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ARTÍCULO ORIGINAL

Detection of Cryptosporidium oocysts by auramine and Ziehl Neelsen staining methods

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ABSTRACT

Cryptosporidium *spp is a common intestinal pathogen of animals and humans. It may have an important economic impact on farms and cause potentially zoonotic infections. Fecal specimens were collected from 331 domestic animals (81 beef cattle, 50 sheep, 100 pigs and 100 dogs) and checked for the presence of* ***Cryptosporidium*** *oocysts by way of Ziehl Neelsen and auramine staining methods. An overall positivity rate of 7.5% (25/331) was found, with rates of 10% (10/100) among the dogs and 18.5% (15/81) among the beef cattle. The feces of sheep and pigs tested negative. In beef cattle, 15 and 12 positive samples were detected by the auramine and Ziehl Neelsen staining techniques, respectively, with no statistically significant difference between the two methods. In dogs, the same number of positive samples was found by both techniques.*

Key words: Cryptosporidium*, domestic animals, auramine, Ziehl Neelsen method.*

INTRODUCTION

Members of the genus *Cryptosporidium* are eukaryotic organisms, including obligate and intracellular parasites. *Cryptosporidium* has a complex life cycle, including both sexual and asexual reproduction, an auto-infectious cycle, and the ability to complete its development within a single host. The transmission form is a robust, environmentally resistant oocyst, excreted in the stool, which can exist for long periods of time in the environment. Because animals, in particular domesticated livestock, are its primary host, human infection is usually zoonotic[1]. Those at greatest risk are immunocompromised adults and children, especially those with AIDS, children in day care, travelers to endemic regions, dairy or cattle farm workers or their families or contacts, household contacts of cases or carriers, and possibly owners of infected dogs or cats or their neighbors[2-4].

The genus *Cryptosporidium* includes 13 species that are currently considered valid, distributed among domestic and wild mammals, birds, reptiles, and fish. Other morphologically distinct species have been found in fish, reptiles, birds, and mammals, but have not been named[5]. Five *Cryptosporidium* species (C. *parvum*, C.

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hominis, C. *canis*, C. *meleagridis* and C..*felis*) are the causative agents of infection in humans [6]. *C. parvum* is a zoonotic pathogen composed of genetically distinct but morphologically identical genotypes. Although large-scale studies of *Cryptosporidium* infection in dogs have been performed in several countries, the isolates were not accurately identified because of the lack of a method for molecular analysis. It is important to identify the isolates harbored in dogs, which come in close contact with humans, in order to control human cryptosporidiosis[7, 8].

Cats are a source of cryptosporidiosis for humans, regardless of whether they are immunocompromised or not; however, only one human case has been described, in which *C. felis* was identified using the molecular method[9].

In pigs, the prevalence of *Cryptosporidium parvum* has been investigated worldwide, ranging from 1 to 33%[10], and the following rates have been found: Germany (1.4%), Spain (21.9%), Japan (33.2%) Canada (11%), United States (7.1%)[11, 12]. Preweaning diarrhea, caused by a complex of protozoan organisms including *Isospora suis* and *Cryptosporidium spp*., remains a major problem in pigs worldwide[13]. Yet, due to the inadequacies of conventional diagnostics, little is known about the prevalence and significance of *Cryptosporidium* in pigs[14].

It is one of the main causes of diarrhea in newborn ruminants, in cattle, sheep, deer[15], and goats[16, 17]. *Cryptosporidium* infection in livestock may cause important economic impact to farmers because of its high morbidity and sometimes high mortality rates among farm animals[18], and disease is well documented. Comparatively, there is less information on the occurrence of cryptosporidiosis in sheep and goats, although the infection is common in these animals with prevalence rates that vary from country to country, often causing death in lambs[12,17,19-21].

Few studies have been conducted in dogs and a prevalence rate of 9.7% for cryptosporidiosis among Korean dogs while they were monitoring sources of environmental contamination[22] and rate of 9.3% among dogs was demonstrated in Osaka, Japan[8]. By using a molecular method, the authors identified all isolates as *C. canis*. A study conducted in São Paulo, Brazil, revealed a prevalence rate of 6.8% for cryptosporidiosis among street dogs 23 and of 2.41% in Rio de Janeiro[24].

The present study was performed to investigate the status of cryptosporidiosis in beef cattle, sheep, pigs and dogs in the rural and urban regions of Lages, state of Santa Catarina, southern Brazil, using two diagnostic methods.

MATERIAL AND METHODS

Among 331 fecal samples, 81 were collected from extensively managed beef cattle, 50 from free-grazing sheep, 100 from intensively farmed pigs, and 100 from owned dogs in Lages, state of Santa Catarina, Brazil. Among the beef cattle, 32 were younger than 12 months (17 females and 15 males) and 49 females were up to12 months. Of the dogs, 40 were female and 60 were male, either young or adults, and all of them lived on the outskirts of the urban area. Among the pigs, there were 80 sows and 12 gilts, one male older than 12 months and 7 males aged up to 12 months. Among the sheep, there were 49 ewes and one ram, all of them older than 12 months.

The samples were collected from the rectum of each animal using disposable latex gloves. The samples were submitted to sedimentation of oocysts by centrifugation, in which the sediment was fixed with methanol for 5 minutes and then used for the smearing procedure. The specimens were smeared onto glass slides and stained using the modified Ziehl Neelsen and auramine techniques[25,26].

The samples stained by the Ziehl Neelsen technique were examined under light microscopy (1,000 X). The auramine-stained smears were analyzed by fluorescence microscopy, after a previous screening (100 X) and later confirmation (400 X).

Fisher's exact test (Graphpad Software, v2.04) was used for the comparison between the two diagnostic methods. An alpha error probability of less than 5% ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

The overall positivity rate was 7.5% among 331 fecal samples examined for *Cryptosporidium* sp. The comparison between both techniques demonstrated that 25 (7.5%) and 22 (5.7%) samples were positive for the auramine and Ziehl-Neelsen methods, respectively.

Of 100 fecal samples collected from dogs, 10 (10%) were positive, five in female and five in male animals. When these animals were assessed according to age, *Cryptosporidium sp.* was found in 4 (40%) dogs younger than 12 months and in 6 (60%) adult dogs. The 10% infection rate observed is in agreement with others authors[8,23].

Among the 15 fecal samples of cattle that tested positive for cryptosporidiosis, 7 (46.6%) belonged to oxen and 8 (53.3%) to cows. In terms of age, positive results were observed in 12 (80%) cattle younger than 12 months and in 3 (20%) cattle older than 12 months.

Auramine detected 25 (100%) positive samples, whereas the Ziehl-Neelsen method detected 22 (80%) positive samples, with no statistically significant difference.

The fecal samples collected from sheep and pigs were negative for *Cryptosporidium* oocysts.

Several methods, including both flotation and sedimentation, are used for the detection of *Cryptosporidium* oocysts; however, neither of the methods shows any difference[2]. Auramine has a greater affinity for the *Cryptosporidium* oocyst wall than fuchsin, a red dye used in Ziehl Neelsen staining technique[25]. Auramine-stained oocysts withstand discoloration for 5 minutes, but oocysts stained by the Ziehl Neelsen technique exhibit complete discoloration within the same time frame. Auramine staining has more advantages over the Ziehl Neelsen method, i.e., it is quicker to perform and read, and ideal for population-based studies. Stained slides, if protected from light, can last for months, and can be later stained by the Ziehl Neelsen technique.

The results of the age distribution in this study possibly reflected a bias due to the deviated population structure toward aged animals in rural and urban areas of our city. Direct contact with infected animals is suggested to be an important mode of transmission of *Cryptosporidium*, which is possibly present in every domestic beef cattle herd in the world with asymptomatic infections and prolonged oocyst excretion by cattle recognized as a major and continuous source of environmental contamination[27,3].

At least 13 *Cryptosporidium* species are currently recognized; this is based on genotyping and on a limited number of transmission experiments. *C. parvum* has recently been known to have several different genotypes such as genotype 1, found exclusively in humans and a few other primates, and genotype 2, found in most mammals, including humans[27] although *C. hominis*, found exclusively in humans, has been well described[5,28].

CONCLUSIÓN

The present survey demonstrated that *Cryptosporidium* infection of calves is important and that further studies are needed to show its relative importance, mainly in the neonatal diarrhea syndrome.

We did not evaluate the genotype of *C. parvum* in the animals of this study. Future studies will be necessary to verify the infection status of these animals. The positive rate in beef cattle and dogs suggested that these animals could be a source of human infection. Because *C. parvum* is a major waterborne protozoan pathogen, water contamination should be investigated to protect public health from the risk of transmission of the pathogen.

In addition, these results also highlight the importance of investigating the possibility that other animals also act as reservoir hosts for *Cryptosporidium*.

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