim of the project was to enhance knowledge of the epidemiology of these diseases, capacity to control them, in order to minimise their impacts on both animals and humans.

The project team included scientists from the School of Veterinary and Biomedical Sciences, Murdoch University, the National Agriculture and Quarantine Inspection Authority (NAQIA) in PNG, the Regional Animal Health Service of the Secretariat of the Pacific Community and the WHO Reference Centre for Leptospirosis, Brisbane. The project was lead by from Murdoch University which has experience implementing research projects on livestock diseases in the Asia Pacific region. In PNG the project was led by NAQIA which is the only agency with the necessary capacity to perform research on animal health and zoonotic infections. The SPC Regional Animal Health Service provides advice and technical assistance to small PICT's that do not have their own veterinary service. The majority of activities in PNG occurred in the Markham Valley in Morobe Province that is the centre for cattle production and crocodile farming.

The development and validation of an antibody detection ELISA to detect infection with Leptospira in cattle is a significant output in PNG because existing tests (microscopic agglutination test) requires the use of live and potentially infectious strains of Leptospira, which make it impractical for use in PNG. This is the first time an ELISA test has been rigorously optimised and validated in PNG

The project has demonstrated by way of collaborative surveys between the SPC and the Fiji Ministry of Health that the epidemiology of human leptospirosis in Fiji may involve atypical reservoir hosts such as dog, horse, and even mongoose. This is important because it suggests that livestock (cattle, pigs, goats etc.) are not a significant reservoir for human leptospirosis and that vaccination of dogs in rural villages may reduce the incidence of human disease.

An epidemiological study of leptospirosis in livestock (mainly cattle) in PNG provided strong evidence that the disease is a significant cause of infertility in female cattle on large commercial farms. The disease is, however, not a significant constraint to cattle production in smallholder cattle. Data collected from longitudinal surveys of cattle in PNG also showed that the recommended vaccination program (a single booster each year) does not provide sufficient protection to prevent infection and stop transmission of Leptospira. The outputs of this research are sufficient to recommend that all female cattle in PNG are vaccinated every 6 months.

Smallholder cattle grazing adjacent the large farms were not shown to be a reservoir of infection for commercial cattle or people living in the surrounding areas. Indeed, this work has shown that there is very little evidence of leptospirosis in any livestock (horses, pigs and cattle) or companion animal species (dogs), which suggests that these animals do not pose a significant zoonotic risk to humans in PNG

Information confirming that infection with Trichinella papuae (the causative agent of all Trichinellosis in PNG) has a greater geographic distribution in pigs and possibly people in

PNG than at first thought is important for the crocodile industry. This is because the industry sources live crocodiles from a broad geographic area and these animals can become infected when village collectors feed them pig meat prior to sale to the farm. Knowledge of the distribution of Trichinella in PNG helps the industry reduce the risk of introducing infected crocodiles onto their farms. This is important because the crocodile industry must prove that all fresh meat products are free from infection prior to export to Australia to prevent entry of Trichinella into Australia. There is little evidence that Trichinella is present in PICT's such as Fiji, Tonga and Kiribati.

A significant impact of the project has been to increase the technical capacity of the National Veterinary Laboratory in PNG. This was achieved through provision of equipment and training for NVL staff. One NAQIA staff member obtained a PhD under a John Allwright Fellowship. His research enabled the project to gain a better understanding of the epidemiology of leptospirosis in cattle in PNG by performing comparative studies in Western Australia and PNG. He is the first member of NAQIA to achieve a PhD. His position as Manager of the NVL will allow him to transfer relevant skills and knowledge to local staff.

3 Background

The need to address the zoonotic disease implications of increasing numbers of livestock being raised in close proximity to human populations was identified at several Pacific Island regional forums during the 1990's. Zoonotic infections were identified as a high priority during the 14th Regional Conference for Permanent Heads of Agriculture and Livestock Production Services (PHALPS, Nadi, Fiji, 30th April – 4th May 2001). They recommended that funding be sought to reduce the impact of zoonotic diseases on both animal production and human health. The zoonotic infections of leptospirosis, trichinellosis and angiostrongylosis were identified as the priority diseases for this project based on preliminary data from surveys completed by the SPC Animal Health Service. Leptospirosis is a significant cause of infertility in cattle, which has a direct impact on farm income, especially in PNG, and the main impact of trichinellosis is through trade restrictions. The overall aim of the project was to enhance knowledge of the epidemiology of these diseases, capacity to control them, in order to minimise their impacts on both animals and humans.

Most Pacific Island Countries and Territories (PICT's) are experiencing a period of increasing human population and standard of living that has lead to an increased demand for animal products. The livestock population is increasing resulting in overstocking and, coupled with increasing human population density, greater contamination of the environment with animal waste and the consequent impact this has on public health. Increased intensification of animal production in many PICT's with limited land resources has increased the possibility of human-animal contact and the resultant risk of zoonotic disease.

The identification of a number of pigs serologically positive for Trichinella spp. infection has led to the erection of trade barriers between Pacific countries (S. Angus, pers. comm.) Sensitive diagnostic techniques are required to differentiate between species of Trichinella found in the region in order to reduce the likelihood of false trade barriers. Similarly, there is a need to determine the prevalence and host range of Angiostrongylus in the Pacific Islands. Although there is much evidence of its widespread distribution and public health significance in the region, appropriate diagnostic tools have not been available to determine the impact of this parasite on livestock production. This is important information given the increasing number of reports of the disease in livestock in the region.

Human leptospirosis caused by various species of Leptospira is an emerging disease in the Pacific Region with outbreaks of human infections in Fiji, New Caledonia, Federated States of Micronesia and Palau occurring in the last year. A single zoonotic disease, leptospirosis, was implicated in the deaths of 48 people in Fiji in 2000, a figure not including the number of non-fatal infections (Department of Health, Fiji). This compares with 9 new cases of HIV/AIDS in the same period and a cumulative number of HIV/AIDS cases over twelve years of 62 with 12 attributable deaths (WHO).

Previous Animal Health /Status surveys in various PICT's have identified a significant prevalence of Leptospira infection in pigs and cattle (where present). Angiostrongylus cantonensis is also being more frequently recognised as a disease of livestock and man in the Pacific (S. Angus, pers. comm.).

Infertility is a significant problem in beef cattle in PNG and may be suppressing calf production by up to 10% or more. This would have a sizeable effect on sales and farm incomes. Evidence from the project indicates that Leptospirosis is likely to be a major factor in this infertility. The beef cattle industry would appear to have a very good future but expansion will be very dependent on growth in the smallholder sector. This is because the bulk of suitable land (estimated to be at least 500,000 hectares) is in customary ownership and is therefore unlikely to be available for large scale commercial

development. However, cattle production in the smallholder sector was boosted during the life of the project through improved market access provided by several large commercial producers supplying to the newly-established live export trade to SE Asia (Almost 4,000 cattle were exported in 2004). The importance of remunerative market access for smallholders can not be overstated.

Information on the epidemiology of bovine leptospirosis in PNG will enable the development of effective control programs for Leptospirosis in cattle. A more productive national herd will mean additional animals for live export as well as increased quantities of lower grade beef from culled breeders. This latter category of beef will partly substitute for the substantial imports of red meat from Australia and NZ and also provide raw material for canning domestically. In addition, information on the likely zoonotic potential of livestock leptospirosis will provide important information that will assist the cattle industry in PNG manage the public health risks posed by increasing cattle production.

The continued exports of crocodile meat from PNG to Australia support the local crocodile industry, which is a major source of income to many rural dwellers that depend heavily on eggs and live crocodiles collected from the wild for their living. The presence of T. papuae in wild pigs naturally raises concerns about the situation in commercial pigs, from both a national and potential export perspective. In the past there has been interest by other PICs in possible pork imports from PNG. Although no exports have yet been made and Trichinella does not appear to be present on the commercial farms, possible future exports of fresh pork from PNG will be dependent on confirmed “farm freedom from Trichinella infection”. The priority for PNG is to develop a rational framework that will enable them to achieve the status of freedom from infection in commercial properties as well as providing important information about the distribution of Trichinella in the country.

The priority for Australia was to maintain freedom from the presence of pathogenic Trichinella sp. including T. papuae. Information on the distribution of T. papuae and the availability of well validated tools for its detection will assist Australia remain free from infection. This is important because it will be impossible to eradicate or control once it is present in the feral pig population in Australia.

Abbreviations

FAO	Food and Agricultural Organisation of the United Nations
AQIS	Australian Quarantine and Inspection Service
ELISA	Enzyme-linked Immunosorbent Assay
Ab-ELISA	Antibody-detection ELISA
MAT	Microscopic Agglutination Test
NAQIA	National Agriculture and Quarantine Inspection Authority
NVL	National Veterinary Laboratory
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
SPC	Secretariat of the Pacific Community
WHO	World Health Organisation

4 Objectives

The objectives of the first phase of the project were:

1. To develop the capacity for effective surveillance of Trichinella, Leptospira and A. cantonensis infection in the South Pacific region through the development and transfer of diagnostic technologies

2. To determine the prevalence of Trichinella, Leptospira and A. cantonensis infection in livestock in Fiji and Kiribati and risk factors associated with their transmission

3. To determine which species of Trichinella are present in Fiji and Kiribati

4. To investigate the pathogenesis of T. papuae in pigs and Angiostrongylus cantonensis in ruminants

5 To determine the epidemiology of bovine leptospirosis in PNG (additional objective included as part of Peter Waiín's PhD program)

The objectives of the project extension were:

6. Determine the economic benefit of vaccination to control bovine leptospirosis in commercial and smallholder cattle herds

7. Determine the epidemiology of public health significance of leptospirosis (non-bovine) in rural villages in PNG and in occupational groups associated with the livestock industry

8. To determine the prevalence of Trichinella, Leptospira and infection with enteric protozoa in livestock in Kiribati and risk factors associated with their transmission

9. To assist NAQIA prove that commercial and semi-commercial pig producers are free from Trichinella infection

5 Methodology

Objective 1. To develop the capacity for effective surveillance of Trichinella, Leptospira and A. cantonensis infection in the South Pacific region through the development and transfer of diagnostic technologies

This work was completed in 2 stages. Firstly, attempts were made to develop or modify ELISA-based assays to detect each pathogen and secondly, attempts were made to transfer each suitable assay to the NVL and SPC. The optimisation and validation of serological tests was performed at Murdoch University because the necessary expertise and laboratory equipment was present. Activities to transfer the relevant technologies to the NVL and SPC were performed in PNG and Fiji respectively.

1.1 Development of diagnostic assays for Trichinella, Leptospira and A. cantonensis infection in livestock

Leptospira

A genus-specific ELISA for Leptospira was developed using crude antigens extractedfrom cultures of L. biflexa serovar Patoc (a non-zoonotic species) and L. interrogans serovar Pomona (a zoonotic species associated with pigs) using ultrasonication. Horseradish peroxidase conjugated Protein G was used as the secondary antibody in the ELISA to enable serum from multiple host species to be tested. The 2 antigens were used to construvct Ab-ELISAs, which were optimised and partically validated using a positive control serum obtained from five pigs experimentally infected with Leptospira interrogans serovar Pomona at Murdoch University. A sample of pig serum donated by the Animal Health Laboratory of the Department of Agriculture and Food, Western Australia with no MAT titre was used as a negative control. A collection of sera from pigs from a farm in Western Australia were tested with the MAT and a reference collection of 21 positive and 96 negative pig sera were chosen based on a cut-off titre of <50 to determine negative from positive. Results from the preliminary validation showed that the ELISA using the L. Interrogans serovar pomona antigen was superior in terms of its overall accuracy (measured by calculating the area under the ROC curve) so all further validation was performed with this test.

Sera from three populations of cattle and pigs were used to validate the Ab-ELISA. They were:

1. A total of 200 serum samples were collected from beef cattle on Kimbe island east of mainland Papua New Guinea that had reported episodes of infertility characterised by abortion, stillbirth and weak calves that died shortly after birth. The presence of infection with Leptospira Hardjo was confirmed using the MAT, with antibody titres ranging from 50 to 6,400. A further 200 samples were obtained from cattle from two farms on the mainland of PNG that had not experienced signs of leptospirosis and did not have any MAT titres greater than 50.

2. A total of 159 serum samples were collected from cattle from a farm in Pinjarra, Western Australia that had experienced an outbreak of leptospirosis based on the presence of typical clinical signs and a high prevalence of MAT titres ranging from 50 to 3,200.

3. 1,465 sera from eight cattle populations from PNG and WA and the results were compared with the MAT to evaluate the performance of the two tests. Two of these cattle farms reported clinical signs of leptospirosis while five farms did not. The eighth group was from cattle slaughtered at the Lae abattoir.

The level of agreement between the MAT and ELISA was determined by calculating values for the Kapppa statistic and Spearman's rank correlation coefficient. The sensitivity and specificity of the test were determined using a Bayesian framework (non-

gold standard approach). In addition, the repeatability of the ELISA and the stability of the antigen preparation were determined empirically.

Trichinella

Two antigens were evaluated for the detection of antibodies to Trichinella in pigs using an Ab-ELISA. A native Trichinella spiralis excretory-secretory antigen (ES) was provided by the International Trichinella Reference Centre (ITRC), Rome, Italy and the synthetic glycan b-tyvelose antigen, which purchased from Heska Corporation. The test was optimised initially using positive control sera provided by the ITRC and later using sera from pigs in PNG that were naturally infected with T. papuae. Partial validation was performed for use in pigs and rodents from Australia and the Pacific Islands using serum from small numbers of infected pigs from PNG and serum from uninfected feral pigs, from north Queensland, and from laboratory-bred rodents at Murdoch University.

A. cantonensis

At Murdoch University attempts were made to develop an Ab-ELISA to detect A. cantonensis in ruminants based on published reports of a similar test used to detect human infections (Chye et al. 2000). Partial optimisation and validation of the modified ELISA was performed using serum from experimentally infected rodents and sheep and from uninfected ruminants and rodents from Australia and Pacific Islands.

1.2 Transfer of equipment and diagnostic tests to NVL and SPC

Laboratory equipment necessary to improve the capacity for detection of Leptospira and Trichinella were provided to the SPC and NAQIA. The NVL received one binocular and one compound microscopes, a water bath, electronic scales, tissue macerator and minor laboratory equipment. In addition, reagents necessary for the detection of Trichinella larvae was provided. The capacity of the NVL to conduct diagnosis of Leptospira was enhanced by the provision of a microplate (ELISA) reader and transfer of the Ab-ELISA developed and validated at Murdoch University. At Murdoch University one technician from NAQIA were be trained for two weeks in the use of the ELISAs developed for the detection of Trichinella and Leptospira infection. The diagnostic capacity of the SPC in Fiji was improved by provision of 2 microscopes and training in techniques to detect Trichinella infection in pigs at the NVL.

Objective 2. To determine the prevalence of Trichinella, Leptospira and A. cantonensis infection in livestock in Fiji and Kiribati and risk factors associated with their transmission

2.1 Trichinella

The testing of samples from the SPC serum bank was performed at Murdoch University because the serological tests necessary had been established there. Serum stored in the SPC freezer was shipped to Murdoch to test using the Trichinella ELISA. New cross-sectional surveys were performed in Fiji by Dr Steve Angus and in PNG by Peter Waiín and Nime Kapo.

Data held by the Animal Health service of the SPC was reviewed to identify key villages or regions in Fiji and Kiribati that had significant sero-prevalences of Leptospira infection. A collection of serum stored at SPC from livestock in Fiji and Kiribati were shipped to Murdoch University and tested for the presence of anti-Trichinella antibodies using the Ab-ELISA optimised in 1.1 above. The apparent prevalence and 95% confidence intervals were calculated for each country. Raw optical densities were also plotted and examined visually to detect apparent clusters of sero-positive animals. This was necessary because the selection of an appropriate cut-off OD value for the Trichinella ELISA was problematic, which confounded the interpretation of the prevalence estimates. It was not possible to determine the sero-prevalence of A. cantonensis because attempts to develop an effective ELISA were unsuccessful.

A cross-sectional survey was conducted in areas of Fiji where recent outbreaks of human leptospirosis had occurred (Naduri) and a matched village with no history of leptospirosis (Burebusaga) to determine the prevalence of infection in livestock, companion and wild animals (mongoose). Serum collected from each animal in each village was sent to the WHO Reference Centre for Leptospirosis in Brisbane for testing using the MAT. The prevalence of each infecting serovar was determined and compared with data provided from the testing the human population of the outbreak village (provided by collaborators in the Fiji Department of Health).

Trichinella infection in PNG

Two separate studies have been undertaken in PNG to determine the prevalence, distribution and impact of Trichinella infection. Firstly, Dr Nime Kapo performed a retrospective serological survey of 479 people living in the Balimo area to determine the prevalence of infection, estimated using the Ab-ELISA developed at Murdoch. Surveys of the human population provide data that can be used to determine a reasonable estimate of the gross level and distribution of Trichinella infection in wild pigs in an area where it is virtually impossible to obtain a representative sample from pigs. Serum and tissue samples were also collected from 31 pigs and 15 rats in the Goroka area and tested using the Ab-ELISA and pepsin-acid digestion respectively.

Following the discovery of Trichinella zimbabwensis in crocodiles in Zimbabwe AQIS has required PNG to confirm freedom from Trichinella infection in its crocodile meat exported to Australia from Mainland Holdings Crocodile Farm in Lae. Samples of muscle from 10 crocodiles (Crocodilus porosus) are collected each time a group of animals is slaughtered and sent to the NVL to be tested by the digestion method for the presence of Trichinella larvae.

2.2 Leptospira in PNG and Fiji

Surveys to determine the prevalence and identity of infecting Leptospira serovars in PNG in cattle began in 2003. The first property sampled was Numundo Beef (West New Britain Palm Oil). A cross-sectional survey was conducted by the livestock manager to collect serum from female cattle from three age groups (<2 yrs, 2-5yrs and >5 yrs) and sent to Lee Smythe at the Leptospirosis Reference Centre in Brisbane for testing using the MAT. The remainder of the serum was sent to Murdoch University for use in the validation of the Ab-ELISA. In addition, a sample of serum from cows that had either aborted or lost a calf soon after birth were included to determine if leptospirosis was the cause of their infertility. Subsequent surveys in PNG are incorporated into Objective 5.

Kidneys were collected from cattle at the abattoirs in Lae, Ramu beef , Numundo beef and in Port Moreaby and from pigs slaughtered at the abattoir in Port Moresby that originated from Hagen Planters piggery. Samples of kidneys collected from cattle and pigs were placed into sterile plastic bags and stored in an insulated box with ice bricks. The kidney samples were cultured within 3 to 4 hours of collection. A 1-2 mm3 piece was excised from the medullary region of each kidney and placed into EMJH semi-solid agar containing 5-fluorouracil and a similar sized portion was stored in 10% dimethyl sulfoxide (DMSO) and saturated sodium chloride (NaCl) solution for subsequent PCR analysis. All EMJH cultures were incubated at 28oC for 3 to 4 days. After this time approximately 5 ml of the culture medium was sub-cultured aseptically into fresh EMJH semi-solid agar containing 5-fluorouracil and incubated at 28oC for a further 21 days. After that all cultures were sent to the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis in Brisbane for identification and serovar typing of any leptospires isolated.

Objective 3. Determine which Trichinella species are present in Fiji and Kiribati

Work towards this objective was not pursued because there was insufficient serological evidence of Trichinella infection observed in the pig sera collected from Fiji and Kiribati in past animal health surveys performed by the SPC (Objective 2).

Objective 4. Investigate the pathogenesis of T. papuae in pigs and A. cantonensis in ruminants

Research on T. papuae in pigs was completed at the NVL because the parasite is indigenous to PNG and isolates and techniques were already established there. Work to determine the pathogenesis of A. cantonensis in ruminants was undertaken at Murdoch University because isolates of the parasite were available from a collaborator in Sydney and facilities to perform the experimental infections in sheep were available.

T. papuae

A piglet was infected with Trichinella papuae larvae and killed 5 months later. The pig carcase was divided into sections, enclosed in strong wire-mesh baskets, and placed on a platform about 6 feet above the ground in a fenced area. Two or three samples of muscle tissues (each about 10g) were taken from the pig carcase and digested on day 4, 7, 9, 11 and 14 after the pig was killed. Larvae were recovered from the exposed pig carcase for up to 14 days. Only very few larvae were present on day 14, when this part of the trial was ended and the carcase disposed of. On each occasion, larvae recovered from the digests were counted and inoculated into one or two mice, as a means of checking larval viability. The infected mice were killed, and their whole carcases digested, between day 72 to 76 post infection.

A continuously-recording temperature probe was placed inside the pig carcase to record daily. Adjacent maximum and minimum atmospheric temperatures were also recorded and relative humidity records obtained from a local meteorological station.

A. cantonensis

At Murdoch University the life cycle of A. cantonensis was established including maintenance of infected snails (intermediate host) and infection of rats (definitive hosts). Two experiments were conducted to determine the pathogenesis of A. cantonensis infection in sheep. In each experiment 6 infected and 3 uninfected wether merino sheep were observed visually for the development of clinical evidence of eosinophilic meningitis (evidenced by abnormal head carriage, inappetence and pyrexia) and blood and faeces were collected 7 days after infection and a complete blood count and faecal egg count for nematodes performed respectively.

The first group were infected with 10 larvae per head given per os and the second 100 larvae per head. In both experiments two infected and one uninfected animal were slaughtered and post mortem examination conducted at timed intervals after infection. In the first experiment sheep were slaughtered 2, 4 and 5 weeks post infection and the second at 1, 2 and 3 weeks after infection.

A third experiment was conducted to ensure that the negative results obtained from the above study design were not related to infecting dose, sex of the host and method of preparation of A. cantonensis larvae. Two merino cross-bred ewes were infected with at least 1,000 L3 larvae of A. cantonensis (comprising snail tissue with minimal maceration) and one ewe was kept uninfected. Animals were examined daily for signs of neurological disorder and blood and faeces were collected 7 days after infection and a complete blood count and faecal egg count for nematodes performed respectively. All sheep were euthanased 10 days after infection and a full post mortem performed. Brain and spinal cord were removed and sliced into 2mm thick sections to visually detect the presence of nematode larvae.

Differences in the parameters measured between groups was assessed using Analysis of Variance and the significance of any difference determined using Tukey's test for statistical significance.

The criteria for deciding if A. cantonensis has had a pathogenic effect will be onset of clinical signs of a central nervous system disorder and a significant peripheral eosinophilia and histological evidence of eosinophilic meningitis.

A. cantonensis infection in intermediate hosts

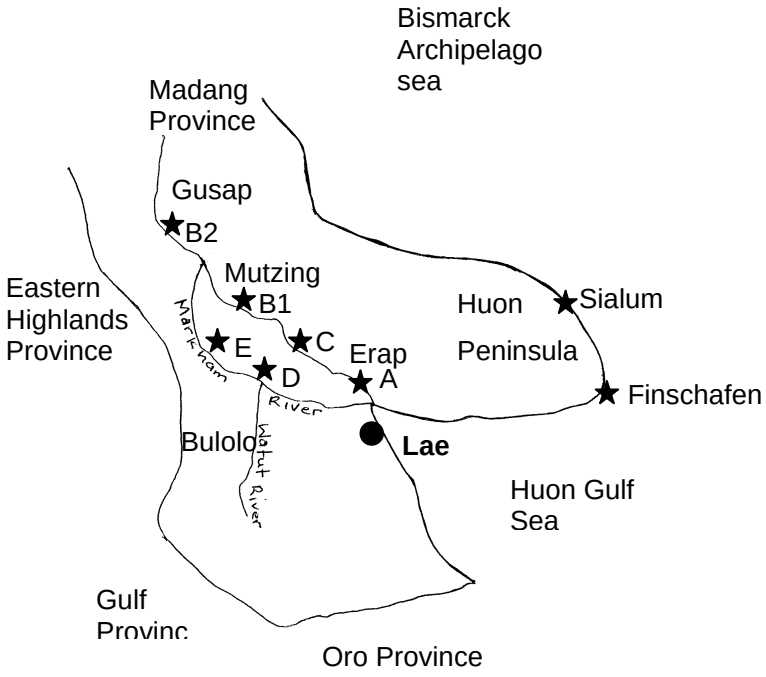
Additional research was conducted as part of a BSc. Honours program by Anna le Souef, a veterinary undergraduate student. The susceptibility of a range of different intermediate invertebrate hosts to A. cantonensis infection. This was determined by bathing each species in a suspension of L1 larvae from the faeces of an infected rat and examining the tissues of the invertebrate microscopically for evidence of larval development.

Objective 5. To determine the epidemiology of bovine leptospirosis in PNG

Data and field samples were collected from farmers in PNG and analysed at Murdoch University. Samples were tested at Murdoch University and at the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis in Brisbane.

The survey was undertaken from March to April of 2004 on cattle farms in Lae and Kimbe. Three large commercial farms (Trukai farm, Ramu beef and Rumion), and two smallholder enterprises (Prhalda and Rearaguntu) in Lae and one large commercial property in Kimbe (Numundo beef) were included in the survey (Figure 5.1). Collectively these farms represent approximately 90% of the total cattle population of PNG. In addition to samples from live cattle, samples of blood and kidneys were collected from cattle slaughtered at abattoirs in Lae, Kimbe and Port Moresby.

Figure 5.1 Sketch of Lae showing farms located in the Markham–Ramu Valley Plains. A: Trukai Industries; B1 and B2: Ramu Beef; C: Rumion; D: Prhalda and E: Rearaguntu



Serum was collected from the coccygeal artery or vein of a total of 1,300 female cattle from Trukai, Ramu Beef, Rumion, Prhalda, Rearaguntu and Numundo Beef farms. Each herd was stratified into 3 age groups (<2, 2-5 and >5 years) which were systematically sampled to provide approximately equal numbers of samples from each age stratum. In addition, 79 sera were collected from cattle during slaughter at the Lae abattoir.

Serum was collected from pigs originating from commercial farms which were killed at the abattoirs in Lae and Port Moresby. The two commercial pig farms in Lae were Rumion piggery and Pelgens piggery and both are located outside Lae township. Hagen Planters piggery is located in Port Moresby. Ninety four blood samples from pigs from Rumion farm were collected at the central abattoir in Lae and 111 blood samples were collected from

pigs killed at Pelgens piggery abattoir in Lae. Seventy eight sera were collected from pigs originating from Hagen Planters piggery at the abattoir in Port Moresby.

All sera were tested for the presence of anti-leptospiral antibodies using a reference panel of 21 leptospiral serovars: Pomona, Hardjo, Tarassovi, Grippotyphosa, Celledoni, Copenhageni, Australis, Zanoni, Robinsoni, Canicola, Kremastos, Szwajizak, Medanensis, Bulgarica, Cynopteri, Ballum, Bataviae, Djasiman, Javanica, Panama and Shermani using the MAT. The MAT was performed at the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis in Brisbane. Sera with titres to more than one serovar were regarded as seropositive for all those serovars unless the serovar was known to cross-react, in which case the sera were scored as positive to the serovar with the highest titre (Black et al., 2001).

Three of the commercial farms, Numundo beef, Trukai Industries and Ramu beef routinely vaccinate female cattle with a leptospiral vaccine (Ultravac 7:1 from CSL, Australia) that contains serovars Hardjo and Pomona. The vaccine contains a combination of leptospiral serovars Hardjo and Pomona and antigens of five clostridial species which commonly affect cattle. For this study a MAT titre of > 400 was used as the cut-off to classify a vaccinated animal as infected (NerVig et al., 1980) while a titre of < 400 was considered to be due to vaccination (Trueba et al., 1990). The other three farms (Rumion, Prhalda and Rearaguntu) did not vaccinate cattle so a titre of > 50 was used as the cut-off to classify animals as seropositive. To measure the rate of active infection a titre of > 400 was used on the six farms (Faine et al., 1999).

Apparent seroprevalences were calculated for all of the 21 leptospiral serovars. Age-specific seroprevalences of the most common serovars from the six farms and abattoirs in the study were calculated. Cattle in each age group from Trukai farm were further allocated into two groups according to their origin, determined by their brand (i.e. Trukai-born, non-Trukai born), and the seroprevalences within age groups calculated. The program SPSS Ver. 14.0 (SPSS Inc) was used for descriptive data analysis including chi-square tests and Excel 2000 (Microsoft) was used to plot graphs. The 95% confidence intervals were also calculated.

A cohort of approximately 50 unmated and unvaccinated heifers were selected randomly and individually identified (ear tag, brand) and 10 maintained unvaccinated as sentinel animals. Serum was collected from each animal every month for 12 months and tested for the presence of anti-Leptospira antibodies using the MAT (at WHO Leptospirosis Reference laboratory in Brisbane). The serological data were evaluated to determine the rate of active infection (MAT >1:400) and the rate at which unvaccinated sentinel animals sero-converted (i.e. MAT >1:50).

Objective 6. Determine the economic benefit of vaccination to control bovine leptospirosis in commercial and smallholder cattle herds

This objective could not be completed because project staff encountered difficulties in enrolling farms in the study. Work to determine the longitudinal dynamics of Leptospira infection in commercial farms was completed as part of Objective 5.

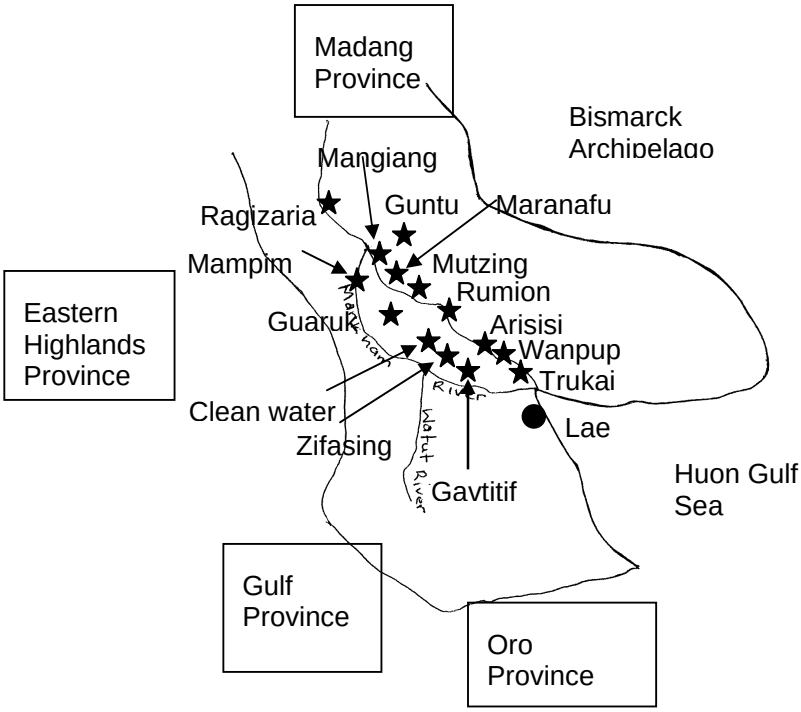
Objective 7. Determine the epidemiology of public health significance of leptospirosis (non-bovine) in rural villages in PNG and in occupational groups associated with the livestock industry

The survey was conducted at the end of the wet season in June 2006. Smallholder villages were selected based on the results of the survey in 2004. Two groups of villages were selected based on their proximity to the commercial farms. The first group were close to Trukai farm (Lower Markham) and the second were close to Ramu Sugar farm in the Upper Markham. The villages in these areas sell animals to either Trukai or Ramu.

The smallholder villages in the Markham Valley were identified from information supplied by the Regional Veterinary Officer in Lae. A total of 11 villages were selected with the assistance of the President of the Smallholder Cattleman's Association in the Markham

Valley and the Trukai farm manager. Seven villages were selected in the Upper Markham Valley, Mampim, Maranafun (smallholder cattle farmer), Ragizaria, Mutzing, Guntu, Guaruk (smallholder cattle farmer) and Mangiang. Four villages were selected in the lower Markham Valley, Wanpup (smallholder cattle farmer), Arisisi (smallholder cattle farmer), Gavtitif and Zifasing. In addition, Rumion piggery, Clean Water cattle farm in Zifasing and Trukai farm were included. The locations of the villages and farms are shown in Figure 7.1.

Figure 7.1 Location of villages and farms in the Markham Valley



In addition to cattle, blood samples were also collected from pigs and dogs in all the villages except Maranafun (only cattle) and Guaruk (horses were also sampled). Only cattle were bled in Wanpup and Arisisi villages and Clean Water farm. Horses were bled at Trukai farm. Blood samples were collected from pigs from Rumion piggery when they were slaughtered at the Lae abattoir.

In order to provide a point of reference for use in interpretation of the serovar data from PNG sera were collected from 159 of 250 heifers from a herd of cattle on a property in Pinjarra approximately 150 kms south-west of Perth that were suspected of having leptospirosis. Heifers from the group that aborted were also included.

All serum specimens were tested at the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis in Brisbane. A panel of 22 live antigens were used in the MAT. This differed from the standard panel used by the addition of Leptospira borgpetersenii serovar Ballum, substituted for L. borgpetersenii serovar Arborea, and the new serovar L. interrogans serovar Topaz. The panel of 22 leptospiral serovars from 19 serogroups represented those identified previously in the Western Pacific Region. In this study an agglutination titre > 50 was indicative of seropositive for Leptospira.

Objective 8. To determine the prevalence of Trichinella, Leptospira and infection with enteric protozoa in livestock in Kiribati and risk factors associated with their transmission

No further work was completed towards achieving this objective

Objective 9. To assist NAQIA prove that commercial and semi-commercial pig producers are free from Trichinella infection

This work was not pursued because of time constraints and the relocation of the PNG collaborator Dr ilagi Puana to Fiji for a 3 year posting with the SPC.

THE ROLES AND RESPONSIBILITIES OF EACH MEMBER OF THE PROJECT TEAM WERE:

Australia

Simon Reid is a veterinary parasitologist/epidemiologist who maintained overall responsibility for project implementations and was involved in the design and analysis of work in PNG and Australia. Dr Reid was the principal supervisor for Peter Waiín. Stan Fenwick is a veterinary microbiologist who was involved in project activities related to Leptospirosis and he co-supervised Peter Waiín and Terri Cheow. Ian Robertson is the Associate Professor of epidemiology at Murdoch University and he was involved in the design and analysis of the research on Leptospirosis. Dr Lee Smythe is the Head of the WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis, Western Pacific Region. He provided expert input into research on Leptospirosis

Papua New Guinea

Dr Ilagi Puana is the Chief Veterinary Officer of PNG He was responsible for oversight of all aspects of the project in PNG. Dr Nime Kapo is the veterinary office with NAQIA based in Lae (currently Acting CVO). He was involved in research activities on Leptospirosis in PNG. Dr Peter Waiín is the Manager of the NVL in PNG. He was involved in the development and transfer of diagnostic methods to the NVL and he undertook some activities in PNG as part of his PhD. Dr Ifor Owen is a retired parasitologist who performed all project activities related to T. papuae in PNG with the assistance of Columba Awui the parasitology laboratory technician.

SPC

Steve Angus was the Senior Veterinary Epidemiologist with the Regional animal Health Service of the Secretariat of the Pacific Community (SPC). He was involved in the design and implementation of all project activities in Fiji.

6 Achievements against activities and outputs/milestones

Objective 1: To develop the capacity for effective surveillance of Trichinella, Leptospira and A. cantonensis infection in the South Pacific region through the development and transfer of diagnostic technologies

no.	activity	outputs/ milestones	completion date	comments
1.1	Develop and validate serological techniques for the detection of Leptospira (and PCR), Trichinella and A. cantonensis infection in livestock and rodents: (A)	Well validated and standardised serological assays for the detection of Leptospira, Trichinella and A. cantonensis infection in livestock	Dec 2003 (Trichinella) Dec 2004 (Leptospira)	Attempts to produce an antigen for a. cantonensis were unsuccessful so no further work was pursued for that test. Results generated using the Trichinella ELISA was difficult to interpret because of the high levels of background reaction that was observed. The results from the validation of the ELISA for Leptospira were above expectations. The test was robust, repeatable and accurate.
1.2	Training workshop at NVL, PNG (PC)	Transfer of skills to partner institutions necessary to perform Trichinella detection techniques and post mortem examination of rodents to collect parasites and serum and	Dec 2003	The workshop was held at the NVL. However, there was only one participant from SPC because no laboratory assistant had been recruited at the time of the workshop.
1.3	Training at Murdoch (A) to transfer ELISA technologies	Establishment of diagnostic capability for Trichinella, Leptospira and A. cantonensis infection at the SPC, Fiji and NAQIA	September 2003	One laboratory technician from NVL attended a workshop to gain knowledge of the Trichinella and Leptospira ELISAs . there was no laboratory technician employed by the SPC so the workshop was held with only PNG staff represented
1.4	Establishment of ELISA capability in the SPC, Fiji (PC)	Provision of equipment, and laboratory consumables required to conduct ELISA for the detection of infection with Trichinella, Leptospira and A. cantonensis	July 2003	This equipment was not provided as described in the project documents because it was agreed that the SPC laboratory could not support ELISA capability. The equipment was provided to the NVL instead with the view that this laboratory could be used as an informal regional reference centre

1.5	Regional training workshop at SPC, Fiji (PC, A)	Acquisition of knowledge of the impact and risks associated with Trichinella, Leptospira and A. cantonensis infection in the Pacific Islands	Not completed	This workshop was not held because no data were available from Kiribati. Instead Project staff contributed several papers at the 2004 commonwealth Veterinary Association meeting held in Lae, PNG.

PC = partner country, A = Australia

Objective 2: To determine the prevalence of Trichinella, Leptospira and A. cantonensis infection in livestock in Fiji and Kiribati and risk factors associated with their transmission

no.	activity	outputs/ milestones	completion date	comments
2.1	Review of existing survey data and test serum stored at SPC with ELISA for the presence of anti-A. cantonensis antibodies (A)	Information on the prevalence and distribution of Trichinella, Leptospira and A. cantonensis in the Pacific Islands	Dec 2004	No attempt was made to determine the serological prevalence of A. cantonensis because a suitable test was not successfully developed. Reviews of serum stored in the SPC freezer was completed as planned
2.2	Surveys for three zoonotic infections in livestock and rodents in Fiji and Kiribati (PC)	Information on the prevalence, geographic distribution, host range and risk factors for transmission associated with Trichinella, Leptospira and A. cantonensis infection in the Pacific Islands	Dec 2004	No work was undertaken to determine A. cantonensis prevalence because of a lack of suitable test. No further work was performed to determine the species of Trichinella present in Fiji and Trichinella because there was no conclusive evidence that Trichinella was present. An extensive body of work was performed to determine the identity and epidemiology of Leptospira sp in PNG and to a limited extent in Fiji.

PC = partner country, A = Australia

Objective 3: To determine which species of Trichinella are present in Fiji and Kiribati

no.	activity	outputs/ milestones	completion date	comments
2.1	Collect and identify isolates of Trichinella from PNG, Fiji and Kiribati (PC)	Information on the species of Trichinella present in the Pacific Islands	Dec 2005	Attempts to collect new isolates of T. papuae were delayed in PNG until the parasite was identified from infected crocodiles from a commercial farm. No further work was performed to determine the species of Trichinella present in Fiji and Trichinella because there was no conclusive evidence that Trichinella was present.
2.2	Survey for T. papuae in PNG (PC)	Collection and identification of new isolates of Trichinella from pigs and rodents in an area in PNG where human infection is suspected	Dec 2005	Attempts to collect new isolates of Trichinella from pigs on the Bula Plain were unsuccessful. The focus of this work shifted to crocodiles once it was identified that farmed animals were commonly affected.

PC = partner country, A = Australia

Objective 4: To investigate the pathogenesis of T. papuae in pigs and Angiostrongylus cantonensis in ruminants

no.	activity	outputs/ milestones	completion date	comments
2.1	Investigate the pathogenesis of T. papuae infection in pigs (PC)	Information on the pathogenic effect of T. papuae in pigs and the diagnostic indicators for parasite recovery from pig carcasses	Dec 2004	The scope of the work was necessarily altered once it was realised that the NVL did not have the capacity for large animal infection studies.
2.2	Investigate the pathogenesis of A. cantonensis in ruminants (A)	Information on the impact of A. cantonensis infection in ruminants	Dec 2004	This work progressed as planned. The results were inconclusive but activities were discontinued because there was insufficient evidence that A. cantonensis had a significant pathogenic effect in ruminants

PC = partner country, A = Australia

Objective 5: To determine the epidemiology of bovine leptospirosis in PNG

no.	activity	outputs/ milestones	completion date	comments
2.1	Surveys to determine factors associated with Leptospira infection in commercially reared cattle in PNG (PC)	Information on the epidemiology of bovine leptospirosis in PNG that would provide evidence for the development of a rational vaccination program	December 2006	This work was largely completed by Dr Peter Wai'in from NVL as part of his PHD program. The objective was completely achieved and the outputs also enabled several other project activities

PC = partner country, A = Australia

Objective 6: To determine the economic benefit of vaccination to control bovine leptospirosis in commercial and smallholder cattle herds

no.	activity	outputs/ milestones	completion date	comments
6.1	Longitudinal survey of Leptospira infection in cattle and other livestock species (PC and A)	Quarterly sample collection completed	May 2007	The longitudinal survey was completed with sera collected from 2 cohorts of cattle on Trukai farm. It was not possible to recruit a second farm into the study despite repeated attempts. Therefore the study was modified and a cross sectional survey was completed at Markham farms where no vaccination is practised
6.2	Data collection for production indices (PC and A (in design phase))	Completed 1 survey of 4 field sites in Lae. Collected economic and production data	Not completed	This activity was not completed because of the inability to recruit a second farm
6.3	Testing of serum and urine for the presence of antibodies (ELISA) and leptospires (PCR) respectively (A)	Serovar and PCR data available for analysis	May 2007	Serovar data are available. No PCR work was completed because the scope of the study had become too limited to provide useful interpretation. The results are included as part of Objective 5 because this provides better context.
6.4	Workshop to disseminate results of economic analysis and epidemiological study	Workshop completed in Lae and manual prepared	Not completed	This workshop was not held because detailed economic data was not available

PC = partner country, A = Australia

Objective 7: To determine the epidemiology of public health significance of leptospirosis (non-bovine) in rural villages in PNG and in occupational groups associated with the livestock industry

no.	activity	outputs/ milestones	completion date	comments
7.1	Collection and testing of serum from animals and people in beef cattle producing enterprises (PC)	Prevalence of infection with Leptospira determined in each class of animals and in people, analysis reveals public health significance	March 2007	A survey of cattle, pigs, dogs and horses in villages in the Markham valley was conducted and the results showed a low prevalence of infection with Leptospira sp in all species. There was limited evidence of the presence of serovars of Leptospira that commonly infected humans. No medical counterpart could be identified to perform the human surveys.

PC = partner country, A = Australia

Objective 8: To determine the prevalence of Trichinella, Leptospira and infection with enteric protozoa in livestock in Kiribati and risk factors associated with their transmission

no.	activity	outputs/ milestones	completion date	comments
8.1	Collection and testing of serum and faeces from livestock on Kiribati(PC)	Results of survey analysed and report to Government of Kiribati prepared	Not completed	This work was not completed because the PC counterpart lacked sufficient time to complete the work

PC = partner country, A = Australia

Objective 9: To assist NAQIA prove that commercial and semi-commercial pig producers are free from Trichinella infection

no.	activity	outputs/ milestones	completion date	comments
9.1	Collection of samples from commercially produced pigs in PNG (PC). Testing of samples for the presence of anti-Trichinella antibodies (A)	Results of Trichinella ELISA available, data analysed and report prepared for NAQIA	Not completed	This work was not completed because changes in the counterpart agency reduced their capacity

PC = partner country, A = Australia

7 Key results and discussion

Objective 1. To develop the capacity for effective surveillance of Trichinella, Leptospira and A. cantonensis infection in the South Pacific region through the development and transfer of diagnostic technologies

1.1 Development and validation of antibody-detection ELISAs to detect infection with Leptospira, Trichinella and A. cantonensis in livestock in the Pacific region

Leptospira ELISA

Preliminary validation of the ELISA showed that the antigen extracted from L. Interrogans serovar Pomona using ultrasonication was superior in terms of its repeatability and accuracy as evidenced by its sensitivity and specificity (using the reference panel of pig sera) and the AUC of the ROC.

There was no significant difference between the sensitivity and specificity of an L1 Ab–ELISA for the detection of antibodies to Leptospira in cattle and pigs (Table 1.1).

Table 1.1 Sensitivity and specificity of the L1 Ab-ELISA for the detection of antibodies to Leptospira in sera from 200 seropositive and 200 seronegative cattle from PNG, 159 seropositive cattle from WA and 96 seropositive pigs from WA and 196 seronegative pigs from PNG

Species	Repeats	% Sensitivity*	% Specificity*
PNG cattle	1	89.0 (84.7, 93.3)	98.5 (96.8, 100)
	2	84.0 (78.9, 89.1)	98.5 (96.8, 100)
WA cattle	1	76.1 (69.5, 82.7)	98.5 (96.8, 100)
	2	76.1 (72.9, 85.6)	99.0 (97.6, 100)
WA pigs	1	87.5 (80.9, 94.1)	96.9 (94.5, 99.4)
	2	87.5 (80.9, 94.5)	96.9 (94.5, 99.4)

**95% confidence intervals are given in parentheses*

There was a significant overall correlation (Spearman correlation coefficient = 0.68) between the Ab-ELISA and the MAT titre in all eight cattle populations ($P < 0.01$) (Figure 1.1). There was also significant correlation between the MAT and Ab-ELISA results from the 8 cattle populations when the animal ages were stratified into three groups ($P < 0.01$) (Table 1.2) and from cattle on 6 farms and slaughtered at the Lae abattoir (Table 1.3).

Figure 1.1 Correlation between MAT titres and Ab-ELISA OD of 8 cattle populations

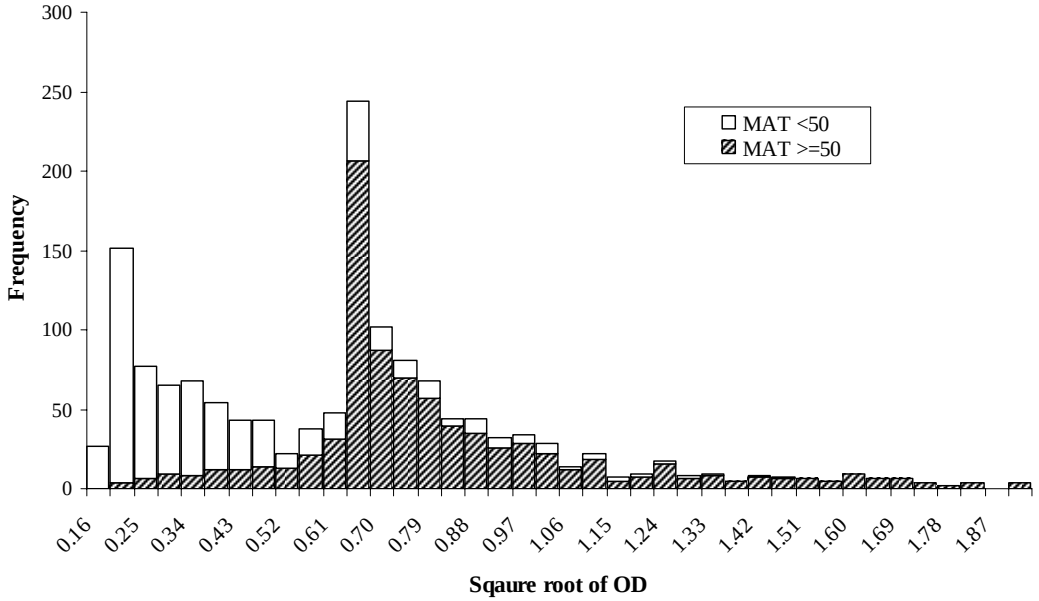


Table 1.2 Correlation between the results of the MAT and the Ab-ELISA to test serum from cattle on 8 properties that were stratified by age

Age (years)	Number of sera	Spearman Coefficient
<2	418	0.72*
2-5	571	0.66*
>5	475	0.66*

** Correlation is significant (P < 0.01)*

Table 1.3 Correlation between the results of testing cattle from 6 farms and an abattoir in PNG using the MAT and ELISA

Farms	Number of sera	Spearman Coefficient
Numundo	200	0.32*
Trukai	369	0.77*
Ramu	224	0.89*
Rumion	172	0.82*
Prhalda	134	0.49*
Rearaguntu	128	0.18
Abattoir	79	0.39*

** Correlation is significant (P < 0.01)*

The median estimate of the sensitivity and specificity of each test data is shown in Table 1.4. The Ab-ELISA was significantly more sensitive compared to the MAT for the detection of antibodies to Leptospira in cattle ($P < 0.05$). There was no significant difference in the specificity between the two tests.

Table 1.4 Median (95% CI) sensitivity and specificity of the Ab-ELISA and MAT for detection of antibodies to Leptospira in cattle estimated using Bayesian analysis

Tests	% Sensitivity (95% CI)	% Specificity (95% CI)
Ab-ELISA	97.3 (95.1, 99.4)	90.1 (86.0, 93.2)
MAT	90.4 (87.5, 92.9)	84.1 (80.4, 87.2)

This is the first time a test to detect Leptospira infection has been fully validated for use in PNG, or indeed anywhere in the world. The MAT is not suitable for use in PNG because it is laborious, hazardous (requiring the handling of live leptospires), requires the continuous culture of leptospires and is subjective. In addition, the assay may be less sensitive for detecting chronically infected animals (Faine et al., 1999). The Ab-ELISA is therefore probably the preferred assay since it is accurate, safe and inexpensive to perform and it measures serum antibody objectively. However, the Ab-ELISA does have a major limitation in that it can only detect infection to the genus level and is not serovar specific. It addition, it cannot differentiate vaccinated from the un-vaccinated animals. The utility of the Ab-ELISA was demonstrated by testing sera from a cattle farm in WA after an outbreak of bovine leptospirosis during the study. There was a high level of agreement between the results of the ELISA and the MAT which reiterates the practicality of using the Ab-ELISA to detect Leptospira infection in cattle in PNG. Most clinical lepto+f these limitations are taken into account then the assay can be easily transferred to PNG and other Pacific Island countries.

1.1.1 PCR-based tests for the detection of Leptospira and A. cantonensis infection.

A PCR to detect Leptospira infection in urine of pigs was also developed at Murdoch University during a parallel project. Preliminary evaluation showed that it was robust and highly sensitive. The PCR was successfully used to detect Leptospira in kidneys from infected animals in PNG. However, the test is not suitable for transfer to the NVL because the necessary equipment does not exist there.

Two methods used to extract DNA from urine were compared. The results showed that there was no significant difference in the results obtained after extraction of DNA from urine using heat lysis or the QIAamp Mini kit. However, the heat lysis method is simple and inexpensive, which again makes it suitable for use in PNG. The multicentre study between the four Australian laboratories using either version of the Rotor-Gene that showed the TaqMan® assay is robust and can be easily transferred to other laboratories for Leptospira detection. The real-time PCR is not recommended for diagnostic test in an animal health laboratory in PNG because it is not feasible for routine use

1.1.2 Trichinella ELISA

Two antigen preparations (ES antigen and Tyvelose) and a collection of reference serum for an ELISA to detect anti-Trichinella antibodies have been provided by Dr Darwin Murrell form the Centre for Experimental Parasitology, Denmark. Each antigen has been used to develop an ELISA using Protein G that is able to detect antibodies from rodents and pigs. Preliminary validation results show that the ELISA is able to correctly identify pigs infected with T. papuae (serum from pigs from Bula Plain that were shown to be infected with T. papuae and positive reference serum provided by Darwin Murrell) and that the specificity is approximately 95% when used to test serum from feral pigs from north Queensland.

1.1.3 A. cantonensis ELISA

Attempts to develop an antibody detection ELISA to detect infection with A. cantonensis were unsuccessful. All attempts to produce an antigen with acceptable specificity failed, most likely because serum from domestic animals contains a large array of cross-reacting antibodies that may be present following infection with several species of gastrointestinal nematodes.

Objective 2: To determine the prevalence of Trichinella, Leptospira and A. cantonensis infection in livestock in Fiji and Kiribati and risk factors associated with their transmission

2.1 Trichinella

Results of the human survey showed that the true prevalence of antibodies to T. papuae in humans in Balimo was 29.5% (95% CI: 27.3-31.7%) (calculated using a specificity of 93.7%, which was determine using sera from healthy Australians, and a sensitivity of 100% (Bruschi et al., 2001)). Antibody prevalence did not differ according to sex but there was a significant difference between age groups,17.7%, 30.5% and 51.6% respectively for <15 years old (n=130), 16-35 years old (n=190) and >36 years old (n=159) ($p<0.05$). Antibody prevalence also appeared to differ significantly according to geographic region and age (see table 2.1).

Table 2.1 Prevalence of anti-Trichinella antibodies in people of different ages in the Western, Central and Eastern Balimo regions

		Prevalence within Balimo regions (%) (numbers tested in parentheses)		
Age group	*n*	*Western*	*Central*	*Eastern*
<15 years	130	19.2 (73)	17.9 (39)	11.1 (18)
16-35 years	190	48.8 (84)	20.0 (45)	13.1 (61)
≥36 years	159	77.1 (35)	51.4 (74)	34.0 (50)

These results (and those from other surveys) show hat T. papuae is probably widely distributed in the south coastal area of PNG at least as far east as Gulf Province.

Results from examination at the NVL of muscle digests from 31 pigs and 15 rats from the Goroka area confirms that T. papuae is probably not present. In addition, muscle samples from 4 wild pigs and 11 deer shot on Bula plain as part of a NAQS survey were tested. Two pigs were shown to be infected with very low numbers of larvae, too low to enable infection of rats at the NVL.

T. papuae in Crocodiles

Following the discovery of Trichinella zimbabwensis in crocodiles in Zimbabwe AQIS has required PNG to confirm freedom from Trichinella infection in its crocodile meat exported to Australia from Mainland Holdings Crocodile Farm in Lae. Samples of muscle from 10 crocodiles (Crocodilus porosus) are collected each time a group of animals is slaughtered and sent to the NVL to be tested by the digestion method for the presence of Trichinella larvae.

The crocodile industry in PNG is substantial. Some 6,000 saltwater crocodiles are slaughtered each year for their skins and their meat exported mainly to Australia. Approximately 50% of these crocodiles are either sourced from the wild as juveniles or are hatched from eggs collected from private land.

A total of 448 crocodiles were tested for the presence of T. papuae during the life of the project. Of these 340 were wild caught mainly from the Kikori area in Gulf Province and a small number originate from other coastal areas of PNG. One hundred and fifty one crocodiles were born on the Mainland Holdings site. A total of 60 animals were found to have Trichinella larvae in their muscles. The majority of these animals (n=43) originated from Kikori, 12 were from other locations (Ambunti, Angoram, Murik Lakes, Wewak and Popondetta) and 5 were born on the farm. Larvae from the crocodiles from Ambunti (Sepik) were analysed at the International Trichinella Reference Centre, Rome and found to be genetically identical to larvae isolated crocodiles from Kikori. These data suggest that Kikori was the only source of infected crocodiles at Mainland Holdings. In addition, meat from 3 wild pigs from Ambunti was also negative for Trichinella infection. Muscle form the foreleg most commonly infected.

Information that infection with T. papuae has a greater geographic distribution in pigs and possibly people in PNG than first thought (an area extending eastwards from the south-western border to Gulf Province (Kikori). Knowledge that crocodiles from Kikori in the Gulf province have been infected with T. papuae and that the source of infection was pig meat that is fed to them in captivity (the pig meat was obtained from the market and may be a source of infection for humans). The ongoing testing of wild-born crocodiles from the crocodile farm allows PNG to check if infection occurs elsewhere in PNG.

Trichinella may not be highly prevalent in Fiji (only 1 mongoose and 3 pigs were shown to have high levels of anti-Trichinella antibodies) and it appears to be absent from the samples tested from Tonga and the Solomon Islands. No Trichinella larvae were recovered from tissues from mongoose captured in Fiji.

2.2 Leptospira

2.2.1 Leptospira spp. in PNG cattle

A total of 247 serum samples were collected from female cattle on Numundo farm. Results from testing sera with the MAT show that there is a high prevalence of Leptospira infection in cattle older than two years of age (see Table 2) and that the predominant infecting serovars were hardjo, pomona, swajizack and medanensis. Other serovars identified include kremastos, tarassovi, australis, panama and bataviae (plus other minor serovars).

Table 2 Prevalence of Leptospira infection in female cattle from Numundo Beef, PNG

		Prevalence of infecting serovar			
Age	n	hardjo	pomona	swajizack	medanensis
<2 yrs	84	36.9	26.2	25	16.7
2-5 yrs	83	84.3	41	65.1	27.7
>5 yrs	80	92.5	27.5	67.5	33.8

These data show the high prevalence of active Leptospira infection may indicate that it is a factor in the infertility observed in cattle in PNG. Current information suggests that hardjo and pomona are not usually associated with abortion in cattle, although there are no data from PNG to support this. It is difficult to interpret the significance of the other two serovars (swajizack and medanensis) because information on their clinical significance is not available.

There was a high seroprevalence of serovar Tarassovi in cattle but no evidence for clinical disease was reported. The source of Tarassovi infection in cattle is unknown as pigs do not seem to be the reservoir host in PNG. Small marsupials and rodents could be the source of infection for cattle. The low seroprevalence of serovar Pomona in pigs is

surprising because pigs have been implicated as the source of infection in other studies (Chappel et al., 1998; Mason et al., 1998; Faine et al., 1999). The low seroprevalence of Pomona in cattle is important as a number of animals from commercial properties were vaccinated for Pomona. This could suggest a failure in the vaccine to induce a high antibody response or that there was no challenge with this serovar from the environment.

Significant cross-reaction was seen between serovars Hardjo, Szwajizak and Medanensis in this study. Serovars Szwajizak and Medanensis are reported to be rodent-associated and may be involved in bovine leptospirosis because many rodent-associated serovars can cause overt disease in cattle (Thiermann, 1982; Faine et al., 1999). This study confirms the presence of a number of leptospiral serovars in livestock that are potentially significant to public health.

The newly isolated serovar Topaz was detected in cattle and horses for the first time in PNG (Monash, http://www.med.monash.edu.au/microbiology/staff/adler/tsc-minutes-november-2005/ accessed 2006). In the villages surveyed in the Markham Valley pigs do not appear to play a role in leptospirosis; however dogs may be a source of infection for humans.

Leptospires were successfully cultured from 2 of the 73 kidney samples collected from cattle slaughtered at the central abattoir in Lae. Both isolates were typed and identified as L. borgpetersenii serovar Hardjo. All the other cultures of bovine kidneys were either negative or contaminated. All the 79 pig kidneys cultured in EMJH medium were negative for leptospires.

The isolation of leptospires from kidney or urine is essential proof that an animal is infected with Leptospira. However, the sensitivity of leptospiral isolation techniques used to achieve such proof is limited. Culture is the most practical test to use but its sensitivity is very low as a result of contamination with extraneous bacteria and the inherent requirement for leptospiral survival during transportation to the laboratory. The PCR-based assay is highly sensitive and is a more useful test for PNG. Storage of kidney samples in DMSO and NaCl at room temperature has been shown to be suitable for preserving samples from remote locations such as PNG.

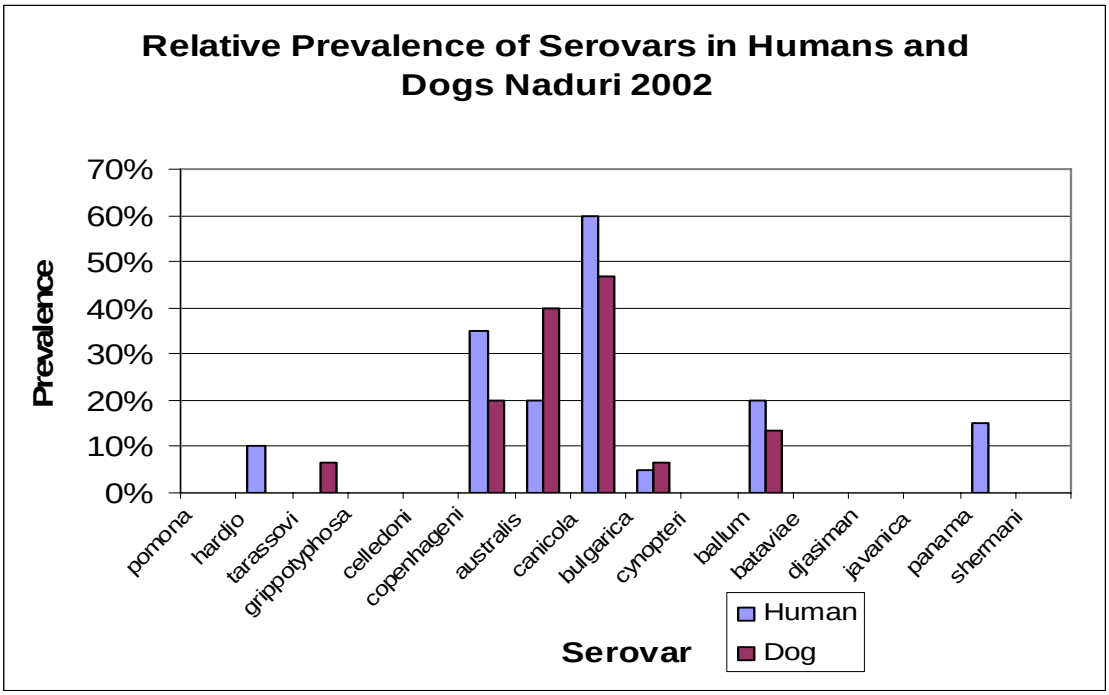
2.2.2 Leptospira infection associated with outbreaks of human leptospirosis

The results of the cross-sectional surveys in Naduri and Burebusaga showed that there was a significant prevalence of antibodies to Leptospira in all animal species except pigs. Serological data from testing all people in each village was provided by the Fiji Department of Health. A comparison of the two data sets revealed that, although the order of magnitude of the prevalence of leptospira spp antibodies is about 25% more in the village experiencing human cases of leptospirosis (Naduri) compared to Burebasaga the ranking of prevalence in the domestic species of animal remained the same (Table 2.1). In addition, the pattern of serovars affecting the human population most closely resembles the profile found in domestic dogs (see graph below). This opens up the possibility of interventions by vaccination of village dogs or by modifying the contact between the species.

Table 2.1: Results from testing serum from livestock and companion animals in Naduri and Burebusaga villages in Fiji for the presence of anti-leptospira antibodies using the microscopic agglutination test (MAT)

	Naduri		Burebusaga	
Horse	No. sampled	Prevalence	No. sampled	Prevalence
Cattle	20	85 (69-100)	3	67 (13-100)
Dog	130	73 (65-81)	50	50 (36-64)
Goat	25	60 (41-79)	8	38 (4-71)
Pig	13	38 (12-65)		
Cat	41	22 (9-35)	38	16 (4.2-27)

Figure 2.1: Relative prevalence of Leptospira serovars in humans and dogs from Naduri, Fiji



Objective 3. To determine which species of Trichinella are present in Fiji and Kiribati

There are no results from this objective because there was insufficient serological evidence to suggest that Trichinella infection is widespread in either Fiji or Kiribati. In addition, field studies would have been difficult because there was no commercially available rapid test for Trichinella.

Objective 4: To investigate the pathogenesis of T. papuae in pigs and Angiostrongylus cantonensis in ruminants

4.1 Information on the pathogenic effect of T. papuae in pigs and the diagnostic indicators for parasite recovery from pig carcasses

The results of work towards completion of this objective are limited because there were lengthy delays in obtaining fresh isolates of T. papuae, that were originally only available from wild-shot pigs from the western Province of PNG.

Experimental studies to determine the viability of T. papuae in pig meat revealed that larvae remain infective in for up to 26 days at 5°C and for 9 days in meat maintained outdoors at ambient temperature (32°C). These results mean that when an infected wild pig dies on the open Bula Plain, the carcase could remain infectious for 9 days to any scavenger that was susceptible to infection with T. papuae. It is also important because it shows that surveys to recover Trichinella from animals in remote locations are possible if tissue samples can be refrigerated.

In addition, the highest number of T. papuae larvae was present in the muscles of the neck and diaphragm

4.2 Information on the impact of A. cantonensis infection in ruminants

The life cycle of A. cantonensis was established at Murdoch University including maintenance of infected snails (intermediate host) and infection of rats (definitive hosts). Two experiments were conducted to determine the pathogenesis of A. cantonensis infection in sheep infected with 10 and 100 larvae per head given per os. There was no clinical or pathological evidence of eosinophilic meningitis in the sheep experimentally infected with A. cantonensis in both experiments.

These results were not considered conclusive because the failure to elicit neuro-angiostrongylosis in these sheep could be due to a number of factors including; the isolate used is not infective for sheep, the dose given is not sufficiently high to elicit an immune response, the method of preparation of larvae reduced their infectivity, sheep are refractory to A. cantonensis infection. There is no data on infectivity of A. cantonensis for ruminants but anecdotal evidence suggests that ruminants are susceptible. There are also no data available on the infective dose of A. cantonensis for ruminants. The infecting doses used in these two experiments were chosen to ensure that any disease induced by infection is not immediately life threatening to the host. Two methods were used to prepare larvae for infection, manual dissection and pepsin-acid digestion, both of which have been shown to result in infective material. Sheep used in the experiment were approximately 18 months old. It is possible that they have innate immunity due to prior exposure to other unrelated helminth infections or some other non-specific age-related mechanism.

The results of the third experiment using higher infecting doses of A. cantonensis were also negative. The combined results from the 3 experiments suggest that sheep are not easily infected with A. cantonensis under experimental conditions.

A. cantonensis infection in intermediate hosts

A large range of snail and slug species commonly found in Perth are able to act as intermediate hosts for A. cantonensis. Snail species included small aquatic snails that are likely to be accidentally ingested by ruminants grazing near sources of standing water.

Objective 5: To determine the epidemiology of bovine leptospirosis in PNG

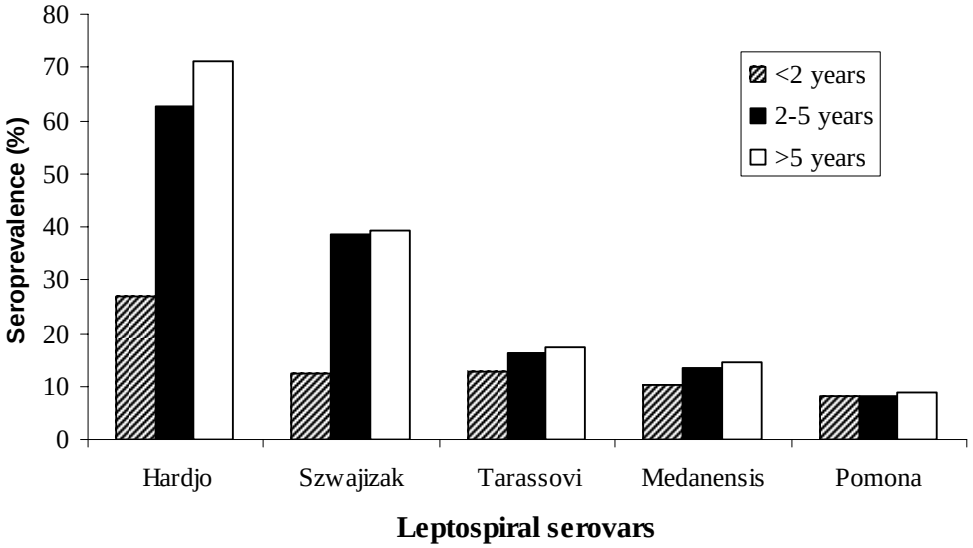
The main serovars detected included Hardjo, Szwajizak, Tarassovi, Medanensis, Pomona, and Kremastos (Table 5.1). Antibodies to other leptospiral serovars were detected but each at an apparent seroprevalence of <2% with exception of Australis at 2.2%. The apparent seroprevalence of serovar Hardjo was significantly higher than all other serovars ($P < 0.0001$).

Table 5.1 Proportion of cattle (n=1379) from the study with antibodies to leptospiral serovars determined using the MAT

Leptospira species	Serovar	No. positive	% positive	95% CI
Leptospira	Hardjo	742	53.7	51.1, 56.4
L .interrogans	Szwajizak	416	30.2	27.7, 32.6
L .interrogans	Tarassovi	214	15.5	13.6, 17.4
L .interrogans	Medanensis	176	12.8	11, 14.5
L .interrogans	Pomona	116	8.4	6.9, 9.9
L .interrogans	Kremastos	91	6.6	5.3, 7.9
L .interrogans	Australis	31	2.2	1.5, 3.0
L. noguchii	Panama	27	2.0	1.2, 2.7
L. santarosai	Shermani	21	1.5	0.9, 2.2
L. interrogans	Bataviae	17	1.2	0.7, 1.8
L. borgpetersenii	Ballum	13	0.9	0.4, 1.5
L. interrogans	Djasiman	11	0.8	0.3, 1.3
L. interrogans	Robinsoni	6	0.4	0.1, 0.4

Age-specific seroprevalences were calculated for each of the three age groups, <2 years, 2-5 years and >5 years (Figure 5.1). There was an increase in the seroprevalence of serovar Hardjo with increasing age and was significant. There was no age-specific difference in the seroprevalence of other serovars except with serovar Szwajizak. The seroprevalence of Hardjo was found to be significantly higher in each age group compared to the other serovars ($P < 0.0001$).

Figure 5.1 Age–specific seroprevalence of the principal leptospiral serovars identified in cattle



The farm-specific seroprevalences of serovars Hardjo, Pomona and Tarassovi in cattle from six farms in PNG are summarised in Table 5.2. The seroprevalence of serovar Hardjo infection was significantly higher in unvaccinated cattle from Rumion compared to the Numundo, Trukai and Ramu farms that vaccinated ($P < 0.05$). There was a low seroprevalence of serovar Pomona infection from cattle in all farms.

Table 5.2 The prevalence of leptospiral serovars Hardjo, Tarassovi and Pomona from cattle on six farms and an abattoir (95% confidence interval in parentheses)

Farm	Total sera	% Seroprevalence		
		Hardjo	Tarassovi	Pomona
Numundo*	273	38.5 (32.7, 44.2)	14.3 (10.1, 18.4)	1.8 (0.2, 3.4)
Trukai*	369	22.5 (18.2, 26.8)	21.4 (17.2, 25.6)	0 (0, 0)
Ramu*	224	19.2 (14.0, 24.4)	4.9 (2.1, 7.7)	0.9 (0, 2.1)
Rumion	172	57.6 (50.2, 64.9)	23.8 (17.5, 30.2)	2.3 (0.1, 4.6)
Prhalda	134	32.8 (24.9, 40.8)	6.0 (2, 10)	0.7 (0, 2.2)
Rearaguntu	128	35.2 (26.9, 43.4)	21.9 (14.7, 29)	0.8 (0, 2.3)
Abattoir	79	19 (10.3, 27.6)	10.1 (3.5, 16.8)	0 (0, 0)

** MAT titre > 400 was used as cut-off for positivity for serovars Hardjo and Pomona in these farms to discriminate infection from vaccination titres.*

The prevalence of leptospiral serovars detected in sera from cattle from Pinjarra, Western Australia using the MAT is displayed in Table 5.3. The most common serovars were Hardjo (seroprevalence 71.3%), followed by Medanensis (47.8%), Szwajizak (29.9%), Tarassovi (20.4%) and Arborea (11.5%). The apparent seroprevalence of serovar Hardjo was significantly higher than all the other serovars ($P < 0.05$).

Table 5.3 Proportion of cattle from the farm in WA with antibodies to leptospiral serovars determined with the MAT (n = 159)

Leptospira species	Serovar	Positive sera	% positive	95% CI
L .interrogans	Hardjo	112	71.3	64.3, 78.4
L .interrogans	Medanensis*	75	47.8	40.0, 55.6
L .interrogans	Szwajizak*	47	29.9	22.8, 37.1
L .interrogans	Kremastos*	1	0.6	0, 1.9
L .interrogans	Tarassovi	32	20.4	14.4, 26.7
L. borgpetersenii	Arborea	18	11.5	6.5, 16.4
L. borgpetersenii	Ballum	3	1.9	0, 4.1
L. santarosai	Shermani	4	2.5	0.1, 5.0
L. interrogans	Pomona	0	0	0

** Cross reacting serovars. When a sera had titres to more than one of these serovars, the highest titre was regarded as the correct one in these animals. When these titres were equal to the Hardjo titre, the sample was recorded as a Hardjo positive. If titres were equal for both Szwajizak and Medanensis the sample was recorded as positive for both (Black et al., 2001).*

The results showed that on the prevalence of Leptospira infection was highest in large commercial herds (Trukai, Ramu and Rumion) compared to smaller properties (Ralda) and smallholder farmers. In addition, there is no evidence that the prevalence of infection in cattle slaughtered at the Lae abattoir is higher than the general population. Three of the properties in PNG have commenced vaccination strategies for their young cattle. The results show that the control programs at Trukai and Numundo appear to have failed but the program at Ramu may have succeeded, as evidence by the lower rate of infection in young animals (<2 years old). Ramu have also introduced a policy to isolate young animals, which may have contributed to the reduction in the prevalence of infection. There is no evidence that pigs are source of infection for cattle. The presence of positive titres for swajizak are difficult to interpret but may be a cross reaction with another serovars (see below).

Results from testing 50 heifers from Trukai farm every month for 12 months showed that there was good antibody response to the vaccinations provided and no evidence of active infection in vaccinated heifers. Unvaccinated heifers remained sero-negative for the duration of the study suggesting that there was little or no circulation of Leptospira in the mob of cattle. Thus it is reasonable to conclude that vaccination was successful in this mob of cattle when used as directed, i.e. 2 initial vaccination just prior to mating and a booster each year.

A cross sectional survey of a the prevalence of antibodies to Leptospira in a herd of beef cattle in Pinjarra, Western Australia was performed to provide a comparison for the data collected in PNG. This was considered necessary to determine the significance of the minor serovars detected in PNG. The clinical history of the herd suggested that clinical leptospirosis was the cause of infertility and abortions. The results showed that the serological profile was similar to that observed in herds in PNG where leptospirosis was clinically important. In addition, the presence of a high prevalence of two minor serovars (swajizak and medanensis) and the high correlation between them and the serovar hardjo

suggested that these were cross reactions because they have only been recorded rarely and in association with rats.

The relatively low prevalence of serovar Hardjo in smallholder cattle showed that there is very little infection with this serovar circulating outside the large commercial farms. The presence of L. borgpetersenii serovar Hardjo in PNG suggests that the main transmission route is through direct contact with contaminated urine because it was shown this serovar does not survive in the environment like L. interrogans serovar Hardjo (Bulach et al., 2006). This could explain why infection is high in commercial properties where management brings cattle into close proximity on many occassions. Cattle from smallholder farms introduced to commercial properties may thus be at risk of becoming infected and developing clinical disease.

No antibodies to the 21 leptospiral serovars were detected in any of the 283 pig sera tested using the MAT.

In conclusion, this study has shown that infections with Leptospira are endemic in the PNG cattle population, with a seroprevalence of over 30%. The main infecting serovar is Hardjo and the seroprevalence was found to increase with age. Isolation of the bacteria and typing confirmed the presence of L. borgpetersenii serovar Hardjo (type hardjobovis) in PNG. Serovar Tarassovi also appears to be prevalent in cattle but its significance in PNG is uncertain. Cattle are potential zoonotic reservoirs for serovars Hardjo, Tarassovi and Pomona. Other findings from this study showed that pigs from commercial piggeries in PNG were not infected with any leptospiral serovars and that pigs are thus probably not a significant reservoir of infection for cattle.

Objective 7. Determine the epidemiology of public health significance of leptospirosis (non-bovine) in rural villages in PNG and in occupational groups associated with the livestock industry

A summary of the number of blood samples collected from the animals is shown in Table 7.1.

Table 7.1 Summary of animal species and number of blood samples collected from the villages in the Markham Valley

Location	Area	Village	Animal	Number sampled
Upper Markham	Leron	Rumion farm	pig	50
Upper Markham	Mutzing	Mampim	pig	2
			dog	1
Upper Markham	Mutzing	Maranafun	pig	3
			dog	2
			cattle	28
Upper Markham	Mutzing	Mutzing	pig	6
			dog	0
Upper Markham	Umin	Ragizaria	pig	3
			dog	0
Upper Markham	Kiapit	Mangiang	pig	1

			dog	3
Upper Markham	Kiapit	Guntu	dog	3
Upper Markham	Leron	Guaruk	dog	5
			cattle	15
			horse	4
Lower Markham	Erap	Wanpup	cattle	23
Lower Markham	Erap	Arisisi	cattle	13
Lower Markham	Zifasing	Gavtitif	pig	1
			dog	5
Lower Markham	Zifasing	Zifasing	pig	3
			dog	3
Lower Markham	Zifasing	Clean Water farm	cattle	32
Lower Markham	Erap	Trukai farm	horse	11

The results of testing sera with the MAT are shown in Table 7.2. The dominant serovar was Hardjo (18.9%) followed by serovars Tarassovi (5.4%), Arborea (3.6%) and Topaz 0.9%). Cattle from Wanpup, Clean Water and Guaruk were seropositive to serovar Hardjo (Table 7.3). Cattle from Arisisi and Maranafun were seronegative.

Of the 22 dogs tested, only one from Maranafun village was seropositive (Table 7.4). This dog had titres to L. interrogans serovar Canicola and L. interrogans serovar Djasiman.

Leptospiral antibodies were not detected in any of the village pigs. Only 1 pig from Rumion piggery had titres to two serovars, Canicola and Djasiman (Table 7.5). Horses from Trukai farm and Guaruk village were positive for serovars Arborea (6/15), Topaz (3/15), Hardjo (2/15), Canicola (1/15) and Grippotyphosa (1/15) (Table 7.6). A summary of the individual serovars identified during the survey is presented in Table 7.7.

Table 7.2 Proportion of cattle from smallholder farmers in the Markham Valley in PNG with antibodies to leptospiral serovars determined using the MAT

Species	Serovar	Number tested	Number positive	% positive	95% CI
Leptospira	Hardjo	111	21	18.9	11.6, 26.2
L. borgpetersenii	Tarassovi	111	6	5.4	1.2, 9.6
L. borgpetersenii	Arborea	111	4	3.6	0.1, 7.1
L. interrogans	Topaz	111	1	0.9	0, 2.7

Table 7.3 Seroprevalence of serovar Hardjo in 111 cattle from smallholder village farms in the Markham Valley in PNG

Location	Village	Number tested	Number positive	% positive	95% CI
Lower Markham	Wanpup	23	9	39.1	19.2, 59.1
Lower Markham	Arisisi	13	0	0	0, 0
Lower Markham	Clean Water	32	7	21.9	7.6, 36.2
Upper Markham	Guaruk	15	5	33.3	9.5, 57.2
Upper Markham	Maranafun	28	0	0	0, 0

Table 7.4 Leptospiral serovars detected in 22 dogs from villages in the Markham Valley in PNG

Location	Village	Number tested	Number positive	Serovars	
				Canicola	Djasiman
Upper Markham	Manpim	1	0	0	0
Upper Markham	Maranafun	2	1	1	1
Upper Markham	Mangiang	3	0	0	0
Upper Markham	Guntu	3	0	0	0
Upper Markham	Guaruk	5	0	0	0
Lower Markham	Gavtitif	5	0	0	0
Lower Markham	Zifasing	3	0	0	0

Table 7.5 Leptospiral serovars detected in 69 pigs from villages and Rumion piggery in the Markham Valley

Location	Village	Number tested	Number positive	Serovars	
				Canicola	Djasiman
Upper Markham	Manpim	2	0	0	0
Upper Markham	Maranafun	3	0	0	0
Upper Markham	Mangiang	1	0	0	0
Upper Markham	Mutzing	6	0	0	0
Lower Markham	Gavitif	1	0	0	0

Lower Markham	Zifasing	3	0	0	0
Upper Markham	Rumion farm	50	2	1	1

Table 7.6 Leptospiral serovars detected in 15 horses from Trukai and Guaruk smallholder farms in the Markham Valley

Farm	Number tested	Number of serovars positive				
		Hardjo	Canicola	Grippotyphosa	Arborea	Topaz
Trukai	11	2	1	1	2	1
Guaruk	4	0	0	0	4	2

Table 7.7 Summary of leptospiral serovars detected in all animals sampled

Species	Number tested	Leptospiral serovars						
		Hardjo	Tarassovi	Canicola	Gripptyphosa	Djasiman	Arborea	Topaz
Pigs	69	0	0	1	0	1	0	0
Dogs	22	0	0	1	0	1	0	0
Horses	15	2	0	1	1	0	6	3
Cattle	111	21	6	0	0	0	4	1

This survey of villages in the Markham Valley showed that seroprevalence in domestic animals like dogs, pigs, horse and cattle is low. The absence of titres in pigs in these villages does not mean that there is no infection with Leptospira. The animals could be infected with other leptospiral serovars that are not included in the panel of serovars used in the MAT. One such example is serovar Icterohaemorrhagiae, which is believed to be associated with dogs (Faine et al., 1999) but which was not included in the panel.

The results of this study highlights that cattle from the smallholder farms in the valley are unlikely to be reservoirs of infection with Hardjo for cattle on commercial farms as was previously assumed because of the practice of buying cattle from smallholders. It is probable therefore that most of the active infections with Hardjo are endemic on the commercial farms and that the greatest risk is for infection of introduced cattle. Preventive measures such as vaccination should be undertaken by commercial farms when new cattle are introduced.

In this study village pigs were not commonly infected with Leptospira and appear not to play a significant role in the transmission of leptospirosis in the Markham Valley. Whether this finding applies to other village communities in Morobe province and other parts of PNG is not known. The role of village dogs in the transmission of leptospirosis to other animals and humans is also not clear. Of the nine villages sampled, only one village had a dog with evidence of infection with serovar Canicola. This dog could play a role in transmitting leptospirosis to humans and village animals due to their close contact with each other. A serological survey performed in several Fijian villages reported that dogs

are commonly infected with serovar Canicola and that this serovar is an important cause of human leptospirosis (S. Angus, pers. comm). To date, there have been no reported cases of leptospirosis in villagers however the risk of infection from dogs should be a concern to the public health authorities.

In addition, there is a lack of knowledge of the part played by the type of reservoir hosts such as rodents and bandicoots, of the leptospiral serovars that they maintain in the different locations in the Markham Valley.

The project was unable to achieve collaboration with a medical agency capable of completing the human studies initially described for this objective. However, the lack of evidence of high prevalences of Leptospira infection in livestock and companion animals in villages near Trukai farm suggest that it is unlikely that non-rodent animals play a significant role in human leptospirosis in these villages.

Objective 9. To assist NAQIA prove that commercial and semi-commercial pig producers are free from Trichinella infection

Results of a retrospective serological survey of 479 people living in the Balimo area showed that the prevalence of infection with Trichinella, estimated using the Ab-ELISA, was 29.5% (95% CI: 27.3-31.7%). Antibody prevalence did not differ according to sex but there was a significant difference between age groups,17.7%, 30.5% and 51.6% respectively for <15 years old (n=130), 16-35 years old (n=190) and >36 years old (n=159) ($p<0.05$). Antibody prevalence also appeared to differ significantly according to geographic region and age. These results (and those from other surveys) show hat T. papuae is probably widely distributed in wild pigs (the most likely source of infection for humans) in the south coastal area of PNG at least as far east as Gulf Province.

Results from examination at the NVL of muscle digests from 31 pigs and 15 rats from the Goroka area confirm that T. papuae was not present.

8 Impacts

8.1 Scientific impacts – now and in 5 years

The following information was gained during this project:

- Leptospira hardjo is a significant cause of infertility in cattle in PNG
- There is limited evidence of Leptospira infection in pigs in PNG
- There is a low prevalence of Leptospira infection in domestic livestock in smallholder villages associated with commercial properties
- Identification that Leptospira sp. infecting cattle in PNG belong to L. borgpetersenii that have been shown to have a limited capacity to persist in the environment, which suggests that transmission is most likely to occur in commercial cattle herds with higher stocking densities and active cattle management. This also suggests that the risks to humans are highest in those groups that are associated with commercial cattle herds and not in villages nearby.
- This is the first time an ELISA for Leptospira and the MAT have been rigorously validated using samples from multiple populations of cattle in PNG and Australia
- The Ab-ELISA developed during this project is a suitable replacement for the MAT in PNG because it is accurate, repeatable and has a high level of agreement with the MAT.
- There is little evidence that Trichinella is present in Fiji or Kiribati
- T. papuae infection is widespread in wild pigs in the southern coastal areas of PNG but there is limited evidence that it exists in the highland and northern provinces.

8.2 Capacity impacts – now and in 5 years

A highlight of the project in terms of capacity building has been the postgraduate training of staff from the Animal Health component of NAQIS. Dr Nime Kapo completed the research component of his MTVSc at James Cook University in collaboration with the project and Dr Peter Waiín was awarded a PhD for his work on the epidemiology of bovine leptospirosis in PNG. Drs Waiín and Kapo are the first successful postgraduate students in the veterinary services in PNG.

The project has contributed significantly to the knowledge and skills of laboratory staff at the NVL with regards to the diagnosis of infection with Leptospira and Trichinella by:

- Development and validation of antibody-detection ELISAs to detect infection with Trichinella and Leptospira in livestock in the Pacific region
- Establishment of capability for serological diagnosis (ELISA) of Trichinella and Leptospira infection at the National Veterinary Laboratory in PNG via a training workshop at Murdoch University and follow up training at the NVL
- Development of a sensitive PCR-based test to detect pathogenic species of Leptospira in urine and kidneys of livestock in the Pacific region
- Provision of training to a staff member from the NVL (Peter Wai'in) in techniques required for the culture and diagnosis of pathogenic Leptospira at the WHO/OIE Reference Laboratory for Leptospirosis in Brisbane

- Transfer of knowledge of the epidemiology, public health and economic significance of Leptospira infection in cattle in PNG to farmers, meat inspectors and livestock technicians via a training workshop held in Lae in November 2004
- Strengthening of the scientific collaboration between the FAO/OIE/WHO Reference Laboratory for Leptospirosis and the NVL and SPC
- Enhancement of the capacity to detect Trichinella infection in pig and crocodile tissues at the NVL through the provision of equipment (water bath, electronic scales, tissue macerator and microscopes) and reagents
- A 2 day workshop on diagnostic techniques for Trichinella infection was held at the National veterinary Laboratory in PNG conducted by Dr Ifor Owen. It was attended by Dr Stephen Angus (SPC, Fiji), Dr Isagani Majuelo (PNG) and three laboratory technicians from the NVL, Columba Awui, Asi Matuka and Frexie Maneh.
- The project was able to financially support the employment and training of laboratory personnel at the NVL and SPC

8.2.1 Equipment

Provision of laboratory equipment to the NVL included (2 microscopes, a water bath, electronic scales, tissue macerator and minor laboratory equipment) and reagents for the diagnosis of Trichinella has upgraded the parasitology laboratory to the necessary standard required to continue testing crocodile meat for the presence of Trichinella, which is a requirement the industry must meet to continue exporting crocodile meat to Australia. The capacity of the NVL to conduct diagnosis of Leptospira was enhanced by the provision of a microplate (ELISA) reader and transfer of the Ab-ELISA developed and validated at Murdoch University.

The diagnostic capacity of the SPC in Fiji was improved by provision of 2 microscopes and training in techniques to detect Trichinella infection in pigs.

8.2.2 Postgraduate study

One PhD was awarded during the project to Dr Peter Waiín, Manage of the National Veterinary Laboratory, PNG with support from a John Allwright Fellowship. His thesis is entitled: "Epidemiology of Infection with Leptospira Species in Livestock in Papua New Guinea"

This project assisted Nime Kapo (NAQIA veterinarian) to complete research in part fulfilment of the degree of MTVSc at James Cook University. His thesis is entitled “Observations on Trichinellosis in Goroka and Balimo areas in Papua New Guinea and north Queensland”.

The project also enabled two BSc. Honours students to complete projects which evaluated the ability of several potential invertebrate hosts to support the development of A. cantonensis larvae and assisted in the development of a PCR-based test to detect Leptospira:

Anna Le Souef –	Characterisation of Angiostrongylus in known and potential hosts
Terri Cheow –	Rapid detection and identification of pathogenic Leptospira using PCR

8.3 Community impacts – now and in 5 years

Improving livestock production in smallholder farms and villages will lead to improved prosperity for those involved. Whilst this project did not have a direct impact on the productivity of smallholder cattle it did significantly improve the capacity for NAQIA to respond to problems that are likely to occur in the future as the livestock industry develops. This is important because this project has clearly shown that as the scale of cattle production is increased so to does the risk of adverse production impacts from leptospirosis.

8.3.1 Economic impacts

The main economic impact during the reporting period arises from the recognition that the there is a low risk that Leptospira is having an impact on the fertility of domestic livestock in smallholder villages in the Markham valley. This obviates the need for cattle to be vaccinated to prevent infection with Leptospira, which would have been a relatively significant cost. The major constraints to livestock production in these villages are poor genetics and poor nutrition.

8.3.2 Social impacts

The project did not have any direct social impacts. Knowledge that livestock and domestic animals are not a reservoir for human leptospirosis may have benefits in the long term if this disease becomes a problem and attempts are made to locate the source

8.3.3 Environmental impacts

The project had no adverse or positive environmental impacts

8.4 Communication and dissemination activities

A workshop on Leptospirosis was held in Lae, PNG in 2004 to disseminate information from the project and promote control programs for Leptospirosis to smallholder and large commercial cattle farmers.

Dr Reid and Wai'in visited each farm and in villages included in the cross-sectional survey in the Markham valley to verbally disseminate the results of the survey on leptospirosis.

9 Conclusions and recommendations

9.1 Conclusions

The capacity of the NVL to conduct serological surveillance and monitoring for pathogenic Leptospira infection in livestock has been significantly enhanced during the project. The Ab-ELISA that was developed is the first of its kind to be fully validated. The ELISA platform is ideal for countries such as PNG and Fiji that have limited laboratory capacity.

There is little or no evidence that Trichinella sp are widespread in Fiji and Kiribati. In PNG it appears that T. papuae is still only restricted to wild pigs living in the southern coastal provinces. T. papuae remains a stubborn problem for the commercial crocodile industry in PNG mainly because of the requirements imposed on them by AQIS to test 10% of each batch of animals slaughtered for export to Australia. The project has provided valuable assistance to the NVL to enhance and maintain its diagnostic capacity for T. papuae.

It was not possible to conclusively prove that Angiostrongylus cantonensis had a pathogenic effect on ruminant livestock. Thus it is unlikely to be a significant constraint to ruminant productivity.

Leptospirosis is a significant constraint to livestock production in most cattle producing areas of the world. There was a high prevalence of infection in all large commercial herds, especially those herds that were less well managed. There was evidence that vaccination and management programs, such as segregation of younger animals from older cows, which were designed to reduce infection with Leptospira, were effective in some cases, especially where both approaches were combined. However, some large and well managed farms appear to have continuing clinical leptospirosis despite rigorous vaccination programs. This may be due to reduced vaccine efficacy as a result of improper transport of vaccine from Australia. This is a significant problem in PNG and stems from the many logistical obstacles that exist in supply chains in that country. Despite these difficulties there is direct evidence from longitudinal studies that vaccine available on mainland PNG remains efficacious in young heifers.

There was evidence that at least one outbreak of human leptospirosis in Fiji may be associated with Leptospira infection in dogs. This is important because effective vaccines are available for use in dogs. It may be worthwhile considering a vaccination program for dogs if further evidence is found suggesting that dogs are a reservoir for other outbreaks of disease in humans.

There is little evidence that domestic and companion animals in villages in PNG are reservoirs for human leptospirosis.

It is often difficult to undertake large and complex research projects in countries of the Pacific region such as PNG. It is especially difficult to rely on the capacity of national agencies to provide the human resources necessary for completion of projects because these organisations are often poorly staffed. An alternative approach is to work closely with organisations such as Unitech in Lae that have staff and students working on Agricultural related topics. However, this is problematic in the livestock field because livestock are not given a high priority by national agricultural bodies. However, the intellectual capacity of staff in countries such as PNG to complete world-class postgraduate degrees is extremely high.

9.2 Recommendations

It is extremely difficult to accurately measure the impacts of zoonotic infections on livestock production and the health and prosperity of the communities in which they exist.

It would be useful if research could be undertaken to design a framework that can be used to determine the impact of zoonoses, which would provide valuable outputs for policy makers.

Further work on zoonoses in SE Asia and the Pacific should focus on gaining a better understanding of the drivers for establishment of new and emerging diseases. PNG has experienced new pig-related diseases such as Japanese Encephalitis and is likely to encounter more as diseases emerge in Asia and spread south-west through Indonesia.

Future projects in countries such as PNG should consider including academic organisations to ensure transfer of knowledge to the broader undergraduate community. In addition, where possible project teams should include paid national staff in partner organisations to assist them deliver on their obligations.

The intellectual capacity of staff in countries such as PNG is extremely high. We would recommend that capacity building in the form of post graduate education is a part of any future projects in the Pacific region.

Livestock industries such as cattle production have a bright future in PNG, which does not have many trade-limiting diseases present in its trading partners. Significant benefit would be gained from research to identify the constraints to the development of livestock industries and to suggest solutions. These projects would require multidisciplinary approaches that include activities in animal health, trade and market access, genetics and nutrition and social networks and cooperative farming approaches.

10 References

10.1 References cited in report

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10.2 List of publications produced by project

The following scientific publications were published over the course of the project:

Pozio, E., Owen, I.L., Marucci, G. & LaRosa, G. (2004) Trichinella papuae in saltwater crocodiles (Crocodylus porosus) of Papua New Guinea. Emerging Infectious Diseases 10, 1507-1509.

Owen IL, Gomez Morales MA, Pezzotti P, Pozio E (2005) Trichinella infection in a hunting population of Papua New Guinea suggests an ancient relationship between Trichinella and human beings. Trans R Soc Trop Med Hyg. 99(8):618-24.

Owen IL. (2005) Parasitic zoonoses in Papua New Guinea. J Helminthol. 79(1):1-14.

Pozio E, Owen IL, Marucci G, La Rosa G. (2005) Inappropriate feeding practice favors the transmission of Trichinella papuae from wild pigs to saltwater crocodiles in Papua New Guinea. Vet Parasitol. 28;127(3-4):245-51.

Owen IL and Reid SA Survival of Trichinella papuae muscle larvae in a pig carcass maintained under simulated natural conditions in Papua New Guinea. J Helminthol. 81, 429–432

The following publications have been prepared in draft form for submission to a peer-reviewed journal:

Peter Wai'in, Ian Robertson, Stan Fenwick, Lee Smythe and Simon Reid. Epidemiology of leptospirosis in livestock in Papua New Guinea

Peter Wai'in, Stan Fenwick and Simon Reid. Validation of an antibody-detection ELISA for the detection of Leptospira infection in cattle using non-gold standard approaches.

Peter Wai'in, Stan Fenwick, Ian Robertson, Lee Smythe, Eric Taylor and Simon Reid. The investigation of an outbreak of bovine leptospirosis in Western Australia.

The following conference and workshop presentations were made:

Three oral papers were presented on project activities at the CVA conference held in Lae 2004

Peter Wai'in: Progress report on the leptospirosis project in PNG

Nime Kapo: A survey of trichinellosis in Balimo and Goroka, PNG

Steve Angus: Zoonoses in the Pacific region

Peter Wai'in presented a poster entitled "Distribution of leptospiral serovars in the beef herd in PNG" at the Postgraduate poster day at Murdoch University.

Peter Wai'in presented an oral presentation entitled "Seroprevalence of Leptospirosis in cattle in Papua New Guinea" at the 4th International Leptospirosis Society in Chiang Mai, Thailand in November 2005