Prevalence Of Cryptosporidium Oocysts From Calves In Kurdistan Province, Of Iran

F Esmail, H Ataallah

Citation

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Abstract

Prevalence of Cryptosporidium spp. oocysts in calves (n=412) in five selected farms in Saghez, Iran, was determined by using the modified Ziehl Nielsen staining technique. Of 412 bovine faecal samples, 8.5% were positive for Cryptosporidium spp. The prevalence of Cryptosporidium in less than 3-month age's calves was higher than weaned calves and adults. Cryptosporidium spp. oocysts were detected in both diarrheic and non-diarrheic animals, but there was a significantly higher prevalence ($p<.05$) of oocyst shedding in diarrheic than in non – diarrheic animals. Confirmation of the diagnosis was performed using anti-Cryptosporidium monoclonal antibody specific for Cryptosporidium muris, Cryptosporidium parvum and Cryptosporidium baileyi.

INTRODUCTION

Cryptosporidium has emerged as one of the most important zoonotic coccidian parasites affecting a wide range of domestic animals and man. Although the parasite was first reported in 1907 (1), its importance was only appreciated in the early 1970s when infections were associated with diarrhea in calves (2). Cryptosporidium has been reported to be second only to rotavirus as a cause of diarrhea in newborn calves in Britain (3, 4) with prevalence of 80% and 62.4% in calves and adult beef cattle, respectively (5). Profuse and severe life-threatening diarrhea occurs in neonates and immunocompromised individuals, especially those with acquired immunodeficiency syndrome. However, self-limiting or sub clinical disease occurs in adults and immuncompetent animals and humans (6, 7). Cattle are a major source of C.parvum, which is responsible for neonatal diarrhea in calves and for zoonotic contamination (8). Bovine cryptosporidiosis is widespread and prevalence studies show a wide range of oocyst shedding from 14 to over 80% depending on the age, clinical situation and breeding regime of the animals. Overall, prevalence studies in cattle ranging from 3 days old to adult usually give results of appreciatively 20% of animals shedding oocysts, while prevalence in neonates is higher, especially when diarrheic calves are concerned (9). Although several studies on a prevalence of Cryptosporidium spp. have been conducted in developed and developing countries (7,10, 11,12,13,14), little is known on the occurrence of the parasite in Iran. Little information has been published about bovine cryptosporidiosis in Iran and epidemiological studies have not yet been conducted on a wide scale and in large number of animals. The aim of this study was, therefore, to determine the prevalence of Cryptosporidium spp. oocysts in cattle on selected farms within Saghez county of Iran.

MATERIAL AND METHODS

SOURCE OF ANIMALS

In the present study cattle from five medium-scale dairy farms (range 50-150 animals per farm) and 20 small-scale dairy cattle units (each with an average of ten animals) within Saghez County, NW of Iran were used. Saqqez or Saghez is a city in Kurdistan Province of Iran. It had a population of around 132,100 in 2010. Kurds have lived in Saqqez and the surrounding region since approximately 1,000 B.C., and many believe that the city's first known inhabitants, the Medes, are direct ancestors of the present-day people of western Iran. At an altitude of 1,493 meters (4,898 feet), Saqqez has variable climate, with cold, snowy winters and hot, dry summers, during which there is a large daily temperature range due to the combination of thin, dry air produced by the high elevation and low summertime humidity. Saqqez is situated at 36°15 N 46°16 E (Map1). The selection of herds was based on the willingness of the farmers. Most of the animals used in our study were crosses of exotic and indigenous breeds but some were pure exotic breeds. Three farms belonged to Agriculture University of Kurdistan. These animals were kept in houses with concrete floors that were cleaned regularly. Animals in two privately

owned medium-scale farms, farm “C” animals kept in non-concrete floor houses that were rarely cleaned, and farm “M” animals kept in clean, concrete-floored houses, were also sampled. Animals in the medium-scale farms grazed in fenced paddocks, whereas 15 out of 20 small-scale dairy units practiced zero grazing, and the remaining five farmers tethered their animals near homesteads. In most herds, calves were left to suckle for the first week of birth and thereafter were bucket-fed.

Cattle were categorized as calves (<3months old), weaners (3-8 months old) and adult (>8 months old). The presence or absence of diarrhea in the animals at time of sampling was recorded; animals passing soft and fluidly faces were considered as diarrheic.

FAECAL SAMPLING

Faecal specimens (412) were collected per rectum from a randomly selected 50% of the cattle on in medium-scale farms, whereas all animals in the small-scale dairy units were screened. The randomization was achieved by assigning real numbers to all animals in the medium-scale farms visited and selecting animals with numbers drawn from a Casio FX 3400 calculator until 50% of the animals in the farm had been selected. Faecal specimens were preserved in 2.5% (W/V) potassium dichromate solution at a refrigeration temperature until examined.

CRYPTOSPORIDIUM OOCYST DETECTION TECHNIQUE

The modified Zieh-Neelsen (MZN) staining technique (15) was used for detecting of Cryptosporidium spp. oocysts in faecal specimens from cattle. An oocyst concentration technique was performed for all faecal specimens from cattle.

A modified formol – ether sedimentation (16) was employed for concentrating Cryptosporidium spp. oocysts. A specific anti-Cryptosporidium monoclonal antibody technique (Immunological Direct. Com- Cryptosporidium Cel Reagent FITC Conjugated test kit; Oxford Biotechnology) was used for confirmation of all the positive faecal specimens.

STATISTICAL ANALYSIS

Data was computed using Epi Info Version 6 statistics system (17). A Two – tailed chi – square test was used to compare the differences on the prevalence of Cryptosporidium oocysts between farms, age – groups of cattle at 5% level of significance. The 95% confidence intervals on overall prevalence were also computed.

RESULTS

The overall prevalence of cryptosporidial infection in cattle was 8.5%. Medium-scale unites had 11.8% of 212 animals, positive for Cryptosporidium spp.oocysts. Cryptosporidium oocysts were detected in 5% of the animals in the small-scale dairy units (Table 1). Oocyst shedding was recorded in bovines ranging from 3 days old to adults, although infection rates were significantly higher than calves (41%) in the remaining age groups ($p<0.0001$), (Table 2).

The fecal consistency of Cryptosporidium spp-positive and-negative samples was described in Table 3.

While 60% of the oocyst-positive samples were diarrheic (watery and loose or both), only 9% of the oocyst-negative samples were this form ($p<0.05$). Thus, it was clarified that Cryptosporidium infection is associated with the occurrence of diarrhea in calves. Between 21 diarrheic animals, 13 animals were less than 3-month ages calves and nine (75%) of these were positive for Cryptosporidium spp. oocysts.

Cryptosporidium spp. oocysts from cattle appeared as pink to red spherical to ovoid bodies against a green or purple background under MZN staining and all the positive specimens showed positive reaction with specific anti – Cryptosporidium monoclonal antibody. Because of the limited amount of Cryptosporidium monoclonal antibody we had, none of the negative samples were double-checked for the presence of the oocysts.

Figure 1

Fig1:Map of Kurdistan Province of Iran and green colored is area of interest

Figure 2

Table 1 Prevalence of cryptosporidia infection by dairy calves' distribution centers survey in Saghez County of Iran (2009-2010).

Distribution Centers Of Saghez County	Number of Positive Calves	Total Calves Tested	Prevalence (%)
Medium scale dairy (No.=5)	25	212	11.8
Small scale dairy (No.=20)	10	200	5
Total	35	412	8.5

Figure 3

Table 2. Prevalence of Cryptosporidium infection according to the age range in Saghez County of Iran (2009-2010).

Age range	No. Studied	Cryptosporidium	
		No. Infected	%
< 1.5 months	44	18	41
1.5 –3 months	87	7	8
3 – 8 months	96	5	5
Adults	185	5	3
Total	412	35	8.5

Figure 4

Table 3. Relationship between Cryptosporidium spp. infection and diarrhea in calves of Saghez County, NW Iran (2009-2010)

Cryptosporidium spp.	No. Of samples	Diarrheic	Formed
		Watery and Loose	
Positive	35	21(60)	14(40)
Negative	377	9(2.4)	368(97.6)

DISCUSSION

The prevalence of Cryptosporidium oocysts in cattle in this study were relatively lower than that reported in other countries. For instance, in Germany, the examination of field samples from different parts of Germany showed that the prevalence of cryptosporidia in calves (up to the age of 6 months) ranges mostly from 20 to 30%, with 35–36% monoinfections. Considering the fact that C. parvum is most prevalent in calves up to 4 weeks age. This age group probably has considerably higher prevalence's (12), whereas, in Britain, up to 80% of calves and 62.4% of adult beef cattle have been reported as shedding Cryptosporidium spp. oocysts (5). Little in known on the prevalence of bovine cryptosporidiosis in Middle East (Asia) and Africa countries for comparison purposes. However, studies on human cryptosporidiosis have shown prevalence of 2-6% in Iran (17), 3.1 – 17.6% in Guinea Bissau (18), 0.4-8.2% in Korea (10) and 32% in Zambia (19). Chai et al. (20) reported 7.9% positive rates of cryptosporidiosis among the 3,146 people examined. The study showed a remarkable difference in positive rates between rural area and urban area, from 10.6% to 0.5%.

The high prevalence of Cryptosporidium spp. in young calves was similar to previous reports that Cryptosporidium infection was common in newborn calves. (3, 4).

The occurrence of high infection rates in this age category was attributed to the poor immunity in the newborn calves and the easiness of oocysts contamination through bucket – feeding. Of the screened farms, farm 'C' had the highest prevalence of Cryptosporidium spp. with up to 11.8% of animals shedding oocysts. The high prevalence rate may be attributed to the poor management or hygiene on the farm. The presence of a dirty and muddy environment could lead to the persistence of Cryptosporidium spp.oocysts on the farm, leading to increased risk of animals being exposed to the parasite. This low prevalence of infection in farms owned by Kurdistan University could be attributed to the good level of hygiene. Similarly, only ten (5%) out of 200 animals screened on 20 small – scale dairy units were positive for Cryptosporidium spp. oocysts. The low prevalence of Cryptosporidium spp. on the small – scale dairy units could be due to relatively good management and hygiene facilitated by small numbers of animals kept per household.

The prevalence of Cryptosporidium spp. oocysts in the cattle may result in these animals playing an important role as a source of Cryptosporidium spp. infection for livestock and man. However, further studies are required to ascertain this likelihood of this mode of transmission of the disease and to determine the pathogenicity of Cryptosporidium spp. isolates from cattle and other animals for man.

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Author Information

Fallah Esmail
Department of Parasitology, Faculty of Medicine, Tabriz University of Medical Sciences

Hajihasani Ataallah
Department of Parasitology, Faculty of Medicine, Tabriz University of Medical Sciences