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 zoonotic1. 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 neighbors2-4.

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 named5. Five Cryptosporidium species (C. parvum, C.
**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*.

**REFERENCES**


