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Short communication

Prevalence and distribution of *Neospora caninum* in water buffalo (*Bubalus bubalis*) and cattle in the Northern Territory of Australia

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ABSTRACT

The seroprevalence of *Neospora caninum* infection in water buffalo (*Bubalus bubalis*) and domestic cattle in the Northern Territory (NT) of Australia has never been determined. A total of 480 serum samples from water buffalo and 192 serum samples from cattle, collected by the NT Government from 1993 through to 2001, at 18 different survey sites throughout the Northern Territory were tested by commercial ELISA for anti-*N. caninum* antibodies. The water buffalo samples demonstrated a seroprevalence of 88.3% (95% CI ± 2.9%), while 31.8% (±6.1%) of the cattle sera tested positive for *N. caninum* antibodies. Individual buffalo from the same herd, sampled over years, showed considerable fluctuations in S/P ratios. Overall, seropositivity was consistent across buffalo herds, and showed a slight decline over the years. The study presents evidence for the first time that *N. caninum* infection in water buffalo in the Northern Territory is a highly endemic and that infection rates are higher than those for cattle. This is important for an understanding of any potential sylvatic life cycle of *N. caninum* in Northern Australia. This survey also tests cattle from that territory for the first time for evidence of *N. caninum* infection and makes an important contribution to the understanding of disease management issues for the beef industry in the region.

Neosporosis, caused by the coccidian parasite *Neospora caninum*, is one of the leading infectious causes of abortion in cattle worldwide. It is estimated to cost the Australian beef industry up to $US139.5 million annually [1]. *N. caninum* has been identified in a wide range of hosts, both domestic and wild, and is maintained in a bovid–canid lifecycle [2]. Canids, including the Australian dingo [3] act as definitive hosts, acquiring infection though the ingestion of intracellular tachyzoites or tissue cysts, found in the central nervous system of infected intermediate hosts. Oocysts are passed in the faeces of the definitive host and once sporulated, become infective to intermediate hosts when ingested. Tachyzoites are transmitted transplacentally to the foetus during pregnancy and may cause abortion. Currently, no economically viable method of controlling the disease has been established [4].

The existence of a sylvatic cycle of *N. caninum* complicates the management of the disease in domestic herds [5]. *N. caninum* has been identified in wild canids and dingo in Australia [3,6], and, world-wide in many wild ruminant species, including water buffalo (*Bubalus bubalis*) [7]. Water buffalo have tested positive for *N. caninum* in multiple countries of Asia [8–12], Europe [13], South America [14,15] and Africa [16]. From the worldwide literature it would appear that water buffalo generally show a higher seroprevalence than domestic cattle [2].

In Argentina, *N. caninum* infection has been identified in co-existing domestic cattle and buffalo populations [15]. These buffalo had a higher calving and weaning rate than the co-habiting domestic cattle, despite a higher seroprevalence for *N. caninum*. Only few studies, have, thus far, shown links between naturally acquired *N. caninum* infection and abortion in *B. bubalis* [8,13], although experimentally abortion can be induced readily [17].

There are a number of tests available for the serological diagnosis of *N. caninum* in bovid species [18]. The indirect fluorescent antibody test (IFAT) is considered the gold standard for the diagnosis of neosporosis. ELISA testing is more suitable for large-scale testing. While ELISA testing of cattle for *N. caninum* antibodies has become the *de-facto* standard [19], there are few examples of this technology being used in the testing for *N. caninum* testing in water buffalo. Competitive ELISA testing to identify *N. caninum* antibodies in *B. bubalis* serum samples has been validated by quantifying the ELISA results against an IFAT performed on the same samples [20].

The aim of this study was to assess the prevalence of infection with *N. caninum* in water buffalo and cattle in the Northern Territory (NT) of Australia, where feral and farmed buffalo co-exist with extensively farmed domestic beef cattle populations. This is the first survey of this nature in Australia.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFAT, indirect fluorescent antibody test; S/P ratio, sample to positive ratio.

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A total of 480 water buffalo (*B. bubalis*) serum samples were obtained through the Berrimah Veterinary Laboratory, Darwin from 18 different survey sites in the NT, north of Tennant Creek. These samples had been collected by the NT Government as part of an ongoing disease surveillance scheme at varying intervals, from 1993 through to 2001. The samples had been kept frozen and stored at −82°C until testing. Data for the year and location of each sample taken was collected. However, there was no information available regarding the age or sex of the buffalo, and only limited information whether they were wild caught or taken from animals in domestically managed herds. Some animals were sampled multiple times from the same locations over many years. Buffalo generally reside in the coastal, tropical wet regions of the Top End of the Northern Territory.

One hundred ninety two samples were collected from domestic cattle from similar regions as the buffalo, spanning the same timeframe.

The serum samples were analysed using an indirect enzyme-linked immunosorbent assay (ELISA) technique. For the testing of buffalo sera, the IDEXX Neospora Ab test [1] was used in this study, as it uses an anti-ruminant IgG conjugate, and has been validated to identify anti-*Neospora* antibodies in sheep in New Zealand [21].

In an initial study, the ELISA was used to identify anti-*Neospora* antibodies in 60 *B. bubalis* sera from Pakistan [20] (42 positive, 18 negative) and results validated by comparing ELISA results against an indirect fluorescent antibody test (IFAT). The results of that study (not shown) were used to arrive at a cut-off value of a Sample to Positive (S/P) ratio of 21% for ELISA seropositivity (equivalent to an IFAT tire of 1:200), which was used in the present survey to maximise the test's diagnostic sensitivity at 97.6% (95% CI ± 2.4%) (also resulting in a diagnostic specificity of 100% ± 0%). This is a deviation from the manufacturer's recommended cut-off value for ruminants of 40% for this assay (there S/P ratios between 30% and 40% results are classed as suspicious), but approximately double the modified cut-off threshold that had been previously established for sheep [21].

To analyse the domestic cattle samples, the IDEXX Neospora X2 Ab test was used, which uses an anti-bovine IgG conjugate [2]. The recommended cut-off value stated by the manufacturer is an S/P ratio of 50%. In order to maximise the assay's diagnostic sensitivity, an S/P ratio of 21% was chosen for the present survey as previously described [22], which was shown to be approximately equivalent to an IFAT titre of 1:200.

For the buffalo samples, survey sites where less than 10 samples had been collected were excluded from further analysis for location prevalence, leaving a total of 6 survey sites and 437 sample results to be analysed further. For the annual seroprevalence calculations, years in which less than 10 samples were collected were also excluded, leaving 420 samples included in analysis for seroprevalence over time.

95% confidence intervals of the proportions were calculated according to the following formula [23]:

$$\textit{Confidence Interval}\ (95\%) = 1.96\sqrt{\frac{p(1-p)}{n}}$$

where n is the number of samples and p is the proportion of positive samples in the sample population (n).

Statistical significance of the differences between the proportions of sero-positive cattle versus water buffalo was tested for by using the z-test statistic: (http://epitools.ausvet.com.au/content.php?page=z-test-2):

Antibodies S/P ratios against *N. caninum* in water buffalo sera ranged from −2.1% to 157.0%. With the newly established cut-off threshold of an S/P ratio of 21%, 424 out of 480 water buffalo samples were regarded as positive, resulting in a total seroprevalence of 88.3% (95% CI ± 2.9%) (Table 1).

Of the cattle sera, 61/192 were identified as seropositive at a threshold of 21% [22], resulting in an overall seroprevalence of 31.8% (95 CI ± 6.1%) (Table 1). Histograms of the frequency distributions of S/P ratios across the two sample populations are shown in Fig. 1a and b.

If manufacturer-recommended cut-off thresholds were applied to the buffalo or cattle sera respectively, the overall seroprevalence value was only slightly reduced in water buffalo, to 85.4% (95% ± 1.6%), at a threshold of 40% (the manufacturer's recommended one signifying positivity), but reduced to 17.7% (95% ± 2.7%) (at an S/P ratio of 50%) in cattle, respectively. Only nine (1.9%) water buffalo sera would have fallen in the manufacturer's suspicious range, between 30% and 40%.

Seropositive animals were identified at all of the 18 survey sites (Fig. 2). Twelve survey sites (where $n < 10$) were excluded from further analysis. Three hundred eighty five of the remaining 437 samples from 6 survey sites were considered positive and the revised seroprevalence was calculated to be 88.1% (±3.0%). There was little variation in seroprevalence across these herds. When years during which $n < 10$ were excluded from further analysis, 369 out of 420 samples were seropositive (87.9% ± 3.1%). There was a trend for a slight decrease in average seroprevalence from 1994 to 2001.

Table 1
Test results of 480 water buffalo and 192 cattle samples from the NT that were regarded positive (plus total numbers, percentage and 95% CI) from each of 18 locations tested for *Neospora caninum* antibodies using the IDEXX *Neospora* Ab ELISA [1] and IDEXX Neospora X2 Ab ELISA [2], respectively regarding a cut-off value for a Sample to Positive Ratio of 21% as being positive.

	Buffalo				Cattle			
	Positive (n)	Total (n)	Prevalence (%)	95% CI (%)	Positive (n)	Total (n)	Prevalence (%)	95% CI (%)
Berrimah ARC [a]	1	3	33.3	53.3	8	23	34.8	19.5
Cape Arnhem	1	1	100.0	0.0				
Cobourg Peninsula	13	14	92.9	13.5				
Beatrice Hill [a]	275	298	92.3	3.0	16	57	28.1	11.7
Daly River Port Keats LT	5	5	100.0	0.0	8	23	34.8	19.5
Darwin Rural Area [a]	4	4	100.0	0.0	4	15	26.7	22.4
Koolatong	2	2	100.0	0.0				
Maningrida	4	4	100.0	0.0				
Marrakal	1	1	100.0	0.0				
Milingimbi	1	1	100.0	0.0	12	34	35.3	16.1
Murgenella	6	6	100.0	0.0				
Nathan River	1	1	100.0	0.0				
North East Arnhem	30	50	60.0	13.6				
North West Arnhem	18	20	90.0	13.1				
Numbulwar	7	8	87.5	22.9				
South East Arnhem	14	14	100.0	0.0				
Tiwi ALT	35	41	85.4	10.8				
Walker River	6	7	85.7	25.9				
Other NT					13	40	32.5	14.5
Total	424	480	88.3%	2.9%	61	192	31.8	6.6

[a] Farmed.