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Review

A review of *Neospora caninum* in water buffalo (*Bubalus bubalis*)

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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
					Individual	Farm			
Asia	China	2005	40	0	0.0		ELISA (not given)		(Yu et al., 2007)
	India	2004	32	16	50.0		c-ELISA		(Meenakshi et al., 2007)
		2008	341	34	10.0		c-ELISA		(Sengupta et al., 2012)
	Iran	2014	122	76	62.3		ELISA (IDEXX)		(Hamidinejat et al., 2015)
	Pakistan	2010	300	164	54.7		ELISA		(Nasir et al., 2011)
	Philippines	2007	105	4	3.8		ELISA (not given)		(Konnai et al., 2008)
	Thailand	2015	628	57	9.1	16.7	IFAT (anti-bovine)	1:100	(Kengradomkij et al., 2015)
	Vietnam	1995	200	3	1.5		ELISA/IFAT (anti-bovine)	1:640	(Huong et al., 1998)
Europe	Italy	1999	1,377	476	34.6		IFAT (anti-buffalo)	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		IFAT (Bovine)	1:100	(Campero et al., 2007)
		2012	500	211	42.2		IFAT (anti-bovine)	1:100	(Konrad et al., 2013)
		2013	1,350	584	43.3		IFAT (anti-bovine)	1:100	(Moore et al., 2014)
	Brazil	2000	222	142	64.0		NAT/IFAT (anti-bovine)	1:25	(Fujii et al., 2001)
		2004	196	139	70.9		IFAT (anti-bovine)	1:25	(Gennari et al., 2005)
		2013	4,796	2,662	55.5		ELISA/IFAT (anti-bovine)	1:100	(da Silva et al., 2014)
		2014	192	169	88.0		IFAT (not stated)	1:50	(Chryssafidis et al., 2015)
Africa	Egypt	1995	75	51	68.0		NAT	1:20	(Dubey et al., 1998)
	Kenya[a]	2001	4	2	50.0		NAT	1:80	(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 (*Syncerus 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). Aborting 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.

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