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Cryptosporidiosis in Dairy Calves from Aguascalientes, Mexico: Risk Infection in Relation with the Season and Months of Sampling

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Abstract: The objective of this study was to estimate the risk infection of cryptosporidiosis in dairy calves from Aguascalientes, Mexico, in relation with the season and months of sampling. During a period of 12 months, fecal samples were collected every 14 days, which were processed by Kinyoun staining of fecal smears. Seasonal distribution of *Cryptosporidium* sp. prevalence was described and in order to establish the risk of infection during each season of the year and the months of sampling, a logistic regression analysis was performed. The highest prevalence (48 and 45%, respectively) was observed during summer and autumn and during spring and winter the lowest (33 and 36%), there were no differences between seasons ($p>0.05$) general prevalence was 40%. Distribution of monthly prevalence showed fluctuations from March to July, with a peak in August (57%) and it slowly tapered down until December (43%). It was not possible to identify any season of the year as a possible risk factor in the infection by *Cryptosporidium* sp. nevertheless, sampling months of August to November had odds ratios in a range between 2.3 and 1.4 ($p<0.05$).

Key words: *Cryptosporidium*, dairy calves, risk factors, season, Aguascalientes, Mexico

INTRODUCTION

Cryptosporidiosis is a parasitic disease caused by a protozoan of the genus *Cryptosporidium* (Apicomplexa: Cryptosporiidae) that was identified in the beginning of the last century and colonizes epithelial cells that are found along all the length of the digestive tract of a ample variety of vertebrates, including domestic and wild animals as well as humans (Ramirez *et al*., 2004). In cattle, 2 species have been recognized, *C. parvum*, that infects the intestine of neonate calves and *C. andersoni* that infects the abomasum of young and adult animals; during the last few years, two new species have been detected, *C. bovis* and *C. ryanae* (Anderson, 1998; Fayer *et al*., 2000, 2006, 2008; Lindsay *et al*., 2000; Feltus *et al*., 2008); likewise, numerous sub-genotypes of *C. parvum* have been reported (Fayer *et al*., 2005, 2006; Slapeta, 2006; Wielinga *et al*., 2007; Brook *et al*., 2009; Xiao and Fayer, 2008). Cryptosporidiosis is especially important in dairy calves up to 30 days of age, with most of the prevalence detected between 8 and 14 days of age (De Grafft *et al*., 1999; Castro-Hermida *et al*., 2002; Santin *et al*., 2004; Brook *et al*., 2008), being the probable main cause of severe diarrhea symptoms, by its role as sole or opportunist agent in intestinal bacterial or viral origin infections, in immuno-compromised animals and besides, adult cattle may act as asymptomatic carriers of this protozoan, representing a source of permanent infection for young animals in the herd. *C. parvum* is recognized as a zoonosis (De Grafft *et al*., 1999; Ramirez *et al*., 2004; Xiao and Feng, 2008).

In Mexico, the presence of the disease was first documented in dairy cattle in 1983 and has since been reported mainly from dairy herds in the central region of the country (Gonzalez *et al*., 1983; Maldonado *et al*., 1998; Vazquez-Flores, 2000), nevertheless, there is limited

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information about the disease epidemiology in the dairy calves maintained in a dairy cattle intensive rearing system.

The objective of this study was to estimate the risk infection of cryptosporidiosis in dairy calves from Aguascalientes, Mexico, in relation with the season and months of sampling.

MATERIALS AND METHODS

This study was carried out in the State of Aguascalientes, located in the North-Central region of Mexico, at an average altitude of 1885 m above sea level. The State has an average temperature of 16.9°C and 475 mm rainfall per year. Rainfall is seasonal and happens during the summer. Three dairy farms of the Aguascalientes dairy region (Cruz-Vazquez *et al*., 1999) were selected, in which Holstein calves are lodged in a special nursery with individual elevated pens and concrete floors, the management and feeding practices are the same in the dairies included in the study. During a 12 months period (March/2005 to February/2006), the dairies were visited every 14 days to collect fecal samples directly from the rectum of all the dairy calves, with ages between 0 and 28 days, that were present during the day of the visit not considering if the animals had diarrhea or not; each sample was identified with the name of the dairy farm, number of calf ear tag and age, the samples were transported in refrigeration to the laboratory for processing, any calve were sampled in 2 occasions. Each sample was homogenized, taking 10 g of feces diluted 1:1 in water to prepare a slide containing 6 fecal smears of the same sample; these were previously dried at room temperature during 24 h and then processed by the acid fast Kinyoun stain method (Bernal *et al*., 1998). In order to minimize false positive readings, a sample was considered positive only when after having completely examined the six smears ≥ 5 oocysts per smear were detected. Records were kept of animals positive to *Cryptosporidium* sp. by sampling month during the study period, with said information general prevalence was estimated and seasonal distribution of parasitic infestation. Chi square (χ^2) test was used to compare proportions of calves with and without parasitic infestation between the seasons of the year ($p<0.05$). To establish the risk of infection by *Cryptosporidium* sp. in each of the four seasons of the year and 12 months of sampling (independent variables), a logistic regression analysis was carried out (Hosmer and Lemeshow, 2000), where the dependent variable was parasitic infestation status. Selection of independent variables was carried out by backward step by step, whereby, one by one of the variables that does not have $p<0.05$ to the Chi square (χ^2) test is excluded. Odds Ratio (OR) was estimated for independent variables that showed statistical significance in the multivariate analysis ($p<0.05$). The analysis was done with the Statistics Data Analysis Program (STATA) v. 9.1.

RESULTS AND DISCUSSION

Prevalence distribution per season is shown in Table 1, where it is observed that in summer and autumn prevalence was highest (48 and 45%, respectively) and spring and winter the lowest (33 and 36%), nevertheless, there were no differences between the four seasons ($p>0.05$). General prevalence during the study was 40% (669/1658). Monthly prevalence distribution recorded during the study is shown in Fig. 1, fluctuations were detected from March to July with a peak during the month of August, (57%) and then prevalence slowly tapered down until December (43%); the highest prevalence months were at the end of summer and in autumn, while, the lowest prevalence ones were during winter and spring; the lowest prevalence was detected in May and January (29 and 30%, respectively). No season could be established as a risk factor for the infection by *Cryptosporidium* sp. nevertheless, the following sampling months were identified as a risk factor: August (OR = 2.3), September (OR = 1.6), October (OR = 1.5) and November (OR = 1.4) (Table 2).

In this study, it was not possible to identify seasonality in the presentation of infection nevertheless, seasonal distribution showed highest prevalence during summer and autumn, while, the lowest happened during

Table 1: Seasonal prevalence distribution of infection by *Cryptosporidium* sp. in dairy calves (0-28 days of age) in Aguascalientes, Mexico

Season	Examined	Positive	Prevalence (%)
Spring	483	162	33a
Summer	391	186	48a
Autumn	441	197	45a
Winter	343	124	36a
Total	1658	669	40

Values in the same column followed by the same letter are not different p>0.05

Table 2: Logistic regression analysis results for the sampling months

Risk factor	Odds ratio	95%	Confidence interval	p-value
January	0.97	0.56a	1.69	0.942
February	1.27	0.86a	1.87	0.227
March	1.25	0.87a	1.80	0.214
April	1.37	0.88a	2.13	0.155
May	1.02	0.60a	1.73	0.928
June	1.36	0.93a	0.20	0.109
July	0.98	0.48a	1.96	0.955
August	2.31	1.59a	3.36	0.000*
September	1.62	1.15a	2.29	0.005*
October	1.51	1.04a	2.21	0.031*
November	1.42	1.01a	2.00	0.042*
December	1.41	0.98a	2.03	0.059

*(p<0.05)

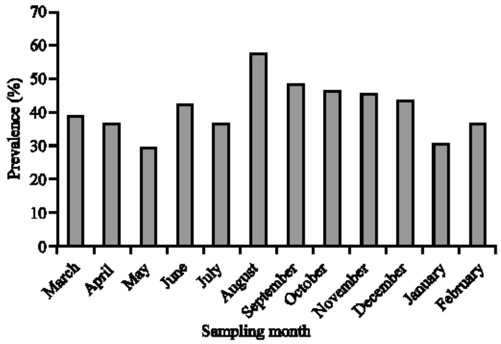

Fig. 1: Prevalence of infection by *Cryptosporidium* sp. in dairy calves (0-28 days of age) in Aguascalientes, Mexico, according to the sampling month

spring, also, there were no statistical differences between the four seasons; prevalence was high during all the year (>33%). However, in different conditions, other studies have found comparable results, reporting summer and spring as the seasons of highest and lowest prevalence, respectively, but without showing infection seasonality or effect of seasons on prevalence (Garber *et al*., 1994; Becher *et al*., 2004; Paul *et al*., 2008) general prevalence during this study was higher that what has been reported previously in the central region of Mexico (Maldonado *et al*., 1998). On the other hand, no season was identified as risk factor for infection by *Cryptosporidium*, this observation coincides with results obtained by Starkey *et al*. (2005), who didn't find association between sampling season and infection incidence in lactating animals; likewise, other studies have reported summer as the season with highest risk of infection by *Cryptosporidium* (Garber *et al*., 1994) and spring as the one with lowest risk (Mohammed *et al*., 1999). In beef cattle reared in grazing fields, infection seasonality is associated with the calving season (Mann *et al*., 1986). Prevalence distribution per sampling month permitted the identification of August to December as the period of highest prevalence (Fig. 1), with August to November as risk factors (Table 2), corresponding to the end of summer and autumn. Other studies indicate that some months of summer and autumn (August to October) are associated to the presence of *Cryptosporidium* (Huetink *et al*., 2001; Saha *et al*., 2006), while, Starkey *et al*. (2005), report the highest infection incidence during the months of autumn, while Mohammed *et al*. (1999), mention the period of October to December, as the one of highest infection risk. Seasonality of infection by *Cryptosporidium* sp. has been mentioned as an important element in the disease epidemiology, even though it is possible to see in what has been mentioned that influence has not been demonstrated in similar manner by the different studies, due mainly to the sampling protocol, to the specific characteristics of the lodging and managing of production units included in the research and/or the geographical location of the same. Even though, seasonality of infection has not been clearly established in dairy calves of dairy farms according to our results and the other reports mentioned previously, it could be stated that certain months of summer and autumn may be more propitious for infection and development of disease.

CONCLUSION

The high prevalence observed in dairy stables studied during the four seasons suggests that measures taken to prevent the infection by *Cryptosporidium* should be carried out during all the year, nevertheless, it is important that more emphasis be placed on said measures during the months of highest risk, that is, in August to November.

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