Comparison of capture ELISA and modified Ziehl-Neelsen for detection of Cryptosporidium parvum in feces of camel (Camelus dromedarius) in Iran

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Abstract. Cryptosporidium parvum is widespread among nonhuman hosts and is the most frequently reported zoonotic species in genus Cryptosporidium. Data related to Cryptosporidium parvum infection in camels in Iran is scarce and Cryptosporidium spp., reported from most of the studies have been identified only by oocyst morphology. It is not known how many of the reported isolates are actually C. parvum. The purpose of this study was to compare the capture ELISA and modified Ziehl-Neelsen techniques regarding the detection of Cryptosporidium parvum in 85 camels fecal samples in Kerman province of Iran. Four samples (4.7%) were positive for cryptosporidiosis by two methods. Two samples (2.4%) were positive for C. parvum antigen by capture ELISA, and no oocysts were observed in these samples. Two other samples (2.4%) were positive for Cryptosporidium oocysts by modified Ziehl-Neelsen. At modified Ziehl-Neelsen method, the oocysts were ellipsoidal in shape with a mean length 7.3 x 5.3 µm with the mean shape index of 1.37. The dimensions and morphology of these oocysts were comparable with C. muris and C. andersoni. It is concluded that capture ELISA is more efficient than the modified Ziehl-Neelsen technique, especially for detecting Cryptosporidium parvum in fecal samples.

Keywords: Cryptosporidium parvum; Capture ELISA; Camels; Iran.

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Introduction

Humans are exposed to various infectious diseases. These infectious organisms can be naturally transmitted between vertebrate animals and humans by direct and indirect contacts or by vectors (Taylor et al., 2001; Shakespear, 2002). Among protozoan parasites that parasitize humans, 65% are zoonotic (Shakespear, 2002). Among them, cryptosporidiosis is caused by various species of genus Cryptosporidium. During the last century, since the parasite was discovered, 20 valid species and more than 40 genotypes have been described (Fayer, 2004; Medema et al., 2006; Šlapeta, 2006; Wang et al., 2008). Several
of these species and genotypes are considered important zoonotic parasites, especially in immunocompromised humans (Šlapeta, 2006; Medema et al., 2006; Pirestani et al., 2008; Razavi et al., 2009; Chalmers and Davies, 2010; Abdel-Wahab and Abdel-Maogood, 2011).

Among Cryptosporidium species, C. parvum is the most medically and veterinary important. Traditionally, cattle have been considered to be a primary reservoir for human Cryptosporidium infection (Morgan-Ryan et al., 2002). This protozoan parasite is one of the most important causes of death due to severe diarrhea in AIDS patients (Xiao et al., 1998; Haileyesus et al., 2005; Pirestani et al., 2008; Wang et al., 2010). Among the different genotypes, only the cattle genotype of C. parvum which was recently named C. pestis has zoonotic potential. This genotype infects a wide range of hosts including humans, cattle, camel and sheep. Infected hosts, especially newborns, shed oocysts in very high numbers.

The oocysts of C. parvum was found in variety of foodstuffs including: milk, meat products, and vegetables that contaminated with animal and human feces. In developed countries, outbreaks of cryptosporidiosis have been associated with waterborne transmission via recreational and drinking water supplies (Shakespear, 2002; Leav et al., 2003; Thompson et al., 2003; Abdel-Wahab and Abdel-Maogood, 2011). Cryptosporidiosis in livestock causes severe diarrhea with high morbidity and sometimes high mortality rates, so this infection is an important economic impact for farmers (Soltane et al., 2007; Razavi et al., 2009).

Infected hosts shed up to \(10^{5-7}\) oocysts per gram of feces at the peak of the infection. The oocysts are immediately infective and because of their tough protective wall they can survive more than 120 days in soil and for many months in moist conditions and water without losing their infectivity (Fayer, 2004; Medema et al., 2006). In humans, cryptosporidiosis can become chronic and even life-threatening, especially in undernourished infants and immunocompromised patients (Thompson et al., 2003).

It should be noted that other species and genotypes of Cryptosporidium might infect humans and pathogenicity of the parasites vary with the species of Cryptosporidium and species, age and immune status of the host (Thompson et al., 2003; Fayer, 2004; Šlapeta, 2006).

The most obvious symptom of the disease is diarrhea, characteristically voluminous and watery, which can lead to dehydration (Thompson et al., 2003; Fayer, 2004). In immunologically healthy patients, the illness lasts 2-26 days (Shakespear, 2002; Thompson et al., 2003; Fayer, 2004; Pirestani et al., 2008).

Although infected cattle are thought to be the most important animal reservoir of C. parvum, other species of animals, including reptiles, birds, and other kind of livestock such as camels have been shown to harbor the parasite.

In Iran, upon the annual report of Iranian Veterinary Organization (IVO) in 2008, the average population of camels was 153,000. Around 100 are two-humped camels (Camelus bactrianus), and the rest are one-humped dromedary camels (Camelus dromedarius). Molecular characterization of camel Cryptosporidium revealed that C. parvum, C. muris and C. andersoni can infect camels. Cryptosporidium parvum infection in young camels can lead to severe diarrhea, emaciation, dehydration and death (Wernery and Ruger Kadden, 2002; Abdel-Wahab and Abdel-Maogood, 2011).

One of useful methods in epidemiological studies is ELISA method which is a highly sensitive technique designed as a diagnostic tools for detecting and measuring antigens in the samples.

This study investigated the prevalence of cryptosporidiosis by capture ELISA and modified Ziehl-Neelsen methods in a dromedary camel population (Camelus dromedarius) from Shahrebabak region.
Materials and methods

Samples

This research was performed in Shahrebabak region, Kerman province (Southeast of Iran). The camels (Camelus dromedarius) of different sexes and ages were randomly selected from 1200 camels and visited during the summer of 2011. A total of 85 fecal samples from camels (37 females, 48 males) were collected directly, using disposable gloves. Collected fecal samples were labeled and transported in a plastic container, containing 10% formalin to laboratory in Kerman University.

Modified Ziehl-Neelsen staining

Presence of Cryptosporidium spp. oocysts in fecal samples was detected using the modified Ziehl-Neelsen staining technique as described by Shore Garcia (1999). The fecal samples were concentrated by formalin-ether sedimentation technique. Fecal smears were prepared on a microscope slide, air dried at room temperature, and fixed with 96% methanol. Fixed smears were stained with diluted carbol-fuchsin (1:10) for 3-5 minutes and washed with tap water. Smears were decolored using 3% acid alcohol (3% HCL in ethanol) for 10-15 minutes, and then counterstained with 0.5% malachite green solution for one minute. Smears were washed with tap water, air dried, and then examined under the microscope at x1000 magnification.

Cryptosporidium oocysts appear as pink to red, spherical to ovoid bodies against a green to purple background. In each positive sample the lengths and widths of at least 50 oocysts was measured with an eyepiece micrometer and the shape index (the ratio of length to width) of them calculated.

Capture ELISA

The fecal samples were tested by Capture ELISA with a commercial kit (Pourquier, France) for the detection of Cryptosporidium parvum antigens. The kit was used to test samples as instructed by the manufacturer. The wells of microtiter plates are coated with anti Cryptosporidium parvum polyclonal antibody. Fifty µl of undiluted fecal samples and then 50 µl of diluted buffer were added to each well. One hundred µl of each positive and negetive controls were added to the appropriate wells. The contents of microtiter wells was then heamogenized by using a microplate shaker, and incubated for 30 min at 25°C. All the wells were washed and 100 µl of diluted anti-Cryptosporidium antibody conjugated to HRP was added to each well. The plates were incubated for 30 min at 25°C. After incubation, the wells were washed to remove unbounded enzyme, then 100 µl of tetramethylbenzidine (TMB) substrate was added to each well to develope the reaction. The color developed by incubating the wells in a dark place at 25°C for 10 minutes. The color development was stopped by adding a 100 µl volume of stop solution to each well. The absorbance value was determined by reading the optical density at a 450 nm wavelength using a colorimeter.

Statistical analysis

Statistical analyses included chi-square test. Statistical significance was defined at P≤0.05 (two-tailed).

Results

The modified Ziehl-Neelsen and capture ELISA tests revealed a positivity of 4.7% (4/85) (table 1).

<table>
<thead>
<tr>
<th>Method used</th>
<th>Modified Ziehl-Neelsen</th>
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<tr>
<td></td>
<td>Positive</td>
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<td>ELISA</td>
<td>0</td>
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<tr>
<td>Negative</td>
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<td>Total</td>
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Two (2.4%) of the four samples were found positive for Cryptosporidium parvum with capture ELISA, while other two (2.4%) of the four samples were positive for Cryptosporidium spp. oocysts with modified Ziehl-Neelsen staining. In modified Ziehl-Neelsen the oocysts were ellipsoidal in shape and the mean dimensions of oocysts were 7.3 x 5.3 µm, with the mean shape index of 1.37. The dimensions and morphology of these oocysts were comparable with C. muris and C. andersoni as
described by Wang et al. (2008). The Capture ELISA test appears to be a significantly more sensitive technique (chi-square test, P<0.05) than the Ziehl-Neelsen staining in the diagnosis of Cryptosporidium parvum.

Discussion

Epidemiological study of zoonoses in animals and humans has attracted a lot of interests in recent years because of their importance in immunosuppressed patients, especially AIDS patients (Shakespear, 2002). Cryptosporidium parvum bovine genotype or C. pestis plays an important role in zoonotic cryptosporidiosis and causes human health disorders of varying severity in both developing and developed countries. In the developed countries, cryptosporidiosis occurs mainly in sporadic outbreaks and epidemics but in developing countries, the disease is endemic and it is one of the most common causes of persistent diarrhea among children (Leav et al., 2003; Šlapeta, 2006). This parasite is known to infect more than 150 species of animals and causes disease in laboratory personnel, veterinary students and livestock farm workers. Villous atrophy, shortening of microvilli and sloughing of enterocytes are the major pathological changes associated with the disease.

This parasite is also an opportunistic infection in AIDS patients and there is no effective chemotherapeutic substance against it (Leav et al., 2003; Thompson et al., 2003; Medema et al., 2006; Šlapeta, 2006). In animals, the infection by Cryptosporidium has been associated with economic losses. Death of livestock can be high, especially when the infection occurs as a mixed infection with enteropathogenic Escherichia coli or rotaviruses.

Our results demonstrated that capture ELISA can be used as the “golden” test and it is capable method in detecting C. parvum coproantigens with high sensitivity and specificity compared with conventional methods like modified Ziehl-Neelsen. Oocysts cannot be readily identified in microscopical examination of stained smears, even in heavily infected specimens, and are difficult to be precisely distinguished from yeast cells or fungal spores, particularly when the oocysts are present in low numbers. However, concentrating oocysts in fecal samples can increase the sensitivity of detection (Wernery and Ruger Kadden, 2002; Leav et al., 2003; Brook et al., 2008). Morphology is not adequate by itself and should not be the sole criterion for identify the Cryptosporidium species, especially C. parvum. Oocysts of many species are virtually identical in size, and similarities in oocyst structure have even caused confusion for identification of several Cryptosporidium spp. (Wernery and Ruger Kadden, 2002; Thompson et al., 2003; Šlapeta, 2006; Keshavarz et al., 2009; Abdel-Wahab and Abdel-Maogood, 2011). Infectivity, host specificity and biochemical differences can also be used as one criterion in defining Cryptosporidium spp (Xiao et al., 2004).

In modified Ziehl-Neelsen methods the two positive samples were probably Cryptosporidium muris or Cryptosporidium andersoni. Cryptosporidium muris oocysts are morphologically indistinguishable from Cryptosporidium andersoni oocysts and separated only by molecular procedures (Kváč et al., 2004; Medema et al., 2006; Šlapeta, 2006).

These species are also the major species in dairy cattle and affect the digestive glands in adult cattle and in some cases cause reduction in weight gain and milk yield. However, infected animals are usually clinically normal and do not develop diarrhea (Palmer et al., 2003; Thompson et al., 2003; Medema et al., 2006; Wang et al., 2008; Wang et al., 2010). Moreover, the species is zoonotic.

Oocysts might not be detectable in clinical samples from all cryptosporidiosis cases, and the absence of oocysts in repeated submissions of samples from symptomatic hosts does not necessarily indicate the absence of infection. In these instances, and particularly when clinical suspicion is high, oocyst negative feces samples should be subjected to antigen detection, as sufficient Cryptosporidium antigen from asexual life cycle forms should be present (Smith, 2008). This is a major advantage of coproantigens detection immunoassays.
The prevalence of cryptosporidiosis in this study (4.7%) was lower than those reported in similar studies carried out by Razavi et al. (2009) 37.9% in slaughtered camels in Isfahan province, Nazifi (2010) 16.9% in Qeshm Island, South of Iran and Sazmand et al. (2012) 20.33% in Yazd Province. Our results are nearly similar with Borji et al. (2009) 1.9% in Mashhad slaughterhouse.

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References


