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Article

# Prevalence and risk factors associated with *Cryptosporidium* spp. in dairy cattle in Chiquinquirá (Colombia)

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**Abstract:**

Cryptosporidiosis is a disease characterized by episodes of diarrhea in cattle worldwide, caused by a protozoan parasite of the genus *Cryptosporidium* spp. of the phylum Apicomplexa and Family Cryptosporiidae. It is responsible for important economic losses, and, in addition to this, it generates an impact on human health, as it can parasitize humans. The objective of the study was to determine the prevalence of and risk factors associated with *Cryptosporidium* spp. in cattle in Chiquinquirá (Colombia). A descriptive cross-sectional study with simple random sampling was carried out, with a sample size of 1,044 head of cattle, including males and females of different breeds and age groups, using the WinEpi statistical software. Fecal samples were taken directly from the rectum and processed with the modified Ziehl-Neelsen (ZN) technique for the identification of parasite oocysts using a 100X objective. The data were processed with the Epi Info® statistical software. An overall

prevalence of 7.3 % (73/1000) was found; females, 2 to 4-yr-old bovines, and crossbred cattle were the most prevalent. No significant statistical association was found between breed, age, and sex of the individuals evaluated, and protozoan positivity ($P \geq 0.05$). The purchase of animals and larger productions were considered risk factors for parasitosis. Protozoan prevention and control plans should be designed and implemented based on sanitary practices to prevent the dissemination of oocysts found in fecal matter.

**Keywords:** *Crypstoporidium* spp., Cryptosporidiosis, Cattle.

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# Introduction

*Cryptosporidium* spp. is a protozoan, coccidian, zoonotic, obligate intracellular parasite that is part of the phylum Apicomplexa and the family Cryptosporiidae; it is distributed across the world[1-5]. The parasite affects the gastrointestinal tract of vertebrate species such as cattle, birds, small ruminants, rodents, canines, felines, rabbits, squirrels, and even humans[6-9]. Recent reports indicate that more than 40 species of *Cryptosporidium* spp. have been described, among which *C. parvum*, *C. bovis*, *C. ryanae*, and *C. andersoni* are routinely found in cattle[5].

Parasites of this genus cause a serious gastrointestinal disease known as cryptosporidiosis[7,8] that impacts both human health and animal health[1,5,6]. Cattle, especially calves, have been identified as one of the most common reservoirs of this protist[1,4], which is one of the main causes of morbidity and mortality in calves aged 1 mo or less worldwide[10]. However, there is a wide variety of hosts that can act as reservoirs of the parasite, favoring the persistence of *Cryptosporidium* spp. in the environment for long periods of time as oocysts and, therefore, increasing the risk of their transmission to susceptible hosts[6,7].

*Cryptosporidium* spp. infections constitute a substantial public health burden and are responsible for economic losses in livestock herds worldwide[11]. Therefore, the reduction of disease and shedding of *Cryptosporidium* spp. oocysts is considered an important objective in livestock productions, by inhibiting the transmission of the protist through direct contact with infected animals, or ingestion of feed and water contaminated with animal feces[9]. The diagnosis of the protozoan is based on the identification of oocysts at the laboratory, where

it is a common practice to carry out a microscopic observation of the oocysts applying a Ziehl-Neelsen (ZN) stain with an acid alcohol solution, auramine with phenol, or immunofluorescent stain to fecal smears[12].

The therapeutic options available to treat cryptosporidiosis are limited[11]. Despite the substantial interest in this type of parasite, progress in terms of treatment development and understanding of most of the life cycle of this unusual organism is scarce[7]. So far, in the Department of Boyacá there are no recent studies on the identification of parasite oocysts in fecal material by microscopy, nor the analysis of different variables[13]. Therefore, the objective of this research was to determine the prevalence and risk factors associated with *Cryptosporidium* spp. in cattle in Chiquinquirá (Colombia).

# Material and methods

## Geographical location

Boyacá has four municipalities specialized in milk production (Chiquinquirá, Caldas, San Miguel de Sema, and Saboyá), reaching a volume of 70,000 L per day derived from approximately 50,000 cows destined for milk production[14]. According to national government data, livestock farming in Chiquinquirá represents an important part of the economy of the municipality, which is located at 5°36'48" N and of 0°15'21" W of the meridian of Bogotá, at an altitude of 2,000 to 3,200 meters above sea level, having an average temperature of 15 ºC[15].

## Sample size

In the year 2022, Chiquinquirá reported 33 398 head of cattle, according to the National Livestock Census of the Colombian Agricultural Institute (*ICA*)[16]. Based on the reported data and following the formula obtained from the WinEpi statistical program, a sample size of 947 female and 47 male cattle of various age groups and breeds with dairy potential was determined. In addition, a confidence interval of 95%, an accepted error of 5%, a sampling fraction of 1.15% and an expected prevalence of 50% were considered.

$$n = \left( \frac{Z_{\frac{a}{2\sqrt{p(1-p)}}}}{E} \right) = \frac{Z^2 \alpha/2 \cdot p(1-p)}{E^2}$$

Where: n= sample size; E= accepted error; p= expected value of the ratio; α= queuing probability.

## Sample collection and processing

A total of 2 to 5 g of fecal material were taken directly from the rectum by rectal palpation. The samples were labeled and stored in refrigeration coolers to be transported to the Veterinary Parasitology Laboratory of the Pedagogic and Technological University of Colombia (Universidad Pedagógica y Tecnológica de Colombia, UPTC) for processing. For the identification of *Cyptosporidium* spp. oocysts in bovine feces, the modified Ziehl-Neelsen (ZN) or Kinyoun cold staining technique was utilized. A thin smear of fecal material was made on the slide and allowed to air dry. The slides were then placed in staining racks where they were stained for 10 min with ZN fuchsin. The slides were then placed in staining racks where they were stained for 10 min with ZN fuchsin. The slides were examined microscopically using a 100x objective with immersion oil. In these samples, *Cryptosporidium* spp. oocysts stained bright red were considered positive[17].

## Statistical analysis

The identification of *Cryptosporidium* spp. oocysts in bovine fecal material and the data obtained in the epidemiological survey were consolidated and filtered. Among the evaluated factors, it is important to mention that reference is made to the absence or presence of management practices; large herds were those with more than 10 animals in production, while small herds were those with 10 animals or less. In terms of the water sources, the only one that provided potable and treated water was the aqueduct. The results were analyzed with the Epi Info® statistical software, version 7.2.4.0.

The proportion of individuals affected by *Cryptosporidium* spp. and exposed to the factors evaluated in the study were compared with the same proportion of a population not exposed to that factor to estimate prevalence ratios (PR). The PR was employed to measure the association between cryptosporidiosis and the hypothesized causal factors, as well as the significance of these associations using Fisher's exact test[18].

PR values above 1 (lower 95% confidence interval < 1) and with $P<0.05$ were considered risk factors, whereas PR values below 1 (upper 95% confidence interval < 1) and with $P<0.05$ were regarded as protective factors. The dependent variable included the modified ZN results, while the independent variables were all the determinant variables established in the epidemiological survey applied during sampling. Once these factors were established, a logistic regression was performed[19].

## Ethical considerations

The study was conducted per Resolution 8430 of the Colombian Ministry of Health and Social Protection and the 1989 Law No. 84. These set out the standards that are appropriate for the welfare of animals during research. In addition, before blood sampling, an informed consent form was signed by the owners of the cattle.

# Results

An overall prevalence of 7.3 % (73/1000) was determined in the municipality of Chiquinquirá. Females were more prevalent than males, with a prevalence rate of 7.39 (70/947) and 6.98 % (3/43), respectively. Cattle aged 2 to 4 yr and crossbred cattle had a higher presence of *Cryptosporidium* spp. oocysts (Table 1). No significant statistical association was found between the breed, age, or gender of the individuals evaluated and protozoan's positivity ($P\geq0.05$).

**Table 1:** Prevalence of *Cryptosporidium* spp. by age group and breed in cattle in the municipality of Chiquinquirá, Boyacá

| Variable | N | Positive *Cryptosporidium* spp. | Prevalence (%) |
|---|---|---|---|
| | | Age groups | |
| < 2 years | 304 | 20 | 6.58 |
| 2-4 years | 84 | 10 | 11.90 |
| > 4 years | 612 | 43 | 7.03 |
| | | Breeds | |
| Ayrshire | 138 | 11 | 7.97 |
| Crossbreed | 95 | 9 | 9.47 |
| Holstein | 767 | 53 | 6.91 |

Regarding the assessed variables, management practices such as the presence of cattle belonging to other owners (*P*=0.0018), pasture leasing (*P*=0.0010), and the purchase of animals (*P*=0.0062) were statistically significantly associated with the occurrence of the parasite in the evaluated cattle (Table 2).

**Table 2:** Analysis of management practices as potential risk factors associated with *Cryptosporidium* spp. infections

| Variable | Category | PR | Confidence interval (95%) | *P*-value |
|---|---|---|---|---|
| Management practices | Pen | 0.9769 | 0.9416 - 1.0135 | 0.1234 |
| | Other owners' livestock | 0.9427 | 0.9136 - 1.0729 | 0.0018 |
| | Other species | 0.9352 | 0.8351 - 1.0474 | 0.1113 |
| | Lease of pastures | 0.9455 | 0.9138 - 1.0783 | 0.0013 |
| | Purchase of animals | 1.0472 | 1.0118 - 1.0839 | 0.0062 |
| | Damaged fences | 1.0056 | 0.9696 - 1.0430 | 0.4254 |
| | Deworming | 0.9352 | 0.8351 - 1.0474 | 0.1113 |

The results are presented as prevalence ratio (PR) and 95% confidence interval (CI).

The association between diarrhea and the presence of *Cryptosporidium* spp. oocysts in the analyzed fecal samples was statistically significant. Herd size too was statistically significant: large herds were considered as a potential risk factor, while small herds were established as a preventive factor against the occurrence of the parasite. On the other hand, when analyzing the source of drinking water, the aqueduct and the stream exhibited a statistically significant association with the positivity of the protozoan, and the stream was established as a potential risk factor, while the aqueduct was a protective factor (Table 3).

**Table 3:** Analysis of clinical manifestations, herd size, and drinking water source as potential risk factors associated with *Cryptosporidium* spp. infections

| Variable | Category | PR | Confidence interval (95%) | *P*-value |
|---|---|---|---|---|
| Clinical manifestations | Diarrhea | 0.9552 | 0.9208 - 1.0909 | 0.0078 |
| | Fever | 0.9699 | 0.9366 - 1.0044 | 0.0545 |
| Herd size | Large herd | 1.051 | 1.0169 - 1.0863 | 0.0081 |
| | Small herd | 0.9515 | 0.9206 - 0.9834 | 0.0072 |
| Water source | Aqueduct | 0.9496 | 0.9175 - 0.9829 | 0.0023 |
| | Cistern | 0.9694 | 0.9364 - 1.0035 | 0.0657 |
| | Gully | 1.0589 | 1.0122 - 1.1078 | 0.0041 |

The results are presented as prevalence ratio (PR) and 95% confidence interval (CI).

The analysis of the variables that were determined as potential risk factors through logistic regression allowed determining that the purchase of animals and production units with more than 10 animals as risk factors for the occurrence of *Cryptosporidium* spp. oocysts in the evaluated cattle (Table 4).

**Table 4:** Analysis of variables as potential risk factors associated with infections by *Cryptosporidium* spp.

| Variable | Odds ratio | LCI | UCS | *P*-value |
|---|---|---|---|---|
| Purchase of animals | 2.252 | 1.3358 | 3.7965 | 0.0023 |
| Large herd | 2.6677 | 1.2593 | 5.651 | 0.0104 |
| Gully | 1.5773 | 0.9484 | 2.6232 | 0.0791 |

LCI= lower confidence interval; UCS= upper confidence interval.

# Discussion

Enteric protozoan infection in cattle can pose a threat to the productivity and survival of the animals, resulting in negative impacts on the livestock industry[20]. Within this group of pathogens affecting animal health, *Cryptosporidium* spp. is an obligate intracellular parasite transmitted by the fecal-oral route after ingestion of oocysts that can contaminate, persist, and resist disinfection in the water and food[21]. The published literature on the parasite is extensive, providing details of its distribution in most regions of the world[22]. Prevalence rates of 52.2 % in Algeria[10], 16.2 % in Ethiopia[4], 53 % in Latvia[23], and 64 % in cattle sampled in the Lagoon region of Mexico have been reported[24].

Similarly, at the national level, there are prevalence rates of 22 % in the Central Savannah province (Cundinamarca)[25], 22 % and 7 % in Chiquinquirá[26,27], and 48 % in bovines in Boyacá[28]; microscopic diagnosis revealed that 115 calves (26.6 %) from 44 farms (59.5 %) in a central area of Colombia (Antioquia, Boyacá, Cundinamarca, and Meta) tested positive[29], these rates being higher than those found in the present study. The reported variations may be caused by different environmental conditions, management practices, and the number of animals in the farms; therefore, the role of the environment in direct and indirect contamination should be considered, mainly the accumulation of oocysts having occurred previously in animals of the herd, which facilitates the fecal-oral transmission route among the cattle and can thus modify the prevalence of infection by the parasite[30].

In the present study, cattle aged 2 to 4 yr had the highest prevalence of the parasite, unlike in Africa[6,10], Asia[20], and South America[29], where a higher infection rate was detected in young cattle compared to adult animals. No significant statistical association was found

between cattle age ($P \geq 0.05$) and the prevalence of *Cryptosporidium* infection in cattle from central Ethiopia[31]; however, in cattle from dairy farms in Colombia[28], United States[32], and India[2], reports have detected an association between the age of the bovines and the excretion of oocysts in fecal matter. Although age was not considered a risk factor for the occurrence of the protozoan in the present study, bovines aged <12 mo were associated with the excretion of *Cryptosporidium* spp. oocysts in Colombia[28]. In this regard, it should be taken into account that nursing calves are more predisposed to acquire infection by the parasite; in addition, the clinical disease may be influenced by the immune status of the host[33].

Das *et al*[2] report that there is statistical significance between positivity to *Cryptosporidium* spp. and the sex of the cattle, which was not evident in the present study; however, similarly to our results, in Ethiopia[31] and Nigeria[6] no significant statistical differences in the prevalence of *Cryptosporidium* infection were found between males and females. On the other hand, crossbreeds had the highest oocyst excretion of the protozoan, with no statistical association between cattle breed and parasite occurrence ($P \geq 0.05$). Likewise, in Addis Ababa and its surroundings[31], the prevalence of infection is likely due to the potential occurrence of the coccidian in beef and dairy cattle[2] regardless of the breed of the animals.

The risk factors for *Cryptosporidium* spp. are mainly associated with the handling and sanitary condition of the animals[31]. The presence in the herds of bovines belonging to other owners, the leasing of pastures, and the purchase of animals whose health and deworming history was unknown were associated with the presence of oocysts in the evaluated samples ($P \leq 0.05$). This is because the transmission of cryptosporidiosis is mainly due to management practices that allow the dissemination that oocysts found in the environment or in diseased animals or susceptible hosts. Similarly, the purchase of animals was identified as a risk factor for the presence of the parasite, possibly because *Cryptosporidium* spp. is not specific to the host. Thus, an environment contaminated with oocysts during an outbreak in cattle can lead to the infection of other species that subsequently use the same grazing area; the unknown health history of individuals can also increase the potential transmission of the protozoan[2].

Herd size was associated with *Cryptosporidium* spp. oocyst excretion, where smaller herd sizes were considered a protective factor, and larger herds were established as a risk factor for infection, consistently with the positive association between higher cattle population density and fecal excretion of *Cryptosporidium* spp. in Africa[31], Asia[20], Europe[34], and North America[32]. Likewise, individual calf rearing reduces the potentiality of infection by the protozoan by approximately 2.5 times compared to group calf rearing[31], as the rate of oocyst shedding differs between housing systems, exhibiting a higher prevalence in calves kept as a group compared to the individual system. However, this will depend on the age of the animals[34] which demonstrates the importance of the facilities used in intensive farms with higher animal densities[29].

The aqueduct differed significantly ($P \leq 0.05$) from the stream as a source of drinking water, the aqueduct having been established as a protective factor against parasite positivity. Farms with drinking water sources such as wells, rivers, or streams acquired 2.4 and 2.9 times more *Cryptosporidium* than farms using tap water to provide water to cattle[31]. Likewise, herds that dispose of wastewater in the field compared to farms that discharge wastewater into nearby wells may also be more likely to be infected with the protozoan[31]. Infection by *Cryptosporidium* spp. is also significantly associated with the symptoms of the infected animals[2], as in the case of the presence of diarrhea in the evaluated individuals ($P \leq 0.05$). However, previous studies showed no association between the presence of diarrhea and oocyst shedding[6,34,35], as well as only a slightly higher prevalence in diarrheic cattle compared to non-diarrheic cattle[6]. Despite this, the rate of infection by *Cryptosporidium* spp. in Colombia[27,28] and Algeria[10] is higher in animals with diarrhea compared to those that do not have it, consistently with the findings in Chiquinquirá, which highlight that the risk of occurrence of this symptom in bovines may decrease as they reach adulthood[23].

There is currently no vaccine or drug in the market for the treatment and control of cryptosporidiosis in ruminants, which makes its prevention difficult. In this sense, it is necessary to implement strategies to reduce the spread of infection in herds, including good disease management practices, such as the separation of cattle with diarrhea; cleaning and disinfecting the facilities before introducing animals; the removal and disposal of fecal matter or wet garbage; good hygiene of the feed and water troughs, and adequate supply of colostrum to newborns, as well as the development of strategies to reduce humidity in the herds[36].

## Conclusions and implications

A moderate prevalence of infection by *Cryptoporidium* spp. was found in cattle in Chiquinquirá, where females, cattle aged 2 to 4 yr, and crossbreeds were the most prevalent. Although infection by the protozoan occurs more frequently in calves, adults can become a source of dissemination of the parasite; therefore, its prevention and control in herds should be paramount. The purchase of animals and larger productions were considered as risk factors for parasitosis; in this sense, sanitary and management practices should be adjusted to minimize the excretion of oocysts in fecal matter in extensive systems and in those where animals whose sanitary history is unknown are allowed to enter.

## Conflict of interest

The authors of this article declare that they have neither conflict of interest nor any economic, personal, political, financial, or academic relationship that might influence their judgment.

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