SHORT NOTE [NOTA CORTA]

PREVALENCE OF *Cryptosporidium* spp. AND ASSOCIATED RISK FACTORS IN FEMALE CALVES IN THE CENTRAL REGION OF VERACRUZ, MEXICO

[PREVALENCIA DE *Cryptosporidium* spp. Y FACTORES DE RIESGO ASOCIADOS EN BECERRAS DE LA ZONA CENTRO DE VERACRUZ, MÉXICO]

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SUMMARY

The objective of the study was to determine the prevalence of *Cryptosporidium* spp. and its associated risk factors in female calves in central Veracruz, Mexico. A cross-sectional study with a convenience sampling was conducted. One fecal sample was obtained from each of 120 female calves. The lateral flow immunochromatographic (LFIC) and the Ziehl-Neelsen (ZN) tests were performed. A questionnaire was applied in each farm to obtain individual and herd information. Overall prevalence was 3.33% ($CI_{95\%}$ 1-8) through LFIC and 12.50% ($CI_{95\%}$ 8-20) through ZN. Prevalence by municipality was 0 to 9.1% ($CI_{95\%}$ 0.03-0.24) through LFIC and 0 to 30.43% ($CI_{95\%}$ 16-51) through ZN. Prevalence by age was 0% at 31-45 days and 9.10% at 1-15 days through LFIC, and 0% at 31-45 days and 18.8% at 1-15 days through ZN. The calves with diarrhea had the highest prevalence, which was 14.3% ($CI_{95\%}$ 3-51) through LFIC and 57.1% ($CI_{95\%}$ 25-84) through ZN. The protective factors were calves housed in individual stalls, compared with those in common stalls but separated one from the other (OR=0.27; 0.09-0.85, P<0.04), as well as calves that ingested colostrum 30 to 60 min after birth, compared with those that ingested colostrum 2 to 3 h after birth (OR=0.22; 0.05-0.87, P<0.04). In conclusion, *Cryptosporidium* spp. was present in the suckling female calves from the farms surveyed, and the protective factors should be considered when establishing a program for controlling diarrhea in calves.

Key words: Prevalence; *Cryptosporidium* spp.; female calves; diarrhea.

RESUMEN

El objetivo del estudio fue determinar la prevalencia de *Cryptosporidium* spp. y sus factores de riesgo asociados en becerras de la zona centro de Veracruz, México. Fue un estudio transversal con muestreo de conveniencia. Se obtuvo una muestra fecal de cada una de 120 becerras. Se realizaron las pruebas de inmunocromatografía de flujo lateral (LFIC) y Ziehl-Neelsen (ZN). Se aplicó un cuestionario por rancho para obtener datos individuales y de hato. La prevalencia general fue 3.33% ($IC_{95\%}$ 1-8) por LFIC y 12.50% ($IC_{95\%}$ 8-20) por ZN. La prevalencia por municipio fue 0 a 9.1% ($IC_{95\%}$ 03-24) por LFIC y 0 a 30.43% ($IC_{95\%}$ 16-51) por ZN. La prevalencia por edad fue 0% a 31-45 días y 9.10% a 1-15 días por LFIC, y 0% a 31-45 días y 18.8% a 1-15 días por ZN. Las becerras con diarrea fueron las que tuvieron la prevalencia más alta, que fue 14.3% ($IC_{95\%}$ 3-51) por LFIC y 57.1% ($IC_{95\%}$ 25-84) por ZN. Los factores protectores fueron becerras en corraletas individuales, comparadas con becerras en corraletas comunes pero separadas unas de otras (RM=0.27; 0.09-0.85, P<0.04), así como becerras que recibieron calostro 30 a 60 min después de nacidas en comparación con las que lo recibieron 2 a 3 h después de nacidas (RM=0.22; 0.05-0.87, P<0.04). En conclusión, *Cryptosporidium* spp. estuvo presente en becerras lactantes de los ranchos estudiados, y los factores protectores deben ser considerados al establecer un programa de control de diarreas en becerras.

Palabras clave: Prevalencia; *Cryptosporidium* spp.; becerras; diarrea.

INTRODUCTION

Cryptosporidiosis is a zoonosis caused by a protozoan parasite of the genus *Cryptosporidium* in the phylum Apicomplexa, which can infect a wide variety of animals, including humans (Fayer and Ungar, 1986; Chako *et al*., 2010). *Cryptosporidium hominis* (formerly *C. parvum* genotype 1) is human-specific, whereas *C. parvum* (formerly *C. parvum* genotype 2) is present in different species that can act as reservoirs, including cattle (Plutzer and Karanis, 2009). *Cryptosporidium parvum* can cause the disease in humans, who get the infection from the cattle. In immunocompromised people (children, AIDS patients), *C. parvum* infection can be lethal (Houpt *et al*., 2005) In cattle, *Cryptosporidium* infection is transmitted by the fecal-oral route; transmission is easy, since the oocysts can persist for long periods in an adequate environment (Castro-Hermida *et al*., 2002), and even a low number of oocysts can produce infection in susceptible hosts (Ramirez, *et al*., 2004). Clinical manifestation of cryptosporidiosis has been reported in a variety of animals, including calves as young as 4 days-old (Del Coco *et al*., 2008).

In Mexico, *Cryptosporidium* spp. infection in humans and animals is common (Maldonado-Camargo *et al*., 1998). However, in studies conducted in young female calves kept under different production systems the results have been very variable due to the different diagnostic techniques used, management and feeding of the animals. As a result, the information on the prevalence of the disease, its distribution and its impact on these replacement populations is not clear. Therefore, the objective of this study was to determine the prevalence and the risk factors associated with *Cryptosporidium* spp. in female calves at 1 to 60 days of age in cattle farms in the central region of the state of Veracruz, Mexico.

MATERIALS AND METHODS

The study was carried out at four municipalities (Naolinco, Acatlán, Miahuatlán and Landero y Coss) of the central region of the state of Veracruz, Mexico. A cross-sectional study (Kelsey *et al*., 1986) was conducted in female calves at 1 to 60 days of age, which belonged to dairy cattle herds. The sample size was calculated using the statistical package Win Episcope ver 2.0 (Thrusfield *et al*., 2001); an expected prevalence of 50% was used, as well as an error of 10% and a confidence level of 95%. The sample size was 120 female calves. Only the female calves were sampled, since the male calves are sold as veal.

From each female calf a fecal sample was obtained directly from the rectum; the samples were kept refrigerated at 4 ºC until analyzed. For the diagnosis of *Cryptosporidium* spp. in feces the techiques used were the modified Ziehl-Neelsen (ZN) test (Casemore, 1991) and the lateral flow immunochromatographic (LFIC) test for *Cryptosporidium parvum* (BioX Diagnostics®; Jemelle, Belgium).

The ZN test was used in order to observe the oocysts of *Cryptosporidium* spp. For this purpose, the feces were processed at a concentration 1:1 water:ether, and were sieved using the Ziehl-Neelsen modified acid method (Casemore, 1991; Leventhal and Cheadle, 1992). One sample was considered as positive when one oocyst of *Cryptosporidium* spp. was observed.

The LFIC test was performed following the instructions of the commercial kit used (Crypto STRIPS BIO K 155®, Laboratorios Bio-X Diagnostics), which has a specificity of 95% and a sensitivity of 94.1%. The assay was made immediately after collecting the feces at the farm. The advantage of this technique is that it detects *C. parvum* oocysts via specific monoclonal antibodies.

At each farm a questionnaire was applied in order to obtain the following information: location of the farm (municipality), age and housing of the female calves, colostrum ingestion by the female calves, presence of diarrhea in the female calves, and feces consistency. The data were registered in an Excel® sheet and the results were analyzed through descriptive statistics. The differences among proportions were determined with the Chi-square test, using the SPSS® ver. 17.0. The confidence intervals were calculated at 95%, and the risk factors were determined using the Odds Ratio (OR), with the statistical package Vassar Stats®.

RESULTS AND DISCUSSION

Of the 120 female calves sampled, 15 were positive to *Cryptosporidium* spp. through the ZN test, for a prevalence of 12.50% ($CI_{95\%}$ 8-20), whereas four female calves were positive through the LFIC test, for a prevalence of 3.33% ($CI_{95\%}$ 1-8), finding statistical difference between both prevalences ($P<0.01$). This could be explained because the LFIC test detects *C. parvum* oocysts through a specific monoclonal antibody, and the ZN test is based on the detection of oocysts according to their morphological characteristics, so its diagnostic sensitivity for the detecion of *C. parvum* is low and the prevalence could increase if different species of *Cryptosporidium* coexist in the process of infection in the female calves. In the central region of Veracruz and in beef cattle, Aguilar *et al*. (2008) obtained a prevalence of *Cryptosporidium* spp. of 77% through the ZN test, higher than the prevalence found in the present study and than the prevalence of 35.7% reported by Cano *et al*. (2011) in the same region.

According to the origin of the samples by municipality, the highest prevalence was observed in the female calves from Acatlán through the ZN test, and in the female calves from Miahuatlán through the LFIC test (Table 1). No prevalence was found through any of both tests used in Landero y Coss (Table 1).

Table 1. Prevalence of *Cryptosporidium* spp. in female calves in four municipalities of the central region of the state of Veracruz, Mexico, using two diagnostic tests.

Municipality	Number of samples	Prevalence % ZN (positive)	$CI_{95\%}$ (ZN)	Prevalence % LFIC (positive)	$CI_{95\%}$ (LFIC)
Acatlán	23	30.43 (7)	16-51	4.35 (1)	0.8-21
Landero y Coss	31	0 (0)	0	0 (0)	0
Miahuatlán	33	15.15 (5)	7-31	9.10 (3)	3-24
Naolinco	33	9.10 (3)	3-24	0 (0)	0
Total	120	12.50 (15)	8-20	3.33 (4)	1-8

ZN = modified Ziehl-Neelsen test; LFIC = lateral flow immunochromatographic test; CI= confidence interval.

According to age, the prevalence was from 0 to 18.18% ($CI_{95\%}$ 7-39) through ZN and from 0 to 9.10% ($CI_{95\%}$ 3-28) through LFIC, which indicates that in this region there is a greater presence of other species of the genus *Cryptosporidium* different than *C. parvum.* This prevalence was similar to that reported by Aguilar *et al*. (2008) of 14 to 47% in beef calves at 1 to 6 months of age. However, the prevalence of the present study differed from the report by Castelán *et al*. (2011), who found prevalences of 72.4 and 74.4% in cattle farms in the central region of Veracruz, finding no statistical difference by age.

Fayer *et al*. (2006), Santín *et al*. (2008) and Coklin *et al*. (2009) have reported the presence of diarrhea in female calves when they reduce their milk intake and start foraging, as well as when the calves share their space with older animals. In the present study, the prevalence in female calves at 1 to 15 days of age was 18.18%, at 16 to 30 days of age it was 12%, and at 31 to 45 days of age the prevalence was 0%. However, in female calves at 46 to 60 days of age the prevalence was 22.90%, this probably due to the change in the feeding regime that produces stress in the animals, making them more susceptible to infections.

Maldonado-Camargo *et al.* (1998) reported that most of the infections occur at around two weeks after birth, period during which the clinical manifestations are more frequent. These data suggest that the calves get the infection during their first days of life (Uga *et al*., 2000), therefore, the measures practiced to reduce the morbility and spread of *C. parvum* should be directed to this high-risk group.

Regarding the consistency of the feces, in female calves producing normal feces the prevalence was 8.90% ($CI_{95\%}$ 4-17) through ZN and 1.30% ($CI_{95\%}$ 0.2-7) through LFIC. In female calves producing pasty feces the prevalence was 12% ($CI_{95\%}$ 4-30) through ZN and 8% ($CI_{95\%}$ 2-25) through LFIC. In female calves that produced feces with a semi-liquid consistency the prevalence was 11.10% ($CI_{95\%}$ 2-43) through ZN, and through LFIC none calf was positive, which might indicate that such consistency is due to *Cryptosporidium* spp. In the case of feces with a liquid consistency, the prevalence was 57.10% ($CI_{95\%}$ 25-84) through ZN and 14.30% ($CI_{95\%}$ 3-51) through LFIC (Table 2).

In female calves that produced feces with a liquid consistency the prevalence increased significantly through both tests (ZN 57.10% and LFIC 14.30%), compared with the prevalences for the feces with other consistency. These results differ from those reported by Hamnes *et al*. (2006), since they observed that the presence of diarrhea during the first 14 days of life, as well as the consistency of the feces at the time of collection, are not significant ($P>0.05$), as they could be due to the presence of other enteropathogenic microorganisms or to environmental factors that cause a change in the feces consistency.

Regarding the risk factors, the age was not significant in any strata. The stratum age from 1 to 15 days was a protective factor, but very low though (OR=0.03; $CI_{95\%}$ 0.006-0.15 $P=<0.05$), which differs from other studies, since it has always been reported that age is a factor that predisposes to infection. The two protective factors with the highest significance were the female calves housed in individual stalls, compared with those housed in common stalls but tied to keep them separated one from the other (OR=0.27; 0.09-0.85, $P <0.04$), since in the first ones the fecal-oral transmission decreases, and the second protective factor was the female calves that ingested colostrum within 30 to 60 min after birth, compared with those that ingested colostrum within the first 2 to 3 h after birth (OR=0.22; 0.05-0.87, $P<0.04$), since during the first 60 min of life the colostrum is best absorbed in

the intestine (Table 3). The only risk factor found was the presence of diarrhea (OR=7.33; 2.32-23.18, P<0.05) in female calves, compared with those that had no diarrhea. It is very likely that *C. parvum* causes diarrhea in early-age calves, and that it is one of the clinical manifestations of the disease.

Table 2. Prevalence of *Cryptosporidium* spp. according to the consistency of the feces collected in female calves from the central region of Veracruz, Mexico.

Consistency of feces	Number of samples	Prevalence % ZN (positive)	$CI_{95\%}$ (ZN)	Prevalence % LFIC (positive)	$CI_{95\%}$ (LFIC)
Normal	79	8.90 (7)	4-17	1.30 (1)	0.2-7
Pasty	25	12.0 (3)	4-30	8.0 (2)	2-25
Semi-liquid	9	11.10 (1)	2-43	0 (0)	0
Liquid	7	57.10 (4)	25-84	14.30 (1)	3-51
Total	120	12.50	8-20	3.33	1-8

ZN = modified Ziehl-Neelsen test; LFIC = lateral flow immunochromatographic test; CI= confidence interval.

Table 3. Odds Ratio for the risk factors associated with the presence of *Cryptosporidium* spp. in female calves in the central region of the state of Veracruz, Mexico.

Factor	N	Prevalence* (%)	OR	$CI_{95\%}$	*P*
Age (days)**					
16-30	25	12.00	1	-	-
1-15	22	18.18	0.03	0.006-0.15	0.05
46-60	62	12.90	0.92	0.22-3.79	0.38
Presence of diarrhea					
No	94	6.45	1	-	-
Yes	26	33.33	7.33	2.32-23.18	0.05
Housing of the calves					
Individual stalls	73	6.85	1	-	-
Common stalls with the calves tied individually	47	21.27	0.27	0.08-0.85	0.04
Colostrum ingestion (time after birth)					
30 to 60 min	108	10.19	1	-	-
2 to 3 h	12	33.33	0.22	0.05-0.87	0.04
Consistency of the feces					
Normal	79	8.86	1	-	-
Pasty	25	12.00	0.71	0.16-2.99	0.44
Semi-liquid	9	11.11	0.77	0.08-7.15	0.59
Liquid	7	57.14	0.07	0.01-0.39	0.05

*The prevalence used was that obtained by the modified Ziehl-Neelsen test.
**The calves at 31 to 45 days of age are not considered because all tested negative (N=11).

The presence of diarrhea was a statistically significant factor through the ZN test as a protective factor (OR=0.14; $CI_{95\%}$ 0.04-0.44; P=<0.05); this might be because after manifesting diarrhea, the calves acquire partial immunological resistance to the reinfection by *Cryptosporidium* spp.

The risk factor feces consistency had statistical significance, compared with the feces with normal consistency as protective factor (Table 3). Rajkhowa *et al*. (2006) indicated that the animals which presented diarrhea had higher prevalence (94%) than the animals that did not have diarrhea (51%). Likewise, Saha *et al*. (2006) proved that *Cryptosporidium* spp. causes diarrhea in cattle, since throughout two years these authors analyzed fecal samples from diarrheic animals and from asyntomatic animas at 1 day to 12 months of age, and obtained a prevalence of 17.9 and 18.0% in

the first and second year, respectively. These results agree with the report by Diaz *et al*. (2004), who found an association between the presence of *Cryptosporidium* spp. and diarrhea in calves at 2 to 30 days of age in one herd of dairy cattle and in another herd of dual-purpose cattle; the results showed that 65.8% of the dairy calves and 43.1% of the dual-purpose calves excreted *Cryptosporidium* spp. oocysts, which indicates a high prevalence of this disease in newly born calves, and it suggests that the protozoan may have a clinical significance.

As for the housing of the female calves, either in an individual stall or in a common stall but tied to be kept separated one from each other, the fact of being in an individual stall resulted a protective factor (OR=0.27; $CI_{95\%}$ 8-85; P=0.04) through the ZN test, because if the calves are tied one next to the other, the direct infection could occur, which is coincident with the report by Starkey *et al*. (2006), who found that having the calves one close to the others could be a risk factor.

The ingestion of colostrum by the female calves within the first 30 to 60 min after birth turned out to be a protective factor, in comparison with the ingestion of colostrum 2 to 3 h after birth (OR=0.22; $CI_{95\%}$ 0.05-0.87; P=0.04). However, according to Hamnes *et al*. (2006), Starkey *et al*. (2006) and Trotz-Williams *et al*. (2008), the ingestion of colostrum by the calves within the first 30 to 60 min after birth was not significant as a protective factor.

CONCLUSION

Cryptosporidium spp. was present in the suckling female calves from the farms surveyed. The only risk factor found was the presence of diarrhea. The ingestion of colostrum by the calves during the first 30 to 60 min after birth and the housing of the calves in individual stalls might be useful when establishing a program for controlling diarrheas in female calves.

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