Prevalence of *Cryptosporidium* spp. in lactating calves from cattle ranches in the temperate zone of Central Veracruz, Mexico

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ABSTRACT

Objective: to determine the prevalence of *Cryptosporidium* spp. in a population of lactating calves from one to 60 days of age.

Design/Methodology/Approach: this was an under convenience cross-sectional epidemiological study in cattle ranches located in five municipalities in the temperate zone of Veracruz, Mexico. The complete sample size was 500 animals (Win Episcope© 2.0), 100 per municipality. A feces sample was taken directly from the rectum with a latex glove and transported in refrigeration (4 °C) to the laboratory for analysis. The samples were analyzed using modified the Faust centrifugation method, and the Ziehl-Neelsen technique. Statistical analyses were performed in STATA® 14.0. at a 95% confidence interval.

Results: all municipalities presented *Cryptosporidium* spp. An overall prevalence of 62.6% was found, within a range of 49% to 83% according to the municipality. In regard to sex, a prevalence of 78.4% ($_{95\%}$CI 69.9-85.0) of *Cryptosporidium* spp. was found in males. Regarding age, the highest prevalence of *Cryptosporidium* spp. 75.6% in living tissue was found in calves one to 15 days old ($_{95\%}$CI 69.8-80.7). The highest prevalence 78.7% was found in liquid feces ($_{95\%}$CI 71.6-84.6), which proves that diarrhea sometimes fetid, is the main sign of *Cryptosporidium* spp. presence in lactating calves.

Limitations/ Implications of the study: the study did not consider other pathogens that also can cause diarrhea in lactating calves.

Findings/Conclusions: *Cryptosporidium* spp. is present in lactating cattle in the temperate zone of central Veracruz, Mexico. The highest prevalence was found in lactating calves one to 15 days old, in males, and in those with liquid stools.

Keywords: bovine, diarrhea, protozoan, Ziehl-Neelsen.

INTRODUCTION

Cryptosporidium spp. is a single-celled protozoan that inhabits the intestinal villi of calves, lambs and other animal species including humans. Transmission is fecal-oral by ingestion of sporulated oocysts (Yang *et al.*, 2021). Also, indirectly by water and food contaminated with fecal material, by contact with contaminated environmental surfaces, or person-to-person contact (Pezzani, 2023). The life cycle of this protozoan begins with the ingestion and decystation of oocysts that involves asexual and sexual phases, and ends with the infective phase of the parasite, which is eliminated in large quantities in the feces (Gunasekera *et al.*, 2020). Oocysts are environmentally resistant, and remain viable for a long time (Olson *et al.*, 2004).

There are 29 mammalian species of *Cryptosporidium* (Ryan *et al.*, 2021), of which six have been found infecting cattle, the most common being *Cryptosporidium parvum*, widely distributed in intensively produced dairy cattle (Thomson *et al.*, 2017) and has been identified as one of the primary etiological agents of neonatal diarrhea (Wells and Thomson 2014; Santin, 2020). Clinical cryptosporidiosis is characterized by profuse watery diarrhea, loss of weight yield, and death (Thompson *et al.*, 2016). In calves (less than six weeks of age) it reduces daily weight gain. Young calves can shed a large quantity (3.89×10^{10}) of oocysts in six to 12 days (Shaw *et al.*, 2020). Older calves (≥6 months) and adult cattle may be naturally infected with *C. parvum* and excrete oocysts, while remaining subclinical or asymptomatic (Shaw *et al.*, 2021).

Cryptosporidiosis is recognized as endemic in cattle worldwide and is one of the leading causes of neonatal enteritis in calves around the world. In various studies implemented in Europe, North America and Egypt, it has been reported that in calves from meat production systems the prevalence is lower than in dairy calves; with prevalence values 6-78% of *Cryptosporidium parvum* in calves from dairy herds (Garro *et al.*, 2016 Brainard *et al.*, 2020; Weldemariam *et al.*, 2024). This behavior has also been reported in meta-analyses of *Cryptosporidium* spp. in ungulates (Hatam-Nahavandi *et al.*, 2019). In South America, prevalence values from 7.3% in Colombia (Bulla-Castañeda *et al.*, 2024) to 51.75% in Brazil (Candeias *et al.*, 2022) are reported. Various risk factors have been identified, such as contact with other species (goats and sheep), use of a semi-intensive breeding system, absence of hygienic conditions —fecal contamination of food and water (Conceição *et al.*, 2021) and high animal density in hot and humid climate (Brainard *et al.*, 2020; Bulla-Castañeda *et al.*, 2024).

In Mexico, in the states of Coahuila and Durango, prevalence values 56.41% and 87.17% of *Cryptosporidium* spp. have been reported in adult animals and calves. This suggests that the parasite is endemic and represents a risk to the dairy industry; in addition to risks to public health if manure is applied as fertilizer in agricultural lands (López *et al.*, 2020). In the northern region of the state of Veracruz, a prevalence 78% was reported in calves from one-day old to six months of age in a beef cattle production system (Aguilar *et al.*, 2007). Similarly, another study in dual-purpose production systems found an overall prevalence 73.6% of *Cryptosporidium* spp. in calves from three to 12 months of age from three ecological regions of central Veracruz (Castelán-Hernández *et al.*, 2011).

In Mexico there is a wide variety of calf breeding systems in the different dairy regions of the country. In specialized livestock management in the state of Veracruz there are no reports of *Cryptosporidium* spp. in calves. Therefore, it is important to know the situation of livestock farming and thus be able to establish appropriate control strategies for the disease caused by this parasite. The objective of this research was to determine the prevalence of *Cryptosporidium* spp. in calves ≤60 days of age in cattle ranches; under the hypothesis that a prevalence higher than 50% can be found in calves from 1 to 60 days of age, in the temperate zone of Veracruz, México.

MATERIALS AND METHODS

Study site

The study was implemented across the municipalities Acatlan (19° 4' N; 96° 50' W), Landero y Coss (19° 44' N; 96° 51' W), Miahuatlan (19° 43' N; 96° 53' W), Xico (19° 25' N; 97° 00' W), and Naolinco (19° 39' N; 96° 51' W), in the temperate zone of central Veracruz.

Experimental design and protocols

The collection of samples was based on an under convenience cross-sectional epidemiological study with lactating calves from one to 60 days of age, regardless of sex, presence or absence of diarrhea, in cattle ranches located in the five municipalities above mentioned. The number of calves included in the study was estimated with the statistical software Win Episcope© 2.0 (Thrusfield *et al.*, 2001), considering a 50% prevalence, a 5% margin of error, and a confidence level at 95%, the animal sampling size was set in N ≤500 animals. The study protocol was approved by the Bioethics and Animal Welfare Commission of the Faculty of Veterinary Medicine and Zootechnics under Universidad Veracruzana, in compliance with the Mexican Standard (NOM -062-ZOO-1999).

Collecting and transporting of samples

From each one of the municipalities, 100 animals were selected in order to obtain samples from 500 lactating calves. Samples were collected from February to July 2023, in the ranches where producers agreed to participate. A feces sample was taken directly from the rectum of each calf, using a latex glove and deposited in a bag. Individual samples were identified with the ID-number of the animal, date of birth, and the number or name of the mother. The fecal samples were kept refrigerated (4 °C) and transported to the Parasitology Laboratory of the Diagnostic Unit Ranch "Torreón del Molino" of the Faculty of Veterinary Medicine and Zootechnics, under Universidad Veracruzana.

Diagnose laboratory techniques

The samples were analyzed using the modified Faust centrifugation method and the modified Ziehl-Neelsen technique (Figueroa-Castillo *et al.*, 2015), in order to detect the presence of *Cryptosporidium* spp.

Statistical analysis

Data was stored in a Microsoft Excel® database. Overall and specific prevalence by age was calculated according to age class (1 to 15 days, 16 to 39 days, and 40 to 60 days); also by sex, municipality and the structural consistency of the feces (normal, pasty, semi-liquid, or liquid). For the data analysis, the statistical software STATA® 14.0 was used. A Chi-Square test (χ^2) with a 95% confidence interval was performed to determine the relationship between the presence of *Cryptosporidium* spp., and the sex, age, or consistency of the feces.

RESULTS AND DISCUSSION

Table 1 shows the prevalence of *Cryptosporidium* spp. per municipality. Positive samples were obtained in the five municipalities, with an overall 62.6% prevalence within a range 49% to 83% (χ^2=27.08 y p<0.001) depending on the municipality (Table 1). In addition, the actual 64.08% prevalence was obtained by means of the formula developed by Ameni *et al.* (2008) for the Zielh-Neelssen test.

These results coincide with other studies, such as those in the state of Veracruz and other Mexican southern states, and the Lagunera Region of Mexico. In Veracruz, 12-20% prevalence values have been reported per municipality (Aguilar *et al.*, 2007); also an overall prevalence up to 73.6% (Castelán-Hernández *et al.*, 2011). Whereas in the Lagunera Region, a 71.79% prevalence of *Cryptosporidium* spp. is reported in cattle (López *et al.*, 2020). Variation in prevalence may be due to possible differences in facilities, management, or diet in each study. Likewise, it has been described that environmental factors such as temperature and humidity influence the survival and transmission of *Cryptosporidium* spp. oocysts, the hot climate with a high humidity content favor the persistence and propagation of this parasite. In addition, *Cryptosporidium* oocysts are extremely resistant to the action of commonly used chemical control agents. Oocysts can survive in ambient conditions, maintaining their infective capacity in the environment for prolonged periods about three months or more (Ikiroma and Pollock, 2021; Weldemariam *et al.*, 2024).

In this study, according to the sex of the evaluated animals, a 78.4% prevalence was observed in males, higher than 66.1% found in females, statistically significant (χ^2=4.58; p=0.032). In regard to the age of the animals, significant differences were found among

Table 1. Prevalence of *Cryptosporidium* spp. found with the Zielh-Neelssen technique in lactating calves per municipality sampled in Veracruz, Mexico.

Municipality	Lactating calves	Positive(+) Lactating calves	Prevalence (%)	*$_{95\%}$CI
Acatlan	100	57	57.0	46.7-66.7
Landero y Coss	100	49	49.0	38.9-59.1
Miahuatlan	100	83	83.0	73.8-89.5
Xico	100	59	59.0	48.7-68.6
Naolinco	100	65	65.0	54.7-74.0
Total	500	313	62.6	58.1-66.8

*Confidence Interval at 95%.

the age classes (χ^2=41.5; p<0.001). The highest prevalence 75.6% of *Cryptosporidium* spp. was found in calves of 1-15 days of age. However, animals aged 16-39 days and those aged 40-60 days also had a moderate prevalence of 53.1% and 41.1%, respectively (Table 2).

Some studies have indicated that there may be differences in the prevalence and severity of infections between male and female calves. In a previous study in Veracruz, the prevalence was 78.7% for females and 45.5% for males; Although the prevalence was considerably higher in female calves, it was suggested that could be due to female sampled population, which was larger than that of males (Castelán-Hernández *et al.*, 2011). In our study, despite we got a larger population of females than males, the trend was opposite, we found a higher prevalence of *Cryptosporidium* spp. in males than in females (78.4% *vs*. 66.1%, respectively). Some studies mention that there is no relationship between a greater or a lesser presence of *Cryptosporidium* spp. when comparing females and males (Bulla-Castañeda *et al.*, 2024). However, the variability in the results in different studies related to the sex of calves focuses more on the management given to them on farms (Silverlås *et al.*, 2009).

Regarding age, López *et al.* (2020) observed a higher prevalence in weaned calves 87.17% than in adult cattle 56.41%. However, in studies on calves, other authors indicate that higher prevalence values and higher odds ratios (OR) are found in animals <20 days of age (Thomson *et al.*, 2019; de Alba *et al.*, 2023). This high prevalence in calves under three weeks-old is because they are more susceptible to *Cryptosporidium* infection due to their immature immune systems. Also, because there is a higher probability of exposure to contaminated environments (Bilata and Shimelis, 2023). In another study, Zeleke *et al.* (2017) mentioned that the prevalence of *Cryptosporidium* infection decreases as the age of the animal increases, which is consistent with what was found in this study, because calves aged 1-15 days had a 75.6% prevalence, whereas in those aged 40-60 days, prevalence was reduced to 41.1% (Table 2).

Regarding the consistency of the feces and prevalence of *Cryptosporidium* spp., we found that there were significant differences (χ^2=77.8; p<0.001). Table 3 shows that the highest prevalence was in the consistency of semi-liquid stool 70.7%, and liquid stool 78.7%; This proves that the main sign of *Cryptosporidium* spp. in lactating calves is the

Table 2. Prevalence of *Cryptosporidium* spp. by the Zielh-Neelssen technique in lactating cattle according to sex and age classes (1-15, 16-39, and 40-60 days) in Veracruz, Mexico.

Sex	Lactating calves	Positive(+) Lactating calves	Prevalence (%)	*$_{95\%}$CI
Female	325	215	66.1	60.6–71.2
Male	175	98	78.4	69.9–85.0
Age (days)				
1-15	255	193	75.6	69.8–80.7
16-39	160	85	53.1	45.1–61.0
40-60	85	35	41.1	30.7–52.3
Total	500	313	62.6	58.1–66.8

*Confidence Interval at 95%.

Table 3. Prevalence of *Cryptosporidium* spp. by the Zielh-Neelssen technique in lactating calves related to the consistency of the feces.

Consistency of the feces	Lactating calves	Positive(+) Lactating calves	Prevalence (%)	*$_{95\%}$CI
Normal	58	15	25.8	15.6-39.2
Pasty	89	35	39.3	29.3-50.2
Semi-liquid	188	133	70.7	63.6-77.0
Liquid	165	130	78.7	71.6-84.6
Total	500	313	62.6	58.1-66.8

*Confidence Interval at 95%.

presence of diarrhea, which sometimes, can be fetid. That is why an empirical diagnosis of cryptosporidiosis could be done by observing the consistency of the feces, since the presence of this protozoan could be suspected through the appearance of the stools.

The high prevalence of *Cryptosporidium* spp. reported in this study could indicate that feces of liquid consistency are unequivocal sign related to the distinct presence of this parasite. In Italy, Diaz *et al.* (2018) associated the presence of liquid feces with *Cryptosporidium*. However, it is possible that the consistency of the feces is caused by other pathogens that weaken the immune system of calves, making them more susceptible to *Cryptosporidium* infection (Conceição *et al.*, 2021). Diarrhea caused by this parasite is associated with the excretion of a large number of oocysts, but it is not evident that such an excretion always occurs at the same time of diarrhea (Castro *et al.*, 2002).

Nonetheless, the relative risk between the presence of *Cryptosporidium* spp. oocysts and the occurrence of diarrhea is significant in calves. It is demonstrated there is a relationship between them (Aguirre *et al.*, 2013), since those animals that had feces of liquid consistency had a higher prevalence (78.7%). This also coincides with Hernández-Valdés (2018) who reported the highest prevalence 75.5% in the semi-liquid feces of dual-purpose cattle in the central region of the state of Veracruz. Both examples are similar to our findings in this study.

In order to reduce the presence of *Cryptosporidium* in calves, better sanitary practices should be implemented; some of which are that calves got adequately access to colostrum; to separate calves apart from adult animals; to isolate those animals that excrete diarrhea; as well as an efficient cleaning when new animals are brought into management areas. Better management practices are relevant because *Cryptosporidium* presence in lactating calves has a negative impact on growth. In addition, because that is a parasite highly resistant to environmental conditions, it can contaminate water, feed and feed management; thus affecting other animal species, even people.

CONCLUSIONS

The infection caused by *Cryptosporidium* spp. infection was confirmed in lactating calves in cattle ranches of five municipalities located in the temperate zone of central Veracruz, Mexico. The highest prevalence of *Cryptosporidium* spp. occurred in newborn calves from one to 15 days of age, particularly in males, and in those that excreted liquid feces.

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