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Effects of Age and Breed on the Prevalence of *Neospora caninum* in Commercial Dairy Cattle from Pakistan

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ABSTRACT: *Neospora caninum* is a major cause of bovine abortion worldwide. A serological survey was carried out to determine the seroprevalence of exposure to *N. caninum* in dairy cattle based on age and breed from Punjab and Sindh provinces, Pakistan. Serum samples from 641 animals from 12 herds from Punjab (n = 7) and Sindh (n = 5) provinces were tested for antibodies against *N. caninum* using a commercially available competitive enzyme-linked immunosorbent assay. Positive reactions to *N. caninum* were seen in 277 (43%) of the 641 of the samples. Seropositive animals were present in all 12 herds. Animals over 2 yr of age (47%) and crossbreds (55%) were more likely to be seropositive than the other cattle examined. These results indicate that *N. caninum* infection is widespread among dairy cattle in Pakistan.

Neospora caninum is an important cause of abortion in dairy cattle, causing significant economic losses to the industry worldwide (Dubey and Lindsay, 1996). The parasite was recognized in dogs in Norway in 1984 as a cause of neuromuscular degeneration, leading to hind limb paralysis (Bjerkas et al., 1984). Because of structural and antigenic differences from *Toxoplasma gondii*, it was described as a new species in 1988 (Dubey, Carpenter, et al., 1988) and isolated from naturally infected dogs that same year (Dubey, Hattel, et al., 1988). In 1989 *N. caninum* was first reported as a cause of bovine abortion (Thilsted and Dubey, 1989). Domestic dogs are definitive hosts for *N. caninum* (McAllister et al., 1998; Lindsay, Dubey, et al., 1999; Lindsay, Upton, et al., 1999). Horizontal transmission of *N. caninum* can result in abortion storms (Thilsted and Dubey, 1989), but vertical (transplacental) transmission of the *N. caninum* is the most economically important outcome of infection (Björkman et al., 1996; Paré et al., 1996; Schares et al., 1998). The majority of congenital infections result in the birth of apparently healthy calves. Cows may abort repeatedly due to *N. caninum* or give birth to apparently healthy calves, calves with subclinical *N. caninum* infection, or uninfected calves. Most congenitally infected calves remain clinically asymptomatic. Most *N. caninum*-induced abortions occur at 5–6 mo of gestation (Dubey, 1999).

Horizontal transmission occurs when cows ingest sporulated *N. caninum* oocysts shed by dogs (McAllister et al., 1998). Domestic dogs are natural definitive hosts for the parasite (McAllister et al., 1998; Lindsay, Dubey, et al., 1999; Lindsay, Upton, et al., 1999). It has been demonstrated that they can become infected by consuming *N. caninum*-infected tissues (Gondim et al., 2002) or bovine placenta (Dijkstra et al., 2001). Experimentally infected dogs excrete few oocysts (Lindsay et al., 2001), and the role of dogs and *N. caninum* oocyst excretion is an area of active research in the epizootiology of the disease (Bandini et al., 2011; Cavalcante et al., 2011; King et al., 2012).

Dairy herd health is extremely important to the economy of Pakistan because it is the third largest milk-producing country in the world (FAO, 2009). We have recently reported the seropositivity rate of *N. caninum* in dairy herds with a history of high rates of abortion (Shabbir et al., 2011). *Brucella abortus* was the most common agent found in aborting cattle (77%), while *N. caninum* was present in 47% of aborting animals, and

dual infections with these agents were present in 28% of the animals. However, *N. caninum* was found in 39% of nonaborting cattle, while *B. abortus* was found in 27% of nonaborting cattle (Shabbir et al., 2011).

We became interested in determining if horizontal or vertical transmission is more important in maintaining the parasite in the cattle population in Pakistan. In the present study, we examined animals from 3 age groups to consider this question. Additionally, we examined the effects of breed on prevalence to determine if genetic factors may play a role in susceptibility to *N. caninum*. The present study was done in healthy dairy cows from farms in Punjab and Sindh provinces, Pakistan.

Pakistan is an agricultural country, located in southern Asia with hot dry summers, during which temperatures reach over 40 C, and mild winters with average temperatures between 15 and 20 C. Pakistan is located at latitude between 24° and 37° north and longitude between 61° and 76° east, and has 803,940 km² of land, a human population of 1.8 million, and a cattle population of 24.2 million. Between January 2009 and August 2010, serum samples were taken from 641 clinically normal cattle on 12 dairy farms, 7 from Punjab and 5 from Sindh provinces (Tables I–III). Information regarding abortion history was available for only 2 farms (10 and 11). Farm 10 reported abortions in the last year, while farm 11 reported abortions over the last 4 yr. Only 6 cows aborted in herd 10 in the previous year; in herd 11, 21 of the 37 cows had no history of abortion, and the remaining 16 had aborted previously. Some of the cows may have had repeated abortions, but no supporting data were available. Samples were selected from clinically healthy cows of different breeds and different age groups using animal numbers provided by the producers by an individual who had no knowledge of animals status. We sampled 3 age groups of cows, including calves (≤8 mo of age), heifers (>8 mo to 2 yr old), and adult lactating cows (>2 yr old) to determine the variations of serological prevalence against *N. caninum* between different age groups (Table II). Cattle included crossbred (local × exotic), non-descript (no cross-discernable based on animal appearance), and purebred (Table III). Water and feed were available ad libitum.

Blood was collected from the jugular vein; serum was separated at the Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan, and stored at –20 C until assayed. Serum reactivity to *N. caninum* was evaluated using a commercially available competitive enzyme-linked immunosorbent assay kit (cELISA, VMRD, Pullman, Washington). The procedures were performed according to the manufacturer's instructions, and results were expressed as percent inhibition.

Pearson's χ^2 test was used to compare seropositive status with age and breed using (SPSS for Windows, version 13.0; SPSS, Chicago, Illinois), and $P < 0.05$ was considered significant.

The cELISA indicated that 277 (43%) of the 641 cows were seropositive to *N. caninum*. A significant difference ($P < 0.05$) of seropositivity was observed between different herds (Table I). The age of the cows were categorized into 3 groups, below 8 mo of age, 8 mo to 2 yr of age, and above 2 yr old. *Neospora caninum* seroprevalences were 39% (37/94), 36% (59/162) and 47% (181/385), respectively. Age-wise prevalence, though not statistically significant ($P > 0.05$), was greatest in animals older than 2 yr of age (Table II). The prevalence was significantly different ($P < 0.05$) between purebred, crossbred, and non-descript animals. However, no

TABLE I. Prevalence of *Neospora caninum* in cattle from 12 dairy farms from Pakistan.

Number of herds	Number of cows in the herd	Number of cows tested	Positive	Percentage
1	407	68	31	46
2	963	41	13	32
3	392	88	33	38
4	576	54	19	35
5	289	61	33	54
6	724	27	16	59
7	267	49	18	37
8	1,271	18	07	39
9	875	83	47	57
10	219	53	20	38
11	86	37	26	70
12	694	62	14	23
Total	6,763	641	277	43

significant difference ($P > 0.05$) was found between sub-breeds, i.e., exotic and local breeds.

The prevalence was not significantly different ($P > 0.05$) within the exotic breeds, i.e., Jersey and Freisian, and local breeds, i.e., Sahiwal, Rojan, Dajal, and Dhanni (Table III). Seroprevalence was highest in crossbred animals (Table III).

The high prevalence of exposure to *N. caninum* (43%) even in apparently healthy animals indicates that *N. caninum* may be an important risk factor of abortions in these provinces of Pakistan. The seroprevalence of *N. caninum* varies within countries, between regions in countries, and between beef and dairy cattle in areas examined (Dubey et al., 2007). Seroprevalence depends largely on management and environmental conditions of the country being studied (Dubey et al., 2007). The overall high prevalence of *N. caninum* among dairy cattle herds corresponds to previous observations made by Shabbir et al. (2011) who reported high prevalence in aborting animals and in those that were at risk. The prevalence of *N. caninum* in all the age groups strongly suggests transplacental and horizontal transmission (Dubey et al., 2007), but the rate of transmission may be different in different scenarios (Bartels et al., 2007; Dijkstra et al., 2008; Williams et al., 2009).

Sadrebazzaza et al. (2004) concluded that there is no significant relationship between *N. caninum* seropositivity and age. However, Guimaraes et al. (2004) reported that *N. caninum* seropositivity was higher in cattle above 2 yr of age and that horizontal transmission could be responsible for this situation. In the present study, the highest prevalence in age groups over 2 yr agrees with this study and observations made by others that seroprevalence increases with animal age (Dubey et al., 2007; Eiras et al., 2011).

The results of our study showed a significant difference in prevalence of *N. caninum* among different breeds of cattle in our cattle populations and that it was significantly ($P < 0.05$) higher in crossbreds. There are many

TABLE II. Prevalence of *Neospora caninum* antibodies based on cattle age.*

Age group	Number of cows tested	cELISA	
		Positive	Percentage
≤8 mo old	94	37	39
>8 mo to 2 yr	162	59	36
>2 yr	385	181	47

* None of the age groups were significantly different ($P > 0.05$) from each other.

TABLE III. Prevalence of *Neospora caninum* antibodies by cattle breed.

Breed	Number of cows tested	cELISA			
		Positive	Percentage		
Purebred	Exotic	Freisian	77	29	38
		Jersey	45	22	49
Local	Sahiwal	Rojan	147	59	40
		Dajal	24	7	29
		Dhanni	19	6	32
		Dhanni	31	11	36
Total purebred*		343	134	39	
Crossbreds†		213	117	55	
Non-descript‡		85	26	31	

* All 3 breed groups were significantly different ($P < 0.05$) from each other.

† Local breed crossed with purebred.

‡ Not possible to define breed makeup.

reports worldwide demonstrating differences in seropositivity of different cattle breeds in *N. caninum* prevalence studies (Armengol et al., 2007; Dubey et al., 2007; Duong et al., 2008; Munhoz et al., 2009). However, these reports should be interpreted carefully because production systems may be a possible explanation rather than breed-related susceptibility. The extent of abortion and immune responses after *N. caninum* infection is likely affected by the breed of cattle (Armengol et al., 2007; Almeria et al., 2009; Romero-Salas et al., 2010; Santolaria et al., 2011). Experimental studies need to be conducted with different bovine breeds before the influence of breed can be accurately evaluated and its importance in the epizootiology of bovine *N. caninum* infections can be determined.

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