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Prevalence and risk factors associated with infection by *Cryptosporidium* spp. in suckling calves in Aguascalientes, Mexico

Dolores García Romo[a]
Carlos Cruz Vázquez[b]
Teódulo Quezada Tristán[c]
Enrique Silva Peña[d]
Arturo Valdivia Flores[c]
Sonia Vázquez Flores[e]
Miguel Ramos Parra[b]

[a] Centro de Bachillerato Tecnológico Agropecuario No. 205, Manuel de Velazco No. 601, Colonia Solidaridad, 20400, Rincón de Romos, Aguascalientes, México.

[b] División de Estudios de Posgrado e Investigación. Instituto Tecnológico El Llano Aguascalientes, Km. 18 carretera Aguascalientes a San Luis Potosí, 20330, El Llano, Aguascalientes, México.

[c] Centro de Ciencias Agropecuarias. Universidad Autónoma de Aguascalientes, Av. Universidad 940, Ciudad Universitaria, 20231, Aguascalientes, México.

[d] Facultad de Medicina Veterinaria y Zootecnia. Universidad de Colima, Km. 40 carretera Colima a Manzanillo, 28100, Tecomán, Colima, México.

[e] Instituto Tecnológico de Estudios Superiores de Monterrey, Campus Querétaro, E. González 500, Fracc. San Pablo, 76130, Querétaro, México.

*Corresponding author:
Tel: + 52 55 4499161251 ext. 116
Email address: cruva18@yahoo.com.mx

Abstract

The aim of this study was to identify the prevalence of and certain risk factors associated with infection by *Cryptosporidium* spp. in suckling calves that were maintained in confinement in three dairy farms in Aguascalientes, Mexico. Over a period of 12 months, 1658 fecal samples were taken from 0- to 28 day-old calves. These samples were processed by a Kinyoun stain of the fecal smears to identify the presence of parasite oocysts. A survey was conducted to identify the different management factors present in the dairy farms, and a risk analysis was performed using logistic regression. The overall prevalence of *Cryptosporidium* spp. infection was 40%; the 8- to 14-day-old age group of calves had the highest prevalence of infection (81%), while the lowest prevalence of infection (21%) was observed in the 22- to 28-day-old age group. The identified risk factors for infection by *Cryptosporidium* spp. included the 8- to 14-day-old group (OR = 15.2; 95% CI 11.2 - 20.6; P < 0.000) and the 15- to 21-day-old group (OR = 2.5; 95% CI 1.9 - 3.2, P < 0.000); the use of sawdust bedding (OR= 1.8; 95% CI 1.1 - 2.8, P < 0.011); the plastic container from which calves drink milk (OR = 1.4; 95% CI 1.1 - 1.7, P < 0.000); the low level of immunoglobulins (OR = 1.8; 95% CI 1.1 - 2.9, P < 0.009); and calving care by non-specialized personnel (night watchman: OR = 2.5; 95% CI 1.6 - 3.9, P < 0.000; nursing area worker: OR 1.7; 95% CI 1.2 – 2.5, P < 0.001).

Keywords: *Cryptosporidium* spp.; Dairy cattle; Suckling calves; Prevalence; Risk factors.

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Introduction

Cryptosporidium spp. are protozoan parasites that cause gastrointestinal infections in a wide variety of vertebrate hosts, including domestic and wild animals as well as humans (Ramírez *et al.*, 2004; Cacciò and Pozio, 2006; Ng *et al.*, 2012). In cattle, at least four species have been identified, including *C. parvum*, *C. andersoni*, C. bovis and *C. ryanae*; the first species listed is found most frequently in suckling animals, while the others are more common in weaned and adult animals (Lindsay *et al.*, 2000; Fayer *et al.*, 2005, 2007, 2008). Cryptosporidiosis is particularly important in suckling animals that are younger than 30 days of age, in which abundant diarrhea, fever, anorexia, weight loss, electrolyte imbalance and eventually death can be observed (Ramírez *et al*., 2004; Cacciò and Pozio, 2006). The group with the highest risk to contract an infection are calves 8 to 14 days of age (Castro-Hermida *et al*., 2002a; Santín *et al*., 2004; Feitosa *et al*., 2004; Trotz-Williams *et al*., 2005), because the prevalence and parasitosis intensity tend to be reduced when the animals are older (Castro-Hermida *et al*., 2002b; Ortolani and Soares, 2003; Starkey *et al*., 2006; Castro-Hermida *et al*., 2006; Bhat *et al*., 2013). However, infection is very rare in animals younger than four days (Uga *et al*., 2000).

Animals infected with *Cryptosporidium* spp. may excrete feces with a high number of oocysts that may be easily disseminated in the environment in such a manner that different management factors have been associated with infection by *Cryptosporidium* spp. These factors should be considered in prevention programs for this parasite, particularly because there is a lack of effective pharmacological treatments (Cacciò and Pozio, 2006). Nevertheless, some of these factors vary across regions and in their production systems and geographical characteristics, while others may be present in any farm. Among the most frequently cited factors are age, multiple birthing areas, a low frequency of cleaning of the premise, the materials or types of bedding, the use of contaminated water for the cattle to drink, the technical level of the production system, sand-floor pens and the rainy season (Maldonado *et al*., 1998; De la Fuente *et al*., 1999; Mohammed *et al*., 1999; Castro-Hermida *et al*., 2002b; Trotz- Williams *et al*., 2007; Brook *et al*., 2008; Almeida *et al*., 2010; Muhid *et al*., 2011; Silverlås and Blanco-Penedo, 2013; Bhat *et al*., 2013). In Mexico, cryptosporidiosis has been reported in several cattle raising regions (Maldonado *et al*., 1998; Castillo-García *et al*., 2009; Castelan-Hernández *et al*., 2011; Cano-Romero *et al*., 2011), but numerous topics still required research regarding its epidemiology in the different production systems.

The aim of this study was to identify the prevalence and some of the risk factors associated with infection by *Cryptosporidium* spp. in suckling calves maintained in confinement in three dairy farms in Aguascalientes, México.

Materials and methods

Study site

The study was carried out in the state of Aguascalientes, which is located in the north central region of Mexico at an average altitude of 1885 masl. The state has an annual mean temperature of 16°C and an average annual rainfall of 475 mm which occurs primarily during the summer.

Dairy farms

In this study, three dairy farms were selected by convenience; two were located in the central northern part of the Pabellón de Arteaga municipality, and the other was located in the eastern part of the Rincón de Romos municipality. Holstein cattle were found on all three dairy farms. Each farm had a rearing area for female calves and used similar management practices; likewise, in the three farms, the suckling calves were lodged in rearing rooms with concrete floors.

Sampling

The dairy farms were visited every two weeks for a year to collect fecal samples directly from the rectum of all female calves between 0 and 28 days of age. These samples were collected from the calves that were present on the day of the visit, without considering whether the calves had diarrhea. Each sample was individually identified by recording the date of birth, and no animals were included twice in the study. Additionally, blood samples were taken by venipuncture with a new vacutainer in the female calves that were 1 to 7 days old. All of the samples were refrigerated for transportation. In the laboratory, the blood samples were centrifuged at 2500 rpm for 15 minutes to obtain the serum, which was deposited in polypropylene vials and preserved at -20ºC until further use. Due to the study protocol, approval by the Committee of Use and Care of Animals was not required.

To determine the parasitosis distribution in the study population, the calves were classified into four age groups: 0 to 7, 8 to 14, 15 to 21 and 22 to 28 days of age.

Fecal diagnosis

The fecal samples were processed using a Kinyoun stain of the fecal smears to identify the parasite oocysts. From each sample, a slide was prepared with 6 smears; after the smears were stained, they were observed with a microscope. To minimize false positive readings, a sample was considered positive when > 5 oocysts were found during the complete examination of the 6 smears (Castillo-García *et al*., 2009).

Determination of the total amount of immunoglobulins in serum

The quantity of immunoglobulins was determined by the zinc sulfate turbidity test to establish the amount of immunoglobulins that was transferred by colostrums to the calves within the first 7 days of life. The number of zinc sulfate turbidity units (UTSZ) corresponds to the mg of total immunoglobulins per ml of serum, so levels below 10 UTSZ indicate a lack of passive antibody transfer from the mother to the offspring; a range of 10 to 20 represents an acceptable but insufficient level; and more than 20 is considered the necessary level to achieve successful nursing of the neonate (Martinez and Ortega, 2011).

Survey

To establish the hypothetical risk factors associated with infection by *Cryptosporidium* spp., data were obtained from a survey given to the people responsible for the rearing areas in the studied dairy farms; this survey asked questions regarding the characteristics of the facilities and management practices. Five groups were formed with the hypothetical risk factors, and each group was integrated with different variables and their respective categories; this was used as a basis for the risk analysis (Table 1).

Table 1. *Cryptosporidium spp.* infection prevalence for the different categories of each variable and the group of characteristics under study.

Variable	Categories	n	Positive	Prevalence (%)
Group of individual characteristics of the female calves and their mothers				
Age	0 to 7 days	506	107	33.5
	8 to 14 days	399	324	81.2
	15 to 21 days	408	164	40.1
	22 to 28 days	345	74	21.4
Feces type	Normal	1362	501	36.7
	Diarrheic	246	144	58.5
	Semi-liquid	50	24	48.0
Calving type	Dystocial	284	108	38.0
	Normal	1324	541	40.8
	Non - determined	50	20	40.0
Calving number	1st to 4th	1509	604	40.0
	5th or more	149	65	43.6
Group of management characteristics of calving and nursing				
Person that takes care of calving	Night watchman	395	145	36.7
	Worker of the nursing area	512	219	42.7
	Technician on guard	751	266	35.4
Type of colostrums consumed by the calves	Fresh	1328	553	41.6
	Refrigerated	282	12	36.8
	Non- determined	48	104	25.0
Anti-diarrhea treatment	No treatment	608	257	42.2
	With treatment	169	79	46.7
	Non- specified	881	333	37.7
Vaccine against diarrheic diseases	No vaccine	751	266	35.4
	Vaccinated	907	403	44.4
Level of immunoglobulins	Acceptable	12	0	0.0
	Medium	113	21	18.5
	Low	202	42	20.7
Group of hygiene characteristics of equipment and facilities				
Type & hygiene of bedding and/or floor of lodging	Sawdust changed every 5 days	287	145	50.5
	Rubber with daily removal of excrement and washed every week	108	39	36.1
	No bedding, slats type floor with daily removal of excrement	1263	485	38.4
Washing and disinfection of individual lodgings	When vacated, lodgings are washed and lime is applied.	395	184	46.5
	When vacated, lodgings are washed with ammonium quaternary salts	512	219	42.7
	When vacated, lodgings are washed with chlorine	751	266	35.4
Frequency & manner of washing of milk or milk substitute drinking containers	Daily with water plus detergent	395	184	46.5
	Daily with water and iodide soap	512	219	42.7
	Daily with water, every third day with iodide soap	751	266	35.4

Table 1. *Continued*

Variable	Categories	*n*	Positive	Prevalence (%)
Washing of the colostrum feeding bottles	Rinse after use by each calf With soap plus iodide after use by each calf	1573 85	642 27	40.8 31.7
Frequency & manner of washing the floor in the rearing room	Weekly with water Daily with water Daily with iodide soap	395 427 836	184 192 293	46.5 44.9 35.0
Group of characteristics of the type of equipment and facilities				
Type of rearing room	Closed Semi-closed Open	1037 395 226	395 184 90	38.0 46.5 39.8
Individual lodging	Floor pen Metallic high	395 1263	184 485	46.5 38.4
Type of container for milk or milk substitute drinking	Plastic Galvanized sheet Both of the above	907 226 525	403 90 176	44.4 39.8 33.5
Group of characteristics of dairy farm operation and administration				
Size of the herd	≤1000 head of cattle >1000 head of cattle	395 1263	184 485	46.5 38.4
Other animal species in the dairy farm	None Present	1057 601	413 256	39.0 42.5
Age of the rearing area workers	≤ 20 years of age From 21 to 30 years > 31 years of age	226 920 512	90 360 219	39.8 39.1 42.7
Incentives given to workers	None Given	907 751	403 266	44.4 35.4
Origin of breeding cattle	Same dairy farm Same state Foreign	751 512 395	266 219 184	35.4 42.7 46.5

Statistical analysis

The general parasitosis prevalence was estimated for each age group and for each variable and category of the hypothetical risk factors. To establish the risk of infection by *Cryptosporidium* spp., a logistic regression analysis was performed (Hosmer *et al*., 2013) in which the dependent variable was the parasite infection status. The independent variables were selected using a "backward step by step" method, in which the variables that were not statistically significant ($p<0.05$) according to the Chi square test were excluded one by one. The odds ratios (OR) were estimated using the independent variables that showed statistical significance in the multivariate analysis ($p<0.05$). The analysis was carried out using the Statistics Data Analysis (STATA) v. 9.1 software.

Results and Discussion

The general prevalence of *Cryptosporidium* spp. infection in the studied population was 40% (669/1658). Table 1 shows the prevalence for each category within the different variables.

Table 2. Risk factors for infection by *Cryptosporidium* spp. identified in three dairy farms in Aguascalientes, Mexico.

Variable	Category	OR	95% Confidence Interval	p-value
Age	Group of calves aged 8 to 14 days	15.2	11.2 - 20.6	0.000
	Group of calves aged 15 to 21 days	2.5	1.9 - 3.2	0.000
Person that cares for calving	Night watchman	2.5	1.6 - 3.9	0.000
	Nursing area worker	1.7	1.2 - 2.5	0.001
Level of immunoglobulins in calves	Low level	1.85	1.1 - 2.9	0.009
Type & hygiene of bedding and/or floor of the lodging	Sawdust bedding, replaced every 5 days	1.8	1.1 - 2.8	0.011
Type of container used to drink milk or milk substitute	Plastic	1.4	1.1 - 1.7	0.000

The risk analysis identified 5 variables as risk factors for infection by *Cryptosporidium* spp., with the variable of the age of the calves being the most significant, particularly in the group of calves aged 8 to 14 days (OR=15.2; 95% CI 11.2 - 20.6; p<0.000), followed by the group of calves aged 15 to 21 days (OR= 2.5; 95% CI 1.9 - 3.2, p<0.000). The other variables that were identified as risk factors were related to the person in charge of the care of calving, the level of immunoglobulins, the type and hygiene of the bedding and/or floor of the lodging area, and the type of container in which the milk or milk substitute was given. Table 2 shows the categories identified as risk factors. None of the variables related to the operation and administration of the dairy farms were shown to be risk factors. In the variable related to the person in charge of the care of calving, the technician on guard proved to be a protective factor (OR= 0.73; 95% CI 0.5828 - 9237, p<0.008).

In this study, the general prevalence of infection by *Cryptosporidium* spp, in suckling calves (≤28 days of age) was found to be 40%, while in a study conducted in the same state that included animals from 8 to 14 days old, the prevalence of infection was found to be 75% (Castillo-García *et al*., 2009). In the dairy farms of Aguascalientes, the abundant presence of the parasite is clearly evident. A similar situation has been observed in the central region of Mexico (Hidalgo, Jalisco and the state of Mexico) and in the state of Veracruz, where other authors have reported general prevalences of 25, 35.7 and 73.6%, respectively; they have also found positive animals in at least 90% of the studied production units (Maldonado *et al*., 1998; Castelan-Hernández *et al*., 2011; Cano-Romero *et al*., 2011). However, the public health implications of zoonotic transmission in Mexico are uncertain because very few studies regarding human criptosporidiosis have been conducted (Valenzuela *et al*., 2014). Several studies on the cryptosporidiosis prevalence in suckling calves have been carried out in cattle raising regions of other countries using different diagnostic techniques, with a wide variety of results ranging from a prevalence of 17 to 47.9% (Lefay *et al*., 2000; Castro-Hermida *et al*., 2002a Joachim *et al*., 2003; Ortolani and Soares, 2003; Santín *et al*., 2004; Kváč *et al*., 2006; Brook *et al*., 2008; Bhat *et al*., 2013); this testifies to the ample geographic distribution and elevated frequency of this parasite.

Infection by *Cryptosporidium* spp. in dairy cattle is more frequent and has a greater impact in suckling calves; additionally, the magnitude of the disease is influenced by diverse factors such as age and the immunological status of the calves as well as the climate and other factors related to management practices during rearing. Therefore, the epidemiology of this parasite has specific characteristics for each unit and production system (Fayer *et al*., 2000). In this study, calves that were 8 to 14 days of age had a *Cryptosporidium* spp. infection prevalence of 81%; this was the highest of the 4 age groups investigated in this study. This result is similar to a previous report in Aguascalientes, in which the same age group had a 75% prevalence rate of infection (Castillo-García *et al*., 2009); in the other 3 age groups, the parasite prevalence rate ranged from 21 to 40%. In Mexico, a higher prevalence has been reported in calves under 4 months of age (Cano-Romero *et al*., 2011; Castelan-Hernández *et al*., 2011), with a higher rate of oocyst excretion between days 15 to 19 after birth (Maldonado *et al*., 1998). The prevalence according to age group is variable in suckling calves; nevertheless, it has been shown that the calves are the most affected between 8 and 15 days of age (De la Fuente *et al*., 1999; Ortolani and Soares 2003; Santín *et al*., 2004, 2008; Feitosa *et al*., 2004; Avendaño *et al*., 2010). This coincides with the fact that in this study, the 8- to 14-day-old age group was identified as the most important risk factor for infection by *Cryptosporidium* spp. (OR = 15.2), followed by the 15- to 21-day-old age (OR = 2.5). Other authors have found similar results (Maldonado *et al*., 1998; De la Fuente *et al*., 1999; Santín *et al*., 2004; Trotz-Williams *et al*., 2007; Brook *et al*., 2008), which indicates that the age range of 8 to 21 days has the highest risk of infection and excretion of parasite oocysts, although this risk is reduced as the age increases (Maldonado *et al*., 1998; Santin *et al*., 2004, 2008), as observed in our study. It is inferred that contamination with *Cryptosporidium* spp. occurs after the calves come into contact with other animals, the initiation feeder and drinking water. As Maldonado *et al*. stated (1998), the indicated initiation rations that are contaminated by rodents or birds act as fomites and become risk factors; similarly, drinking water has been referred to as a contamination risk due to contamination of either the liquid ducts (Sischo *et al*., 2000) or the drink containers (Almeida *et al*., 2010). Thus, age should be considered a risk indicator because it is an individual trait (Silva-Ayçaguer, 2005).

The variable represented by the person who cares for the births of the offspring, was identified as a risk factor when the births were overviewed by a night watchman or when calving was overseen by the same worker who took care of the nursing area. In the instances in which the calving was overseen by the technician on guard (OR = 0.73), this variable was not a risk factor but served as protection because the main job of the technician on guard is precisely to care for night births and calving on holidays. Similarly, when births under similar circumstances were cared for by the area worker or the custodial staff, it is inferred that the care is not as appropriate because in addition to caring for births, these individuals have other chores to perform; information on this topic was not found in the literature.

Concerning the type and hygiene of the bedding and/or floor of the lodging area, the sawdust bedding category that was changed every 5 days was identified as a risk factor (OR = 1.80), perhaps because of the physical characteristics of the material: it tends to accumulate humidity and excrement, which is ideal for the parasite oocysts to survive and contaminate the material. In contrast, Maldonado *et al*. (1998) and Mohammed *et al*. (1999) did not find significant relationships between parasitosis and wood shavings serving as bedding. In this context, it seems that independent of the type of bedding, the cleaning frequency has a greater influence because longer time periods between cleanings promote the development of the parasite in the feces and the possibility that the calves or their feed have contact with that material. Similarly, floor slat lodgings and pens with sand bedding were reported as risk factors (Muhid *et al*., 2011). In both cases, the presence of discarded fecal matter, as occurred in our study, represented a source of oocyst contamination.

Regarding the type of container used to provide the calves with milk or milk substitute, plastic materials were found to be a risk factor in this study (OR = 1.4). This could be related to deficient washing because the plastic materials may be splashed with diarrheic feces that are most likely contaminated with the parasite; the containers are then licked by the calves, thereby causing infection. This is feasible because even though the calf lodgings in the studied dairy farms are individual, they do not offer total isolation; therefore the calves may lick the containers of their neighbors at any time. Further, Almeida *et al.* (2010) reported a significant association of the water supply tray with the risk of infection by *C. parvum*; in this case, they noted that liquid was an important reservoir of protozoan oocysts when contaminated with fecal matter. However, Maldonado *et al*. (1998) demonstrated that no type of container or feeding bottle presented a risk of infection by *Cryptosporidium*.

The prevalence of infection was higher in animals that were vaccinated against diarrheic diseases; Trotz-Williams *et al* (2008) found a similar result in both cows and calves that had received such prophylaxis. Therefore, vaccination has been perceived as a risk factor for infection by *Cryptosporidium*. However, Trotz-Williams *et al* (2008) noted that vaccination is not as much a risk factor as it is a measure taken in response to a high frequency of calf scours because vaccines marketed for calf scours have no known impact on the risk of cryptosporidiosis. In fact, vaccines would be ineffective against scouring caused by Cryptosporidium infection.

In this study, the analysis of the level of immunoglobulins as a risk factor was conducted separately from the general model because the test was performed only in calves that were 1 to 7 days of age. Of these calves 61.7 % had insufficient levels of immunoglobulins and the immunoglobulin levels were therefore considered a risk factor (OR = 1.85). It was not possible in this study to establish a relationship between the level of immunoglobulins in the calves and the birth number of the mother, though there is some evidence that this has an influence on the concentration of G immunoglobulin in colostrums (Kehoe *et al*., 2011). Another study has evaluated the level of immunoglobulins in nursing calves and found serum protein values below 5.2 g/dL; these values were significantly related to infection by *Cryptosporidium* spp. (Trotz-Williams *et al*., 2007). Because cryptosporidiosis is associated with young and immunosuppressed animals, these results suggest that for the calves examined in this study, the colostrum feeding process was not sufficiently effective because newborns require the ingestion of colostrums in the first 6 hours of life to guarantee the intestinal absorption of immunoglobulins; additionally, serum amounts of antibodies below 10 mg/mL may cause up to 60% mortality caused by gastrointestinal infections (Weaver *et al*., 2000). In the dairy farms included in this study, the offspring were reported to consume 4 or more liters of the colostrums, but there were no data proving that the first take of colostrums occurred within the first 6 hours after birth; therefore, deficiencies in the postnatal feeding process are inferred.

Despite the present findings it must be noted that this study was undertaken in only 3 dairy farms; therefore, these results may not be generalizable to other farms in Mexico.

Conclusions

In this study, the age of the calves is reported to be a risk factor for acquiring infection by *Cryptosporidium* spp.; in particular, there was an association between infection and the age group of 8 to 14 days that was higher than the results reported in other studies. Therefore, the calving and the care of calves during birth and nursing should be performed by personnel who are dedicated exclusively to those chores to ensure optimum feeding with colostrums; this would also ensure an appropriate immunoglobulin level that may protect calves from infection by this parasite and other diseases. The results of this study suggest that cleaning the lodgings of the calves should be more stringent, particularly when using sawdust, and that feces and humidity should be removed constantly to avoid parasite proliferation. Additionally, containers for drinking milk or milk substitute should be cleaned perfectly to avoid the risk of infection because it seems that type and frequency of cleaning the premises and equipment is more important than the materials that are used.

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Conflicts of interest

None of the authors has any conflict of interest in regard with this article.

Author contributions

Dolores García Romo: Experimental design, field sampling, laboratory testing, writing of the article.
Carlos Cruz Vázquez: Coordination of the group, experimental design, epidemiological analysis, drafting of the article.
Teódulo Quezada Tristán: Field work, statistical analysis.
Enrique Silva Peña: Field and laboratory work.
Arturo Valdivia Flores: Field and laboratory work.
Sonia Vázquez Flores: Parasitological test.
Miguel Ramos Parra: Risk analysis.

References

1) Almeida, A.J., Oliveira, F.C.R., Flores, V.M.Q. and Lopes, C.W.G. 2010. Risk factors associated with the occurrence of *Cryptosporidium parvum* infection in calves. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 62 (6): 1325-1330.
2) Avendaño, C., Quílez, J. and Sánchez-Acedo, C. 2010. Prevalence of *Cryptosporidium* in calves in the Ubaté-Chiquinquirá Valley (Colombia). *Revista UDCA Actualidad y Divulgación Científica*. 13 (1): 41-47.
3) Bhat, S.A., Juyal, P.D., Singh, N.K. and Singla, L.D. 2013. Coprological investigation on neonatal bovine cryptosporidiosis in Ludhiana, Punjab. *Journal of Parasitic Diseases*. 37 (1): 114-117.
4) Brook, E., Hart, C.A., French, N. and Christley, R. 2008. Prevalence and risk factors for *Cryptosporidium* spp. infection in young calves. *Veterinary Parasitology*. 152 (1): 46-52.
5) Cacciò, S.M. and Pozio, E. 2006. Advances in the epidemiology, diagnosis and treatment of cryptosporidiosis. *Expert Review of Anti-Infective Therapy*. 4 (3): 429–443.
6) Cano-Romero, P., Alonso-Díaz, M.A., Figueroa-Castillo, J.A. and Trigo-Tavera, J.F. 2011. Prevalence and incidence of *Cryptosporidium* spp. in calves from the central region of Veracruz, Mexico. *Tropical and Subtropical Agroecosystems*. 13: 567-571.
7) Castelan-Hernández, O.O., Romero-Salas, D., García-Vázquez, Z., Cruz-Vázquez, C., Aguilar-Domínguez, M., Ibarra-Priego, N.D.J. and Muñoz-Melgarejo, S. 2011. Prevalence of bovine cryptosporidiosis in three ecological regions from the central region of Veracruz, México. *Tropical and Subtropical Agroecosystems*. 13: 461-467.
8) Castillo-García, C., Cruz-Vázquez, C., López-Revilla, R., Sánchez-Garza, M., Rosario-Cruz, R., Vitela, M.I. and Medina-Esparza, L. 2009. Frecuencia e identificación molecular de *Cryptosporidium* spp. en becerras lactantes mantenidas en confinamiento en Aguascalientes, México. *Técnica Pecuaria en México.* 47 (4): 425-434.
9) Castro-Hermida, J., González, L.Y., Mezo, M.M. and Ares, M.E. 2002a. A study of cryptosporidiosis in a cohort of neonatal calves. *Veterinary Parasitology*. 106 (1): 11-17.
10) Castro-Hermida, J., González, L.Y. and Ares, M.E. 2002b. Prevalence and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). *Veterinary Parasitology*. 106 (1): 1-10.
11) Castro-Hermida, J., Carro-Corral, C., González-Warleta, M. and Mezo, M. 2006. Prevalence and intensity of infection of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Galicia (NW Spain). *Journal of Veterinary Medicine Series B*. 53 (5): 244-246.
12) De la Fuente, R., Luzón, M., Ruiz-Santa-Quiteria, J.A., García, A., Cid, D., Orden, J.A., García, S., Sanz, R. and Gómez-Bautista, M. 1999. *Cryptosporidium* and concurrent infections with other major enterophatogens in 1 to 30-day-old diarrheic dairy calves in central Spain. *Veterinary Parasitology*. 80 (3): 179-185.
13) Fayer, R., Morgan, U. and Upton, J.S. 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. *International Journal for Parasitology*. 30 (13): 1305-1322.

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14) Fayer, R., Santín, M. and Xiao, L. 2005. *Cryptosporidium bovis* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). *Journal of Parasitology*. 91 (3): 624-629.
15) Fayer, R., Santín, M. and Trout, J.M. 2007. Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. *Veterinary Parasitology*. 145 (3-4): 260-266.
16) Fayer, R., Santín, M. and Trout, J.M. 2008. *Cryptosporidium ryanae* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). *Veterinary Parasitology*. 156 (3): 191-198.
17) Feitosa, F.L.F., Shimamura, G.M., Roberto, T., Meireles, M.V., Nunes, C.M., Ciarlini, P.C. and Borges, A.S. 2004. Prevalence of cryptosporidiosis in calves from Araçatuba region, São Paulo State, Brazil. *Ciencia Rural.* 34 (1): 189-193.
18) Hosmer, D.W., Lemeshow, S. and Sturdivant, R.X. 2013. *Applied Logistic Regression*. 3th edition. New Jersey: Wiley.
19) Joachim, A., Krull, T., Schwarzkopf, J. and Daugachies, A. 2003. Prevalence and control of bovine cryptosporidiosis in German dairy herds. *Veterinary Parasitology*. 112 (4): 277-278.
20) Kehoe, S.I., Heinrichs, A.J., Moody, M.L, Jones, C.M. and Long, M.R. 2011. Comparison of immunoglobulin G concentrations in primiparous and multiparous bovine colostrum. *Professional Animal Scientist*. 27 (3): 176-180.
21) Kváč, M., Kouba, M. and Vítovec, J. 2006. Age related and housing dependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. *Veterinary Parasitology*. 137 (3-4): 202-209.
22) Lefay D., Naciri, M., Poirer, P. and Chermette, R. 2000. Prevalence of *Cryptosporidium* infection in calves France. *Veterinary Parasitology*. 89 (1): 1-9.
23) Lindsay, D.S., Upton, S.J., Owens, D.S., Morgan, U.M., Mead, J.R. and Blagburn, B.L. 2000. *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporiidae) from cattle, *Bos taurus*. Journal of Eukaryotic Microbiology. 47(1): 91-95.
24) Maldonado, C.S., Atwill, E.R., Saltijeral, O.J.A. and Herrera, A.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 (29): 95-107.
25) Martínez, R.A. and Ortega, SJ. 2011. *Manual de Laboratorio de Inmunología Básica y Clínica*. Universidad Autónoma Chapingo: México.
26) Mohammed, H.O., Wade, S.E., and Schaaf, S. 1999. Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. *Veterinary Parasitology*. 83 (1): 1-13.
27) Muhid, A., Robertson, I., Ng, J.S.Y., and Ryan, U. 2011. Prevalence of and management factors contributing to *Cryptosporidium* sp. infection in pre-weaned and post-weaned calves in Johor, Malaysia. *Experimental Parasitology*. 127 (2): 534-538.
28) Ng, J.S.Y., Eastwood, K., Walker, B., Durrheim, D.N., Massey, P.D., Porigneaux, P. and Ryan, U. 2012. Evidence of *Cryptosporidium* transmission between cattle and humans in northern New South Wales. *Experimental Parasitology*. 130 (4): 437-441.
29) Ortolani, E.R. and Soares, P.C. 2003. Aspectos epidemiológicos de la criptosporidiosis en becerros de rebaños lecheros. *Parasitología Latinoamericana*. 58 (3-4): 122-127.

30) Ramírez, N.E., Ward, L.A. and Sreevatsan S. 2004. A review of biology and epidemiology of cryptosporidiosis in humans and animals. *Microbes and Infection*. 6 (8): 773-785.
31) Santín, M., Trout. J.M., Xiao, L., Zhou, L., Greiner, E. and Fayer, R. 2004. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Veterinary Parasitology.* 122 (2): 103-117.
32) Santín, M., Trout, J.M. and Fayer, R. 2008. A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. *Veterinary Parasitology.* 155 (1): 15–23.
33) Silva-Ayçaguer, L.C. 2005. Una ceremonia estadística para identificar factores de riesgo. *Salud colectiva*. 1(3): 309-322.
34) Silverlås, C. and Blanco-Penedo, I. 2013. *Cryptosporidium* spp. in calves and cows from organic and conventional dairy herds. *Epidemiology and Infection.* 141 (3): 529-539.
35) Sischo, W.M., Atwill, E.R., Lanyon, L.E. and George, J. 2000. Cryptosporidia 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.
36) Starkey, S.R., Kimber, K.R., Wade, S.E., Schaaf, S.L., White, M.E. and Mohammed, H.O. 2006. Risk Factors Associated with *Cryptosporidium* Infection on dairy farms in New York State Watershed. *Journal Dairy Science*. 89 (11): 4229-4236.
37) Trotz-Williams, L.A., Brenna, D.J., Wayne, M.S., Kenneth, E.L. and Peregrine, S.A. 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.
38) Trotz-Williams, L.A., Wayne-Martin, S., Leslie, K.E., Duffield, T., Nydam, D.V. and Peregrine, A.S. 2007. Calf-level risk factors for neonatal diarrhea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. *Preventive Veterinary Medicine*. 82 (1): 12-28.
39) Trotz-Williams, L.A., Wayne-Martin, S., Leslie, K.E., Duffield, T., Nydam, D.V. and Peregrine, A.S. 2008. Association between management practices and within-herd prevalence of *Cryptosporidium parvum* shedding on dairy farms in southern Ontario. *Preventive Veterinary Medicine*. 83 (1): 11-23.
40) Uga, S., Matsuo, J., Kono, E., Kimura, K., Inoue, M., Rai, S.K. and Ono, K. 2000. Prevalence of *Cryptosporidium parvum* infection and pattern oocysts sheding in calves in Japan. *Veterinary Parasitology*. 94 (1): 27-32.
41) Valenzuela, O., González-Díaz, M., Garibay-Escobar, A., Burgara-Estrella, A., Cano, M., Durazo, M., Bernal, R.M., Hernández, J., Xiao, L. 2014. Molecular characterization of *Cryptosporidium* spp. in children from Mexico. *PloS One*. 9 (4): e96128.
42) Weaver, D.M., Tyler, J.W., VanMetre, D.C., Hostetler, D.E. and Barrington, G.M. 2000. Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine*. 14(6): 569-577.