INVESTIGATIONS INTO THE PREVALENCE OF *NEOSPORA CANINUM* ABORTION IN QUEENSLAND

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Abstract

A number of investigations are beginning to give an insight into the prevalence and effect of *Neospora caninum* on Queensland dairy and beef cattle. This presentation will give a brief overview of these and discuss their implication to the Australian cattle industry.

An investigation on the Atherton Tablelands, north Queensland, was carried out to determine the prevalence of antibodies to *Neospora caninum* in dairy cows, and the relationship between seropositive cows and abortion rates. Ten farms were selected, five with high abortion rates (>10%) in the previous 12 months, and five with low abortion rates (<5%). Twenty sentinel cows were selected at random from each of the high abortion farms and paired with 20 cows from each of the low abortion farms. All farms participated in a monthly herd health program, and all cows on these farms were pregnancy tested. Results will be presented showing the variation in abortion rate between seropositive and seronegative cows, and also between high and low abortion farms.

Antibodies to Neospora were also tested in farm dogs and wild dingoes in the area, as well as 100 rural and town dogs from the local population.

On three Atherton Tablelands farms and one south-east Queensland property, the entire herd was blood sampled, allowing for determination of the degree of vertical and horizontal transmission as well as to compare the abortion rate between seropositive and seronegative herd mates. The results from this will also be presented.

A serological survey of central Queensland beef cattle showed that approximately 15% were seropositive, with 38 of the 40 properties sampled having at least one positive in the 45 animals sampled.

Introduction

A number of surveys were carried out in major beef and dairy area is of Queensland in order to determine the significance and prevalence of *Neospora caninum* as a cause of abortion.

Initially a retrospective study was carried out to determine the geographical distribution of aborted foetuses that had lesions consistent with protozoal abortion

This was followed up by serological investigations in two distinct geographical regions:

1) ten dairy herds on the Atherton tablelands in far north Queensland
2) a dairy herd close to Brisbane

A serological study was also carried out on domestic dogs, farm dogs and wild dogs/dingoes in the Atherton Tablelands.

The Atherton Tablelands, approximately 70km south-west of Cairns, sits on a plateau at an average elevation of 700m above sea level. Atherton, towards the center of the Tablelands, has an average annual rainfall of over 1400mm/year and maximum temperatures ranging between 21°C and 30°C(1). Dairy farming is a major industry of the area, supplying milk to north Queensland and the Northern Territory, as well as producing a wide range of manufactured dairy products.

Calving in the herds is generally year-round and animals are fed on pasture throughout the year. Reproductive failure due to abortion among cattle on the Tablelands has been recognised as a major problem for quite some time. Abortion due to leptospirosis is prevalent, but a large number of mid-term abortions remain undiagnosed.

The area around Brisbane is also an all year-round calving area. A number of dairy farms in this area had experienced high levels of abortion over a short period of time and histopathological investigations implicated *N. caninum* as a possible causative agent. This is a low land area with a subtropical climate and an annual rainfall of 1143mm, primarily in summer.

Materials and methods

Survey of Protozoal Abortions in Queensland

Pathology laboratory records maintained by the Yeerongpilly and Toowoomba labs were searched over a five-year period (January 1994 to June 1999) for cases of suspect protozoal abortion. The stage of

gestation and month of the year was noted for all positive diagnoses.

Serological studies

Collection and analysis of samples

Peripheral blood was collected using vacutainers and standard collection procedures. Blood was allowed to clot for at least six hours at room temperature, centrifuged at 1000g for 10 minutes and the serum collected. Serum was frozen at –20°C until used.

Unless otherwise specified all bovine serology was performed using the IDEXX HerdChek™ Neospora caninum antibody ELISA according to the manufacturer's instructions.

For all canine serology, the Moredun *Neospora caninum* bovine ELISA was used. It was adapted for use with an anti-canine IgG enzyme conjugated antisera (Sigma). The positive control, kindly provided by the Tasmanian Department of Primary Industries, Water and Environment, was serum from a dog with clinical neosporosis and which had a high *Neospora caninum* antibody titre when tested by the indirect fluorescence antibody test (IFAT).

Atherton Tableland Herds

Selection of cows

Herds were selected from those participating in a herd health program, which involved a monthly reproductive management visit. At this visit cows were presented for pregnancy test between five and 10 weeks after service.

Initially the abortion rate for every herd in the herd health scheme was estimated from abortions recorded in 1997 and 1998. Herds were then grouped into low (<5%), medium (5-10%) and high (>10%) abortion herds.

It was decided to determine how *Neospora caninum* was contributing to abortion rates in these herds by selecting five herds from each of the high and low categories, and then selecting representative cows from each herd to monitor over a whole lactation.

The selected herds were paired on herd size and geographical location. Within these herds cows were split into age groups. Four cows were selected at random from each group in the high abortion herds and paired with cows of a similar calving date in the same age group in the low abortion herds, giving a total of 20 monitor cows for each herd. In some herds monitor cows were culled soon after the commencement of the trial and were replaced by a cow in the same age group with the closest calving date.

The study commenced in June 1999 and all monitor cows were sampled at this time and after each subsequent calving or abortion for the next 18 months. Any cow that aborted in these herds also had blood collected for *Neospora ium* serology during this period, and any foetuses that were found were post-mortemed. Unfortunately due to the extensive nature of the dairy farms and the long tropical grass not many foetuses were recovered and submitted for post-mortem examination.

Definition of Abortion

For the purpose of this survey a cow was only considered pregnant after manual diagnosis of that pregnancy. Only cows diagnosed as pregnant were considered eligible to have an abortion event. A cow that had aborted must have been previously confirmed pregnant. An abortion was defined as

1) a cow in which a foetus or membranes were observed,
2) a heat was observed following a positive pregnancy test and that cow was re pregnancy tested and found to be empty
3) a cow is diagnosed pregnant and due calving date estimated but failed to calve and on subsequent pregnancy test was found to be empty
4) a cow was diagnosed pregnant and due calving date estimated but fails to calve by 30 days after her due date and on subsequent pregnancy test was found to be pregnant to a much later service date by a bull
5) a cow was diagnosed pregnant and due calving date estimated but was pregnancy tested at drying off and found to be empty

The date of abortion for the five categories of abortion was determined as follows:

1) the abortion was attributed to that date when membranes or a foetus was observed
2) the abortion was attributed to that day before the heat or service
3) the abortion was attributed to the date midway between the drying off date and the due calving date
4) the abortion was attributed to the day before the estimated conception date for the second pregnancy
5) the abortion was attributed to the day before the negative pregnancy test

Abortion Rate

The abortion rate for the herds involved in the study was defined as the number of cows aborting per 100 cows pregnant per month

The average number of cows pregnant in a herd at any point was determined from a "cow status" report that detailed average cows pregnant and empty on any particular day. This was run on for the median date between the herd health visits for each herd from June 1999 until December 2000. It is assumed that calvings, culls and deaths were spread evenly throughout the observation period.

This information was used to calculate the total number of cow days at risk for each herd and the

incidence of abortion per 100 cows pregnant per month was derived from this figure.

To compare the incidence of abortion between seropositive and seronegative cows a lifetime reproductive history was generated that detailed every pregnancy. Each pregnancy was then recorded as either going to term or aborting.

Whole herd serology

Four herds were selected based on Neospora having been serologically detected on the property, the willingness of the owner to co-operate in the study and the availability of herd health data. Three Atherton Tablelands herds (Properties 1-3) and a South East Queensland herd (Property 4). All cattle on these properties over six months of age were blood sampled and their herd-health data analysed. Sera collected from Property 1 by Christine McClintock in 1993 as part of a leptospirosis study was also examined.

Herd records of all cattle were examined and the age, number of calves carried to term and number of abortions recorded. For each abortion the term of gestation and the year it occurred was noted. Abortions were defined as has been outlined in the previous section, the difference being that instead of the abortion rate being calculated for a specified period, it was defined as the number of abortions divided by the number of gestations, over the life of the animal. The total number of gestations was calculated for each animal by adding the number of calves born, the number of abortions and 0.4 to account for the average current gestation. The current gestation had to be taken into consideration; otherwise the abortion rates would be overestimated.

The pedigrees of all animals from the whole herd investigations were constructed to allow dam-daughter pairs to be examined for evidence of vertical or horizontal transmission. To investigate the role of vertical transmission within these herds, the serostatus of dam-daughter couplings was scored.

Canine Serology

All dogs on the participating dairy farms were sampled for antibodies to *Neospora*. Sera were also tested from any dingoes and wild dogs shot by a professional shooter in the area and 100 sera were tested that had been collected for another study involving rural and town dogs from the local population.

Central Queensland Beef Cattle.

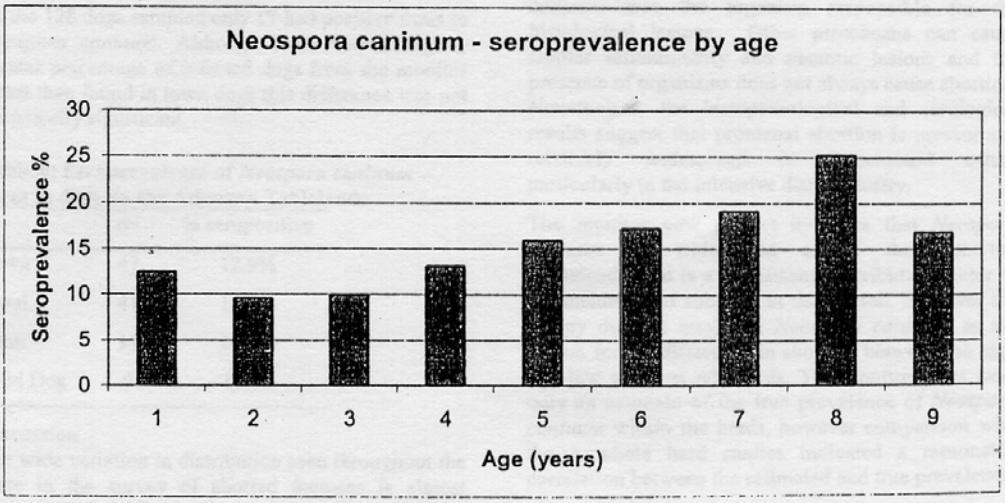
Serum stored by the DPI from the 1997 structure surveillance program was tested by the IFAT for antibodies to *N. caninum*. A total of 1673 samples from 40 properties were examined. Forty-five animals from each property were sampled, but 15 samples were lost from 8 of the properties, and a further 7 samples were also missing. The age-distribution of *Neospora* positive cattle was calculated from the data collected as part of the structure surveillance program.

Statistical analysis

The data was analysed using Microsoft Excel (2). The Chi-squared test was used where appropriate for comparison of proportions and proportions of paired data. All Chi-squared values have one degree of freedom.

Confidence intervals for the abortion rates were calculated using Confint (3)

Figure 1. Seroprevalence by age in central Queensland beef cattle



Results

Protozoal abortions in Queensland

Pathology laboratory records indicate that 30% of aborted bovine foetuses submitted contain lesions consistent with protozoal abortion. The distribution of protozoal abortion coincided well with the dairy farming regions of Queensland. A large number of cases originated from the Atherton Tableland region, as well as from farms just to the north of Brisbane. The dairying regions submitting foetuses were not equally represented.

Of the 127 diagnoses of suspected protozoal abortion made by Queensland Department of Primary Industries, 100 (79%) were in dairy cattle, 19 (15%) were in beef cattle, and 8 (6%) were not specified. Protozoal abortion was diagnosed in foetuses 14 weeks old to term, the majority of abortions examined occurring mid term (mean = 22.9 ± 4.8 weeks).

Abortions were shown to occur through out the year with no obvious seasonal variation (Figure 1)

Study of monitor cows on the Atherton Tablelands

97 cows were sampled in the high abortion herds and 100 in the low abortion herds. All herds had cows with positive titres to *Neospora caninum*, but there was a large variation in the percentage of seropositive cows in each herd (Table 1).

Table 1: Seroprevalence of Neospora titres in the monitor cows

High previous abortion rate	Low previous abortion rate
11%	10%
20%	25%
25%	30%
30%	30%
42%	45%

There was no significant difference in the prevalence of *Neospora caninum* titres between high and low herds. There was no difference in the abortion rate of *N. caninum* seropositive cows between high and low herds (Table 2).

The incidence of abortion

High abortion rate herds did have a higher incidence of abortion in seronegative cows but this was not statistically significant (P=0.11). When all results from the cows were combined irrespective of their herd group, there was a statistically significant difference in the percentage of cows aborting over the study period between the seropositive (26.4%) and seronegative (7.6%) cows (P<0.001).

Table 2: Serological results from the monitor cows

	High previous abortion rate	Low previous abortion rate
No. of cows sampled	97	100
No. seropositive	25	28
No. seronegative	72	72
No. seropositive aborted	7	7
No. seronegative aborted	8	3
Total aborted	15	10

In the 18 months of the study period there were 156 abortions in 144 cows in the high abortion group and 105 abortions in 102 cows in the low abortion herds. Nearly 50% of aborting cows in both herd groups were seropositive for *Neospora caninum* (Table 3)

Table 3: Variation in abortion incidence between high and low abortion rate herds

	High	Low
Mean Herd size	178 (± 44)	216 (± 25)
Total Number of days at risk	230479	265832
Total number of abortions	156	105
Mean abortion rate$^{\Upsilon}$	2.0 (± .7)	1.3 (± .3)
No. (& %) sampled	93 (65%)	57 (56%)
No. (& %) +ve$^{\phi}$	45 (48%)	26 (46%)
No. (&%) –ve	42 (45%)	28 (49%)

Υ Number of abortions per 100 cows pregnant per month.
ϕ Some samples were missing results

Comparison of all aborted cows in the monitor herds

A retrospective study of the outcome of every pregnancy recorded for all cows that had been sampled across all herds, indicated that the seroprevalence of *Neospora caninum* amongst animals that had aborted at some time was 61.2%, compared to 13.2% in animals that had never aborted. The incidence of abortion in seropositive animals was 11.9%, compared to just 2.7% in seronegative animals. This difference translates to a seropositive animal having a 4.4 times higher chance of aborting over her lifetime than a seronegative animal.

Whole herd serology

The seroprevalence on the three Atherton Tablelands (1-3) and the SouthEast Queensland (4) whole-herd bleeds were as follows (Table 4). The variation in abortion rate between seropositive and seronegative animals was statistically significant (P<0.001) in all four herds

Table 4: Results of whole-herd bleeds

Property	Number tested	Percent seropositive	Percent monitor cows seropositive	Number of gestations	Abortion rate[δ] in seropositive animals	Abortion rate[δ] in seronegative animals
1	247	34%	42%	846	17.1%	6.5%
2	244	31%	45%	663	12%	4.4%
3	220	23%	20%	642	14.1%	3.6%
4	186	24%	24%	319	13.7%	2.7%

δ Number of abortions recorded for lifetime of cow/total gestations for all cows tested

Table 5: Pedigree analyses from three herds

Property	Number of pairs examined	Seropositive dams with seropositive daughters	Seronegative dams with seronegative daughters
1	156	66%	82%
2	81	57%	80%
4	64	100%	96%

An association between the serostatus of the dam and daughter was highly significant ($P<0.01$) in all herds. This association indicates that vertical transmission is occurring successfully in these herds. The dam-daughter pairings from herds 1, 2 and 4 are as shown Table 5.

Central Queensland beef cattle serology

Of the 1673 cattle tested from Central Queensland, 14.9% were seropositive for *Neospora caninum*. Of the 40 properties surveyed, only two did not have a seropositive animal in the sample tested.

An increase of seroprevalence of 2-3% per year was seen from 2 to 8 years of age (Figure 2).

The influence of dogs

Of the 128 dogs sampled only 17 had positive titres to *Neospora caninum*. Although there was a slightly greater percentage of infected dogs from the monitor farms than found in town dogs this difference was not statistically significant.

Table 6: Seroprevalence of *Neospora caninum* titres in dogs on the Atherton Tablelands

	n=	% seropositive
Town	47	12.9%
Rural	41	14.6%
Farm	31	16.1%
Wild Dog	9	0.0%

Discussion

The wide variation in distribution seen throughout the State in the survey of aborted foetuses is almost certainly due to a variation in proportion of foetuses submitted for post-mortem in any one area. For example the large number of foetuses submitted from north of Brisbane around 1996 was partially due to a study being conducted on abortion in the region. The number of submissions received may also depend on the attitudes and participation of local veterinary practitioners.

The increased number of foetuses from dairy herds could reflect the more intensive nature of the dairy industry, where the farmers see their cattle at least twice a day and would be more likely to notice an abortion and recover the foetus. In contrast, the first indication many beef producers would have of an abortion is at first muster a few months after calving when cows do not have calves at foot.

Caution must be taken in assuming that *Neospora caninum* was the organism responsible for the histological lesions. Other protozoans can cause similar inflammatory and necrotic lesions and the presence of organisms does not always cause abortion. Nonetheless, the histopathological and serological results suggest that protozoal abortion is present and relatively widespread in Queensland cattle, particularly in the intensive diary industry.

The monitor cow project indicates that *Neospora caninum* is widespread across the Atherton Tablelands, and is a significant contributing factor to the incidence of abortion in this region. However the survey did not implicate *Neospora caninum* as the reason for the difference in abortion between the high and low abortion rate herds. The monitor cows were only an estimate of the true prevalence of *Neospora caninum* within the herds, however comparison with the 3 whole herd studies indicated a reasonable correlation between the estimated and true prevalence.

This study has shown that a seropositive cow is approximately 4 times more likely to abort than a

seronegative cow. This is comparable with the 3-fold increase in abortion found in the Netherlands (4) and a 3.5-fold increase in England and Wales (5). Approximately 50% of the cows that aborted in both high and low abortion herds were seropositive, which would lead to an estimation of the mean seroprevalence of *Neospora caninum* across these herds as 25%.

The pedigree analysis from farms one and two indicated that vertical transmission is occurring, infected dams having a 66% and 57% chance of having infected progeny, compared to a 34% and 31% chance respectively (the seroprevalence in these herds) if vertical transmission was not occurring. The actual success rate of the vertical transmission cannot be determined for a number of reasons, but it appears that rate of success would be comparable to the 80-90% rate reported overseas (6, 7). The pedigree analyses also clearly showed that horizontal transmission was also occurring in this area.

Transmission of *N. caninum* on property four was almost exclusively via the congenital route, as shown by the majority of seropositive animals belonging to four cow families. If horizontal transmission was occurring regularly, more infected animals would be expected in the families of seronegative animals. This farm has no domestic dogs on it and only a few wild dogs and foxes have been noticed. Due to the very small amount of exposure of this property to canids, this low rate of horizontal transmission is not surprising. As the transmission of *N. caninum* on this property was almost entirely vertical, control could be effectively achieved by not breeding replacement heifers from infected cattle. Alternatively, embryo transfer could be used to save the genetics of valuable animals, a technique that has previously been validated in this herd.

The seroprevalence of fifteen percent in the central Queensland Beef Cattle 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 (8). If this 15% infection rate is consistent across the entire state, the reproductive loss to the Queensland beef industry can be estimated at $13.5 million annually*.

Age-related seroprevalence in the central Queensland herds showed that two waves of infection were occurring. Young animals had an infection rate around 10%, a rate that increased by 2-3% per year. This suggests that 10% of all animals are congenitally infected, and horizontal transmission from the definitive host accounts for 2-3% infection per year. This rate is consistent with the rate predicted overseas (9).

Can we eradicate it or prevent its spread?

At present there is no drug therapy or vaccine available in Australia to treat or control Neospora. Control recommendations are difficult to make, as the full biology of this parasite in Australia is not known. It is advisable, where practical, to limit the access of farm and wild dogs to infectious material such as aborted foetuses, stillborn calves and afterbirth, and prevent exposure of 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 (thus cleaning up this afterbirth is also advised, if possible).

Whereas we will probably never eradicate Neospora, effective control within a herd by selective management may be possible. In the central Queensland region, the 2-3% rate of horizontal transmission in an endemically infected herd is much lower than would be expected for most infectious diseases and thus is promising from a control viewpoint, as the disease is not spreading rapidly. The 10% rate of congenital infection is also significant from a control perspective as if it can be reduced by selective breeding (or culling), the prevalence of Neospora within a herd, and the potential loss, can be significantly reduced. Dairy herds can still use infected animals for milk production, but breed them to beef to break the vertical transmission cycle. The transfer of embryos to uninfected animals by routine procedures can also be used to prevent vertical transmission occurring, and was validated in Australia on an animal from Property 4 of this study (10)

A blood test can help determine which animals are infected. This may be especially useful to studs and in pre-purchase testing. The Queensland Department of Primary Industries, Tasmanian Department of Primary Industries, Water and Environment and Agriculture Western Australian all offer serological testing via the sensitive and specific Indirect Fluorescent Antibody Test (IFAT). At present there is no drug therapy or vaccine available in Australia to treat or control Neospora.

Acknowledgments

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* 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

We would also like to thank John Morton for his advise on definitions and data analysis.

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