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Detection of *Cryptosporidium* in Goats in Khartoum State, Sudan

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Abstract

This study was carried out to investigate the prevalence of *Cryptosporidium* infection in goats in Khartoum State – Sudan. A total of 197 goats of both sexes and different ages (> 6 to <12 months) were surveyed in Omdurman locality (No =125) and Sherg Elneel locality (No =72). Faecal samples were randomly collected and classified according to their consistency as diarrhoeic or non-diarrhoeic. All samples were examined for the presence of *Cryptosporidium* sp. oocysts using the Formol-Ether Concentration technique followed by modified Ziehl-Neelsen (MZN) staining method. Results obtained indicated that, the overall prevalence of infection was 20.8%. Infection was widely distributed among goads regardless of faeces consistency, sex, age or the Locality. It is concluded that asymptomatic adult goats are an important source of environmental contamination with *Cryptosporidium* spp. Formol-ether concentration technique followed by modified Ziehl-Neelsen (MZN) staining is useful and reliable for the diagnosis of cryptosporidiosis in faecal samples, and can be used as routine method for diagnosis and surveillances of this protozoan parasite in animal and human.

Keywords: *Cryptosporidium*, Goats, Prevalence, Formol-Ether, Modified Ziehl-Neelsen, Khartoum State – Sudan.

Introduction

Cryptosporidium is one of the most common enteropathogens causing diarrhoea in humans and domestic animals, including livestock, dogs, cats and wildlife (Fayer, 2004). *Cryptosporidium* parasite was first described in the early 20th century*; C. muris* and *C. parvum* were the first species described (Tyzzer 1907, Tyzzer 1912). Both the prevalence and the severity of infection increase in immune-compromised animals, especially in neonates of some animal species such as ruminants. However, the infection is self-limiting in healthy or immunocompetent individuals (Current *et al.*, 1983). The genus is composed of multiple genetically distinct forms, of which *C. parvum* (cattle genotype, type

2) is the most common zoonotic species (Xiao and Ryan 2004). Studies on the prevalence of *Cryptosporidium* in farm animals have revealed that ruminants are an important reservoir for this parasite, although most data are related to cattle. By contrast, the transmission of *Cryptosporidium* in domestic small ruminants has received comparatively little attention. In spite of this, the parasite is considered one of the major enteric pathogens associated with neonatal diarrhoea and mortality in lambs and goat kids (de Graaf *et al*., 1999, Olson *et al*., 2003). Likewise, little is known about the role of sheep and goats as zoonotic reservoirs for human cryptosporidiosis. Diarrhoeic lambs have been reported to excrete as many as 4.8 $\times 10^9$ oocysts per gram of faeces (Bukhari and Smith 1997), which may contribute significantly to human infections by direct contact or by contamination of water sources. These animals represent an agricultural production sector that is even more important than cattle production in many countries (Quilez *et al*., 2008).

To our knowledge, this study is the first extensive and quantitative investigation of *Cryptosporidium* infection in goats in Khartoum State, Sudan. The study aimed at providing basic information on presence of *Cryptosporidium* in goats with regards to age, sex and locality, paves the way for further screening for *Cryptosporidium* species and genotypes in goats in Sudan, and the possibility of zoonotic transmission of parasite.

Materials and Methods

Faecal sampling*:* Faecal specimens were collected during the dry season (June & July) from 197 Sudanese desert goats randomly selected from two localities, Omdurman (No =125) and East Nile (No = 72)). Both males(No = 97) and females(No = 100) of different ages (Less than 6 months, 6 - 12 months and more than 12 months) were included, faecal samples were taken directly from the rectum using sterile plastic gloves in special plastic containers, in accordance to methods described by OIE (2008). The samples were transported to the laboratory in a cool box and then stored for a maximum of 24 h before analysis. The faeces were classified according to their consistency as diarrhoeic or non-diarrhoeic (based on clinical observation).

Samples processing:

***Cryptosporidium* oocyst detection technique**

Faecal specimens were examined for presence of *Cryptosporidium* oocysts using Formol-Ether Concentration technique followed by modified Ziehl - Neelsen (MZN) staining technique (OIE 2008). Briefly: 1gram of faeces, estimated by using an applicator stick, was placed in a clean centrifuge tube containing 7mL of 10% formalin (If the stool is liquid, about 750µLwas dispensed into the centrifuge tube). The sample was thoroughly broken up and mixed with an applicator stick. The resulting suspension was filtered through a tea sieve or gauze into a beaker, and the filtrate poured back into the same tube. 3mL of diethyl ether (or ethyl acetate) was added to the formalinized solution, and the tube was closed with a rubber stopper then the solution was shaken vigorously for 30 seconds. The tube was centrifuged at 1200×g for 2 minutes. The fatty plug was loosed with a wooden stick. The plug and the fluid, above and below it were discarded by inverting the tube, leaving the last one or two drops into the tube. Whole resuspended pellet was transferred onto a microscope slide using Pasteur pipette,

allowed to air-dry then fixed with absolute methyl alcohol for 3minutes and stained with modified Ziehl-Neelsen technique. The *Cryptosporidium* oocysts were screened microscopically using the 40x and 100x objective lens. The parasite species was identified by morphologic and morphometerical criteria (OIE, 2008) using stage micrometer (in conjunction with an eye-piece micrometer, BX51Olympus microscope with DP20- Japan. Infection intensity was quantitatively evaluated; positive and negative specimens were reported. A scoring system for positive samples was used, based on the number of oocysts seen under the 100x objective lens. + = less than 5 oocysts per slide, ++ = 1 to 10 oocysts per field of view and +++ = 11 or more oocysts per field of view (OIE, 2008).

Statistical analysis

Results were analyzed with the aid of the software Microsoft Excel, SPSS version 16.0. Descriptive statistics (crosstabs) was used to determine the prevalence of *Cryptosporidium* infection in goats. Pearson's chi-squared test was used to establish the association between the prevalence of *Cryptosporidium* oocysts and age, sex, locality as well as consistency of faecal specimen. The level of significance was set at 0.05 to achieve 95% confidence. The difference was considered significant when the p value obtained was <0.05.

Results

Cryptosporidium was detected in 41 (20.8%) out of 197 faecal samples examined. The oocysts appeared as spherical pink organisms with clear halos around, measuring 3.53×3.38μm, with range of 2.99- 4.4 μm + 2.82- 4.22 μm and shape index 1.04 (1- 1.18) (Figure 1).

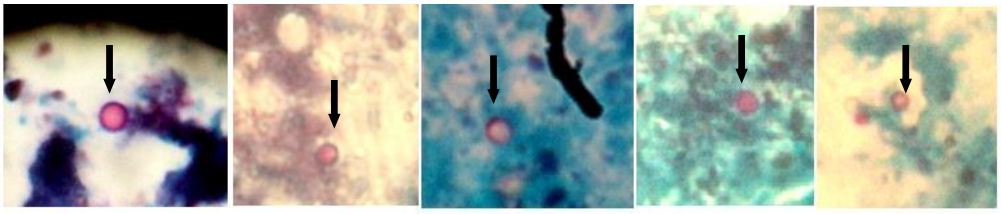

Figure (1): Spherical pink staining *Cryptosporidium* oocysts (arrows) detected in faecal samples of examined goats, MZN, 100X.

The quantitative evaluation showed that all infected animals in all age groups excreted as few as less than five oocysts per gram faeces. The highest prevalence rate (24.1%), of *Cryptosporidium* infection was in adult goats (> 12 months old) followed by kids Less than 6 months old (19.6%) while the lowest prevalence rate (17.2%) was reported in kids between 6 - 12 months old (Table 1); the difference was statistically not significant different (P value = 0.595).

Table (1): The prevalence rate of *Cryptosporidium* infection in goats according to different age groups

Age group	No. positive/No. examined	Infection rate (%)
Less than 6 months	11/56	19.6
6- 12 months	10 /58	17.2
More than 12 months	20 /83	24.1
Total	41 /197	20.8

Regarding the sex of examined goats, the prevalence rate of infection was highest in females (24.0%) compared to males (17.5%), but the difference was not statistically significant (P value = 0.263) (Figure 1).

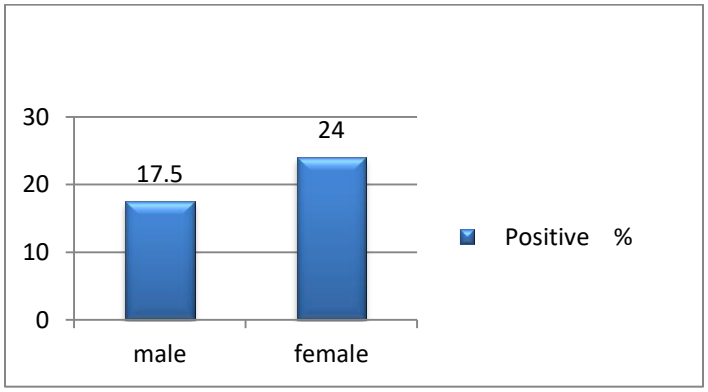

Figure (1): The prevalence rate of *Cryptosporidium* oocysts according to sex of examined goats in Khartoum State, Sudan

Figure (2) shows the prevalence of *Cryptosporidium* oocysts in goats in East Nile and Omdurman localities. Although no significant difference (P value = 0.068) was reported between two localities, the prevalence rate was higher in East Nile (27.8%) than in Omdurman (16.8%).

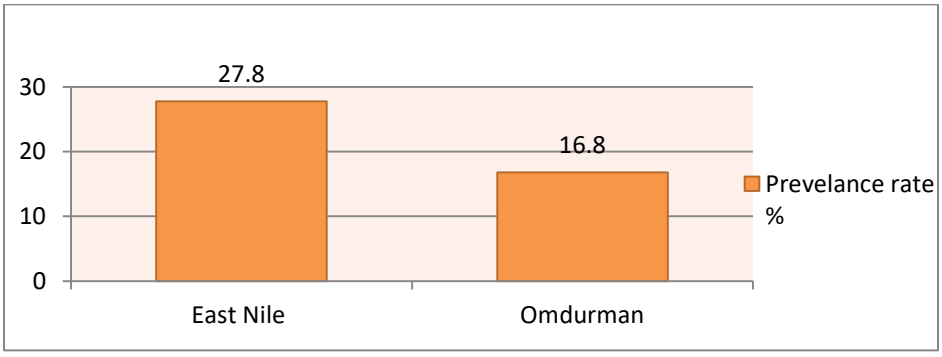

Figure (2) Distribution of *Cryptosporidium* oocysts in goats in East Nile and Omdurman localities

Concerning the consistency of faecal material, the highest prevalence (21.5%) was shown in non diarrhoaeic group, compared to that (12.5%) of the diarrhoaeic group, (Figure 3); the difference was not statistically significant (P value = 0.393).

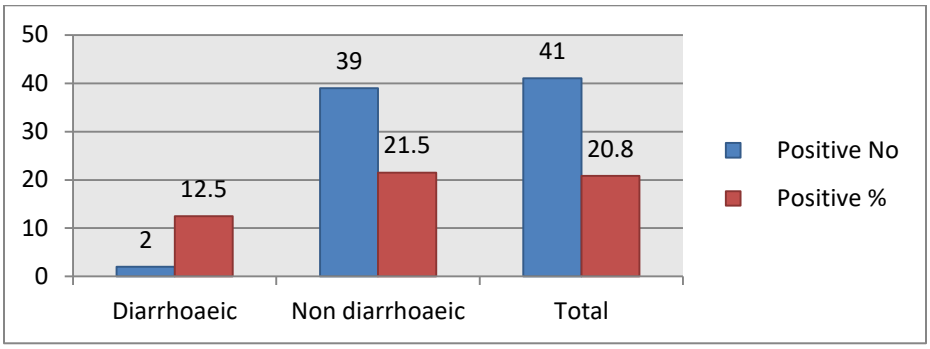

Figure (3): The prevalence rate of *Cryptosporidium* infection in goats according to consistency of faecal material of examined goats

Discussion:

Cryptosporidiosis is a common disease among neonatal ruminants and is most commonly observed in calves. The disease has not been reported as recurrently in small ruminants (Ulutaş and Voyvoda, 2004). *C. parvum* has been known as a cause of outbreaks of diarrhoea in goat kids in several countries (Tzipori *et al*., 1982; Thamsborg *et al*., 1990; Vieira *et a*l., 1997; Johnson *et al*., 1999 and Ulutaş and Voyvoda, 2004).

In the localities in question, this is the initial study on the prevalence of cryptosporidiosis in goats. The study revealed 20.8% (41/197) infection rate in examined animals. Moreover, the measurement of oocysts was within the range reported by Suliman *et al.* (2008) for *Cryptosporidium* in Sudan. The parasite was detected in all ages included in the study. In an international context, our finding is in line with those reported by other authors in goats: 17.7 % prevalence rate in Iraq (Mahdi and Ali, 2002) and 19.1% in Spain (Sanz Ceballos *et al*., 2009). In contrast, the prevalence in Egypt was found to be lower than reported here: 2.12% (El-Manyawe, 1999); 13.34 % (El- Sayed Nasr 2008) and 12.5% (El Manyawe *et al*. 2010). Lower 4.4 % prevalence rate was also reported in Papua Guinea (Koinari *et al*., 2014). Results obtained in the cuurent study showed that the infection intensity was not significantly different ($P > 0.05$) between the three age groups of goats with relatively higher values in adults. This seems to agree with the finding of Alonso-Fres´an *et al*. (2005) in sheep and Romero-Salas *et al*. (2016) in goats and sheep who observed no association between prevalence of infection and age. Similar observation has been reported in cryptosporidiosis in Nigerian cattle (Ayinmode and Fagbemi (2010); Adamu *et al*., 2015). At the same time the present findings disagree with Noordeen *et al.* (2000, 2001); Castro-Hermida *et al*. (2007) and Sanz Ceballos *et al*. (2009) who found strong association between oocyst counts and age; higher *Cryptosporidium* prevalence was seen in goats less than 6months old. The present

study revealed no significant difference in prevalence rate of infection between the male and female goats (P>0.05), though the infection rate was higher among females. This finding is in agreement with some reports on *Cryptosporidium* infection in different animal species (Chalmers *et al*., 1997; Bull *et al*., 1998; Razavi *et al*., 2009; Adamu *et al*., 2015 and Romero-Salas *et al*., 2016). In contrast Ayinmode and Fagbemi (2010) reported a significantly higher infection rate with *Cryptosporidium* spp. in females cattle compared to males. *Cryptosporidium* spp. has been highly correlated with diarrhoea in animals and characterized as the most common contributing agent of neonatal diarrhea syndrome, practically in cattle (Quilez *et al*., 1996; de Graaf *et al*., 1999; O'Handley *et al*., 1999; Bjorkman *et al.*, 2003; Santin *et al*., 2004; Xiao, 2010). Nevertheless, some studies suggested that diarrhoea in *Cryptosporidium* infection may be the result of other enteropathogens that are usually linked with the infection (Anderson, 1998; de Graaf *et al*., 1999). Our results agree with other reports that asymptomatic goats (without diarrhoea) can act as carriers of *Cryptosporidium* and a sources of infection for both human and other animals (Snodgrass *et al*., 1986; Ayinmode and Fagbemi, 2010 and Romero-Salas *et al*., 2016). Additionally, in support of our findings in another study, using the same faecal samples we evaluated modified Ziehl Neelsen and antigen detection via ELISA in diagnosis of *Cryptosporidium spp*. in goats; we found that all positive samples by microscopic MZN were also positive by ELISA and the infection was widely distributed among goads regardless of their faeces consistency, sex, age or the locality (Adam *et al*., 2017, unpublished data).

Conclusion:

The prevalence of *Cryptosporidium* spp infection among goats in East Nile and Omdurman two localities, Khartoum state, Sudan is widely distributed irrespective of sex, age, Locality or faeces consistency. Asymptomatic adult goats contribute to environmental contamination with *Cryptosporidium* spp and constitute a real source of infection to humans and animals. The relatively high prevalence of *Cryptosporidium* oocysts in goats without diarrhoea might be related to factors like herd immunity and absence of concurrent infection. Control and prevention programme of *Cryptosporidium* infection should be considered in both symptomatic and asymptomatic goats. Further studies, involving molecular epidemiology are required to fully evaluate the impact of infection on goat's health and productivity and the zoonotic potential of the parasite. Formol-ether concentration technique followed by modified Ziehl-Neelsen staining is susceptible and reliable for diagnosis of cryptosporidiosis in faeces and can be used as routine method for diagnosis and for surveillances of this protozoan parasite in animals.

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لكشف عن *Cryptosporidium* في الماعز بولاية الخرطوم ، السودان

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1. قسم الباثولوجيا والطفيليات ، كلية الطب البيطري ، جامعة السودان للعلوم والتكنولوجيا (CVM-SUST)
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2. قسم الباثولوجيا ، كلية الطب البيطري ، جامعة الخرطوم

المستخلص:

أجريت هذه الدراسة للتحقق في انتشار الاصابة بطفيل الكربتوسبوريديم في الماعزفي في ولاية الخرطوم- السودان. فحصت جملة 197 رأس من الماعز من كلا الجنسين ومختلف الاعمار(>6 ـ <12شهر) بمحليتي امدرمان(125 رأس) وشرق النيل (72 رأس). جمعت عينات براز بصورة عشوائية وصنفت بناءاً على قوامها الى براز اسهالي وغير اسهالي. فحصت جميع العينات من أجل وجود أكياس بيض الكربتوسبوريديم مستخدماً تقنية التركيز بفورمل ايثر متبع بالصبغ بطريقة زيل نلسين المعدلة.كان معدل انتشار الاصابة الكلية 20.8%. الاصابة منتشرة بصورة واسعة بين الماعز بصرف النظرعن قوام البراز، جنس،عمر او المنطقة. استنتجت الدراسة أن الماعز ذو الاعمار الكبيرة التي لاتظهر أعراضاً مرضية مصدر هام للتلوث البيئ بابواغ الكربتوسبوريديم في المناطق التي تم فيها المسح . تقنية التركيز بفورمل ايثر متبع بالصبغ بصبغة زيل نلسين المعدلة مفيدة وحساسة لتشخيص طفيل الكربتوسبوريديوم في البراز، و يمكن استخدامها كطريقة معتادة لتشخيص ومراقبة هذا الطفيل الأولي في الانسان والحيوان.

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