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## **Investigations into the epidemiology of *Neospora caninum* infection in dairy cattle in New Zealand**

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### **INTRODUCTION**

*Neospora caninum* infection is now considered a significant cause of abortion in dairy cattle in many countries with intensive dairy production around the world. The epidemiology of the infection process is still poorly understood. Transmission occurs vertically from cow to calf, but has been hypothesised to occur horizontally through indirect contact. Reservoir hosts such as farm dogs or other unidentified species may be the cause of new outbreaks. The relative importance of the different transmission paths is not known, and is likely to vary between different types of dairy cattle management systems as well as habitats.

This paper describes the results from two investigations into the epidemiology of *Neospora caninum* infection in dairy cattle in New Zealand. The first part describes the preliminary findings from a longitudinal study which was conducted subsequent to an abortion outbreak in a New Zealand dairy herd. The second part discusses results from a simulation model of the economics of *Neospora caninum* infection in dairy cattle in New Zealand.

### **LONGITUDINAL STUDY**

In 1997 an abortion outbreak occurred in a spring-calving 600 cow crossbred Friesian/Jersey herd. The last recorded outbreak had been about 10 years ago. At the time serum antibodies to

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*Neospora caninum* had been identified in some of the cows involved. Abortions in the current study were confirmed by pregnancy diagnosis during June and July.

A total of 11 leased cows were introduced to the herd in 1996/97. Ten of these animals originated from a herd which did have history of abortions possibly associated with *N.caninum* infection. No data was available on the abortion history of the herd where the remaining cow was sourced from. Yearling Murray Grey bulls were purchased every year from the same breeder.

Blood samples were collected from cows in May, June, July, October 1997 as well as January 1998. An ELISA test developed at Wallaceville Animal Health Laboratory was used to test the samples for presence of antibodies to *N.caninum*. ELISA values greater than 0.15 corrected absorbance at 450nm were classified as positive.

## **RESULTS**

### ***Descriptive analysis***

There were 69 abortions recorded from 608 cows at risk, resulting in an abortion risk of 11.3% per cow. Sera from 8 bulls and 2 steers were collected during the January sampling, and one bull tested positive.

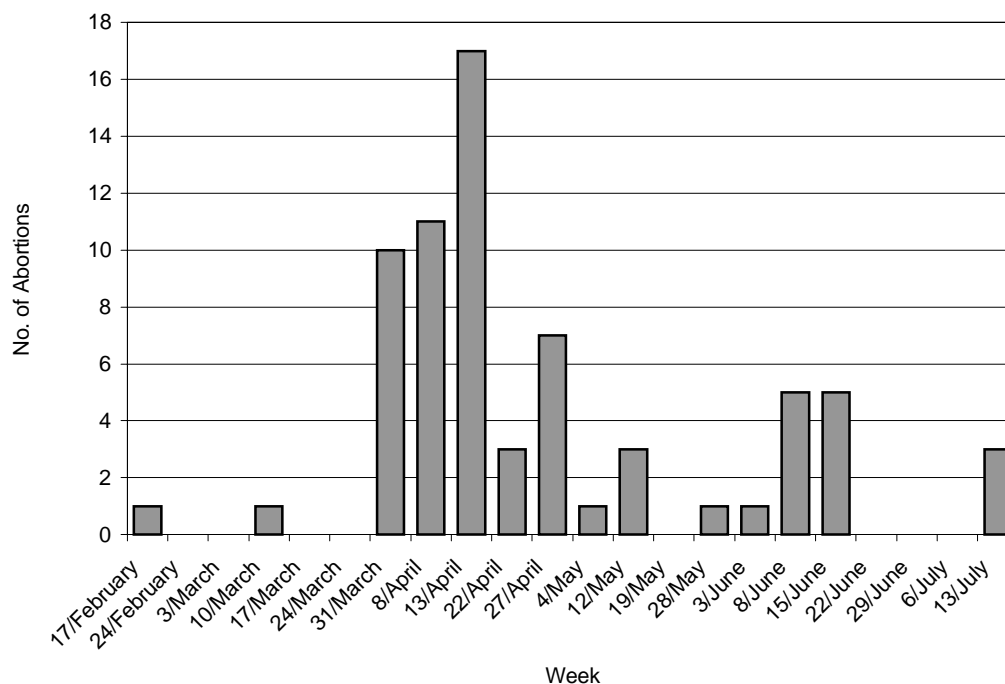
Seventy-seven heifers were leased to replace culls and 38 aborting heifers were carried over. Three dogs on the farm were bled during June and tested for *N.caninum* antibodies using the fluorescent antibody test. Two of the dogs were weakly positive with titres of 1/50.

Examination of faeces from one dog did reveal a coccidial oocyst.

Of the 10 leased cows coming from a property with abortion history likely to be associated with *N.caninum*, 2 were sero-positive in May and 1 subsequently aborted.

### ***Temporal pattern of abortion occurrence***

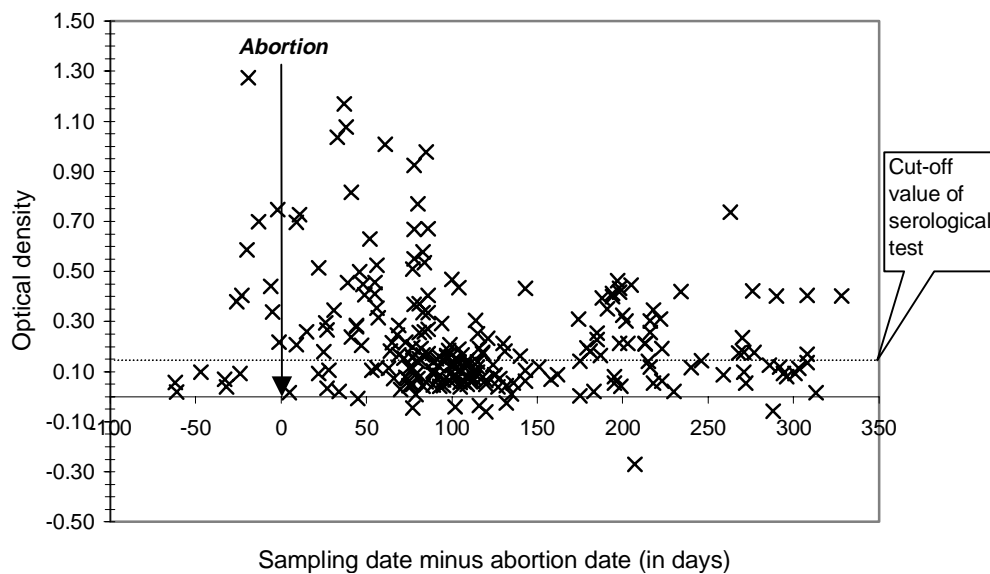
Abortions associated with the outbreak under study began on 17 February, 1997 and continued until 18 July. The peak was reached with 28 abortions occurring during the first half of April (see Figure 1).



**Figure 1: Bar chart of abortion occurrence by week**

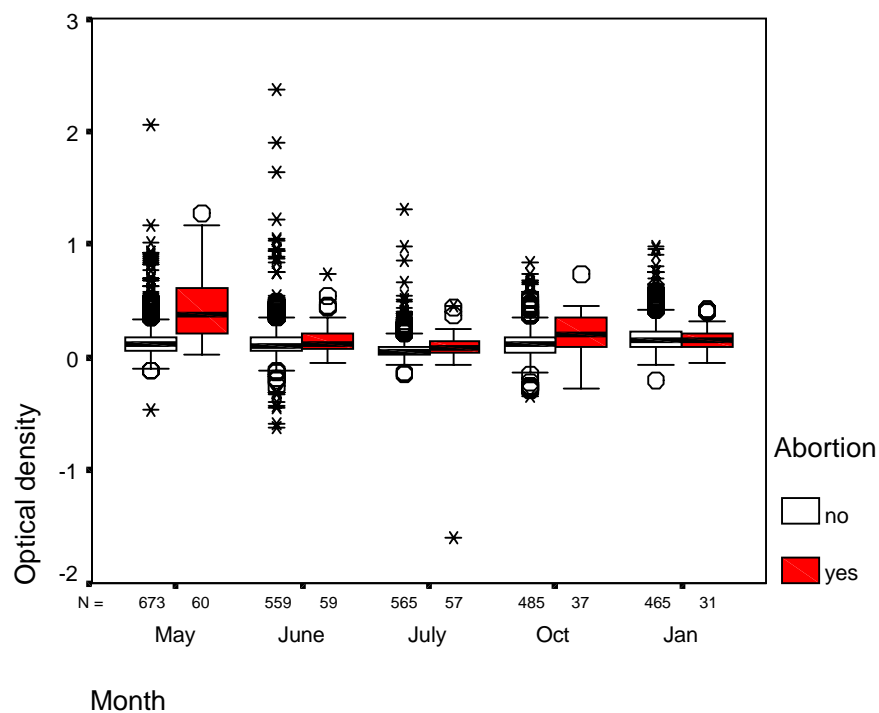
### ***Temporal pattern of serological reactivity***

The pattern of serological reactivity relative to the abortion date of individual cows which had aborted is presented in Figure 2. The scatter plot includes results from sequential samples from the same cows. Reactivity generally increased around the time of abortion, but in the majority of cows decreased to below the cut-off level of 0.15 corrected optical density by about 100 days post abortion.



**Figure 2: Scatter plot of sero reactivity relative to abortion date for cows which had aborted**

In a comparison of the temporal pattern of serological reactivity between aborting and non-aborting cows, it appears that during May cows which aborted did have higher titres than cows that did not (see Figure 3). During the following months the pattern is similar but the difference between the two groups of cows is much smaller.



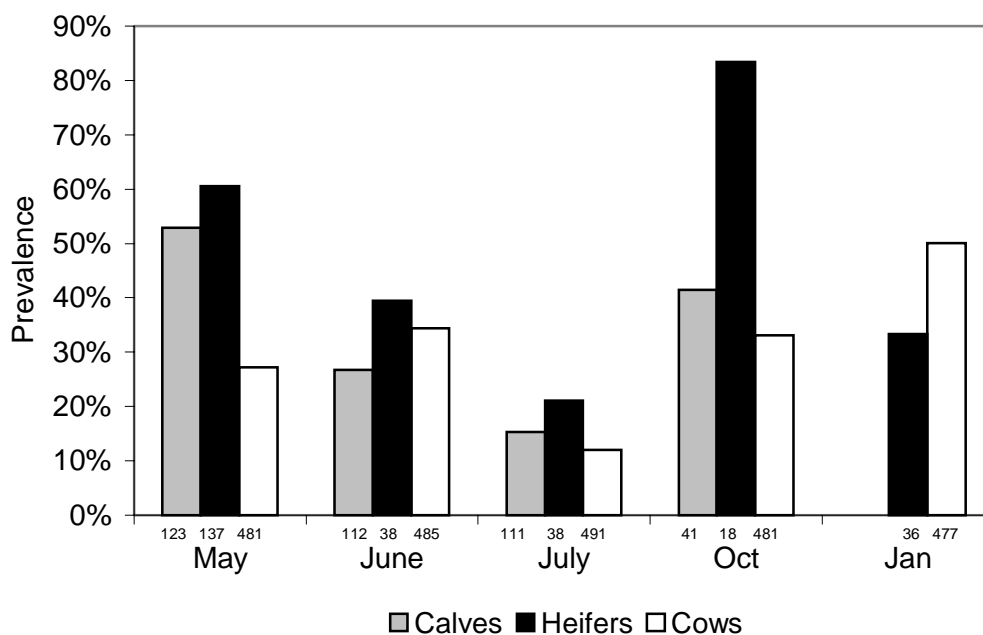
**Figure 3: Box-and-whisker plot for optical density distributions by sample month and abortion status**

Cows which had a serological reactivity of greater than 0.15 at the May sampling were 4.8 times as likely to abort than cows that did have a lower optical density (see Table 1). During the following sampling months, the risk of a sero-positive cow having aborted reduces substantially, except for October when sero-positive cows were 3.5 times as likely to have had an abort than sero-negative cows.

**Table 1: Cross-tabulation of abortion status of cows and serological status by month**

<i>Month</i>	<i>Serology</i>	<i>Abortion</i>		<i>Total</i>	<i>RR (95% CI)</i>
		<i>yes</i>	<i>no</i>		
May	Positive	52	227	279	
		18.6%	81.4%	100.0%	
	Negative	18	446	464	
		3.9%	96.1%	100.0%	
	Total	70	673	743	4.8 (2.9-8.0)
		9.4%	90.6%	100.0%	
June	Positive	24	181	205	
		11.7%	88.3%	100.0%	
	Negative	35	378	413	
		8.5%	91.5%	100.0%	
	Total	59	559	618	1.4 (0.8-2.3)
		9.5%	90.5%	100.0%	
July	Positive	13	65	78	
		16.7%	83.3%	100.0%	
	Negative	44	500	544	
		8.1%	91.9%	100.0%	
	Total	57	565	622	2.1 (1.2-3.6)
		9.2%	90.8%	100.0%	
Oct	Positive	24	157	181	
		13.3%	86.7%	100.0%	
	Negative	13	328	341	
		3.8%	96.2%	100.0%	
	Total	37	485	522	3.5 (1.8-6.7)
		7.1%	92.9%	100.0%	
Jan	Positive	15	229	244	
		6.1%	93.9%	100.0%	
	Negative	16	236	252	
		6.3%	93.7%	100.0%	
	Total	31	465	496	1.03 (0.5-2.0)
		6.3%	93.8%	100.0%	

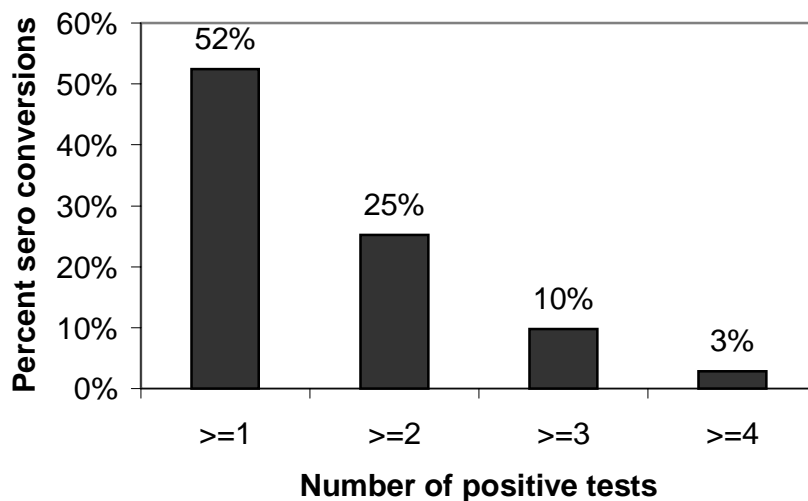
Sero prevalence was recorded as 53% in calves and 63% in heifers during the May sampling (see Figure 4). Prevalence decreased in all three stock classes in June and July, but increased again in October and January.



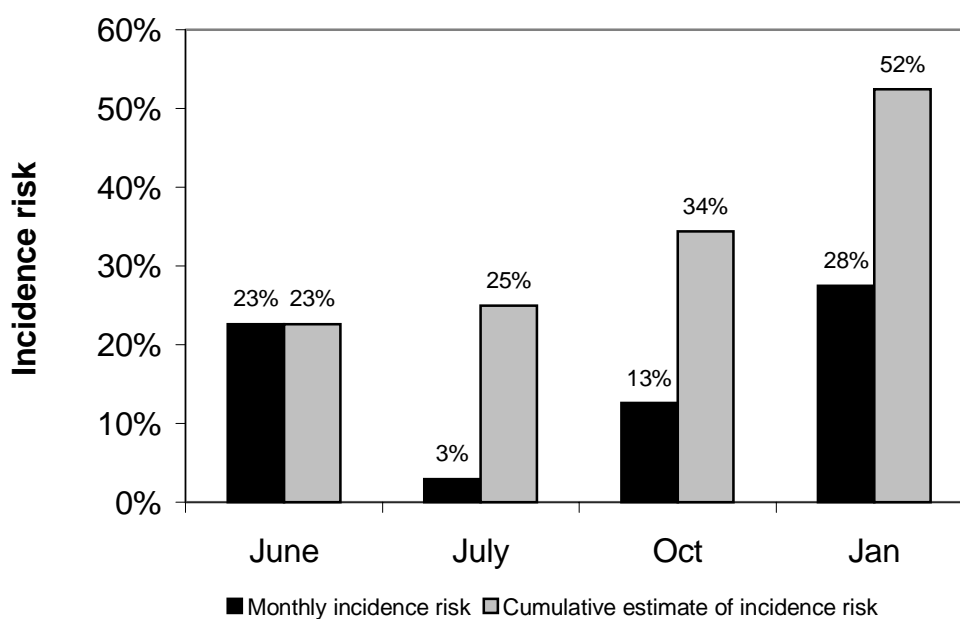
**Figure 4: Bar chart of serological prevalence by stock class and sampling month (including total number of samples)**

### ***Incidence of infection***

New serological reactions categorised as positive in cattle testing negative in the May sampling (N=349) occurred at least once during the following 4 samplings in 52% and 4 times in 3% of these animals (see Figure 5). Serological incidence risk did reach a peak in June and in January with 23% and 28% respectively (see Figure 6).



**Figure 5: Bar chart of percent sero-conversions in cattle testing negative during May sampling (categorised by number of subsequent positive tests)**

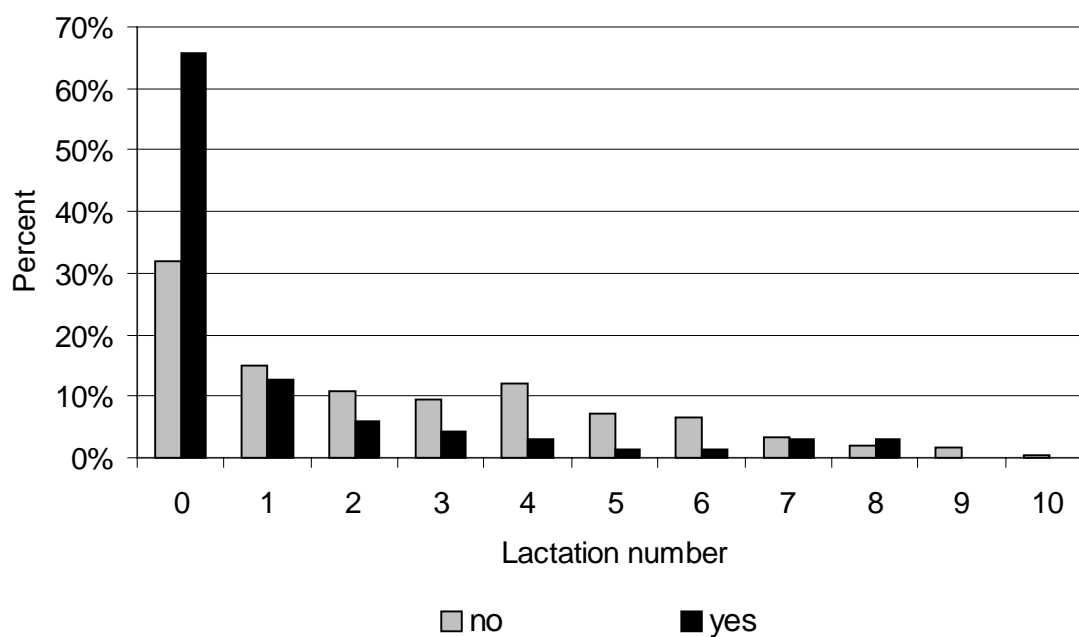


**Figure 6: Bar chart of incidence risk in cattle negative during May sampling (expressed as monthly as well as a cumulative incidence risk - until first positive test)**

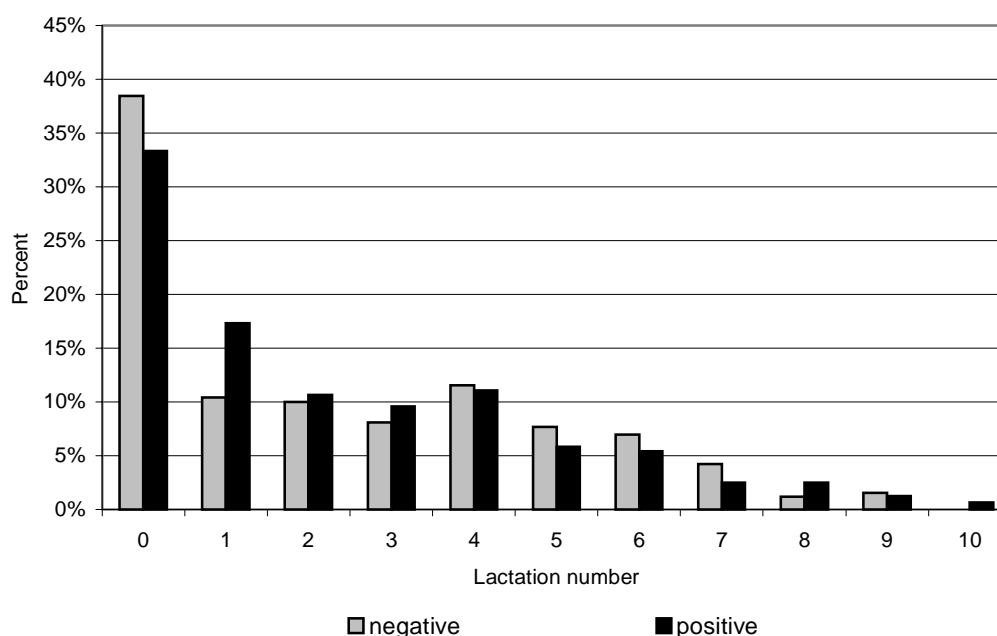
### ***Abortion risk, serological status and lactation number***

The abortion risk was highest in heifers and did reduce with age (see Figure 7). Figure 8 suggest that cows in their second lactation were more likely to have a positive serological

tests result at some stage during the study period than cows in the same lactation category which always tested negative.



**Figure 7: Bar chart of abortion risk by lactation number of cows (expressed as percentage of all aborting and non-aborting cows)**



**Figure 8: Bar chart of serological status of cows by lactation number (expressed as a percentage of total cows testing *positive* (at least once during the study period) and consistently testing *negative*)**

### **Status of dam**

The risk of being serologically positive in calves, heifers and cows was not associated with the serological status of the dam (RR=1.1, 95%CI 0.9-1.3; see Table 2). An analysis of the relationship between the serological status of the dam and abortion in the offspring (heifer or cow) does not indicate the presence of an association (RR=1.6, 95% CI 0.8-3.0; see Table 3).

**Table 2: Cross tabulation of serological status of cattle and their dams by stock class**

<i>Stock class</i>	<i>Dam serology</i>	<i>Serology</i>		<i>Total</i>	<i>RR (95%CI)</i>
		<i>Positive</i>	<i>Negative</i>		
Calves	Positive	45 59.2%	31 40.8%	76 100%	0.9 (0.7-1.2)
	Negative	29 65.9%	15 34.1%	44 100%	
	Total	74 61.7%	46 38.3%	120 100%	
Heifers	Positive	41 66.1%	21 33.9%	62 100%	1.3 (0.9-1.8)
	Negative	21 51.2%	20 48.8%	41 100%	
	Total	62 60.2%	41 39.8%	103 100%	
Cows	Positive	64 68.8%	29 31.2%	93 100%	1.2 (0.9-1.5)
	Negative	36 59.0%	25 41.0%	61 100%	
	Total	100 64.9%	54 35.1%	154 100%	
All	Positive	150 64.9%	81 35.1%	231 100.0%	1.1 (0.9-1.3)
	Negative	86 58.9%	60 41.1%	146 100.0%	
	Total	236 62.6%	141 37.4%	377 100%	

**Table 3: Cross tabulation of abortion status and serological status of dam for heifers and cows**

<i>Stock class</i>	<i>Dam serology</i>	<i>Abortion</i>		<i>Total</i>	<i>RR (95% CI)</i>
		<i>Yes</i>	<i>No</i>		
Heifers	Positive	22 35.5%	40 64.5%	62 100%	1.2 (0.7-2.2)
	Negative	12 29.3%	29 70.7%	41 100%	
	Total	34 33%	69 67.0%	103 100%	
Cows	Positive	8 8.6%	85 91.4%	93 100%	Not defined
	Negative	0 0.0%	61 100.0%	61 100%	
	Total	8 5.19%	146 94.8%	154 100%	
All	Positive	30 19.4%	125 80.7%	155 100%	1.6 (0.8-3.0)
	Negative	12 11.8%	90 88.2%	102 100%	
	Total	42 16.3%	215 83.7%	257 100%	

## DISCUSSION

The results from this longitudinal study demonstrate that in a herd which has experienced an abortion outbreak, a large number of non-aborting cows will show positive serological reactivity to *N.caninum* in the ELISA test. In this particular instance, the serological prevalence was very high in calves and heifers. Potentially, the infection could have been introduced by the heifers which had been leased, but only two of these animals did react serologically. Hence, alternative sources have to be considered as well. The majority of abortions occurred in heifers which could be an indication of an age group specific exposure or that some level of immunity was already present in the older age groups.

Serological reactivity levels in aborting cows did decrease during the 2-3 months following the abortion incidents. Pare *et al.* (1997) found in their study that in a dairy herd with endemic infection 87.5% of sero-positive cows were sero-positive throughout their pregnancy based on monthly blood samples. In the current study antibody response fluctuated strongly, but animals were not sampled throughout their pregnancy, and therefore could not be directly compared with the above result.

Sero-conversion risk in this study was 52% for animals from all three stock classes which had tested negative during the May sampling. Pare *et al.* (1997) did report a sero-conversion rate

of 8.5/100 cows/year in a herd with endemic infection. On conversion of this estimate into a risk, it would result in a much lower risk estimate than what was obtained in the current study. It strongly suggests that in this study an additional source of infection was present, and the herd therefore may not have been endemically infected with the parasite.

The data did not indicate that there was an association between serological status of the dam and abortion risk of heifers/cows or serological status of calves/heifers/cows. Anderson *et al.* (1997) did find in their prospective study that calves from 25 sero-positive heifers did show serological evidence of *N.caninum* infection. A similar result was described by Pare *et al.* (1996). In their study of herds that had abortion outbreaks the lack of an association between sero-positivity of daughter and dam was interpreted by Thurmond *et al.* (1997) as an indication of infection having occurred post-natally, which could well have been the case in the current study.

Data collection and analysis for this longitudinal study has not been completed yet. Additional blood samplings will be conducted and then hopefully allow comparisons between serological status of individual cows prior and post conception in relation to abortion risk in the upcoming 1998 calving period.

## **SIMULATION MODELLING**

From an industry or individual farmer's perspective, the importance of abortions caused by *Neospora caninum* infection has to be measured in economic terms. The losses were believed to be mainly the result of calf losses as well as milk production during the subsequent lactation period. More recently, it has been reported by Thurmond and Hietala (1997) that sero-positive dairy heifers did produce less milk volume as well as fat than sero-negative animals. Computerised spreadsheets can be used to develop simple simulation models representing the factors influencing the economic consequences of infection and abortion due to *Neospora caninum*. Parameters used in such models are often based on a number of assumptions and do not take account of any uncertainty. Hence, the results may convey false impressions as to the real predictive accuracy of these models. Limited knowledge about some the underlying processes or inherent uncertainty due to true between-farm variation can be incorporated into these models by replacing model parameters with stochastic distributions expressing the range of likely values a particular parameter may take on.

## MATERIALS AND METHODS

A computerised spreadsheet simulation model was developed to represent the economic consequences of *Neospora* abortions. The model development evolved in two phases. First a deterministic model was designed incorporating the main parameters determining expected losses through abortion caused by *Neospora caninum*. This model did not contain any uncertainty in its parameter settings. This was implemented in the second phase where this model was converted into a stochastic model.

The spreadsheet simulation model consists of two functional components: one for infection dynamics at the herd level and the other representing the economic consequences of abortion losses at the individual herd and national level. It is a modified version of a model described in Pfeiffer *et al* (1997). Within-herd infection dynamics were simulated and taken up to a national scale based on estimates of the prevalence of infected herds. The within-herd model component of the simulation model uses existing information about individual herd size and infection prevalence in infected herds (see Figure 9). The national herd model based on the same basic parameter settings is described in Figure 10. The economic consequences of abortion are modelled taking into account milk production losses, loss of calves and replacement costs. The input parameters and the resulting economic calculations are presented in Figure 11 and Figure 12 respectively. Assumptions regarding the actual risk of abortion in infected cows had to be made, as only poor or quite variable quantitative data was available. It was decided to use the conservative estimate of 5%. With respect to economic losses the assumption was made that in the case of an abortion, cows would either be carried over or culled. In the case of a carry-over, a full lactation would be lost, but in the case of a cull only a third as the cow would be replaced immediately after the abortion incident (which in turn is most likely to occur during the second or last third of the current lactation period).

The deterministic model described in Figures 1 to 4 was converted into a stochastic model by representing every parameter in the model, which was considered to be subject to variation, through an appropriate probability distribution. Triangular and Beta-PERT distributions were used for parameters such as farm herd size, number of herds, within-herd prevalence, abortion and culling risk (see Figure 13). Events such as number of infected cows, abortions and cows culled were simulated using binomial distributions. Simulation runs based on 1000 iterations were conducted. The output from all iterations was used to generate probability distributions of expected annual economic loss for the dairy cattle industry in New Zealand dollars at a farm as well as at a national level. Sensitivity analyses were conducted to assess the

importance of particular parameter estimates for the model output.

The spreadsheet software Microsoft Excel 97 (Microsoft Corporation, Redmond, WA, U.S.A.) in combination with the simulation add-in software @Risk version 3.5.1 (Palisade Corporation, Newfield, NY, U.S.A.) were used to develop the model.

Parameter	No. cows	Within-herd prevalence	Sero-positive cows	Neospora abortion risk	Within-herd abortions	Proportion cows culled	Cows culled
Setting	200	0.35	70	0.05	4	0.6	2
Formula			=200*0.35		=70*0.05		=4*0.6

Figure 9: Spreadsheet variables in within-herd model

Parameter	No herds	Prevalence of infected herds	No. infected herds	Cows at risk	Sero-positive Cows	Total abortions	Total Cull Cows
Setting	15000	0.3	4500	900000	315000	15750	9450
Formula			=15000*	=4500*200	=900000*	=315000*	=15750*0.6
			0.3		0.35	0.05	

Figure 10: Spreadsheet variables for national dairy cattle herd

Milk payout	Animal values
Fat 160.00 kg	Boner cow value NZ\$ 300.00
Price /kg NZ\$ 6.50	Replacement cow value NZ\$ 900.00
	Lost calf sales NZ\$ 64.00

Figure 11: Spreadsheet variables for economic input parameters

Parameter	National	Farm
Milk	Full season NZ\$ 6,850,480.00	NZ\$ 2,080.00
	Third of season NZ\$ 5,718,398.40	NZ\$ 1,716.00
Animal	Cost culls NZ\$ 14,995,800.00	NZ\$ 4,500.00
	Value culls NZ\$ 4,998,600.00	NZ\$ 1,500.00
	Lost calves NZ\$ 1,487,936.00	NZ\$ 448.00
	Total Loss NZ\$ 24,054,014.40	NZ\$ 7,244.00

Figure 12: Spreadsheet variables for summary economic calculations

Parameter	Minimum	Most likely	Maximum
Size of national dairy herd	14000	14800	15000
Prevalence of infected herds	0.05	0.2	0.6
Herd size	50	200	300
Within-herd prevalence	0.1	0.3	0.5
Abortion risk	0.02	0.05	0.5
Culling probability after abortion	0.6	0.7	0.9

Figure 13: Input parameters used to define BetaPERT distributions

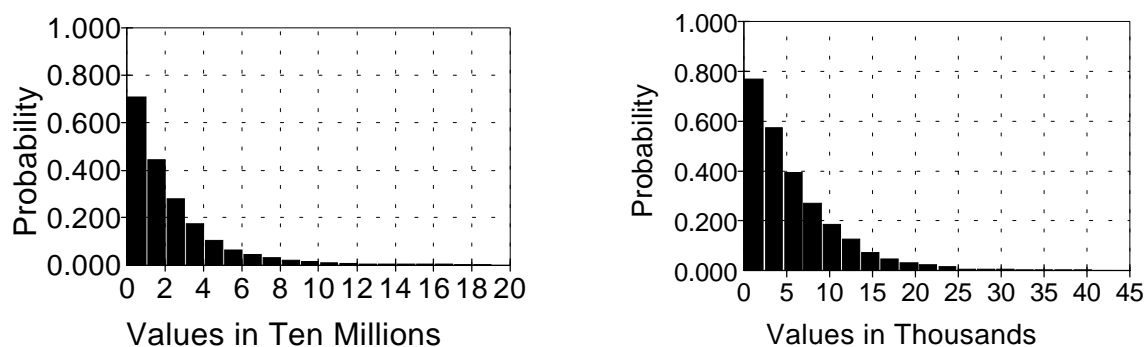
## RESULTS

The deterministic version of the simulation model estimated an average annual loss of about

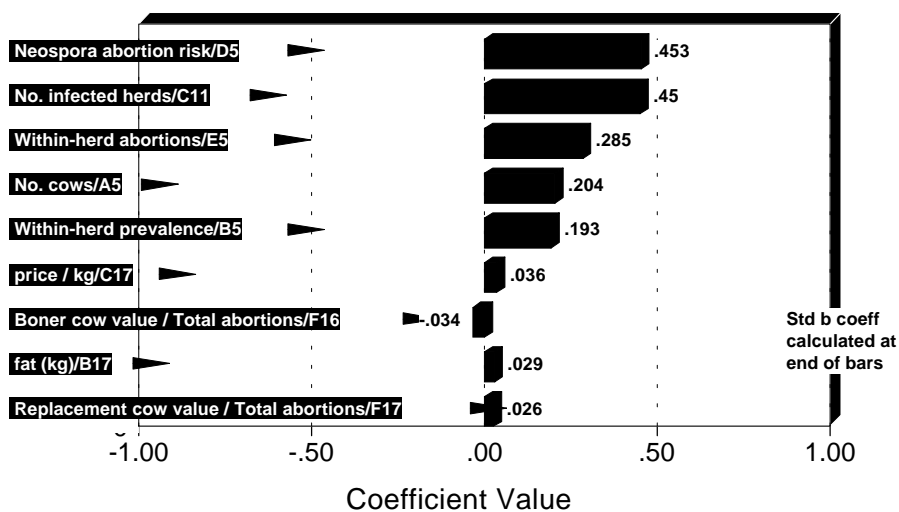
NZ\$ 24 million at a national and NZ\$ 6800 at the farm level (in the case of endemic *Neospora* infection), based on the input parameters defined in Figure 9, Figure 10, Figure 11 and Figure 12. Introduction of stochastic parameters resulted in a distribution for predicted average annual economic loss for the dairy industry with a median value of about NZ\$ 17.8 million and a mode of NZ\$ 11.8 million (see Figure 14A). There was a 70% chance that the loss would exceed NZ\$ 10 million per annum. At the farm level, the distribution of expected losses on farms with endemic or epidemic infection were expected to vary between none and NZ\$ 43000 with a median of NZ\$ 5400 (see Figure 14B). The loss was at least NZ\$ 3000 per year in 70% of infected farms. A model sensitivity analysis revealed that model output was strongly influenced by the probability of an abortion given *Neospora* infection and the prevalence of infected herds as well as the within-herd prevalence (see Figure 15). All three parameters are uncertain as only limited field data is available.

A: National level

B: Farm level



**Figure 14: Cumulative descending histogram distributions for expected economic losses from *Neospora* abortions (expressed as probability of losses exceeding the value plotted on x-axis)**



**Figure 15: Results of sensitivity analysis for national herd presented as a tornado graph**

## DISCUSSION

The design phase of the model was extremely useful, as it was possible to define areas in the epidemiology of *Neospora caninum* infection/abortions where additional field studies were required. These include estimates of the national prevalence of infected herds, as well as within-herd prevalence. There is also a difference between herds which are endemically infected with *Neospora caninum* and those which experience abortion outbreaks. The first group may experience continual losses at a low incidence, and possibly outbreaks every couple of years. The second group experiences an outbreak, and may then become endemically infected. Hence, the risk of an infected cow aborting may depend on a number of factors not currently known. We also need more information about the likelihood of repeated abortions in the same cow. Once these studies have been conducted the model will be used to generate more accurate predictions of economic losses, and to evaluate the benefits of implementing particular control methods.

The results of the simulations strongly support the common belief that *Neospora caninum* abortions are very costly for dairy farming in New Zealand. This was the case although many model parameters were based on conservative estimates. The model can be used to produce output based on different input parameter settings, reflecting different views of the current understanding as to the correct quantitative estimates. Depending on the outcome of further research into the relationship between sero-positivity to *Neospora caninum* and milk production, it may be necessary to introduce this effect into the economic model.

The development of this model demonstrates the usefulness of computerised spreadsheets for designing simple and effective epidemiological models without the need to develop advanced computer-programming skills. Given its simple structure and the use of meaningful input parameters / distributions it was also possible to discuss the model mechanisms and parameters with a group of experienced veterinarians who had only limited computer modelling skills. The model also shows that summarising expected outcome values using single values such as averages can be quite misleading, specifically if they are derived on the basis of a series of input values for whom the true value is unknown.

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