



Review

A review of *Neospora caninum* in water buffalo (*Bubalus bubalis*)Michael P. Reichel^{a,b,*}, Milton M. McAllister^a, Amar Nasir^c, Dadin P. Moore^d^a School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy Campus, Roseworthy, SA 5371 South Australia, Australia^b City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, China^c Department of Clinical Sciences, College of Veterinary & Animals Sciences, Jhang Subcampus, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan^d Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

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ABSTRACT

A number of countries in the world have reported infections with *Neospora caninum* in water buffalo (*Bubalus bubalis*), from Africa to Asia, Europe and South America and recently Australia. In general, clinical manifestations (such as abortion) seem rare, which has raised the prospect that buffalo may be inherently resistant to clinical effects of *N. caninum* infection. Worldwide, the seroprevalence of *N. caninum* infection (as a measure of exposure determined by the detection of antibody) in buffalo is high, at approximately 48%. This reported seroprevalence is three or four times higher than that reported from the world's cattle populations, which have collective seroprevalence rates of 16.1% for dairy cattle and 11.5% for beef cattle. However, there is a lack of standardisation in seroprevalence studies and some studies may well under-estimate the true level of infection. Epidemiologic evidence supports post-natal transmission, and *in utero* transmission has also been demonstrated. The causes for water buffalo to have markedly higher seroprevalence but apparently lower neosporosis abortion rates than cattle warrant further investigation.

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1. Introduction

Neospora caninum has been extensively described as an abortifacient of cattle world-wide, seriously impacting economically both dairy and beef cattle industries (Reichel et al., 2013). Apart from cattle however, a review of the literature suggests that the Asian water buffalo (*Bubalus bubalis*) is a common intermediate

host of this apicomplexan parasite, maybe even more so than cattle. In many countries of the world, the water buffalo (*B. bubalis*) is very important economically – as a draft animal, for its milk and dairy products and its meat. In situations where cattle and buffalo inhabit the same environment, water buffalo appear to show a higher sero-prevalence of infection, despite what should be, at face value, similar potential for exposure (Moore et al., 2014).

N. caninum infections have been reported for thirty years, initially from dogs, then from calves with congenital neurological disease (O'Toole and Jeffrey, 1987; Parish et al., 1987), and subsequently from aborting cattle (Bjerkås et al., 1984; Shivaprasad et al., 1989; Thilsted and Dubey, 1989). The first report of antibodies in water buffalo goes back to 1995 and reported a sero-prevalence of

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Table 1

Records of *N. caninum* infection (as measured by testing for antibody, by Indirect Fluorescent Antibody Testing (IFAT), Neospora Agglutination Test (NAT) or Enzyme-linked Immunosorbent Assay (ELISA)) in water buffalo (*Bubalus bubalis*) in countries around the world (where available, information about conjugate used in brackets).

Continent	Country	Year	n	Number positive	Prevalence (%)	Assay used	Cut-off threshold	References
Asia	China	2005	40	0	0.0	Farm	16.7	(Yu et al., 2007)
	India	2004	32	16	50.0			(Meenakshi et al., 2007)
		2008	341	34	10.0			(Sengupta et al., 2012)
	Iran	2014	122	76	62.3			(Hamidinejat et al., 2015)
	Pakistan	2010	300	164	54.7			(Nasir et al., 2011)
	Philippines	2007	105	4	3.8			(Konnai et al., 2008)
	Thailand	2015	628	57	9.1			(Kengradomkij et al., 2015)
Europe	Vietnam	1995	200	3	1.5	IFAT (anti-bovine) ELISA/IFAT (anti-bovine)	1:100 1:640	(Huong et al., 1998)
	Italy	1999	1,377	476	34.6		1:200	(Guarino et al., 2000)
		2014	908	463	51.0	ELISA (multiple species))		(Auriemma et al., 2014)
South America	Argentina	2006	449	287	63.9			(Campero et al., 2007)
		2012	500	211	42.2	IFAT (Bovine) IFAT (anti-bovine) IFAT (anti-bovine)	1:100 1:100 1:100	(Konrad et al., 2013)
		2013	1,350	584	43.3		(Moore et al., 2014)	
	Brazil	2000	222	142	64.0	NAT/IFAT (anti-bovine) IFAT (anti-bovine) ELISA/IFAT (anti-bovine) IFAT (not stated)	1:25 1:25 1:100 1:50	(Fujii et al., 2001)
		2004	196	139	70.9		(Gennari et al., 2005)	
		2013	4,796	2,662	55.5		(da Silva et al., 2014)	
		2014	192	169	88.0		(Chryssafidis et al., 2015)	
Africa	Egypt	1995	75	51	68.0	NAT NAT	1:20 1:80	(Dubey et al., 1998)
	Kenya ^a	2001	4	2	50.0		(Ferroglio et al., 2003)	
Oceania	Australia	2014	480	424	88.3	ELISA (anti-ruminant)		(Neverauskas et al., 2015)
Total			12,317	5,964	48.4 (95%CI ± 0.9)			

^a African buffalo (*Synacerus caffer*).

1.5% from 200 water buffalo blood samples collected at an abattoir in Vietnam (Huong et al., 1998).

Since then, there have been a number of reports on the prevalence of *N. caninum* antibodies in various countries (summarised in Table 1). Compared to the reported sero-prevalence of infection in dairy or beef cattle (at 16.1% and 11.5%, respectively, as summarised in a recent world-wide review (Reichel et al., 2013)), the overall level of exposure to *N. caninum* in water buffalo appears to be at least three times higher than that reported from cattle. There is also a seemingly rising interest in studies of *N. caninum* in water buffalo, as the number of publications cited on Pubmed is increasing (but still only represents less than 5% of the several hundred publications on *N. caninum* abortions in cattle in the same forum) (Reichel et al., 2013).

In situations where buffalo and cattle inhabit the same area, buffalo were approximately 1.5 times more likely to be sero-positive than their cattle cohort (48.3% versus 28.3%, respectively) (Moore et al., 2014). In Asia, the buffalo is the main milking animal and most farmers have only a few individual animals that are often hand fed. This may be the reason that, in the Vietnamese study, that situation appeared reversed (Huong et al., 1998), however, with cattle in that study having a sero-prevalence of 5.5%, compared to the 1.5% reported from the buffalo. That study, however, also applied an extremely high cut-off threshold in IFAT to signify infection with *N. caninum* in buffalo, and may likely be a gross underestimate (see below and Fig. 1) of the true infection rate.

The isolation of viable *N. caninum* from tissues of naturally exposed buffaloes indicates that water buffalo is an intermediate host for this parasite. Four out of seven dogs fed the brain of infected buffalo in Brazil shed oocysts; oral inoculation of oocysts induced clinical neosporosis in gamma-interferon knockout mice (Rodrigues et al., 2004).

Despite the high observed sero-prevalence, it appears that associations between infection with *N. caninum* (sero-prevalence) and abortion events, as is clearly demonstrated in cattle (Dubey et al., 2006), may be less common in water buffalo. Even in experimental infections that would cause abortion in cattle (usually within three weeks), no water buffalo aborted within a four week observation period, although 1 fetus of 3 had died (Konrad et al., 2012). Abort-

ing water buffalo in Pakistan, however, had an approximately 20% higher sero-prevalence compared with non-aborting ones (Nasir et al., 2011), and in Italy, *N. caninum* DNA has been detected in a few aborted foetuses (Auriemma et al., 2014), hence naturally occurring neosporosis abortions do occur in water buffalo, much as they do in cattle. One problem might be that abortions in buffalo might go unreported, because they tend to be used in regions that are less economically developed and thus abortions are less likely to be noted or thoroughly examined. Another issue might be that buffalo reproduction might be, in general, less efficient than that of cattle, and additional losses from reproductive failure go unnoticed. Also, the reproductive physiology of buffaloes is different from cattle; buffaloes are seasonal breeders (Chryssafidis et al., 2015).

2. Clinical signs and pathology

Descriptions of the lesions caused by *N. caninum* infection in water buffalo are rare, as only a few studies have described associations between *N. caninum* and naturally occurring abortions in buffalo (Auriemma et al., 2014; Nasir et al., 2011). Experimental infections have been induced using intravenous inoculation of tachyzoites between 70 and 90 days of gestation, and histopathological examination of fetuses 4–6 weeks later. The lesions induced were similar to those typical of bovine neosporosis and to lesions previously described in naturally aborted water buffaloes, consisting of nonsuppurative inflammation in placenta, brain, heart and other organs (Dubey and Schares, 2006).

2.1. Naturally observed abortion events

In an earlier study in Italy (Guarino et al., 2000), amongst herds with high anti-*N. caninum* antibody prevalence (34.6%), four aborted buffalo calves were examined histologically on liver, heart, kidney and brain tissue. Three further newborn calves died with neurological signs. Nonsuppurative encephalitis and myocarditis were observed in two of the examined aborted foetuses, and in two fetuses protozoan-like cysts were observed; however their identity was not confirmed.

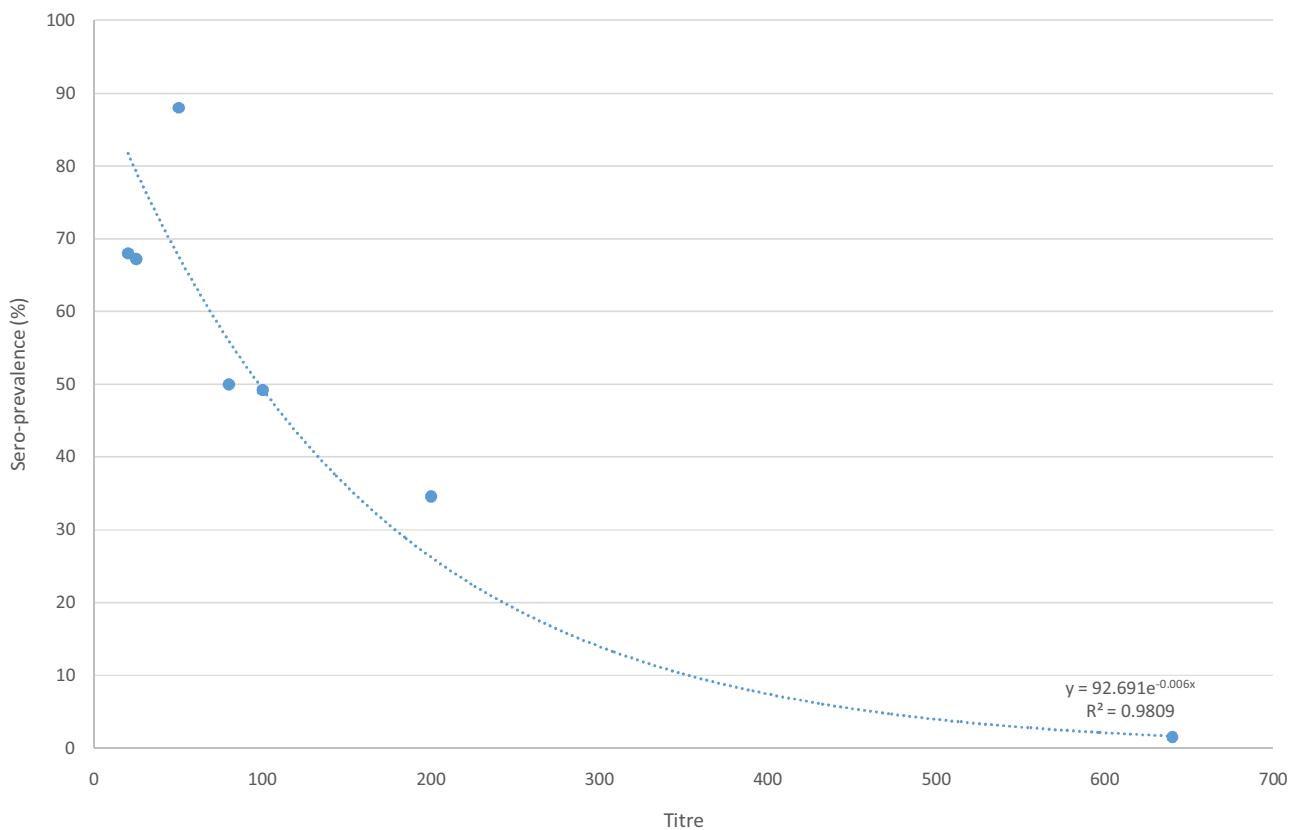


Fig. 1. Sero-prevalence (in%) of *Neospora caninum* in water buffalo (*Bubalus bubalis*) reported in various publications around the world in relation to the cut-off titre (inverse of the serum dilution) chosen in the agglutination assays (IFAT and NAT).

In another Italian study (Auriemma et al., 2014), three abortions were observed over a four month period in two buffalo herds ($n=909$) tested. These occurred, as in cattle, in mid-gestation, at between 4 and 6 months. Lesions observed were confined to the brains (focal necrosis, perivascular cuffing and glial nodules) and hearts (focal infiltration with mononuclear cells with minimal necrosis) of these fetuses. PCR testing detected *N. caninum* DNA in all lesions of all fetuses. No other known abortifacients were observed suggesting that *N. caninum* was the sole cause.

In the Pakistani study (Nasir et al., 2011) ($n=300$; of which 252 were female), in the first instance sero-prevalence for *N. caninum* was high (54.7%), which reached 60% in pregnant animals ($n=115$). Amongst those, dams that had aborted had a significantly higher prevalence of *N. caninum* antibodies at 78.9%, compared to those that had not aborted (55%).

2.2. Experimentally induced infections/abortions

Experimental infection of water buffalo calves with *N. caninum* tachyzoites results in sero-conversion (as measured in IFAT) at between 7 and 11 days post infection (Rodrigues et al., 2005) with titres peaking at about 3 weeks post-infection and declining to almost detection threshold within a year.

Two studies that attempted experimentally to reproduce neosporosis in water buffaloes have been performed. Konrad et al. (2012) did not observe abortion or other clinical signs in any of ten dams after intravenous inoculation with 10^8 tachyzoites of the NC-1 strain at 70 or 90 days of pregnancy, however one of the 70 day fetuses was not viable when examined 3 weeks later. The observa-

tion period post-inoculation was only 28 days in seven of the ten inoculated dams, and 42 days in the remaining three.

Nonsuppurative placentitis was found in 90% of the infected dams. Nine out of ten fetuses had detectable *N. caninum* DNA by PCR, as had eight of the ten placentas. Mononuclear inflammatory foci were observed in brain (but only in 40% of the infected fetuses), while lesions in heart, lung, muscles and liver tissues of infected fetuses were observed in at least 80% of cases. Placental lesions induced in these buffalo appeared to be milder than that observed in similar experiments with cattle (Canton et al., 2013).

By inoculating a higher challenge dose (5×10^8 tachyzoites) of the same *N. caninum* strain, at Day 70 of pregnancy into three dams, Chryssafidis et al. (2014) detected fetal death on Day 35 post inoculation by the absence of fetal cardiac signs at ultrasound examination. Histological lesions were consistent with inflammatory responses (infiltration of lymphocytes) in placenta, fetal brain, heart, lung, kidney and liver. Inoculation of three buffalo dams with the same dose of an inoculum of another (Brazilian) isolate of *N. caninum* showed no lesions or clinical signs pointing to the effects of biological variability and strain differences.

In cattle, birth of clinically normal but persistently infected calves is more frequent than abortion, and there is endogenous (Trees and Williams, 2005) transplacental transmission from generation to generation (Dubey et al., 2007). Naturally occurring *in-utero* infection in water buffalo has also been documented (Chryssafidis et al., 2011), but data about its efficiency and the occurrence of exogenous or endogenous sources of transplacental infection is lacking.

3. Serological assays used for the detection of anti-*N. caninum* antibodies in buffalo

There have been several serological assays used to measure the sero-prevalence and describe the epidemiology of infection in water buffalo. In general, it would appear that most researchers have used assays that had been developed and validated for cattle, with cattle-specific reagents (such as the conjugates) incorporated into the kits. Very little additional validation has been carried out, except recently in two studies where in one, a sub-unit enzyme-linked immunosorbent assay (ELISA) was largely compared to the standard indirect cattle-specific IDEXX ELISA (Hamidinejat et al., 2015) with high values in the kappa statistics, signifying good agreement and another recent study on buffalo that compared the pan-ruminant indirect ELISA produced by IDEXX to IFAT, adjusted the cut-off threshold significantly downwards and arrived at high diagnostic test performance (DSe and DS_p).

Where either the NAT or IFAT were used, the cut-off threshold used varied decidedly across the studies – in some studies IFAT serum dilutions of 1:25 were used, in others serum dilutions of as high as 1:640 being required for an animal to be declared positive, i.e. infected. That latter study, incidentally was the Vietnamese study that detected a low sero-prevalence of just 1.5% – almost certainly an under-estimate (Huong et al., 1998) as not surprisingly, the cut-off threshold chosen has a big influence over the sero-prevalence indicated as seen in the summary of all IFAT studies calculated from Table 1 (Fig. 1). In addition, a longitudinal study after experimental infection by Rodrigues et al. (2005) in buffalo showed a peak serological response in IFAT within the first three weeks of exposure to the parasite, with a return to titres of 1:50, in IFAT, after three months. The majority of the sero-prevalence studies conducted on buffalo (Table 1) used titres of 1:100 or above as their cut-off value and would have missed animals with lower titres.

While it is difficult to demonstrate the same for the ELISA studies, cut-off threshold validation is equally important and the threshold used would influence the sero-prevalence measured.

4. Epidemiology and prevalence

With the caveats mentioned earlier in the chapter regarding the cut-off threshold chosen in the various assays used and its impacts on the sero-prevalence reported, it appears that overall, buffalo populations across the globe, with a composite sero-prevalence of 48.4% (95% CI ± 0.9%) are approximately 3 times more likely to be infected with *N. caninum* than dairy cattle (at globally an estimated 16.1%) and approximately four times more than beef cattle (at 11.5%) (Reichel et al., 2013), leading to suggestions that water buffalo are more susceptible to subclinical infection.

There may be further indications that water buffalo are more susceptible to subclinical infection because they are even displaying this higher sero-prevalence in situations where they co-habit with cattle in the same areas, presumably being at similar risk of infection (Moore et al., 2014).

At the same time, as discussed, reports of naturally occurring abortions due to *N. caninum* in water buffalo are rare, leading to suggestions that they may be more resistant to disease.

Natural infection with *N. caninum* has been reported in a water buffalo fetus which provides evidence of naturally occurring congenital infection (Chryssafidis et al., 2011). There are also several reports indicating increasing exposure to and infection with *N. caninum* of buffalo with age (signifying the importance of post-natal exposure) (Campero et al., 2007; Guarino et al., 2000; Kengradomkij et al., 2015; Moore et al., 2014). Natural transmission from buffalo to dogs (Bandini et al., 2011; Rodrigues et al., 2004) appears

possibly suggested by transmission experiments, and a higher sero-prevalence in buffalo more closely associated with dogs has been described (Nasir et al., 2011). Clearly, more epidemiological work is required to elucidate the relative importance of post-natal and transplacental (both endogenous and exogenous) transmission.

5. Conclusions

Infections of water buffalo (*B. bubalis*) share many features of the infection in cattle, yet there are also striking differences. Sero-prevalence appears to be much higher in buffalo as compared to both dairy and beef cattle. Even when cattle and buffalo co-habit, thus sharing the same environmental risk factors, buffalo appear to show much higher infection rates. Abortions in buffalo, seem infrequent in comparison with cattle.

The above might support suggestions that buffalo are, while more susceptible to infection, also more tolerant of it, and the inflammatory response to infection may be milder. Further study of the interaction of parasite and host seems warranted in buffalo, leading to a better understanding of the interactions and might help us to develop new strategies for control, not only in buffalo, but also cattle populations world-wide.

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