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Frequency of *Cryptosporidium* spp., in Holstein cattle through three diagnostic methods

Frecuencia de *Cryptosporidium* spp., en bovinos Holstein mediante la comparación de tres métodos diagnósticos

Huerta-Magallanes, A.T. , *González-Ruiz, S.** , *Veyna-Salazar, N.P.* , *Cantó-Alarcón, G.J. N.* ,*Bárcenas-Reyes, I.* , *Vera-Ávila, H.R.*

Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro. Av. De las Ciencias s/n; Juriquilla, Delegación Santa Rosa Jáuregui, C.P. 76230, Querétaro, Qro, México..

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ABSTRACT

Cryptosporidiosis is a disease that affects calves, causing acute or chronic gastrointestinal disorders and death. It is also considered one of the main causes of diarrhea in humans. The diagnosis of this disease is made through the identification of oocysts by conventional methods; however, there is a lack of information about the performance of these methods in the field. Therefore, the objective of this study was to determine the most sensitive, practical, economical, and feasible conventional diagnostic method, as well as to determine the frequency of the disease in Holstein cattle, as a preliminary report of the parasite frequency in the State of Queretaro, Mexico. Three diagnostic methods were compared: Ziehl-Neelsen Cold Staining, Safranin-Methylene Blue Stain, and Sheather Sugar Flotation. The best diagnostic method for the identification of *Cryptosporidium* spp., oocysts in feces was the cold Ziehl-Neelsen stain method. A frequency of 43.4 % was obtained, indicating a high prevalence of the disease in cattle. In calves, the frequency was 63.4 %. There was a significant association ($p < 0.05$) between the presence of diarrhea in calves and the presence of oocysts in feces. Our results show the importance of establishing control measures to reduce the impact of this disease on dairy cattle premises.

KEY WORDS: *Cryptosporidium* spp., cattle, diagnosis, Ziehl-Neelsen, public health.

***Corresponding Author:**

Sara González-Ruiz. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro. Av. de las Ciencias s/n; Juriquilla, Delegación Santa Rosa Jáuregui, C.P. 76230, Querétaro, Qro., México. Teléfono (442) 192 1200 ext. 5360.
E-mail: sara.gonzalez@uaq.mx

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RESUMEN

La criptosporidiosis es una enfermedad que afecta especialmente a los becerros, produciendo trastornos gastrointestinales agudos o crónicos, e incluso la muerte de los animales. Se considera una de las principales causas de diarrea en humanos debido a su carácter zoonótico. El diagnóstico de la enfermedad se realiza a través de la identificación de ooquistes por medio de métodos convencionales, sin embargo, existe una falta de información sobre el desempeño de estos en campo, por lo que el objetivo del presente estudio fue comparar distintos métodos diagnósticos convencionales para determinar el más sensible, práctico, económico y viable para los productores a fin de prevenir mayores tasas de morbilidad y mortalidad en los hatos, además de determinar la frecuencia de la enfermedad en ganado Holstein estableciendo un informe preliminar del parásito en el estado de Querétaro. Se compararon tres métodos diagnósticos: Tinción de Ziehl-Neelsen en frío, Tinción Safranina-Azul de Metileno y Flotación con Azúcar de Sheather. Los resultados indican que el mejor método de diagnóstico para la identificación de ooquistes de *Cryptosporidium* spp., en campo fue Ziehl-Neelsen en frío por su facilidad en desarrollo y a la sensibilidad mostrada. Se obtuvo una frecuencia total del 43.4 % indicando una alta prevalencia de la enfermedad, mientras que solo en los becerros fue del 63.4 %. Se determinó una asociación estadísticamente significativa ($p < 0.05$) entre la presentación de diarrea y la detección de ooquistes, por lo que es importante establecer medidas de control y bioseguridad en las unidades de producción para disminuir la presencia de la enfermedad.

PALABRAS CLAVE: *Cryptosporidium* spp., bovinos, diagnóstico, Ziehl-Neelsen, salud pública.

Introduction

Cryptosporidiosis is a disease caused by the intracellular protozoan *Cryptosporidium* spp. which infects the cells of the small intestine of animals and humans causing diarrhea. *Cryptosporidium* represents a public health risk and has important implications in cattle, especially calves, where it causes acute or chronic gastrointestinal disorders such as diarrhea with either mucus or blood, low growth rates, reduction in daily weight gain (GDP), and even death (Brook *et al*., 2008; Tarekegn *et al*., 2021). More than 10 species within the genus *Cryptosporidium* spp., have been identified, and the distribution of these species is related to the age of the host, making evident the misconception that the zoonotic species (*Cryptosporidium parvum*) is present in all ages of the animal (Fayer, 2010). Species distribution can be attributed to different factors such as: the change in intestinal microflora as the animal matures, or dietary changes that may affect the ability of the parasite to infect the intestine (Thomson *et al*., 2017). On the other hand, it has

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been suggested that newborn calves are more susceptible to *Cryptosporidium* spp., infections due to the immaturity of their immune system and that the reduction in prevalence rates with age could be due to the development of a partial protective immune response following multiple infections with the parasite (Diaz *et al*., 2021). The most important species in cattle are: *Cryptosporidium parvum* (*C. parvum*) which causes clinical disease in calves and is zoonotic, *Cryptosporidium bovis* (*C. bovis*) due to its pathogenic potential in adult cattle, and *Cryptosporidium andersoni* (*C. andersoni*) which is strongly associated with the dissemination of oocysts in the environment, contributing to infection of animals (Chalmers & Davies, 2010; Hadfield *et al*., 2011; Kumar *et al*., 2004; Ralston *et al*., 2010; Silverlås *et al*., 2013; Xiao *et al*., 1999). Cryptosporidiosis is distributed worldwide, with prevalences ranging from 6 % to 60 % in different countries such as Canada, United States of America, Mexico, and China (Maldonado-Camargo *et al*., 1998; Nwosu *et al*., 2019; Romero-Salas *et al*., 2016; Ruest *et al*., 1998; Trotz-Williams *et al*., 2006). In Mexico, the disease has been recognized since 1983, when its presence was documented in lactating cattle. Since then some studies have been conducted in different States (Maldonado-Camargo *et al*., 1998), such as Veracruz (73.6 %) (Castelan-Hernàndez *et al*., 2011), Región Lagunera (Durango and part of Coahuila) (71.8 %) (López-Torres *et al*., 2020), Aguascalientes (40 %) (García-Romo *et al*., 2014), Nayarit (26-30 %) (González *et al*., 1997), Chihuahua (30 %) (Castelan-Hernández *et al*., 2011), Guanajuato (35 %) (Castelan-Hernández *et al*., 2011), and Guerrero (3.14 %) (Fitz-Sánchez *et al*., 2013).

The diagnosis of the disease is made by the identification of oocysts in feces by conventional methods: Sheather's Sugar Flotation, Zinc Sulfate Flotation, Ziehl-Neelsen staining, Kinyoun staining, Formalin-ethyl acetate sedimentation, and some negative staining methods using Nigrosin, Light Green, Malachite Green and Carbol fuchsin (Rekha *et al*., 2016); as well as by molecular methods such as those based on PCR (nested PCR, PCR-Restriction Fragment Length Polymorphism, qPCR, ddPCR, multiplex real-time PCR), Loop-Mediated Isothermal Amplification (LAMP) and by serological methods such as Immunofluorescent Assays and Antigen Capture Immunosorbent Assays (ELISA) (Aboelsoued & Abdel, 2022; Khan *et al*., 2016). In addition, commercial kits are available for *Cryptosporidium* spp., antigen detection through Enzyme Immunoassays, Indirect Immunofluorescent Assays (IFA), and rapid tests (Khan *et al*., 2016). Microscopic or conventional methods are considered the methods of choice in the field due to their speed, simplicity, and low cost, compared to molecular methods which are mostly limited to research and specialized laboratories with limited applicability in low-resource settings due to their costs, infrastructure, and high technical expertise involved (McCluskey *et al*., 1995; Omoruyi *et al*., 2014; Santín, 2020; Silverlås et al., 2013). There are also commercial kits that are more expensive than molecular methods and may be inefficient in detecting oocysts in patients with low infection rates (Hawash, 2014). Currently, there is no effective treatment; therefore, the best way to reduce the impact of the disease is the establishment of preventive actions; thus, detecting the parasite in the first days of life in calves is important to prevent the spreading of the disease. To accomplish this, to have a method of diagnosis that is quick, sensitive, and easy to use in the field is relevant.

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Material y Methods

Investigation area

The study was conducted in a herd of Holstein cattle located in the Pedro Escobedo municipality, in Queretaro state, Mexico. The climate conditions are semi-dry temperate, with an average annual temperature of 14-20 °C, rainfall of 500 to 800 mm^2 per year, and 66 % humidity (INEGI, 2010; INEGI, 2020).

Description of the Production System

The dairy unit studied has an intensive production system with approximately 720 animals in line. Animals are separated by age; at birth, calves are separated from cows and housed in individual stalls for about 4 months, then from 4 to 7 months they are moved into groups of different age until the age of heifers and adults.

Study design and population

A cross-sectional study was conducted from June to July 2023. The sample size was determined using a 25 % Maldonado-Camargo *et al.* (1998) hypothetical prevalence, 95 % confidence level and 5 % error.

$$n = \frac{Z\alpha^2 * p * q}{d^2} = \frac{(1.962^2)(0.25)(0.75)}{(0.05^2)} = 288$$

Sample collection

Throughout convenience sampling, feces samples were collected in palpation gloves directly from the rectum of the animals. Due to the susceptibility of younger animals to *Cryptosporidium* spp., the distribution of sampling was as follows: 145 under two months of age, 70 from 4-7 months, and 73 adults. The age of the animal, whether or not they had diarrhea, and the consistency of the diarrhea were recorded. Feces samples were then transferred to the Parasitology Laboratory located at the Faculty of Natural Sciences of the Autonomy University of Queretaro, Mexico, where they were stored at 4 °C until processing.

Analysis of the samples

To establish the best diagnostic method for the diagnosis of *Cryptosporidium* spp., the first 100 samples were analyzed by convenience as follows: Ziehl-Neelsen Cold Staining (ZN), Safranin-Methylene Blue Stain (SMB) and Sheather Sugar Flotation (SSF). All samples were processed fresh due to the urgency of diagnosis for the Production Unit studied. The distribution of the 100 samples analyzed was: 69 samples from calves under two months of age, 24 samples

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from calves 4-7 months, and 7 samples from adults. The samples were analyzed by two different operators simultaneously.

Statistical analysis

To know the level of concordance between the three methods, the Kappa Index was performed. The interpretation of the results was as follows: kappa value (κ): κ ≥0.75=excellent concordance, κ 0.40-0.74= fair to good concordance and κ<0.40= poor concordance. The Chi-square test (X^2) was performed to measure the association between the presence of diarrhea and the incidence of oocysts of *Cryptosporidium* spp., in feces. The final frequency was determined as the proportion of samples positive to *Cryptosporidium* spp., by the presence of oocysts in fecal smears using the Cold Ziehl-Neelsen staining (ZN) method, since as it was the most sensitive, quick, and easy to perform.

Results and Discussion

The best diagnosis method was determined by the highest frequency of oocyst recovery, kappa index value, easy to perform and capacity of visualization of oocysts. Of the 100 samples analyzed to determine the highest sensitivity, a frequency of 53 % was observed for Ziehl-Neelsen Cold Staining, 41 % for Safranin-Methylene Blue and 9 % for Sheather's Sugar Flotation. Kappa (κ) analysis determined a higher concordance between ZN and SMB methods (κ = 0.88), determining that 12 of the animals that were positive to ZN were negative by SMB. A concordance between SMB-SSF of κ = 0.34 and ZN-SSF of κ = 0.26 was obtained (Table 1). The concordance or similarity between the ZN and SMB methods was previously observed by Rubio- Guarín (2010), indicating a higher sensitivity for the ZN test over SMB, which agrees with obtained results. The negative results in SMB, but positive in ZN can be explained by human factors such as the confusion of oocysts with other artifacts such as bacterial spores or fecal debris; as well as the critical heating period present in SMB (Baxby *et al*., 1984).

The SSF method was the lowest in percentage recovery of *Cryptosporidium* spp., oocysts and the most time and training requirements, which is contrary to that described by Rekha *et al*. (2016), reporting that SSF is the most sensitive and specific method for oocyst detection. The low detection rate of *Cryptosporidium* spp., by SSF in the present study may be due to several factors, emphasizing a possible reduced number of oocysts. Fujino *et al*. (2006) indicate that for the SSF method to be reliable, samples should have more than 103 oocysts/ml, which could result in a high number of false negatives in animals with low infection rates, which may have occurred in the present study; moreover, Current & Garcia (1991) mention that the detection of oocysts through this method is considered of greater difficulty than those compared due to the characteristics of the solutions used that can cause the oocysts to collapse or deform if they are left more than 15 minutes in the solution, making the diagnosis less certain. On the other hand, the preparation of the sugar solutions used in SSF requires a very precise specific gravity, which can be a determining factor in the recovery or not of the oocysts (Rojekittikhun *et al*., 2015).

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Table 1. Kappa values to determine concordance between Ziehl-Neelsen, Safranin-Methylene Blue and Sheather Sugar Flotation methods for the identification of *Cryptosporidium* spp., oocysts in Holstein cattle.

Ziehl-Neelsen (ZN)	**Safranin-Methylene Blue (SMB)**		**Kappa index**
	Positives	***Negatives***	
Positives	41	12	κ = 0.88
Negatives	0	47	

Ziehl-Neelsen (ZN)	**Sheather Sugar Flotation (SSF)**		**Kappa index**
	Positives	***Negatives***	
Positives	9	44	κ = 0.26
Negatives	0	47	

Safranin-Methylene Blue (SMB)	**Sheather Sugar Flotation (SSF)**		**Kappa index**
	Positives	***Negatives***	
Positives	8	33	κ = 0.34
Negatives	1	58	

Source: Own elaboration.

None of the ZN-negative samples were positive in the methods used, showing the ability of this to detect oocysts; in addition, allows the possibility of processing multiple samples simultaneously, saves time and it is easy to perform in the field. Therefore, according to the results obtained, the Ziehl-Neelsen Cold Staining method was defined as the most sensitive for the analysis of the total number of samples in this study.

Comparison with molecular or serological methods more sensitive than cold ZN Staining was not performed due to the objectives of the study and the need for rapid diagnosis for the timely treatment of the animals in the production unit studied; in addition, some authors highlight the high costs of using these methods, as well as the need for specialized knowledge on the part of the operators (Omoruyi *et al*., 2014; Santín, 2020; Silverlås *et al*., 2013). In case of requiring investigations about the parasite species affecting the herd, molecular methods are necessary, since microscopic methods such as ZN staining do not differentiate between them despite being cheap and affordable (Rodríguez, 2016; Omoruyi *et al*., 2014). On the other hand, the use of commercial kits was not considered because of the high cost that would involve the diagnosis of the total animals; a comparison study between a staining method (Kinyoun staining) and two enzyme immunoassays determined a greater convenience in the use of the first one due to the practical time required in immunoassays together with the high costs of these (Kehl *et al*., 1995), giving this an overview of the benefit of conventional methods.

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The overall frequency of *Cryptosporidium* spp., in the present study was 43.4 % (Table 2), higher than that reported by Fitz-Sánchez *et al*. (2013) (3.14 %) in the state of Guerrero, similar to a study conducted in the state of Aguascalientes by García-Romo *et al*. (2014) (40 %), and lower than that observed by Castelán-Hernández *et al*. (2011).

The frequency observed in this study can be compared in similarity with Aguascalientes, considering the climatic conditions of both states; however, some national and international studies have evaluated the statistical relationship between Cryptosporidiosis and the climate of each region, determining that, although there is a peak of infection during some months of the year, there is no statistically significant relationship between these two variables (García *et al*., 2009; Starkey *et al*., 2006), being so that the epidemiology of this parasitosis shows particular characteristics in each unit and production system without consider climate; according to Fayer *et al*. (2000) and Thomson *et al*. (2017). The prevalence of infection caused by this parasite varies strongly between countries and between studies due to various causes, including the type of sample examined (diarrheal/non-diarrheal), the diagnostic methods used, the age of the animals along with the hygienic-sanitary conditions of each production unit; thus, the large difference in prevalence observed in the state of Veracruz (76.6 %) and Guerrero (3.1 %) can be explained by the difference of the diagnostic method used; while in Veracruz an oocyst concentration method was used followed by a staining method, in Guerrero only the first one was used, making it difficult to observe the oocysts at diagnosis and underestimating the positive cases of the disease (Castelán-Hernández *et al*., 2011: Fitz-Sánchez *et al*., 2013). Thus, each unit has specific conditions where the frequency may be affected due to risk factors such as lack of hygiene in the maternity area and lack of disinfection of feeding utensils (nipples, esophageal tubes, buckets), and some of these factors may explain the difference between the frequency observed in this study for the state of Queretaro compared to other states in the country (Maldonado-Camargo *et al*., 1998; Sischo *et al*., 2000).

Table 2. Frequency of Cryptosporidium spp., and total of oocysts in Holstein cattle.

Age	*Analyzed samples (n)*	*Positive samples*	*Frequency (%)*
Cattle (≤ 2 months)	145	92	63.45
Calves (4 - 7 months)	70	8	11.43
Adults (> 7 months)	73	25	34.25
Total	288	125	43.40

Fuente: Elaboración propia.

With regards to frequency by age, a higher frequency was observed in calves (63.4 %), followed by adults (34.2 %) and heifers from 4 to 7 months (11.4 %) (Table 2), the frequency

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observed in calves agrees with several studies where it is mentioned that *Cryptosporidium* spp., infection is more frequent in lactating animals (Castelán-Hernández *et al*., 2011; Santín *et al*., 2004; Santín *et al*., 2008). It has been suggested that calves are more susceptible to *Cryptosporidium* spp., infections due to the immaturity of their immune system (Fayer *et al*., 2006) and that the reduction in prevalence rates with age could be due to the development of partial protective immunity following multiple infections with the protozoan (Ares-Mazás *et al*., 1999).

In several studies, it has been determined that the frequency of *Cryptosporidium* spp., decreases with age (Díaz *et al*., 2021; Santín *et al*., 2008), as was observed in the present study where animals from 4 to 7 months of age presented a frequency of only 11.4 %. On the other hand, most studies agree that *Cryptosporidium* spp., infection is less frequent in adult animals (Amer *et al*., 2013; Díaz *et al*., 2021), however, in the present study a frequency of 34.2 % was found, which may be due to the presence of *C. andersoni* that mainly affects animals older than 2 years (Fayer *et al*., 2006; Santín, 2020; Smith *et al*., 2014).

The identification of *C. andersoni* was performed according to the micrometry described in the literature (Elliot *et al*., 1999; Rekha *et al*., 2016) which indicates the size of oocysts of 7.2 ± 0.835 µm long by 5.7 ± 0.835 µm wide against the size of *C. parvum* which is 5.2 ± 0.422 µm long by 4.05 ± 0.052 µm wide (Figure 1). The high frequency of *Cryptosporidium* spp., in adult animals could be explained by the management in the herd, contamination of the pen (cleaning and disinfection), and the immunological status of the animals, among others.

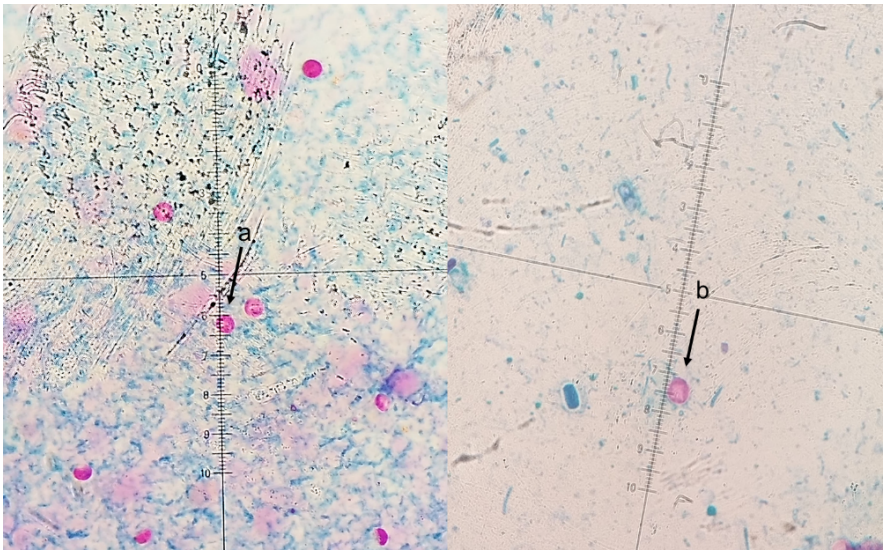

Figure 1. Morphological comparison of *Cryptosporidium* spp., oocysts and oocysts of *C. parvum* (a) and *C. andersoni* (b).

Source: Own elaboration, taken by Atzimba T. Huerta Magallanes.

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Finally, to determine the existence of a statistically significant relationship between the presence of diarrhea and the presence of *Cryptosporidium* spp., the Chi-square test (X^2) was used. The proportion of animals with diarrhea was 24.3 % (39.3 % in calves; 14.28 % in calves aged 4-7 months and 4.1% in adults), observing a decrease in this sign with age. Of the total number of animals with diarrhea, 62.8 % (44/70) had *Cryptosporidium* spp., oocysts at diagnosis, only 9 of these animals had diarrhea with mucus, and 77.7 % of them had a positive diagnosis of the disease (7/9). The X^2 value showed a significant association between diarrhea and the presence of *Cryptosporidium* spp., oocysts in the general population (p = 0.00027) (Table 3); however, it is important to mention that many of the animals without diarrhea were also positive for the presence of *Cryptosporidium* spp., is one of several important factors in the presentation of diarrhea in dairy cattle that is multifactorial. Other studies have previously evaluated the relationship between this clinical sign and *Cryptosporidium* spp., infection agreeing with the reported results (Díaz de Ramírez *et al*., 2007; Lombardelli *et al*., 2019; Rajkhowa *et al*., 2006; Rekha *et al*., 2016; Romero-Salas *et al*., 2016; Saha *et al*., 2006); being so that Trotz-Williams *et al*. (2005) reported a very strong and significant association between infection by this parasite and the occurrence of diarrhea in calves excreting oocysts, determining that these have at least 3 times more risk of suffering from diarrhea than uninfected calves.

Table 3. X^2 and p-values of the association between the presence of diarrhea and the presence of *Cryptosporidium* spp., in feces of cattle.

Stool consistency	***Negative samples***	***Positive samples***	***Total***
Diarrheic stools	26	44	70
Firm stools	137	81	218
Total	163	125	288
X^2			13.221
p-value			p = 0.0027

Fuente: Elaboración propia.

Conclusions

It was determined that the Ziehl-Neelsen Cold Staining was the easiest method to implement, in which the oocysts were observed more clearly and the one showing the best sensitivity. A general frequency of 43.4 % was determined, with animals younger than 2 months having the highest infection (63.4 %). It was possible to determine the presence of *C. andersoni* through micrometry in adult animals. Furthermore, it was found that diarrhea is significantly associated with the presence of *Cryptosporidium* spp., oocysts. The high frequency observed

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indicates the importance of establishing biosafety measures in production units to avoid the risk of infection in humans.

Authors' contribution

Conceptualization of work: Huerta-Magallanes, González-Ruiz; development of the methodology: Huerta-Magallanes, González-Ruiz; software management: Huerta-Magallanes, González-Ruiz, Cantó-Alarcón; experimental validation: Huerta-Magallanes, González-Ruiz, Cantó-Alarcón; analysis of results: Huerta-Magallanes, González-Ruiz, Veyna-Salazar, Cantó-Alarcón, Vera-Ávila, Barcenas-Reyes; data management: Huerta-Magallanes, González-Ruiz; writing and preparation of the manuscript: Huerta-Magallanes, Veyna-Salazar, Cantó-Alarcón, Vera-Ávila, Barcenas-Reyes; writing, review and editing: Huerta-Magallanes, González-Ruiz, Veyna-Salazar, Cantó-Alarcón, Bárcenas-Reyes, Vera-Ávila; project administrator: Huerta-Magallanes, González-Ruiz, Cantó-Alarcón; acquisition of funds: Huerta-Magallanes, González-Ruiz, Cantó-Alarcón.

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Ethical statements

The authors declare prior authorization to carry out the project by the Bioethics Committee of the Faculty of Natural Sciences of the Autonomous University of Queretaro, Mexico with folio number 079FCN2023.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

Aboelsoued, D., & Abdel, K.N. (2022). Diagnosis and control of Cryptosporidiosis in farm animals. *Journal of Parasitic Diseases*, 46(4), 1133–1146. https://doi.org/10.1007/s12639-022-01513-2

Amer, S., Zidan, S., Adamu, H., Ye, J., Roellig, D., Xiao, L., & Feng, Y. (2013). Prevalence and characterization of *Cryptosporidium* spp., in dairy cattle in Nile River delta provinces, Egypt. *Experimental Parasitology*, 135(3), 518-23. https://doi.org/10.1016/j.exppara.2013.09.002

Ares-Mazás, M.E., Fernández-da Ponte, B., Vergara-Castiblanco, C.A., Freire-Santos, F., Quílez-Cinca, J., Causapé-Valenzuela, A.C., & Sánchez-Acedo, C. (1999). Oocysts, IgG levels and immunoblot patterns determined for *Cryptosporidium parvum* in bovine examined during a visit to a farm (northeastern Spain). *Veterinary Parasitology*, 81(3), 185-93. https://doi.org/10.1016/s0304-4017(98)00245-3

Baxby, D., Blundell, N., & Hart, C,A. (1984). The development and performance of a simple, sensitive method for the detection of *Cryptosporidium* oocysts in faeces. *The Journal of Hygiene*, 93(2), 317-23. https://doi.org/10.1017/s0022172400064858

Brook, E., Hart, C.A., French, N., & Christley, R. (2008). Prevalence and risk factors for *Cryptosporidium* spp., infection in young calves. *Veterinary Parasitolgy*, 152(1-2), 46-52. https://doi.org/10.1016/j.vetpar.2007.12.003

Castelán-Hernández, O.O., Romero-Salas, D., García-Vázquez, Z., Cruz-Vázquez, C., Aguilar-Domínguez, M., Ibarra-Priego, N.D.J., & Muñoz-Melgarejo, S. (2011). Prevalencia de Criptosporidiosis bovina en tres regiones ecológicas de la zona centro de Veracruz, México. *Tropical and Subtropical Agroecosystems*, 13(3), 261-267. https://www.redalyc.org/articulo.oa?id=93920942024

Chalmers, R.M., & Davies, A.P. (2010). Minireview: Clinical Cryptosporidiosis. *Experimental Parasitology*, 124(1), 138-46. https://doi.org/10.1016/j.exppara.2009.02.003

Current, W.L., & Garcia, L.S. (1991). Cryptosporidiosis. *Clinical Microbiology Reviews*, 4(3), 325–358. https://doi.org/10.1128/CMR.4.3.325

Díaz de Ramírez, A., Ramírez, I., Lilido, N., Morillo, L., José, G., Barreto, B., & Alejandro, J. (2007). Infección con *Cryptosporidium* spp., y su asociación con diarrea en becerros de ganadería de doble propósito. *Zootecnia Tropical*, 25(1), 29-36. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0798-72692007000100005&lng=es&tlng=es.

Díaz, P., Navarro, E., Remesar, S., García-Dios, D., Martínez-Calabuig, N., Prieto, A., López-Lorenzo, G., López, C.M., Panadero, R., Fernández, G., Díez-Baños, P., & Morrondo, P. (2021). The Age-Related *Cryptosporidium* Species Distribution in Asymptomatic Cattle from North-Western SPAIN. *Animals*, 11(2), 256. https://doi.org/10.3390/ani11020256

Elliot, A., Morgan, U.M., & Thompson, R.C. (1999). Improved staining method for detecting *Cryptosporidium* oocysts in stools using malachite green. *Journal of General and Applied Microbiology*, 45(3), 139-142. https://doi.org/10.2323/jgam.45.139

Fayer, R. (2010). Taxonomy and species delimitation in *Cryptosporidium*. *Experimental Parasitology*, 124(1), 90-7. https://doi.org/10.1016/j.exppara.2009.03.005

Fayer, R., Morgan, U., & Upton, S.J. (2000). Epidemiology of *Cryptosporidium*: transmission, detection, and identification. *International Journal for Parasitology*, 30(12-13), 1305-22. https://doi.org/10.1016/s0020-7519(00)00135-1

Revista Bio ciencias
ISSN 2007-3380

Fayer, R., Santín, M., Trout, J.M., & Greiner, E. (2006). Prevalence of species and genotypes of *Cryptosporidium* found in 1-2-year-old dairy cattle in the eastern United States. *Veterinary Parasitology*, 135(2), 105-12. https://doi.org/10.1016/j.vetpar.2005.08.003

Fitz-Sánchez, E., Rosario Cruz, R., Hernández Ortiz, R., Hernández Castro, E., Rodríguez Bataz, E., & García Vázquez, Z. (2013). *Cryptosporidium parvum*: prevalencia y factores de riesgo en becerros del municipio de Cuajinicuilapa, Guerrero, México. *Revista Veterinaria Y Zootecnia*, 7(1), 49–61. https://revistasojs.ucaldas.edu.co/index.php/vetzootec/article/view/4404

Fujino, T., Matsuo, T., Okada, M., & Matsui, T. (2006). Detection of a small number of *Cryptosporidium parvum* oocysts by sugar flotation and sugar centrifugation methods. *Journal of Veterinary Medical Science*, 68(11), 1191-3. https://doi.org/10.1292/jvms.68.1191

García**,** A.M., Cruz, C., Quezada, T., Silva, E., Valdivia, A., Vazquez, S., & Ramos, M. (2009). Cryptosporidiosis in Dairy Calves from Aguascalientes, Mexico: Risk Infection in Relation with the Seadon and Months of Sampling. *Journal of Advanced Veterinary and Animal Research*, 8(8), 1579-1583. https://www.researchgate.net/publication/287032121

García-Romo, D., Cruz-Vázquez, C., Quezada-Tristán, T., Silva-Peña, E., Valdivia-Flores, A., Vázquez-Flores, S., & Ramos-Parra, M. (2014). Prevalence and risk factors associated with infection by *Cryptosporidium* spp. In suckling calves in Aguascalientes, Mexico. *Veterinaria México*, 1(1), https://doi.org/10.21753/vmoa.1.1.332

González, M., Gómez, E.S., & Aluja, A.S. (1997). Criptosporidiosis en bovinos lactantes (histopatología, microscopía electrónica y de barrido). *Veterinaria México,* 14(1), 12-22. https://biblat.unam.mx/es/revista/veterinaria-mexico/articulo/criptosporidiosis-en-bovinos-lactantes-histopatologia-microscopia-electronica-de-transmision-y-de-barrido

Hadfield, S.J., Robinson, G., Elwin, K., & Chalmers, R.M. (2011). Detection and differentiation of *Cryptosporidium* spp. in human clinical samples by use of real-time PCR. *Journal of Clinical Microbiology*, 49(3), 918-24. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3067739/

Hawash, Y. (2014) Evaluation of an immunoassay-based algorithm for screening and identification of Giardia and *Cryptosporidium* antigens in human faecal specimens from saudi arabia. *Journal of Parasitology Research*, 2014, 213745 https://doi.org/10.1155/2014/213745

Instituto Nacional de Estadística y Geografía [INEGI]. (2010). Compendio de Información geográfica municipal 2010. Pedro Escobedo, Querétaro. https://www.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/22/22012.pdf

Instituto Nacional de Estadística y Geografía [INEGI]. (2020). Panorama sociodemográfico de Querétaro: Censo de Población y Vivienda 2020. https://www.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/702825197957.pdf

Kehl, K.S., Cicirello, H., & Havens, P.L. (1995). Comparison of four different methods for detection of *Cryptosporidium* species. *Journal of Clinical Microbiology*, 33(2), 416–418. https://doi.org/10.1128/jcm.33.2.416-418.1995

Khan, S., Khan, A., Khan, I., & Muhammad, I. (2016). Differential techniques used for detection of *Cryptosporidium* oocysts in stool specimens. *Journal of Parasitic Diseases*, 1(1), 1-11. https://www.researchgate.net/publication/338823938_DIFFERENTIAL_TECHNIQUES_USED_FOR_DETECTION_OF_CRYPTOSPORIDIUM_OOCYSTS_IN_STOOL_SPECIMENS

Kumar, D., Sreekrishnan, R., & Das, S.S. (2004). Cryptosporidiosis in man and animals in Pondicherry. *Indian Journal of Animal Sciences*, 74(3), https://epubs.icar.org.in/index.php/IJAnS/article/view/38362

Lombardelli, J.A., Tomazic, M.L., Schnittger, L., & Tiranti, K.I. (2019). Prevalence of *Cryptosporidium parvum* in dairy calves and GP60 subtyping of diarrheic calves in central Argentina. *Parasitology Research*, 118(7), 2079-2086. https://doi.org/10.1007%2Fs00436-019-06366-y

López-Torres, L.L, López-Cuevas, O., Vázquez-Vázquez, C., Alvarado-Gómez, O.G., Vázquez-Alvarado, R.E., Rodríguez-Fuentes, H., Chaidez-Quiroz, C., & Vidales-Contreras, J.A. (2020). Prevalence of *Cryptosporidium* spp. in dairy livestock from the Laguna Region, Mexico. *Revista Bio Ciencias 7*, e881. https://doi.org/10.15741/revbio.07.e881

Maldonado-Camargo, S., Atwill, E.R., Saltijeral-Oaxaca, J.A., & Herrera-Alonso, L.C. (1998). Prevalence of and risk factors for shedding of *Cryptosporidium parvum* in Holstein Freisian dairy calves in central México. *Preventive Veterinary Medicine*, 36(2), 95-107. https://doi.org/10.1016/s0167-5877(98)00084-1

McCluskey, B.J., Greiner, E.C., & Donovan, G.A. (1995). Patterns of *Cryptosporidium* oocyst shedding in calves and a comparison of two diagnostic methods. *Veterinary Parasitology*, 60(3–4), 185–190. https://doi.org/10.1016/0304-4017(95)00790-4

Nwosu, A., Berke, O., Pearl, D.L., & Trotz-Williams, L.A. (2019). Exploring the geographical distribution of Cryptosporidiosis in the cattle population of Southern Ontario, Canada, 2011-2014. *Geospatial Health*, 14(2), https://doi.org/10.4081/gh.2019.769

Omoruyi, B., Nwodo, U., Udem, C., & Okonkwo, F. (2014). Comparative Diagnostic Techniques for *Cryptosporidium Infection Molecules*, 19(2), 2674–2683. https://doi.org/10.3390/molecules19022674

Rajkhowa, S., Rajkhowa, C., & Hazarika, G.C., (2006). Prevalence of *Cryptosporidium parvum* in mithuns (*Bos frontalis*) from India. *Veterinary Parasitology*, 142(1–2), 146–149. https://doi.org/10.1016/j.vetpar.2006.06.026

Ralston, B., Thompson, R.C., Pethick, D., McAllister, T.A., & Olson, M,E. (2010). *Cryptosporidium andersoni* in Western Australian feedlot cattle. *Australian Veterinary Journal*, 88(11), 458-60. https://pubmed.ncbi.nlm.nih.gov/20973292/

Rekha, K.M., Puttalakshmamma, G.C., & D'Souza, P.E. (2016). Comparison of different diagnostic techniques for the detection of Cryptosporidiosis in bovines. *Veterinary World*, 9(2), 211-5. https://doi.org/10.14202/vetworld.2016.211-215

Rodríguez, A. (2016). Identificación de *Cryptosporidium* en becerras Holstein de 1 día a 12 meses de edad. [Tesis de Licenciatura, Universidad Autónoma Agraria Antonio Narro]. https://repositorio.uaaan.mx/xmlui/bitstream/handle/123456789/8264/ANA%20JANETH%20RODRIGUEZ%20CABRERA.pdf?sequence=1&isAllowed=y

Rojekittikhun, W., Mahittikorn, A., Prummongkol, S., Puangsa-Art, S., Chaisiri, K., & Kusolsuk, T. (2015). Evaluation of sugar flotation and formalin-ether concentration techniques in the examination of GI parasites of refuge dogs and cats in Kanchanaburi province, Thailand. *Tropical Medicine & Parasitology*, 38, 17-24. https://ptat.org/uploads/pdf/journalPdf_38-1-2015-e3.pdf

Romero-Salas, D., Alvarado-Esquivel, C., Cruz-Romero, A., Aguilar-Domínguez, M., Ibarra-Priego, N., Merino-Charrez, J.O., Pérez de León, A.A., & Hernández-Tinoco, J. (2016).

Prevalence of *Cryptosporidium* in small ruminants from Veracruz, Mexico. BMC *Veterinary Research*, 12, 14. https://doi.org/10.1186/s12917-016-0638-3

Rubio-Guarín, D. (2010). Comparación de tres técnicas de tinción para la identificación de *Cryptosporidium* spp., a partir de muestras coprológicas obtenidas en una población infantil de Calarcá Quindío [Tesis de Licenciatura, Universidad del Quindío, Armenia] https://bdigital.uniquindio.edu.co/server/api/core/bitstreams/18cbece2-2357-49f2-8497-0e41dbc093e4/content

Ruest, N., Faubert, G.M., & Couture, Y. (1998). Prevalence and geographical distribution of *Giardia* spp. and *Cryptosporidium* spp. in dairy farms in Québec. *The Canadian Veterinary Journal*, 39(11), 697-700. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1539484/

Saha, S., Sarkar, S., Batabyal, S., Pramanik, A.K., & Das, P. (2006). Observations on the epidemiology of bovine cryptosporidiosis in India. *Veterinary Parasitology*, 141(3–4), 330–333. https://doi.org/10.1016/j.vetpar.2006.06.017

Santín, M. (2020). *Cryptosporidium* and Giardia in Ruminants. *Veterinary Clinics of North America: Food Animal Practice*, 36(1), 223-238. https://doi.org/10.1016/j.cvfa.2019.11.005

Santín, M., Trout, J.M., & Fayer, R. (2008). A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. *Veterinary Parasitology*, 155(1-2), 15-23. https://doi.org/10.1016/j.vetpar.2008.04.018

Santín, M., Trout, J.M., Xiao, L., Zhou, L., Greiner, E., & Fayer, R. (2004). Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Veterinary Parasitology*, 122(2), 103-17. https://doi.org/10.1016/j.vetpar.2004.03.020

Silverlås, C., Bosaeus-Reineck, H., Näslund, K., & Björkman, C. (2013). Is there a need for improved *Cryptosporidium* diagnostics in Swedish calves?. *International Journal for Parasitology*, 43(2), 155–161. https://doi.org/10.1016/j.ijpara.2012.10.009

Sischo, W.M., Atwill, E.R., Lanyon, L.E., & George, J. (2000). *Cryptosporidium* on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. *Preventive Veterinary Medicine*, 43(4), 253–267. https://doi.org/10.1016/S0167-5877(99)00107-5

Smith, R.P., Clifton-Hadley, F.A., Cheney, T., & Giles, M. (2014). Prevalence and molecular typing of *Cryptosporidium* in dairy cattle in England and Wales and examination of potential on-farm transmission routes. *Veterinary Parasitology*, 204(3-4), 111-9. https://doi.org/10.1016%2Fj.vetpar.2014.05.022

Starkey, S.R., Kimber, K.R., Wade, S.E., Schaaf, S.L., White, M.E., & Mohammed, H.O. (2006). Risk Factors Associated with *Cryptosporidium* Infection on Dairy Farms in a New York State Watershed. *Journal of Dairy Science*, 89(11), 4229–4236. https://doi.org/10.3168/jds.S0022-0302(06)72468-7

Tarekegn, Z.S., Tigabu, Y., & Dejene, H. (2021). *Cryptosporidium* infection in cattle and humans in Ethiopia: A systematic review and meta-analysis. *Parasite Epidemiology and Control*, 13,(14), e00219. https://doi.org/10.1016/j.parepi.2021.e00219

Thomson, S., Hamilton, C.A., Hope, J.C., Katzer, F., Mabbott, N.A., Morrison, L.J., & Innes, E.A. (2017). Bovine Cryptosporidiosis: impact, host-parasite interaction and control strategies. *Veterinary Research*, 48(1), 42. https://doi.org/10.1186%2Fs13567-017-0447-0

Trotz-Williams, L. A., Martin, D. S., Gatei, W., Cama, V., Peregrine, A. S., Martin, S. W., Nydam, D. V., Jamieson, F., & Xiao, L. (2006). Genotype and subtype analyses of *Cryptosporidium*

Revista Bio ciencias
ISSN 2007-3380

isolates from dairy calves and humans in Ontario. *Parasitology Research*, 99(4), 346–352. https://doi.org/10.1007/s00436-006-0157-4

Trotz-Williams, L.A., Jarvie, B.D., Martin, S., Leslie, K.E., & Peregrine, A.S. (2005). Prevalence of *Cryptosporidium parvum* infection in southwestern Ontario and its association with diarrhea in neonatal dairy calves. *Canadian Veterinary Journal*, 46(4), 349– 351. https://pubmed.ncbi.nlm.nih.gov/15943123/

Xiao, L., Morgan, U.M., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson, R.C., Fayer, R., & Lal, A.A. (1999). Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. *Applied and Environmental Microbiology*, 65(8), 3386-91. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC91508/