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Risk Factors and Seroprevalence Against *Neospora caninum* in Dual-Purpose and Beef Cattle in Colima, Mexico

[1]S. Arreola-Camberos, [2]L.J. Garcia-Marquez, [1]R. Macedo-Barragan,
[3]E. Morales-Salinas and [1]D. Figueroa-Chavez
[1]Facultad de Medicina Veterinaria y Zootecnia,
[2]Centro Universitario de Investigacion y Desarrollo Agropecuario, Universidad de Colima,
Km. 40 Autopista Colima-Manzanillo, C.P. 28100, Tecoman, Colima, Mexico
[3]Departamento de Patologia, Facultad de Medicina Veterinaria y Zootecnia,
Universidad Nacional Autonoma de Mexico, Ciudad Universitaria, C.P. 04510, Mexico D.F., Mexico

Abstract: A study was conducted with the aim of evaluate risk factors and seroprevalence against *Neosporum caninum* in beef and dairy cattle in Colima, Mexico. Serum samples from 920 heifers and cows were analyzed using ELISA test to detect *N. caninum* antobodies. A survey was carried out to identify potential risk factors related with the presence of the parasite. Association between seroprevalence and each risk factor was estimated based on the odd ratios using a binary logistic regression test. Antibody seroprevalence against *N. caninum* was 16.20% with values ranging from 32.50-6.50%. The following management and reproduction related risk factors were identified: commercial concentrate supplementation (OR: 1.38, 95% IC: 0.71-2.67) and metritis and placental retention history (OR: 2.08, 95% IC: 1.14-3.81 and OR: 1.01, 95% IC: 0.62-1.66, respectively). It is confirmed the presence of serum antibodies against *N. caninum* in the state of Colima, Mexico.

Key words: Beef cattle, dual-purpose cattle, management and reproduction risk factors, *Neospora caninum*, seroprevalence, Mexico

INTRODUCTION

Neospora caninum (Apicomplexa) is a coccidian parasite for cattle and dog and it occasionally causes clinical infections in horses, goats, sheep and deer (Dubey, 2003a). Since, it first recognition in dogs in Norway (Bjerkas *et al*., 1984) and the description of a new genus and species, neosporosis has emerged as serious disease and is considering a major cause of infectious bovine abortion worldwide. As many as 42% of cow may abort due to the infection, economic losses to the cattle industry are estimated in millions of dollars (Dubey, 2003a, b; Dubey *et al*., 2007).

N. caninum causes abortion both dairy and beef cattle. Cows of any age may abort from 3 months gestation to term. Most neosporosis-induced abortions occur at 5-6 months gestation. Fetuses may die in utero be resorbed, mummified, autolyzed, stillborn, born alive with clinical signs or born clinically normal but chronically infected. Abortions may be epidemic or endemic (Wouda *et al*., 1999). In endemic areas as many as 33% of dairy cow fetuses have been reported to abort within a few months. Abortions were considered epidemic if >10% of cows at risk aborted within 6-8 weeks (Anderson *et al*., 1995).

In cattle, vertical transmission (congenital-transplacentary) is the major route involved in the spread of *N. caninum* (Davison *et al*., 1999). Epidemiological evidence also suggests that horizontal transmission can occur due to the ingestion of sporulated oocysts by drinking water or forage contaminated by feces from domestic dogs and coyotes which are considered the definitive hosts of *N. caninum* (McAllister *et al*., 1998; Gondim *et al*., 2004).

Recently, the possibility of venereal transmission in bovine neosporosis has been suggested (Garcia-Vazquez *et al*., 2009) given that *N. caninum* DNA has been reported in fresh and frozen semen of naturally infected bulls (Caetano-da-Silva *et al*., 2004; Ferre *et al*., 2005).

On the other hand, the detection of specific anti-*N. caninum* antibodies in sera of cows using the Enzyme-Linked Immunosorbent Assay (ELISA) has been useful for the diagnosis of the disease and may also,

Corresponding Author: S. Arreola-Camberos, Facultad de Medicina Veterinaria y Zootecnia, Universidad de Colima, Km. 40 Autopista Colima-Manzanillo, C.P. 28100, Tecoman, Colima, Mexico

prove suitable for seroepidemiologic investigations (Morales *et al*., 2001). Besides to understand how the disease is transmitted under typical management environments, it is important to consider risk of infection associated with the various lifetime exposure experienced by cattle (Pare *et al*., 1996). Identify *N. caninum* infection-associated risk factors based on population serological status has been used as an efficient tool in several studies (Dubey *et al*., 2007).

In Mexico, bovine neosporosis was first detected in 1997 and further studies in dairy and beef cattle has confirmed its distribution throughout the country (Morales *et al*., 2001; Garcia-Vazquez *et al*., 2009; Romero-Salas *et al*., 2010; Salinas-Melendez *et al*., 2011). The aim of this study was to evaluate the risk factors associated with seroprevalence to *Neosporum caninum* in dual-purpose and beef cattle in Colima, Mexico.

MATERIALS AND METHODS

Experimental site and sampling: A total of >6 months old 920 females from 90 dual-purpose (*Bos taurus* x Zebu) and beef (Brown Swiss, Simmental, Charolais, Brahman and Angus) herds located in the 10 municipalities that integrate the state of Colima were sampled to detect *N. caninum* antibodies. Colima is located at 19°31'-18°41'N and 103°29'-104°41'W it has a dry warm climate, average temperature is 25°C average rainfall is 1041.8 mm, occurring in summer and the altitude above sea level ranges from 3-940 m.

Blood collection: About 10 mm were collected from the coccygeal vein using Vacutainer devices without anticoagulant. After centrifugation 1500× g from 10 min serum was restored at -40°C until analysis.

Serologic test: Serum samples were submitted to the Laboratory of Pathology of the National Autonomous University of Mexico and tested for antibodies against *N. caninum* using the ELISA test (CIVTEST, Hipra Laboratories, Girona, Spain). The sensitivity and specificity was reported 98.6 and 98.9%.

Risk factors: A written survey was applied to all herd owners with the objective to identify animal characteristics and management practices that could be related with presence of *N. caninum*. Animal information included clinical history of reproductive problems (abortion, placental retention, uterine prolapses, metritis and repeat breeding). Management practices included the presence of final and intermediate *N. caninum* hosts (dogs and wildlife), the origin of replacement heifers (raised or purchased), husbandry system (grazing or pen) and commercial concentrate supplementation.

Data analyses: The seroprevalence rates for *N. caninum* was calculated for each county in relation to the animal data and the association between seroprevalence and each risk factor was estimated based on the odd ratios using a binary logistic regression test where values >1.0 showed and association.

RESULTS AND DISCUSSION

Serology test showed an overall antibody seroprevalence of 16.20 and 72% (65/90) of the herds had at least one serologically-positive animal. By county, the highest prevalence was observed in Comala (32.50%) and the lowest (6.50%) in Tecoman (Table 1).

Commercial concentrate supplementation was a management related potential risk factor (OR: 1.38, 95% IC: 0.71-2.67). Seroprevalence in cattle fed forage plus concentrate was 19.02 and 9.23% in those fed only forage. The breed of cattle, the presence of hosts, the origin of replacement heifers and the husbandry system were not a risk factor (OR<1.00) associated with *N. caninum* prevalence (Table 2).

Metritis and placental retention history were reproduction related potential risk factors (OR: 2.08, 95% IC: 1.14-3.81 and OR: 1.01, 95% IC: 0.62-1.66, respectively). Seroprevalence in cows with metritis history was 23.03 and 16.82% in those with no history. Seroprevalence in

Table 1: Seroprevalence of *Neospora caninum* antibodies among ten counties in Colima, Mexico

County	No. of animals	No. of positives	Prevalence (%)
Comala	80	26	32.50
Minatitlan	48	13	27.08
Manzanillo	80	19	23.75
Ixtlahuacan	80	18	22.50
Cuauhtemoc	80	16	20.00
Armeria	92	16	17.39
Coquimatlan	118	12	10.17
Villa de alvarez	120	12	10.00
Colima	130	11	8.46
Tecoman	92	6	6.52
Overall	920	149	16.20

Table 2: Management related risk factors associated with *Neospora caninum* seroprevalence in dairy and beef cattle in Colima, Mexico

Risk factors	N	Prevalence (%)	OR	CI 95%
Definitive/intermediate hosts				
Yes	86	19.54	0.83	0.55-1.25
No	63	19.09		
Replacement heifers				
Raised + purchased	53	16.56	0.77	0.51-1.17
Raised	96	18.11		
Husbandry system				
Grazing	65	13.54	0.65	0.42-1.00
Grazing + pen	84	22.70		
Commercial concentrate				
Yes	137	19.02	1.38	0.71-2.67
No	12	9.23		

OR: Odds Ratio; CI: Confidence Interval

cows with placental retention history was 20.00 and 15.88% in those cows without history. Repeat breeding abortion and uterine prolapses were not a risk factor (OR<1.00) associated with *N. caninum* prevalence (Table 3).

The overall seroprevalence against *N. caninum* observed in this study (16.20%) was lower than those reported in other states of Mexico, Nuevo Leon 45% (Salinas-Melendez *et al*., 2011), Veracruz 26% (Romero-Salas *et al*., 2010) and Aguascalientes 30 and 57.5% and higher than those reported for Chiapas 15% and Yucatan 11.3% (Garcia-Vazquez *et al*., 2009).

With respect to breed some studies performed in tropical countries have concluded that seroprevalence of *N. caninum* is higher in *Bos taurus* cattle than in *Bos indicus* and their crosses and also, there are reports that indicate differences in seroprevalence among *Bos taurus* breeds (Bartels *et al*., 2006). In Mexico, higher prevalences was found in dairy cattle with lower values for beef cattle (Salinas-Melendez *et al*., 2011; Romero-Salas *et al*., 2010; Garcia-Vazquez *et al*., 2009). Although, a study indicates that the differences observed might be caused by differences in management systems used for each breed and not by breed-related susceptibility to infection (Dubey *et al*., 2007) and other studies report that differences in prevalence among breeds are due to the predominance of one over the others in the herds under study (Kamga-Waladjo *et al*., 2010; Romero-Salas *et al*., 2010).

The lack of association between seroprevalence and the presence of hosts in the herds suggested that the major transmission route of *N. caninum* was transplacental as has been previously described (Pare *et al*., 1996; Dubey and Lindsay, 1996). Nonetheless, the possibility of the horizontal transmission occurring among the beef cattle cannot be denied (Garcia-Vazquez *et al*., 2009), since it has been confirmed the presence of *N. caninum* DNA in the drinking water of all the dairies included in a recent study, representing a high potential risk of infection. Moreover, the contamination with viable oocysts from the feces of dogs and coyotes can occur by the wind and not necessarily by direct deposition of contaminated feces in the water or around water reservoirs. The same study found an association between the presence of poultry (chickens and turkeys) roaming freely in the farm and *N. caninum* prevalence (OR = 2.32). Chickens (*Gallus domesticus*) have been recently reported as natural intermediate host of *N. caninum* (Costa *et al*., 2008). In the matter, husbandry habits of producers and domestic hosts (dogs and poultry) and not hosts quantity could explain the higher prevalence found in cattle fed concentrate in this study. It is common among the producers to use the warehouse where concentrate is stored such as housing for their dogs. Also is common to see poultry fed directly from the bags of concentrate and therefore, defecating in them. As found in this study, a research carried in Venezuela report that cows and heifers fed commercial concentrate had 1.82 more possibilities to be seropositives. Contrary, Rinaldi *et al*. (2005) found that cattle raised on farms having a large number of dogs showed higher seroprevalence that cattle raised on farms having a small number of dogs.

Table 3: Reproduction related risk factors associated with *Neospora caninum* seroprevalence in dairy and beef cattle in Colima, Mexico

Risk factors	N	Prevalence (%)	OR	CI 95%
Repeat breeding				
Yes	31	15.50	0.44	0.26-0.75
No	118	18.15		
Metritis				
Yes	21	23.33	2.08	1.14-3.81
No	128	16.82		
Abortion				
Yes	65	19.69	0.78	0.47-1.29
No	84	16.15		
Uterine prolapses				
Yes	13	13.00	0.62	0.31-1.24
No	136	18.13		
Placental retention				
Yes	68	20.00	1.01	0.62-1.66
No	81	15.88		

OR: Odds Ratio; CI: Confidence Interval

Due to vertical transmission is the main mechanism to spread the infection raising replacement heifers has been associated with increased *N. canine* seropositivity (Barling *et al*., 2001) however coinciding with previous findings (Romero-Salas *et al*., 2010) this association could not be demonstrate in the present study. In literature has been reported that raising replacements perpetuates the infection and promote its endemic status. For this reason it is possible to find farms with higher prevalence raising their own replacements than the ones to purchase it (Otranto *et al*., 2003). It is recognized that replacing cattle with seronegative animals drastically helps to reduce prevalence (Stenlund *et al*., 2003).

Nevertheless, a strong correlation between abortion and *N. caninum* positive status has been reported in Mexico and elsewhere (Morales *et al*., 2001) in the present study abortion was not a risk factor (OR = 0.78). In this regard, abortions recorded in this study could be caused by trauma toxins in plants and by other infectious disease agents such as brucellosis, leptospirosis infectious bovine rhinotracheitis and bovine viral diarrhea which has been previously observed in the state of Colima and Mexico (Segura-Correa and Segura-Correa, 2009). Results showed that placental retention and metritis history as an infection risk factor (OR = 2.08 and 1.01, respectively).

Contrary, literature reports that *N. caninum* induces abortions but rarely results in retention of placenta or development of metritis (Dubey, 2003b). It is possible that these reproductive disordes induce immune depression increasing the risk of infection.

CONCLUSION

This study confirms the presence of serum antibodies against *N. caninum* in the state of Colima, Mexico and identifies three risk factors associated with its presence including concentrate supplementation and metritis and placental retention history.

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