

Studying the prevalence of Cryptosporidiosis among the calves of Sistan area, Iran

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(Received 2 April, 2014; accepted 30 April, 2014)

ABSTRACT

Cryptosporidiosis is a parasitic zoonosis which is of protozoan type and causes long term diarrhea in the cases that their immune systems are weakened. The prevalence of the mentioned parasite among the calves of Sistan has been studied. The feces samples of 120 under 6 months calves in Sistan's cattle sheds were taken from Mehr of 1391 to Mehr of 1392. After delivering samples to the laboratory and concentrating by Formol-Ether method, they were examined by corrected Ziehl-Neelsen staining method and optical microscope in order to determine the existence of *Cryptosporidium* oocytes. The prevalence of the mentioned protozoan in the samples was 11.6%, also 15.78% of male cases and 7.83% of female ones were infected. Regarding the prevalence of *Cryptosporidium* protozoan in Sistan area and its zoonotic aspects in human societies, controlling the above mentioned parasite is of significant importance.

Key words : *Cryptosporidium*, calf, zoonotic, Sistan, Iran

Introduction

Cryptosporidiosis is a parasitic disease common between humans and livestock, and the fact that there is no effective cure for it substantially increases its importance. The pathogenic unicellular organism related to this disease causes long-term diarrhea together with fever, nausea, and weight loss in patients whose immune systems have been weakened, and can even result in death in some cases if patients do not receive the timely care required. However, people with safe immune systems only suffer acute and self-healing enteritis (Anderson, 1982). This unicellular organism is an intracellular, extra-cytoplasmic parasite and has been reported in microvilli of the digestive systems, especially in the epithelial cells of the jejunum area, and in the lungs, the biliary

system, the pancreas, and the lymphatic nodules (Chermette, 1988). The disease appears in two forms: with clinical symptoms accompanied by excretion of oocytes of the parasite, and without clinical symptoms together with the excretion of oocytes. Disease symptoms are similar in humans and livestock but the incubation periods in various animals and in humans are different (Del Coco, 2008). It is an important disease because no effective cure for it has been found yet. On the other hand, because of the small size and the resistance of oocytes, no basic methods have been recommended for their elimination. The economic importance of this animal disease results from the fact that a high percentage of animals, especially livestock, are infected with it. It is more prevalent at earlier ages and causes severe diarrhea (yellow-liquid explosive diarrhea), similar

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to cholera diarrhea, with the production of numerous oocytes in neonates and calves (Brook et al, 2008).

Cryptosporidium is considered one of the most important agents that are transmitted by water and vegetables and cause chronic diarrhea in livestock. Oocytes excreted by infected livestock, including calves, can contaminate environments such as surface water sources (Rosales et al., 1998). Diarrhea caused by this parasite usually heals by itself, but in animals with immune system problems cryptosporidiosis may even result in death. In Iran, calf losses amount to 12-20 percent, about 75 percent of which is caused by diarrhea syndrome (Nadalian, 2008). At present, one of the health problems facing human societies is diarrhea in children caused by various agents, and recognizing each one of these agents is essential for treating, preventing, and controlling this syndrome in children. Moreover, based on available statistics in European countries, every year about 10 percent of newborn calves die of diarrhea, and calf diarrhea inflicts the second largest economic losses on the dairy industry (Mokhber, 2002). The unicellular organism *Cryptosporidium* was first reported in the digestive glands of lab rats by Tyser in 1907 (Sari, 2009) and was named *Cryptosporidium maurice*. Another species of this parasite, isolated from rat small intestine in 1912, was named *Cryptosporidium parvum*. In Iran, cryptosporidiosis was first histopathologically reported in a native rooster (Gharagozleu, 1985) in 1985, and since then it has been reported from various parts of the country. It must be mentioned that the first case of cryptosporidiosis in humans in Iran was reported in 1976 in a 39 year-old man suffering from immune system deficiency (Nime, 1976). Transmission of the parasite to humans takes place through water, food (especially salads), raw milk, and fruit juice (Slifko, 2000). The largest epidemic of cryptosporidium in the world happened in the city of Milwaukee in the United States in 1993 when about 300000 people developed digestive infections (MacKenzie, 1995). Given the distribution of this disease in the world, and to emphasize livestock infected with this parasite that do not exhibit clinical symptoms can play an important role in nature as the source of infection, and considering the role this parasite plays in the health of humans and animals, we conducted this research on this unicellular organism.

Materials and Methods

One hundred and twenty samples of stool spread on slides were prepared from calves less than 6 months old in the villages of Sistan region. Characteristics of the calves, including their age, gender, race, and exhibited clinical symptoms, and particulars of their owners were recorded. Thirty-two of these samples were diarrheal with a watery consistency and the rest were normal. Stool samples were taken with disposable gloves directly from the rectums of the calves (if there was no stool in the rectum, swab samples were taken) and transferred to wide-mouthed plastic containers. In general, infection of calves to this unicellular organism was determined based on isolation of oocytes from stool. Morphological characteristics of isolated oocytes were sufficient to identify the parasite at the genus level. The most reliable method for species identification is to use molecular identification methods. For this purpose, in this research we used the formol-ether concentration technique to concentrate the oocytes present in the stool samples. Half a gram of stool was put in a test tube and six milliliters of formol were added to thoroughly dissolve the stool. Four mL of ether was then added, the top of the tube was covered with a plastic cap, and the tube was vigorously shaken, centrifuged at 2000 rpm for two minutes, and removed from the centrifuge machine. There were four layers in each tube: the top layer was ether, below it undigestible material, formol the third layer, and the sediment at the bottom of the tube. After discarding the top three layers with the help of a Pasteur pipette, we transferred some of the sediment onto a slide and spread the sediment on it. The prepared slide was then fixed with the help of methyl alcohol and stained using the modified Ziehl-Neelsen method. The objective lens with the magnification of 100× was used to examine the slides and identify oocytes of *Cryptosporidium* under a light microscope. The criterion for identification was the observation of red oocytes with the approximate diameter of 3-6. It must be added that if even one oocyte was observed on a slide, the sample would be reported positive. After examining the slides, the positive samples were recorded and the oocytes were counted to find the mean number of oocytes per microscope field. Infection intensities were given the following scores: 1 to 4 oocytes per field: (+), 5 to 25 oocytes per field: (++) , and more than 25 oocytes per field: (+++).

Results and Discussion

Fourteen (11.66%) of the 120 stool samples were infected with *Cryptosporidium* oocytes: four had the infection intensity (+++), four (+), and six (+). Clinically, diarrhea and low body weights were observed in calves that exhibited infection intensity (+++) on stool slides, but calves with lower infection intensities did not show clinical symptoms. As for age of infected calves, 10 were less than two months old, three 2-4 months old, and one 4-6 months old. In our study, 15.78% of the male calves and 7.93% of the female calves were infected with the unicellular parasite cryptosporidium. Thirty samples were examined in each season, three (2.5% of the total 120 samples) were infected in spring, one (0.83%) in summer, two (1.66%) in autumn, and eight (6.6%) in winter.

In this study, 14 of the 120 examined stool samples (11.66 percent) were infected with cryptosporidium oocytes. Identification of diseases, and planning programs of comprehensive control of parasites, will upgrade the level of health and the economic returns, and will substantially reduce incidence of diseases common between humans and livestock too. Cryptosporidiosis is a disease common between humans and livestock and they may acquire it from each other or from sources of infection such as infected water, air, food, and feed. Since cryptosporidium is not host-specific and is transmitted from different places and in various ways, and because no definite cure for it has been found yet, it is important and necessary to observe hygienic principles in order to prevent the occurrence of infections in humans and livestock. *Cryptosporidium* is a unicellular organism of the genus Protozoa and family Apicomplexa that attacks the brush border of intestinal cells of most vertebrates. Until the recent past, it was considered an opportunistic and uncommon parasite, but now it attracts attention as an enteropathogen in diseases common between humans and livestock, especially in those with immune deficiency. More than half of newborn calves of

dairy and beef cattle are exposed to the risk of infection with *Cryptosporidium* and, during the first week of infection, suffer from severe diarrhea, loss of body water, reduced growth; and, in some cases, die. This unicellular organism is mainly transmitted orally by water and materials contaminated with feces. Cows, pigs, and other domesticated animals are the sources of infection for humans. Therefore, people whose occupations put them in contact with animals are more at risk of developing infection. Moreover, transmission from wildlife and zoo animals is also possible, and drinking water may transmit oocytes of this unicellular organism too. Oocytes excreted by infected livestock, including calves, can contaminate the environment, such as surface water sources. Water from rivers and wells that is contaminated with wastewater, contaminated water in grazing grounds of domestic and wild animals, and wastewater used in agriculture play an important role in the transmission of this parasite. Moreover, transmission of oocytes through air resulting in the infection of the respiratory system and transmission by insects such as flies, infected foodstuff (vegetables, fruits, and raw milk), and by personal effects of infected people can also be sources of infection. Six percent of infected people in India and 10 percent in Liberia are asymptomatic (Meisel, 1976). Oocytes of this parasite are very resistant against physical and chemical factors and can survive and remain infective for months, sometimes up to two years, in the open and under favorable conditions. Disinfectants used in labs are not able to kill cryptosporidium oocytes, and chlorine in drinking water cannot destroy them (Quilez, 2008). Depending on the species of the parasite, and the host age and immune system, clinical symptoms and signs of the disease may vary from subclinical to severe. This disease is most common in 2-15 year old children, especially in those less than two years of age. The incubation period is 3-14 days (7-10 days on average). The most obvious manifestation of cryptosporidiosis in humans and various animals is watery diarrhea that in some cases resembles cholera diar-

Table 1.

Age	Total Samples	Positive Samples	(%) +	(%) ++	(%) +++
30 Day	-	-	-	-	-
30-60 Day	60	10	5	2	3
60-120 Day	40	3	1	1	1
120-180 Day	20	1	-	1	-

rhea. In humans, signs and symptoms of the disease are mainly mild fever, muscular pain, lethargy, anorexia, nausea and vomiting (due to possible stomach infection), feeling of discomfort in the stomach, foul smelling diarrhea together with bloat and intestinal gas, dehydration, weight loss (up to 10% of body weight), and corrhiza and coughs (in respiratory infection). Diarrhea lasts from two days up to two months (8 days on average). Severe stomach pain similar to appendicitis pain accompanies infected pancreas and gallbladder (Kenneth, 1990). *Cryptosporidium* infections have been reported from most countries of the world, with reported prevalence of 0.6-20 percent in industrial countries and 4-32 percent in developing countries. The reported rates of infection with cryptosporidiosis in the age group of under five in the Philippines, the Sudan, Liberia, Canada, Bangladesh, and Australia are 8.5, 6.1, 7.9, 4.1, 6.1, and 4.1 percent, respectively (Howard, 1993). Our study showed the prevalence of cryptosporidium in calves in the Sistan region for the first time. Although the presence of cryptosporidium in stools is not always accompanied by cryptosporidiosis, yet numerous studies have shown that this parasite can worsen the course of diarrhea in calves (18). In our study, the highest infection rate (eight percent) was observed in calves with diarrhea that were less than two months old. Of course, most researchers have reported that the greatest numbers of oocytes of cryptosporidium are found in 5-25 day old calves (Brook, (2008).

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