Prevalence of Antibodies Against of *Neospora caninum* in Dairy Cattle in Nuevo Leon, Mexico

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Abstract: The goal of the present epidemiological research was the detection of anti-*Neospora caninum* antibodies in order to determine the presence and prevalence of neosporosis in dairy cattle herds at 8 counties from the state of Nuevo Leon, Mexico. A total of 33 herds and 371 animals were included. Detection of *Neospora caninum* was performed using a commercial kit observed prevalence was 45%. This result allowed the conclusion that animals which are seropositive for the protozoan *Neospora caninum* do exist in Nuevo Leon. Serological evidence was found in animals at milk-production stage indicating that the presence of emerging diseases in the region is a fact therefore, sanitary measurements should be re-evaluated or reinforced in order to keep the cattle free from pathogens that have a negative impact in the production of food from animal origin used for the human consumption.

Key words: Neosporosis, dairy cattle, Nuevo Leon, animal, region, milk production, Mexico

INTRODUCTION

The first neosporosis report was made by Bjerkas *et al.* (1984) at Norway from a canine case showing clinical signs related to encephalitis and myocarditis caused by a protozoan. Dubey *et al.* (1988a) were the first to use the name *Neospora caninum* and were able to prove the Koch postulates in this animal species. That same year neosporosis was experimentally reproduced in canines (Dubey *et al.*, 1988b).

The involvement of *Neospora caninum* as abortion cause in bovines was reported by Thilsted (1989). On the other hand, Bjerkas and Presthus (1988) found that the strains isolated from canines were identical to those isolated from bovines. It has been demonstrated that *Neospora caninum* has a wide range of hosts including cattle, horses, canines and deer (Garcia-Bocanegra *et al.*, 2010; Almeria *et al.*, 2010; Dubey *et al.*, 1992; Dubey and Lindsay, 1989a-c). McAllister *et al.* (1998) were able to identify the dog as the definitive host by showing the presence of oocysts in fecal matter of animals feed with infected tissue of tachyzoites of *Neospora caninum*. Wouda *et al.* (1999) suggested an association among the *Neospora caninum* infection at farms that have dogs and the cattle. In 1991, neosporosis was considered in the

state of California, USA as the largest cause of abortion in cattle causing losses up to 35 million dollars (Anderson *et al.*, 1991). Evidence of natural infection has been found in canines (Cabezon *et al.*, 2010; Dubey *et al.*, 2009a; Bjerkas *et al.*, 1984; Dubey and Lindsay, 1990; Lindsay *et al.*, 1996), felines (Dubey *et al.*, 2002) bovines (Eiras *et al.*, 2011; Brickell *et al.*, 2010; Garcia-Ispuerto *et al.*, 2010; Vanleeuwen *et al.*, 2010; Dubey *et al.*, 1992; Anderson *et al.*, 1991; Barr *et al.*, 1990; 1991a, b; Dubey *et al.*, 1990), ovine (Dubey and Lindsay, 1990), goats (Barr *et al.*, 1992), equines (Dubey and Porterfield, 1990), raccoons (Lindsay *et al.*, 2001), antelopes (Peters *et al.*, 2001) and deer (Dubey *et al.*, 2009a, b, 2008b; Lindsay *et al.*, 2002).

Experimental infections have been induced in mice (Dreier *et al.*, 1999; Lindsay *et al.*, 1992; Yamage *et al.*, 1996), rats (Lindsay *et al.*, 1992), canines (Dubey *et al.*, 2008a, b; Dubey and Lindsay, 1989b), foxes (Bjerkas *et al.*, 1984), goats (Lindsay *et al.*, 1995), cats (Millan *et al.*, 2009; Dubey and Lindsay, 1989a), sheep (Dubey *et al.*, 1996), coyotes (Lindsay *et al.*, 1996), porcine (Jensen *et al.*, 1998), gerbo (Cuddon *et al.*, 1992), rabbits (Dubey and Lindsay, 1996), bovines (Dubey *et al.*, 1996; Ugglia *et al.*, 1998), buffalo (Guarino *et al.*, 2000) and primates (Barr *et al.*, 1994).

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In bovines, the main transmission route is vertical (congenital-transplacental) (Mainar-Jaime *et al.*, 1999; Pare *et al.*, 1996) but it has also been reported the horizontal transmission and experimentally through the lactogenic route (Davison *et al.*, 1999). Transplacental transmission has been experimentally induced in dogs (Dubey and Lindsay, 1989b; Cole *et al.*, 1995b), cats, (Dubey and Lindsay, 1989a), sheep (Dubey and Lindsay, 1990) bovines (Dubey *et al.*, 1992; Barr *et al.*, 1994) and mice (Cole *et al.*, 1995a). Transplacental infection can happen several times in the same animal (Bjerkas *et al.*, 1984; Dubey *et al.*, 1988b).

Clinical signs have been reported in bovines approximately 2 months old with mostly nervous system signs due to alterations of the central nervous system such as ataxia, exoftalmia, loss of conscience and coordination, ocular asymmetry and paralysis. Other possible signs are low birth weight as well as alterations of growth and flexion or extension of anterior or posterior extremities, diminution of patellar reflexes, loss of consciousness and of perception (Thilsted, 1989; Brickell *et al.*, 2010; Dubey *et al.*, 1992; Anderson *et al.*, 1991). The only clinical sign in adult cattle is abortion, occurring mostly among the 4 and 6th month of gestation however, not all seropositive animals abort (Dubey and Lindsay, 1996). Abortion can be explosive, paused or sporadic. Fetuses dying at the uterus can be reabsorbed, mummified or autolyzed and can be born alive but with the disease or be born clinically normal but chronically infected (Dubey and Lindsey, 1996).

In 2001 it was reported in Mexico the presence of neosporosis in dairy cattle at some areas in the Holstein breed whose diagnosis was performed by serology, Immunohistochemistry (IHC) and pathological lesions (Morales *et al.*, 2001). The goal of this study was the detection of antibodies anti-*Neospora caninum* in order to determine the presence and prevalence of neosporosis in milking bovine herds by the competitive ELISA test.

MATERIALS AND METHODS

Sampling: The sampling was performed at 33 ranches of 8 municipalities (counties) from the state of Nuevo Leon, Mexico. Nuevo Leon has a territorial extension of 64,742 km², representing the 3.3% of the country surface. Its maximum altitude is 3700 m above sea level (msnm) and its minimum is of 100 msnm. It has a hot and extreme dry climate. According with governmental data, there are at Nuevo Leon 499,000 bovines from which only 18,312 are dairy cattle (www.campomexicano.gob.mx).

Animals: The animals sampled included 2-4 years old adult females. All thirty three herds were destined to milk production. Breeds included were 85% Holstein-Friesian, 8% Jersey and 7% Holstein-Jersey mix. Animals were sampled at random.

Blood collection: About 5 mL were extracted from each animal (n = 371) from the tail vein using Vacutainer (Becton Dickinson and Co. Franklin Lakes, NJ, USA), tubes (21×11/2) without anticoagulant and with vacuum. The samples were kept refrigerated until and after they were taken to the College of Veterinary Medicine, Nuevo Leon State University. Afterward, samples were centrifuged at 3000 rpm for 15 min in order to completely separate blood serum which was stored at -20°C until analysis.

Laboratory analysis: Diagnosis for the detection of antibodies against *Neospora caninum* was performed by the ELISA technique using the *Neospora caninum* Antibody test (HerdChek Neospora caninum Antibody Test Kit Laboratorios IDEXX, Westbrook Maine 04092, U.S.A) which detects antibodies against *Neospora caninum* in bovine and goats.

RESULTS AND DISCUSSION

Seroprevalence obtained at the different counties from Nuevo Leon is shown in Table 1. All 8 counties sampled had serologically positive animals to *Neospora caninum*. The county with the higher prevalence was Anahuac (78%) followed by Vallecillo (56%) and Zuazua (52%) whereas Paras and Marin had the lowest results with 20 and 30%, respectively. Total frequency for Nuevo Leon was 45%.

Results obtained in the present study on the prevalence of *Neospora caninum* in dairy herds from 8 counties of the state of Nuevo Leon, Mexico are consistent with the previously described by Morales *et al.* (2001) in which they report a prevalence of 47% in dairy cattle whereas in this study was of 45%. The ELISA technique utilized in the present study for diagnosis is according to Pare *et al.* (1996), more consistent, objective, fast, precise as well as more sensitive and specific as compared with others. There are in Nuevo Leon previous data on the serological detection of positive animals to *Neospora caninum*, in both dairy and beef cattle however, the frequency found in those studies is similar to the one observed by Morales *et al.* (2001).

Researchers have to take into account that prevalence variability is influenced by the country, region and diagnostic technique used (Dubey, 2003).

Table 1: Prevalence of antibodies against *Neospora caninum* in dairy cattle in the state of Nuevo Leon, Mexico

Country	No. of samples	No. of herds	Positive animals	%
Anahuac	18	2	14	78
Apodaca	110	8	53	48
Higueras	8	1	3	37
Marin	92	5	28	30
Paras	10	1	2	20
Sabinas	38	5	15	39
Vallecillo	64	8	36	56
Zuazua	31	3	16	52
Total	371	33	167	45

It can be mentioned that results obtained demonstrate a higher frequency in herds destined to dairy production which is similar to data reported by other researchers (Benetti *et al.*, 2009; Venturini *et al.*, 1999; Mainar-Jaime *et al.*, 1999; Dubay and Lindsay, 1996) but too high as compared to the findings of Klevar *et al.* (2010) and Kamga-Waladjo *et al.* (2010). With regard to the disease transmission, it has been recognized that the most common is the vertical (Wouda *et al.*, 1992; Venturini *et al.*, 1999) therefore, herds with no animals with good production traits some times import infected animals in order to increase the productivity and those animals can bring the infection into the herd through healthy carriers (Lindsay *et al.*, 1995). Information about the disease is rare in Mexico due to this, the role of this parasite in the reproductive problems of dairy herds has been poorly studied. The data presented above represent a small amount of the information required for a complete understanding of the *Neospora caninum* role in the reproductive and productive problems for the country livestock. Therefore, more detailed studies are required for this goal.

CONCLUSION

Results obtained in the present study demonstrate immunological evidence which shows a high prevalence of neosporosis in dairy herds at the state.

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