final report

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Investigation of the Prevalence of Neospora caninum in Queensland Beef Cattle

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CONTENTS

PART ONE: ABSTRACT

To define the prevalence of *Neospora caninum* in Queensland cattle, the 1997 structured surveillance serum bank of Queensland beef cattle was tested. *Neospora*-specific antibodies were detected in 14.8% of the 5785 samples tested by the indirect fluorescence antibody test. The results were mapped and regional variations in the parasite's distribution were evident. Possible explanations for the regional variations include wild dog prevalence and also rainfall. This study has defined the prevalence and distribution of *Neospora caninum* in Queensland beef cattle, which will assist with the planning of future research into its biology and ultimately control options.

PART TWO: EXECUTIVE SUMMARY

Neospora caninum is a recently recognised parasite that has been shown to be a major cause of bovine abortion worldwide. In addition to the impact on reproduction, overseas studies have reported decreased weight gain in infected cattle and lower milk production in dairy heifers. Globally, considerable resources have been committed into the understanding this parasite.

Neospora was identified in the MLA funded North Australia Program 1998 Review of Reproduction and Genetics Projects (pp. 76&80) as a pathogen which warranted investigation in Queensland beef herds, but this was not done at that time due to lack of funds and also lack of trade significance. Since that time, there have been indications from countries importing our cattle that neosporosis may be an issue. The Department of Primary Industries, Queensland (QDPI) has fielded questions about our *Neospora* status from the Philippines, Thailand and Indonesia. The serologist from the main government veterinary laboratory in Tasmania has also reported to QDPI that a Victorian cattle exporter was looking into testing cattle for *Neospora* prior to export to Mexico. An increasing number of breeding cattle are being exported from Australia, therefore this parasite has the potential to impact our live cattle trade. We need to be pro-active in defining the *Neospora* status of our beef cattle, not only for the domestic advantages, but also for the risk assessments that may be required by our international trading partners. Queensland, has about 40% of the national beef herd and spans a range of climatic zones and this makes it an ideal state to start such an investigation.

Our preliminary *Neospora* seroprevalence study looked at sera from 45 animals on 32 properties. This study revealed a seroprevalence if 15% in 1400 cattle tested, with only one property not showing infection. This seroprevalence of fifteen percent was much higher than initially anticipated, being much higher then that found in many overseas surveys, for example, New Zealand beef cattle only had a 2.8% seroprevalence.

This project tested 5785 Queensland beef cattle from 154 properties, and found 14.8% of these cattle and 90% of properties were infected with the parasite. The mapping of these results showed distinct areas of infection, the parasite being most abundant along the coastal and northern areas of the state. The inland southern region had the lowest level of infection, which also coincided with a low rainfall level and lower numbers of wild dogs.

This project has provided the first step in defining the *Neospora* situation in the Queensland beef Industry, and will serve as a model for the rest of Australia to structure its research priorities.

PART THREE: MAIN RESEARCH REPORT

Introduction

In the past decade, *Neospora caninum* has emerged as a major cause of abortion in cattle. Considerable resources have been committed globally to understand this parasite, but the situation in Australian beef cattle was previously unknown.

Neospora can be transmitted to a cow one of two ways, congenitally (from dam to calf) or horizontally (from ingesting contaminated faeces from a dog). Cow to calf infection has been shown overseas to be the major route of infection and cycling of the parasite within herds. Usually only a few infected foetuses will be aborted and the calf will be born healthy and then potentially infect her own calf. The infected cow has a 90% chance of infecting her calf before it is born. Transmission from cow to calf is probably the most common route of transmission within herds. Dogs become infected after eating infected foetal tissue or afterbirth, then pass parasite oocysts in their faeces for up to several months. If a dog defecates in stored feed or in water sources, this may result in the infection of a large number of animals and potentially an abortion epidemic. By understanding how the parasite is being transmitted on a local scale, recommendations can be made into what control measures, if any, are appropriate.

This project aimed to provided the first step in defining the *Neospora* situation in the Queensland beef Industry, and be a model for the rest of Australia to structure its research priorities.

Materials and Methods

During 1997, blood was collected from beef cattle across Queensland and stored at -20 ^{o}C as part of a structured animal health surveillance program conducted by the Department of Primary Industries, Queensland, Animal and Plant Health Service. This serological sampling was conducted to provide information to support market access decisions. In addition to blood samples being collected, a questionnaire was also completed by cattle owners which provided information on stock numbers, herd management and a range of other disease related issues. A full description of the herd selection and sampling methodology is provided by Black *et al.* (2001). Briefly, beef properties were selected from each of Queensland's five administrative regions. Only cattle that were bred on the property were sampled. Forty-five female cattle from each property were sampled, irrespective of herd size. Female cattle were selected because they were more likely to remain on the property and be available for follow-up testing if necessary. Fifteen heifers, 1-2 years of age and 30 older cows were sampled in each herd.

Sera were tested at 1:200 dilution by the indirect fluorescent antibody test (IFAT: Paré *et al*., 1995) utilising *in vitro* derived NC-1 tachyzoites as antigen and an anti-bovine IgG (H+L; Sigma-Aldrich, Castle Hill) as the conjugate. Only bright, complete peripheral fluorescence of the tachyzoite was scored as positive.

Results and Discussion

Seroprevalence

Neospora caninum was found to be prevalent throughout Queensland beef cattle. Of the 5785 cattle tested, 858 (14.8%) were seropositive for *Neospora* (Table 1). The mean seroprevalence of individual properties was 13.8% (SD=9.1%). On a global scale, a *Neospora* incidence of 15% is quite high; seroprevalences reported for beef cattle in overseas surveys vary from 2.8% in New Zealand (Tennent-Brown *et al.*, 2000) to 24% in northwestern USA (Sanderson et al., 2000).

Table 1. Seroprevalence of *Neospora caninum* in Queensland, by district.

	Samples tested	Number positive	Overall Percent positive	Number of Properties	Properties with no positives	Inter-Property analysis	
						mean	S.D
North	1589	256	16.1%	36	0	16.3%	9.3%
South	787	43	5%	31	10	5.7%	5.8%
West	826	97	11.7%	20	3	11.4%	10.6%
South East	1146	242	21%	27	0	20.9%	11.1%
Central	1437	220	15.3%	40	2	14.7%	8.6%
Total	**5785**	**858**	**14.8%**	**154**	**15**	**13.8%**	**9.1%**

Of the 154 properties surveyed, only 15 (9.7%) did not have a seropositive animal in the samples tested, showing that infection is also widespread.

Spatial distribution of seroprevalence

The distribution of *Neospora caninum* is represented in figures 1 and 2. A GIS mapping program (Arc Map 8.2) was used to map the serological distribution of *Neospora caninum* infection across Queensland. The centroid of the land parcels where sampling took place was used to map the serological distribution. Arc Map Spatial Analyst was then used to perform a function called “kernel smoothing” to map the serological distribution of *Neospora caninum* for Queensland.

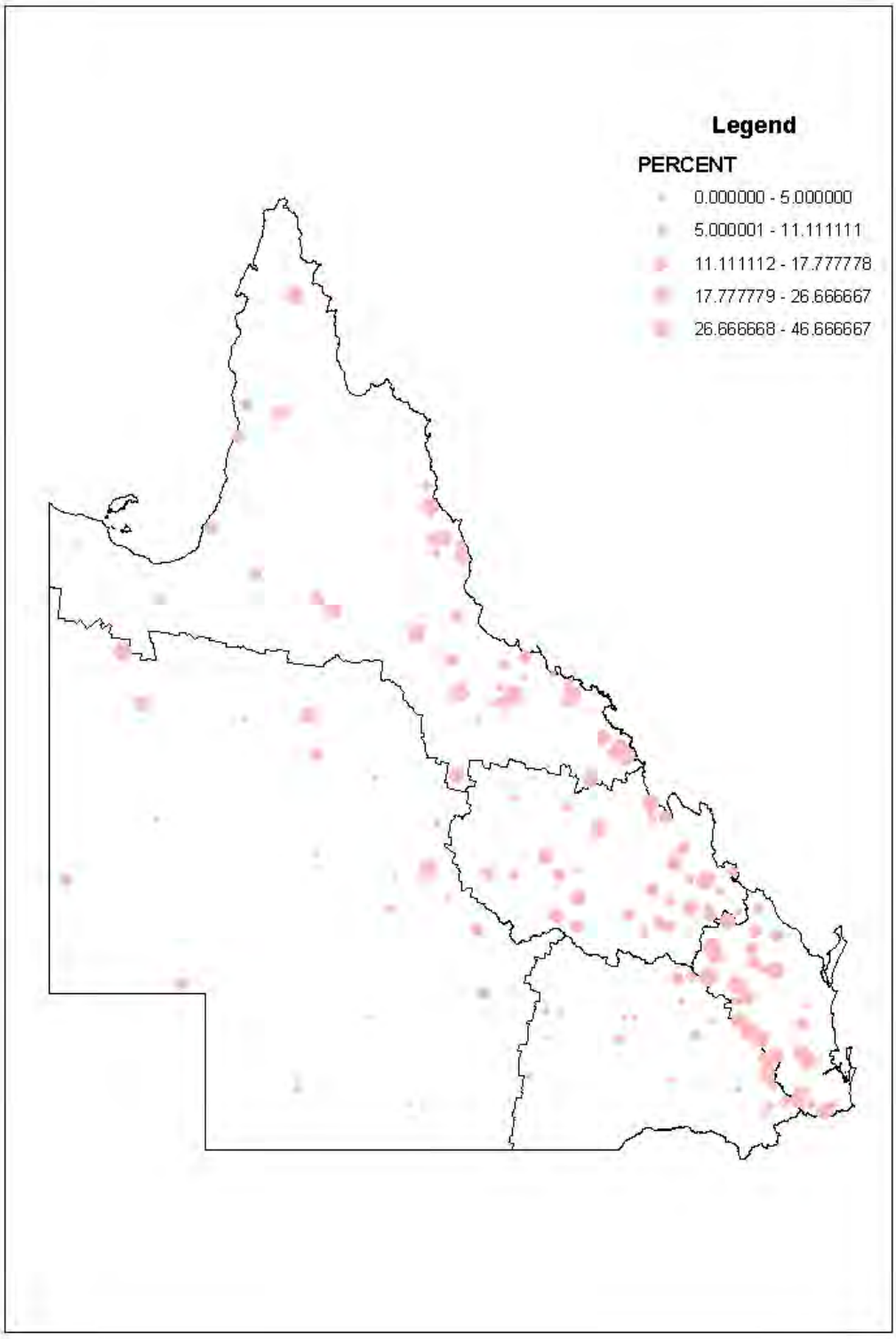


Figure 1. Seroprevalence of *Neospora caninum* on properties surveyed

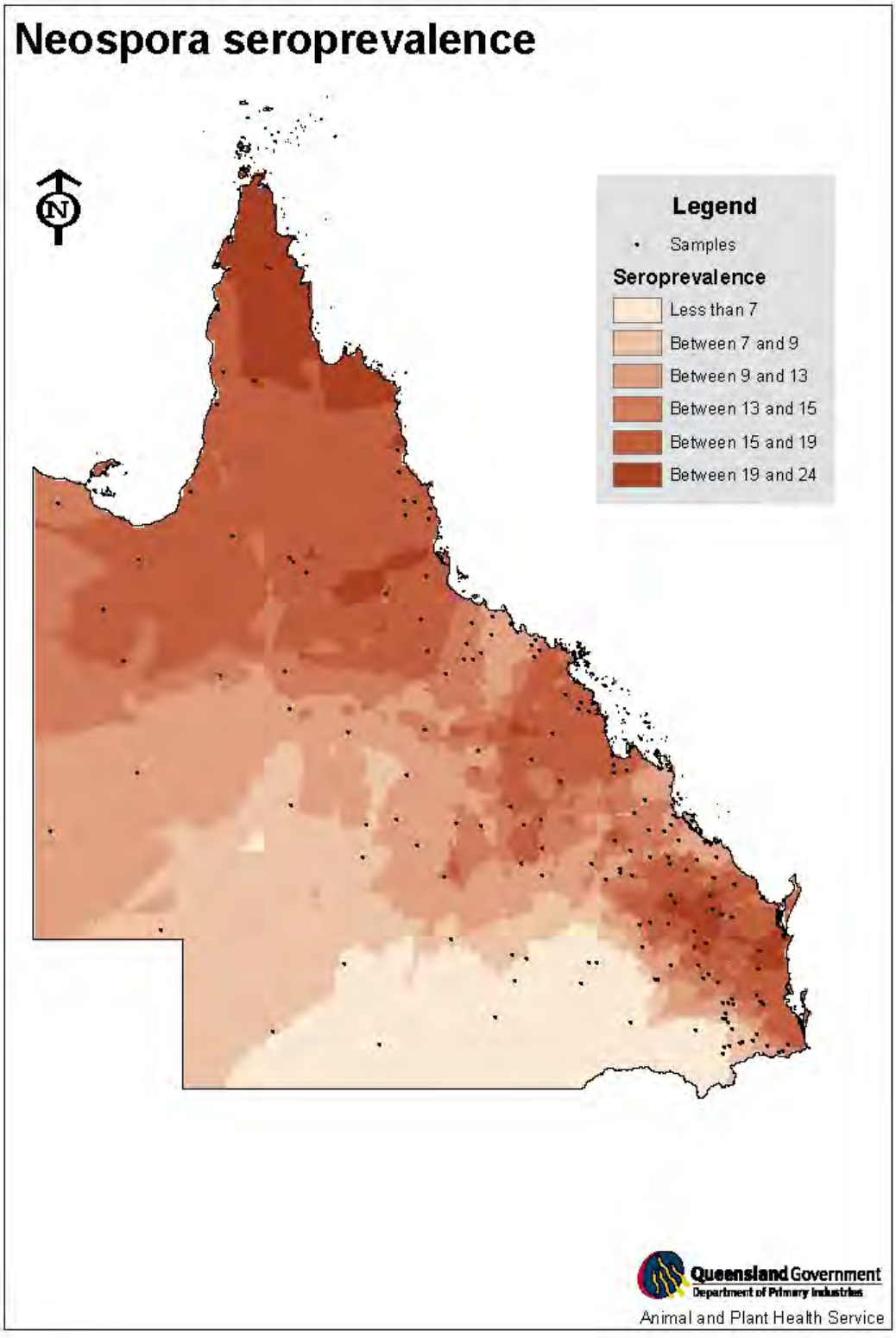


Figure 2. Kernel smoothing projections of distribution of *Neospora caninum* in Queensland cattle.

Factors affecting spatial distribution

Neospora in Queensland cattle is most prevalent along the coast and in tropical North Queensland. Two factors were identified to influence *Neospora* seroprevalence, wild dog populations and rainfall. The first is the distribution of wild dogs in the state. The wild dog and dingo population distribution is shown in figure 3. Most notable is the low seroprevalence in Southern inland Queensland in the area bounded by the dingo fence (Fig 4). While this area does contain some wild dogs, they are generally controlled more effectively than in the rest of the state. The dog is the only known definitive host for *Neospora caninum* and therefore it is logical that a correlation would exist due to horizontal transmission. However, the distribution of *Neospora caninum* seroprevalence is very low in the south-western parts of the state despite wild dogs being classed as “abundant and widespread”. Therefore, factors other than an absence/presence of wild dogs play a part in *Neospora* seroprevalence, such as oocyst environmental survival time.

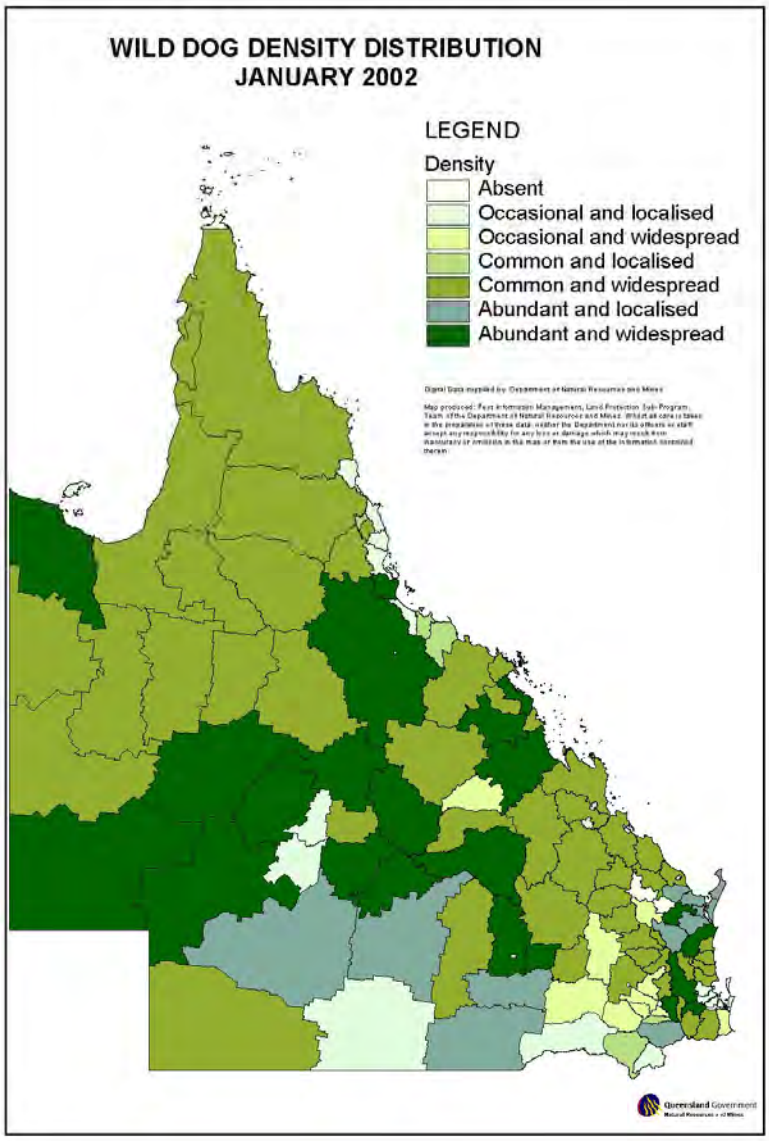


Figure 3. Wild dog density in Queensland

(Courtesy of the Department of Natural Resources and mines, Queensland)

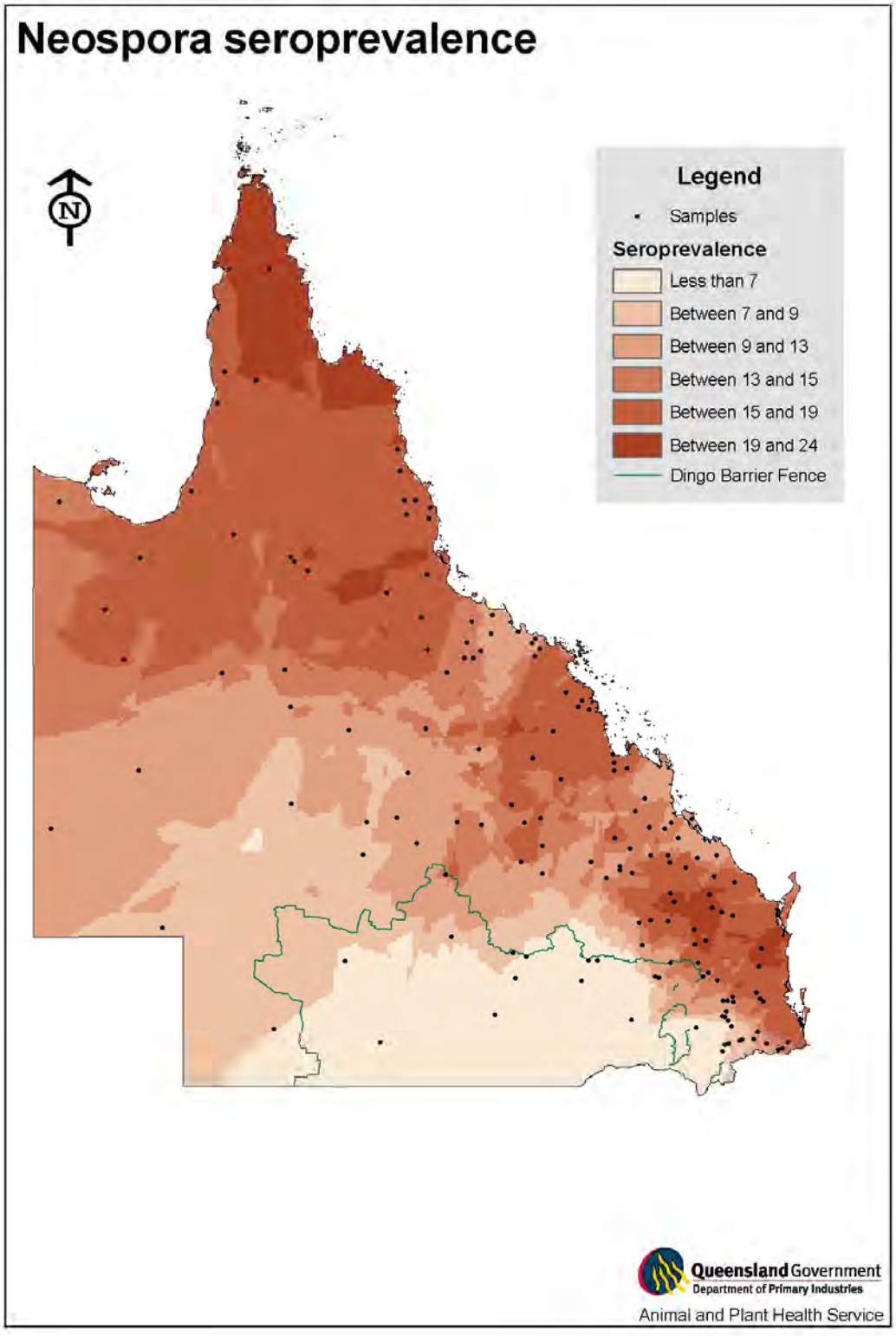


Figure 4. *Neospora* seroprevalence in relation to the dingo fence

Secondly, the distribution of *Neospora caninum* seroprevalence appears to follow the average annual rainfall distribution. The highest seroprevalence corresponds with the highest rainfall areas along the coast, with the 5-600mm isohyet being the demarcation between high and low seroprevalence areas (this is modified somewhat in the south of the state by the presence of the dingo barrier fence). The lowest seroprevalence is in the semi-arid to arid south-west of the state. While stocking rates are generally higher in high rainfall areas, high seroprevalence also occurred in the gulf region, where stocking densities are relatively low. A map showing annual average rainfall for Queensland is shown as Figure 5. Higher rainfall would be advantageous to the environmental survival of the infective parasite oocysts (due to lower desiccation), and this correlation was also noted for the dog-transmitted tapeworm *Echinococcus granulosus*. The distribution of *Echinococcus granulosus* was shown to follow the 500mm rainfall isohyet (Fig 6; Baldock *et al*., 1985), which also approximates the distribution of *Neospora caninum*. Echinococcus lesions in cattle at slaughter come about through cattle grazing areas contaminated with dog faeces. This is also one potential route of transmission of *Neospora caninum*.

The relatively higher seroprevalence of Neospora in beef cattle in high rainfall areas also explains why *Neospora caninum* is such a problem in dairy herds in Queensland as these are generally located in the highest rainfall areas of the state or are using irrigation to produce pasture for grazing cattle.

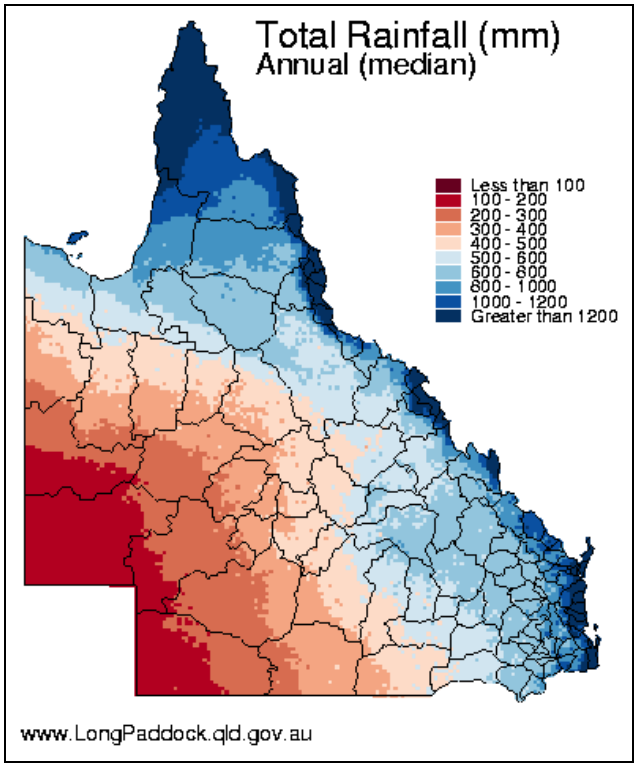
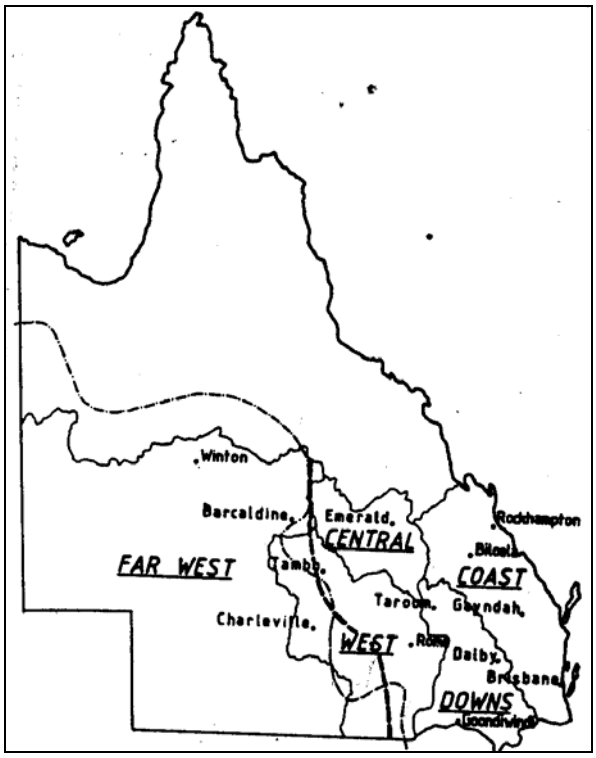


Figure 5. Median annual rainfall for Queensland



----- Western Limit of area of Southern Queensland endemic for bovine hydatidosis

-.-.- 500 mm isohyet

Figure 6. Distribution of bovine *Echinococcus granulosus* in Queensland (Baldock *et al*., 1985)

Effect of infection on branding rate

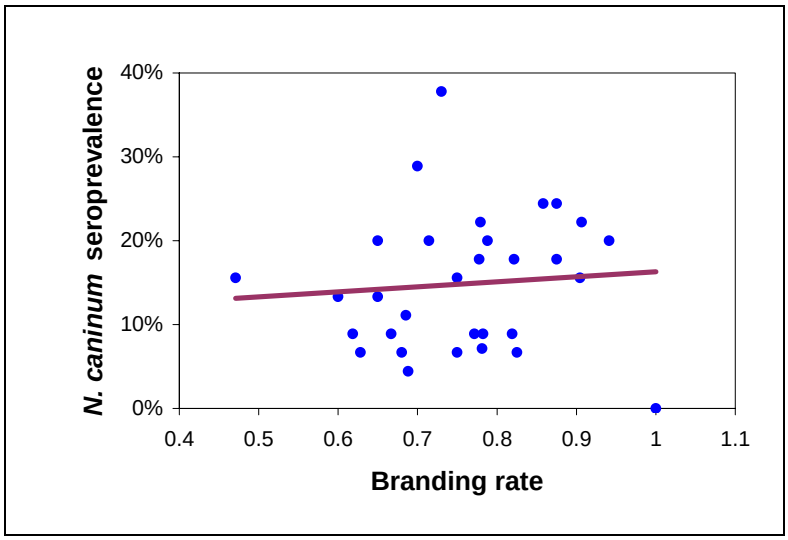


Figure 7. Correlation between branding rate and *Neospora seroprevalence* in Central Queensland beef herds.

There was no association (R^2 = 0.0069) at the herd level between *Neospora* seroprevalence and branding rate (number of calves branded / number of cows mated) in Central Queensland beef herds (Fig. 7). The branding rate was only examined for central Queensland herds as the information available from the other regions was deemed to be unreliable. It was calculated on the basis of questionnaire data which required producers to state the number of calves branded in 1997 and the number of cows bred to produce those calves. Being a branding rate, it would also include components of infertility, abortion, perinatal loss and producer subjectivity.

This is similar to what was found in North Queensland dairy herds where it was shown that the seroprevalence in low abortion herds and the overall seroprevalence in high abortion herds was the same, however analyses of individual animal reproduction records showed that *Neospora caninum* was responsible for a significant proportion of the abortions on these properties (Gunn *et al*., 2002).

Conclusions and Recommendations

This study has shown that *Neospora caninum* is prevalent in Queensland beef cattle. Geographical variations in the distribution of this parasite were noted, and factors such as wild dog populations and rainfall may be factors influencing this.

Fully understanding the impact of *Neospora caninum* to the Queensland and Australian beef industry requires three stages; is it present, is it causing harm and how is it being transmitted. These need to be defined, the first two steps defining the benefit of further investigation, and definition of transmission being required to determine if control is feasible and if so what would be required to implement this.

This study has completed the first stage of understanding, by showing that the parasite is both abundant and widespread throughout the state. From here the actual impact of the parasite should be evaluated, both on reproduction and possibly weight-gain. To determine the impact on reproduction, studies will be required to compare reproduction rates in infected and non-infected animals. The issue of weight gain will require longitudinal studies, following animals on commercial properties from birth through to sale. Focusing solely on animals selected for feedlots, as has been done in overseas studies (Barling *et al*., 2000) would not be suitable for this as too many factors bias the selection of feeder steers.

If we assume 15% of cattle in the state are infected, the reproductive loss to the Queensland beef industry could be estimated at $13.5 million annually (In Queensland, 11 million cattle; 6 million of which are breeding; therefore 900 thousand infected; 1 in 10 gestations abort; therefore 90 thousand abortions at $150 per replacement calf).

Once the impact of *Neospora* has been defined, a better understanding of its biology in the Australian

situation, especially in regards to its mode of transmission, will be required before firm control recommendations can be implemented.

Acknowledgements

The authors thank the Department of Primary Industries, Queensland, staff and also the producers who made the compilation of the serum bank possible. The serology was preformed by Zoe Stoessel, and the maps were generated by John Arrowsmith. Michael McGowan is also thanked for his input in the planning and reviewing of this project.

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Appendices

Appendix 1: First milestone report

Queensland Department of Primary Industries

Tick Fever Research Centre

280 Grindle Rd

Wacol Qld 4076

29 July, 2002

Dr. Peter Rolfe

Veterinary Manager

Meat and Livestock Australia

Level 1, 165 Walker St

North Sydney NSW 2060

Dear Peter,

I am pleased to advise you that we have finished the testing for the project *Investigation of the prevalence of Neospora caninum in Queensland beef cattle* (AHW.013). A total of 4,348 samples were tested serologically, in addition to the 1,437 that were tested previously. The overall seroprevalence was 14.8%; specifically by region this was:

North:	256/1589	16.1%
South:	43/787	5%
West:	97/826	11.7%
South East:	242/1146	21%
Central:	220/1437	15.3%
Total	**858/5785**	**14.8%**

Our research team is now analysing this data, as outlined in our proposal. Additionally, I have attached a copy of an article referring to this work, and MLA's support, that was requested of us by the Queensland Country Life and was published in its Beef Annual.

Yours Sincerely,

Juergen Landmann

Appendix 2: Excerpt from Queensland Country Life

Testing urged for neospora

IT affects between 11 and 30 percent of herds across the country, mainly causing bovine abortion, but until now little has been known about the puzzling and costly disease neospora caninum.

However, research being conducted by the Department of Primary Industries Tick Fever Research Centre and funded by Meat and Livestock Australia has worked to identify and define the problem in Queensland, with more than 16 percent of 5000 beef cattle being infected.

Neospora is a microscopic parasite discovered just more than a decade ago and has been attributed with causing high abortion rates, as well as lower milk production and reduced weight gain.

On the NSW north coast, neospora is believed responsible for more than 30pc of abortions in cattle.

The prevalence by region in Queensland has shown 11.7pc of the western herd is infected, 16.1pc in the north, 15.3pc in Central Queensland and 21pc in the South East. However, the southern region study is still being completed.

Researcher Juergen Landmann said abortion due to neospora usually occurred between months four to seven of gestation, but may occur from three months to full term.

An infected cow has about three times the abortion risk of a non-infected animal and unlike many other causes of abortion, this disease often causes abortions in subsequent gestations.

He said within a herd, abortion due to neospora can be sporadic, with individual animals aborting occasionally in low numbers throughout the herd, or it may occur as an abortion storm.

This could be up to 33pc of the breeding herd aborting within a few months. Some properties could have more than half their cattle infected and never encounter any reproductive problems until an abortion storm occurs.

What triggers these abortion storms however is still not known. What is known now, though, is how cows become infected.

This can take two forms, either from cow-to-calf before birth or by a cow eating parasite eggs defecated by a carnivore, such as a dog.

It is unlikely that a parasite is transmitted by contact, sexually, or through milk.

Mr Landmann said cow-to-calf infection has been shown overseas to be the major route of infection and cycling of the parasite within herds.

"Usually, only a few infected foetuses will be aborted and the calf will be born healthy then potentially infect her own calf," he said.

An infected cow has a 90pc chance of infecting her calf before it is born and transmission from cow to calf is probably the most common route of transmission within herds.

Researchers believe dogs probably become infected after eating infected foetal tissue or afterbirth, then pass the parasites in their faeces for up to several months. If a dog defecates in stored feed or in water sources, this may result in the infection of a large number of animals and potentially an abortion epidemic.

Diagnosis of neospora is best made though a post mortem examination of aborted foetuses.

Therefore, Mr Landmann said, it was vital the foetuses were collected and submitted for the examination as soon as possible.

A blood test is available through DPI, but Mr Landmann said this should be conducted as part of a strategic sampling of the herd.

Control recommendations are difficult to make, as the full biology of this parasite in Australia is still not known.

However, producers can take steps to limit the likelihood of infection and transmission within their herds.

Where practical, Mr Landmann's advice is to limit the access of farm and wild dogs to infectious material such as aborted foetuses, stillborn calves and afterbirth and prevent exposure of exposed pastures and stored feed to dog faeces.

"It should be noted that even afterbirth from non-aborting animals might contain parasites that are infectious to dogs and cattle," he said, stressing the importance of cleaning up afterbirth.

At present, there is no drug therapy or vaccine available in Australia to treat or control neospora. The best method of eradication at this stage is the removal of infected cows from the herd.

AT A GLANCE

- DPI is working to identify and define the problem of neospora caninum in Queensland.
- Microscopic parasite was discovered 10 years ago and has been attributed with causing high abortion rates, lower milk production and reduced weight gain.
- Scientists believe 11.7pc of the western herd is infected, 16.1pc in the north, 15.3pc in Central Queensland and 21pc in the South East.
- While no drug therapy or vaccine is available in Australia to treat or control neospora, producers can contain it by removing of infected cows from the herd and by keeping dogs away from afterbirth.

● For more information contact Juergen Landmann at the DPI's Tick Fever Research Centre, Wacol, on (07) 3898 9654.

DPI research has indicated up to 21 percent of cattle in some regions of Queensland are infected with neospora caninum, a microscopic parasite which causes spontaneous abortion.

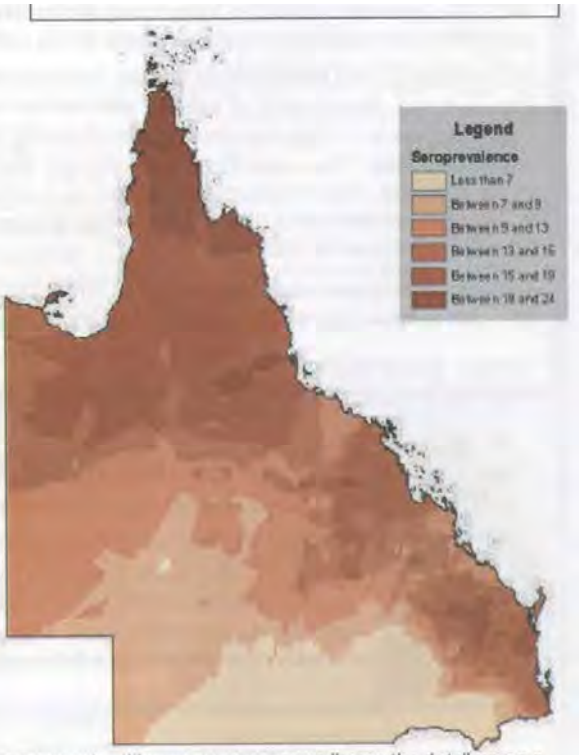
Appendix 3: Exert from DPI Surveillance Snapshots July to September 2002

Neospora seroprevalence in Queensland

Neospora caninum is a parasite that has been shown to cause abortion in cattle throughout the world. Recent research conducted at the DPI Animal and Plant Health Service's Tick Fever Research Centre has allowed further insight into the distribution of *Neospora caninum* in Queensland. Zoe Stoessel, the technician who performed the testing, reported nearly 15% of over 5700 beef cattle tested as positive. The prevalence by region was 11.7% in West, 16.1% in North, 15.3% in Central 5% in South and 21% in South East. This work was funded by Meat and Livestock Australia and supported by AgForce.

Mapping of this data by DPI's John Arrowsmith illustrated regional variations in the distribution of this parasite throughout the state. The distribution roughly follows the 500mm rainfall isohyet and the prevalence was lowest inside the dingo fence (most probably due to the lower incidence of the canine definitive host). The implications of this spatial distribution are being investigated further, but have demonstrated the value of DPI's investment in mapping for disease surveillance.

In the past year, DPI has offered serological testing for *Neospora caninum* to its producers free-of-charge to investigate this parasite as a cause of abortions occurring in Queensland cattle. Additionally, DPI performs health testing for $8.25 per sample. This has proven popular with a number of studs that have used it to screen their valuable breeding stock and reduce the congenital transmission of this parasite within their herds.



NOTE; The map was produced in much the same way that the weekly rainfall maps seen on Landline or in newspapers are produced. Sampling was done at properties around the state, as is done with rainfall measurements. The mapping program "joins the dots" using a process called kernel smoothing to produce the map. The map is intended to show trends only and does not imply that herds in a particular area will have a high seroprevalence for *Neospora*, as significant variation in herd prevalence is found across Queensland, even in high seroprevalence areas.

Contact: Juergen Landmann 07 3898 9654 or Lee Taylor 07 4992 9182

Appendix 4: **Uncorrected proof. (*Aust Vet J*: 81(3): 40-41 – *in press*)**

Prevalence of antibodies to *Neospora caninum* within central Queensland beef cattle

Z STOESSEL[a], LF TAYLOR[b], MR McGOWAN[c], GT COLEMAN[a] and JK LANDMANN[d]

Aust Vet J 2003;81:

Neospora caninum has been shown to be an important cause of abortion in cattle worldwide. Despite the considerable attention this parasite has attracted globally, and Australia's status as a major beef producer, little is known about the epidemiology of *N caninum* in Australian beef herds. The only previous report of *N caninum* in Australian beef cattle was from a diagnostic laboratory report of the aetiological cause of abortion in cattle in northern New South Wales, which found that 41% of all protozoal abortions diagnosed (presumably due to *N caninum* infection) occurred in beef herds.[1] The present study aimed to define the age-specific prevalence of antibodies to *N caninum* in central Queensland beef herds.

The study was conducted in central Queensland, which has a subtropical climate and is a major beef producing area, with over 3 million of Queensland's 11 million beef cattle and more than 10% of the national beef herd.

During 1997, blood samples were collected from 1800 beef cattle and the sera stored at -20°C as part of an animal health surveillance program conducted by the Department of Primary Industries, Queensland (QDPI). A full description of the herd selection and sampling methodology is provided by Black et al[2], albeit for earlier years. Briefly, each year 45 beef properties were selected as representative of the central Queensland administrative region, in 1997 40 of these herds agreed to participate in the survey. Only cattle that were bred on the property were sampled. Forty-five female cattle from each property were sampled, irrespective of herd size. Female cattle were selected because they were more likely to remain on the property and be available for follow-up testing if necessary. Fifteen heifers, 1 to 2 years of age and 30 older cows were sampled in each herd. Cow ages were recorded in years as it is common management practice in most herds for cows to be branded with a number being the last numeral of the year of birth. Fifteen samples were lost from each of eight properties, and a further seven miscellaneous samples were also missing, the total number of samples available for testing was 1673. The missing samples were not from any one particular region or group of properties.

Samples were tested at 1:200 dilution by the indirect fluorescent antibody test,[3] utilising in vitro derived NC-1 tachyzoites as antigen and an anti-bovine IgG (H+L; Sigma-Aldrich, Castle Hill) as the conjugate. Only bright, complete peripheral fluorescence of the tachyzoite was scored as positive.[3]

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E-mail: juergen.landmann@dpi.qld.gov.au

Of the 1673 cattle tested, 249 (14.9%) were seropositive for *N caninum*. The mean seroprevalence of individual properties was 14.7% (SD = 8.6%). Of the 40 properties surveyed, only two (5%) did not have a seropositive animal among those tested. Age-related seroprevalence rates are illustrated in Figure 1 and show a seroprevalence of around 10% in heifers with a trend of increasing seroprevalence with age.

This is the first seroepidemiological report of *N caninum* in Australian beef cattle. The results suggest that *N caninum* is endemic in beef herds across central Queensland. Seroprevalences reported for beef cattle in overseas surveys vary from 2.8% in New Zealand[4] to 24% in northwestern USA.[5] The reasons for the comparatively high seroprevalence in central Queensland cattle are not known, but may include the presence of a significant population of wild dogs and dingoes in this grazing region (which may act as definitive hosts for the parasite[6]), environmental factors (such as factors influencing the survival of oocysts in the environment, the degree of contact between wild canids) and less rigorous culling of cattle that fail to rear a calf every year compared with intensive beef production regions.

Age-related seroprevalence in the central Queensland herds suggests that both horizontal and vertical transmission is occurring (Figure 1). The gradually increasing age-related seroprevalence is suggestive of horizontal transmission, presumably from a definitive host. The seroprevalence of around 10% observed in the younger stock suggests that congenital transmission also plays a part in the natural history of *N caninum* infection in beef herds. The study design meant that in every herd, one third of all samples taken were from 1- to 2-year-old cattle. The comparatively greater numbers sampled in these age groups means that confidence limits on these means are relatively narrow. The determination of the confidence limits did not take clustering within herds or age groups into consideration. However, even considering this, the data do suggest that the seroprevalence in the 1 to 2 year age group is significantly higher than 0.

This pattern of transmission offers promise for parasite control programs. If seropositive animals were removed from breeding groups, then the overall prevalence on a property would be expected to decline significantly because the rate of horizontal infection is quite low. However, a better under-

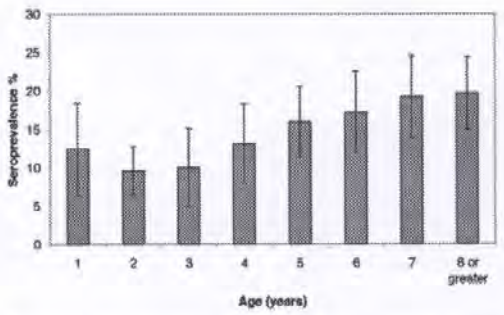

Figure 1. Mean prevalence of antibodies to *Neospora caninum* (and approximate 95% confidence limits) by age in central Queensland beef cattle.

standing of *N caninum* epidemiology is required before optimal control programs can be developed. Aspects of parasite biology requiring further attention include its epidemiology in the canid definitive host, the role of intermediate hosts other then the cow and the influence of environmental factors such as rainfall and stocking rate on maintenance of infection in herds.

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