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Cryptosporidiosis: A Study of Zoonotic Enteric Parasite in Small Ruminants in Sulaymaniyah Province/Iraq

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Abstract

Cryptosporidium species are widely distributed food- and water borne intestinal protozoa that infect a wide range of hosts with zoonotic implications. The lack of data regarding the occurrence of cryptosporidiosis necessitates the current study to find out the prevalence of *Cryptosporidium* among small ruminants in Sulaymaniyah province. Sampling was performed from July to December 2023 involving 215 sheep and 150 goats belonging to a mixed small ruminant herd. A parasitological survey was achieved for protozoan detection from all fecal samples, stained by the Ziehl-Neelsen staining procedure. The observed result confirms the existence of *Cryptosporidium* among a small ruminant population, with a prevalence rate of 22.74% (n=365). A higher prevalence of 24.19% was found in sheep compared to goats at 20.67% (OR =1.2, 95% CI:0.7-1.7). At the flock's level, *Cryptosporidium* oocysts have been detected in all selected sampling herds, with a significantly higher prevalence rate of 29.56% in large herds with more than 200 animals (OR =2.5,95% CI:1.1-2.8). Although no significancy was observed regarding the animals age, a higher prevalence 23.40% was observed in older ones ≥18 months (OR =0.8, 95% CI:0.6-1.4). Season is one of the epidemiological factors, in the current study, a higher infection rate 23.61% was found among animals during wet seasons (OR =0.9, 95% CI:0.6-1.4). The frequency of infection 25.97% was higher among animals that were cohabitated with cattle (OR =0.7,95% CI:0.5-1.2). The study confirms the occurrence of *Cryptosporidium* infection among sheep and goats. The possibility of livestock contributing to human cryptosporidiosis should be established.

Keywords: *Cryptosporidium*, zoonotic, sheep, goat, Sulaymaniyah.

Introduction

Cryptosporidiosis is an anthropozoonosis caused by protozoan parasites belonging to the family Cryptoporididae, which may cause a serious health problems in small aged animal during first weeks of life [1]. *Cryptosporidium* species cause infections in a variety of animals, such as fish, amphibians, birds, and reptiles, in addition to mammals [2]. Based on Molecular data approximately 40 species and over 50 genotypes have been identified [3].

There are currently over ten species of *Cryptosporidium* known to be capable of infecting sheep: *C. xiaoi, C. ubiquitum, C. parvum, C. andersoni, C. fayeri, C. ryanae, C. scrofarum, C. hominis, C. suis,* and *C. bovis* [4]. *Cryptosporidium* species such as *C. andersoni, C. xiaoi, C. parvum* and *C. hominis* had also been isolated from goats [5, 6]. Several species of *Cryptosporidium* have been demonstrated to be host-indigenous, meaning they can infect a broad variety of hosts [7]. Clinical cryptosporidiosis in lambs and goat kids mostly associated with *C. parvum* and sporadically with *C. xiaoi* and *C. ubiquitum* [8, 9].

The parasite is highly infectious enteric pathogen transmission between hosts occur through faecal-oral route by consumption of oocysts released in the faces of hosts that are infected [10] either directly through coming into contact with an infected host's excrement, or indirectly through consuming contaminated food or water or environmental contamination [11].

Due to their high oocyst shedding rate, which results in environmental contamination, ruminants are regarded as significant reservoirs of both host-specific and zoonotic *Cryptosporidium* species among farm animals [9]. Sporozoites are released by

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the oocysts after eating sporulated thick-walled oocysts, each one releases four infectious sporozoites, which adhere to the intestinal epithelial cells' apical surface before actively invading the host cell membrane to create an intracellular but extracytoplasmic parasitophorous vacuole [12].

They habitat primarily in the brush border of the ileum and especially in the dome epithelium covering the Payer's patches, and permanently on the villous epithelium in the jejunum [13]. Through two cycles of merogony and then gamogony, the parasite reproduces. While thick-walled oocysts are expelled to the environment and can cause infection upon oral consumption, thin-walled oocysts excyst within the host's intestine and cause auto-infection [14].

During the four-day prepatent phase, younglings exhibit more pronounced clinical signs [15]. Acute cryptosporidiosis primarily manifests as mild-to-moderate or severe diarrhoea; however, there are additional clinical symptoms that can include abdominal pain, depression, anorexia, dehydration, listlessness, and thriftiness. Although the disease progresses slowly in adulthood and is marked by a steady decrease of body weight, the majority of affected animals show no symptoms [1].

The degree of infection and the animal's age both affect how quickly oocysts are excreted [16]. The majority of environmental pollution comes from young animals. After reaching immunological maturity, the illness goes away; the infection goes away; nevertheless, the recovered animal now becomes a carrier and could infect a vulnerable population [17].

Sheep can raise the danger of *Cryptosporidium* infections for the general public by contaminating water supplies [6]. Drinking water contamination can lead to zoonotic transmission through faecal-oral routes or direct contact [18]. The substantial morbidity and even high mortality rates among animals caused by cryptosporidiosis can also result in large economic losses [19].

The accurate method for detection of *Cryptosporidium* infection is by stool analysis. There are various faecal examination techniques for cryptosporidiosis investigation. All of these techniques have good sensitivity and/or specificity for detecting either oocysts, antigens, or DNA unique to *Cryptosporidium* spp. [10]. Acid-fast and hot-safranin staining techniques are applied procedure for detection of parasitic protozoa belong to the genus *Cryptosporidium* [20].

Raising small ruminants is one of the primary ways that meat is produced. Sheep and goats are an important commodity for smallholder farmers across the study area, additionally, increasing demand on using small ruminant meat necessitate to find out various routes for increasing the small ruminant's productivity. Finding the aetiology of poor health condition in asymptomatic animals is an important step, in which intestinal protozoal infection might be associated. The existence of limited data regarding the occurrence of intestinal protozoa in small ruminants, especially those with potential zoonotic impacts necessitate providing an insight into preliminary data regarding the prevalence of *Cryptosporidium* infection in sheep and goats from Sulaymaniyah province is intended by the current study design.

Material and Methods

Study design

A cross-sectional descriptive study was achieved for detection of *Cryptosporidium* species. Biological samples were collected from indigenous animals including 215 sheep and 150 goats during June to December 2023. Animals were selected randomly from 20 traditional small ruminants' farms in Sulaymaniyah province, animals were kept under traditional rearing condition and in free range environment. The enrolled farms for sample collection were belong to different districts, both male and female animals over six months of age were selected for sampling.

Sample collection and processing

Fresh faecal samples were collected rectally from selected animals using disposable gloves, stored in a container with cup, labelled and transport to the laboratory at Veterinary Medicine College of Sulaimani University. Faecal samples were preserved in 10% formalin till performing the diagnostic test. A questionnaire was completed for each animal, including: age, herd size and cohabitation with bovine host.

Laboratory Identification

All samples were processed for observing *Cryptosporidium* oocysts. Faecal floatation procedure was applied using concentrated NaCl (45%w/v). The mixture was spun for five minutes at 1200 rpm, and the test tube stands held the centrifuge tubes containing the mixtures. The top of the tube was filled to the brim with the concentrated NaCl solution, creating a convex surface. The coverslip was placed over the tube such that the solution came into contact with it. Coverslips were taken off and placed on glass slides after 15 to 20 minutes [21], the slide containing the parasitic stages were examined microscopically.

For additional laboratory investigation employing an a modified acid-fast (Ziehl- Neelsen) staining technique [22]. Faecal smear was prepared from a portion of sediment, let to air dry at ambient temperature. After two minutes of absolute methanol fixation, it was stained for ten minutes with Carbol Fuchsin, cleaned with distilled water, and then left to be stained for two minutes with acid alcohol.

Malachite Green was used again for three minutes, followed by a distilled water washing and drying time [21]. For accurate extent of the parasitic agent slides were observed at X400, oocyst identification was based on the shape, size and acid-fast staining at X1000 magnification using immersion oil objective [23]. Samples were considered positive when the parasitic agent observed by one of the diagnostic procedures.

Data analysis

The obtained data were entered in MS Excel version 2019, and analysed using SPSS version 24. The Chi-square test was used to assess the significance of study variables. The p value < 0.05 was considered statistically significant.

Results

Result of current study revealed that out of the total examined animals 24.74% (n= 83) were harbour intestinal zoonotic protozoa belonging to the genus *Cryptosporidium*, their oocytes was viewed in Fig. 1.

In total, *Cryptosporidium* oocysts was observed in 24.19% of sampled sheep, however 20.67% of examined samples from goats represented oocyst in their faeces (OR =1.2,95% CI:0.7-1.7). At the level of flocks, the oocyst of *Cryptosporidium* has been detected in all selected sampling herds with significant higher prevalence rate of 29.56% from large sized herd with more than 200 animals (OR =2.5,95% CI:1.1-2.8). The impact of various epidemiological risk factors on prevalence rate of *Cryptosporidium* spp. was illustrated in Table 1.

According to the study data, no significant difference was observed regarding the age, season and cohabitation with cattle while higher prevalence was observed in older animals ≥18 months 23.40% (OR =0.8,95% CI:0.6-1.4), during wet months 23.61% (OR =0.9,95% CI: 0.6-1.4), and among animals that co habituated with cattle 25.97 % (OR =0.7,95% CI: 0.5-1.2), an odds ratio (OR) less than 1 represented that defined factor had no effects on cryptosporidiosis prevalence rate.

Discussion

Enteric parasites considered as an associated factor for poor productivity in livestock. In current study the overall prevalence of *Cryptosporidium* infection in small ruminant was 22.74%. Baroudi et al. [24] was reported the overall infection rate of 11.0% in sheep and goats. Prevalence rate of 10.1% was found by Dessì et al. [9] from sheep in Italy via molecular test. In an agreement to current finding via Ziehl-Neelsen staining procedure higher prevalence of 72.5% was reported by Romero-Salas et al. [18], also 25% from Brazil was reported by Paz e Silva et al. [25] using molecular assay respectively. Lower incidence rate of *Cryptosporidium* spp. 2.4% and 3.6% from sheep by microscopy and Nested PCR was reported by Çelik, et al. [4]. In sheep [26] reported 11%, and in goats [21] reported 4% from Iran and Nepal respectively by microscopy. The reasons behind variations in infection rates between studies may include variations in animal age, diagnostic techniques, sample sizes, animal care, and climates [24].

Higher prevalence of 24.19% was found among sheep in compare to goat 20.67% (OR =1.2,95% CI:0.7- 1.7) in an agreement to current study [24] was found 14.5% in lambs and 8.7% in goat kids, similarly [27] was identified Cryptosporidiosis positivity at 19.4% and 13.4% in lambs and goat kids respectively. While in contrast to the current findings [8] was reported higher prevalence rate of.1% in samples taken from goats than in sheep 19.2%. likewise, lower infection rate in lamb 74.4% in compare to higher infection rate 93.8% in goat kids was found in Spain [28] which is dis agree with current findings.

As with sheep, goats eat a variety of vegetation; however, their grazing habits differ from that of sheep, preferring to browse on woody shrubs and weeds rather than on grasses, which means they are less likely to ingest parasites that have been excreted in faces than sheep, in addition whereas sheep tend to stay within the confines area decided by the sheep farmer, goats easily escape from fenced areas and are often good climbers which decrease the chance of infection [29].

This study endeavoured to find out prevalence of *Cryptosporidium* in small ruminants rearing under free range management system, the result data represented higher frequency of infection in small ruminants sharing pasture and cohabitated with cattle 25.97% (OR =0.7,95% CI: 0.5-1.2).

Due to the non-host-specific nature of *C. parvum*, a habitat contaminated with oocysts during an outbreak in calves can cause infection in sheep and goat who subsequently graze in the same area. And it was elucidated that *C. parvum* was the dominant *Cryptosporidium* species among the calves, lambs and goat kids [27]. Previous studies analysing the species and genotypes of *Cryptosporidium* found in livestock and wildlife grazing on a water catchment area with a history of cryptosporidiosis found that farm animals (sheep and cattle) and wildlife (red and roe deer) all shed the same species of *Cryptosporidium* (*C. parvum*), which was found in the local water supply [30].

The impact of cryptosporidiosis on human health have been addressed in various studies from different areas of Iraq [31- 35]. The data represented occurrence of *Cryptosporidium* infection among several population and different age groups at various level, although no information have been documented regarding the source of infection, animals including ruminants may acts as reservoir for

Cryptosporidium species and associated with initiation of human infection.

Animal housing conditions have an impact on cryptosporidiosis prevalence rate in various hosts [36, 37]. In current study significant higher prevalence rate of 29.56% (OR =2.5,95% CI:1.1-2.8) was observed in large sized herds more than 200 animals. Overcrowding might increase the chance of infection as the parasitic oocysts are infectious when they pass in faces of infected hosts.

Season is one of the defined epidemiological risk factors in current study, the hinger infection rate of 23.61% was found among animals during wet season (OR = 0.9, 95% CI: 0.6-1.4). According to Majewska et al. [38] season of the year with environmental conditions such as temperature, rainfall and humidity and sanitary condition were among risk factor that influence the prevalence rate of cryptosporidiosis. The possibility of *Cryptosporidium* oocysts surviving in the environment and contaminating food and water in mud pens will increase since cleanliness and disinfection procedures were less effective [26].

Age of infected host is a relevant factor increasing the chance of infection, animals aged more than 18 months represented 23.40% (OR =0.8,95% CI:0.6-1.4) with no significant difference. Similarly [39] reported higher prevalence of 26.1% in adult sheep. Although some studies designated a decline in the overall prevalence of *Cryptosporidium* with increasing age of the hosts [40].

The association between *Cryptosporidium* prevalence and faecal consistency have been defined in previous studies. The collected faecal samples for current study were with normal consistency, which is harmony with the findings of [41, 42]. Although the faecal consistency of adult ruminants infected with *Cryptosporidium* was normal, there is evidence linking specific *Cryptosporidium* species to clinical illness in livestock. Previously reported data elucidated that, although *C. xiaoi* and *C. ubiquitum* are more frequently identified in healthy lambs or in cases with mild to severe diarrheal cases, *C. parvum* was recorded from clinically diseased lambs [43, 44], moreover [9] reported higher infection rate from diarrheic sheep, Fayer & Xiao [45] demonstrated that cryptosporidiosis prevalence was a strongly associated with diarrhoea.

Based on reported data *Cryptosporidium* species with zoonotic impacts were detected from small ruminants, both *C. parvum* and *C. ubiquitum* have been reported by Sahraouia et al. [46] in Algerian, also Kaupke et al. [8] was reported C. hominis, *C. parvum* and *C. ubiquitum* from small ruminants in Poland. *Cryptosporidium parvum* was also identified in ruminant hosts from Jorden and Turkey by Hijjawi et al. [47] and Taylan-Ozkan et al. [48] respectively. Since all C*ryptosporidium* species are morphologically very similar and have low host specificity [49]. According to Guo et al. [50], small ruminants can transmit the main zoonotic species of *Cryptosporidium* to humans.

Although not all infected livestock exhibit clinical symptoms like diarrhoea, livestock can be a significant source of human cryptosporidiosis [51]. In sheep and goats, cryptosporidiosis does not pose a serious health risk. Nonetheless, diseased animals may serve as human zoonotic *Cryptosporidium* species reservoirs [52]. Because *C. parvum, C. hominis, C. ubiquitum*, and *C. andersoni* are zoonotic species, infections in ruminants are particularly significant [8]. Given that an adult sheep or goat excretes 1-3 kg of excrement per day, there is obviously a significant risk of faecal parasite contamination of the environment, especially water [29].

The study had addressed cryptosporidiosis from randomly collected sheep and goats' faecal samples that confirmed *Cryptosporidium* infection based on flotation and acid-fast staining procedures. Nevertheless, the limitations involve sample size and the applied procedures for parasitic detection, the methods are not as molecular as procedures for detection the involved parasitic species nor genotypes.

Conclusion

The study conducts the existence of zoonotic protozoan parasites habituating the intestinal tracts of sheep and goats in the study areas. Although moderate prevalence rate was documented, their occurrence could be a source for environmental contamination especially water sources, as well as meat contamination during slaughtering is of greater concern. Further investigations for understanding the impact of *Cryptosporidium* on livestock production, and the possible public health implications of cryptosporidiosis are essential by performing large scale studies with advance detection techniques. Livestock may be a factor aggravate human cryptosporidiosis.

Authorship:

SH Abdullah designed the study, literature collection, contributed to sample collection, conducting laboratory works, and writing. AA Mohammed contributed to sample collection, conducting laboratory works, data analysis, and reviewing.

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Ethical of approval

This study follows the ethics guidelines of the Veterinary Medicine College, University of Sulaimani (2024).

TABLE 1. Frequency of cryptosporidiosis in small ruminants from Sulaymaniyah province in association with epidemiological risk factors.

Determinants	Variables	Total examined	No. of positive	% of infection	X^2 value [p value]	Odds ratio [95% CI]	Relative risk [95% CI]
Animal	Sheep	215	52	24.19	[0.622]	1.2	1.1
	Goat	150	31	20.67	[0.4]	[0.7- 2.0]	[0.7- 1.7]
Season	Dry	221	49	22.17	[0.102]	0.9	0.9
	Wet	144	34	23.61	[0.7]	[0.5-1.6]	[0.6-1.4]
Herd size	Large > 200	203	60	29.56	[12.097]	2.5	1.8
	Small< 200	162	23	14.20	[0.0005]	[1.4-4.3]	[1.1-2.8]
Cohabitation with cattle	No	211	43	20.38	[1.586]	0.7	0.8
	Yes	154	40	25.97	[0.2]	[0.4-1.1]	[0.5-1.2]
Age	≥ 6 months	130	28	21.54	[0.165]	0.8	0.9
	>18 months	235	55	23.40	[0.6]	[0.5-1.5]	[0.6-1.4]
Total		**365**	**83**	**22.74**			

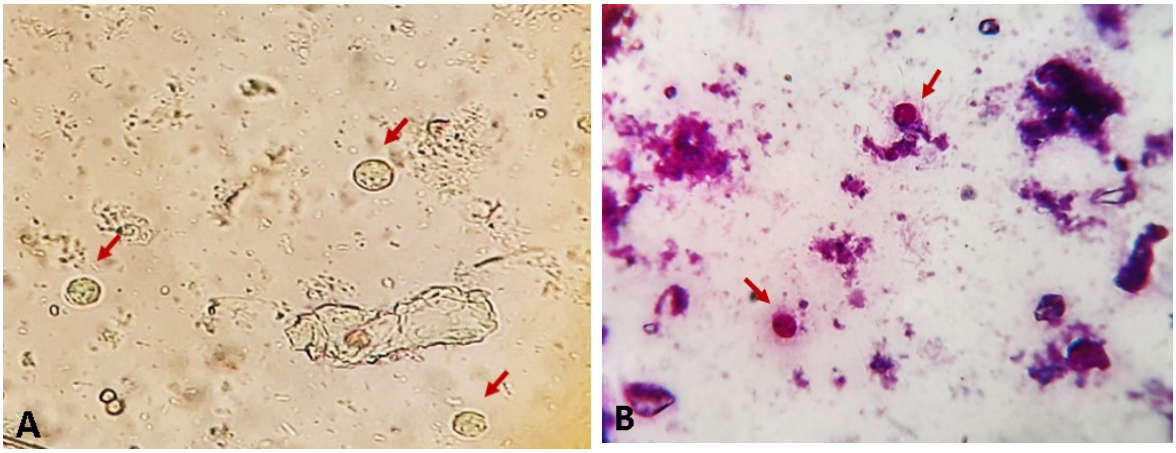

Fig.1. Photomicrograph of *Cryptosporidium* spp. oocysts, A. fecal floatation X40, B. Ziehl- Neelsen stain. X100.

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داء الأ بواغ الاخبيئة: دراسة عن الطفيليات المعوية المشتركة بين الإنسان والحيوان في المجترات الصغيرة في محافظة السليمانية/العراق

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الملخص

تعد طفيليات الكريبتوسبوريديوم من الكائنات الأولية المعوية المنتشرة في الغذاء والماء ، والتي تصيب مجموعة واسعة من العوائل بتأثيرات حيوانية المنشأ. ونظرًا لعدم وجود بيانات بشأن حدوث داء الأ بواغ الاخبيئة ، فإن الدراسة الحالية تتطلب معرفة مدى انتشار داء الأ بواغ الاخبيئة بين المجترات الصغيرة في محافظة السليمانية. تم أخذ العينات من يوليو إلى ديسمبر 2023 من 215 رأسًا من الأغنام و150 رأسًا من الماعز تنتمي إلى قطيع مختلط من المجترات الصغيرة. تم إجراء مسح طفيلي للكشف عن الكائنات الأولية من جميع عينات البراز، ثم تم صبغها باستخدام إجراء تلطيخ Ziehl-Neelsen. تؤكد النتيجة المرصودة وجود طفيليات الكريبتوسبوريديوم بين مجموعة صغيرة من المجترات، بمعدل انتشار 22.74٪. وُجِد معدل انتشار أعلى بنسبة 24.19% في الأغنام مقارنة بالماعز بنسبة 20.67% (OR = 1.2، 95% CI: 0.7-1.7). (OR = 1.2، 95٪ CI: 0.7-1.7).وعلى مستوى القطيع، تم الكشف عن أكياس الكريبتوسبوريديوم في جميع قطعان العينات المختارة، مع معدل انتشار أعلى بشكل ملحوظ بنسبة 29.56% في القطعان الكبيرة التي تضم أكثر من 200 حيوان (OR = 2.5، 95% CI: 1.1-2.8). وعلى الرغم من عدم ملاحظة أي أهمية فيما يتعلق بعمر الحيوانات، فقد لوحظ معدل انتشار أعلى في الحيوانات الأكبر سنًا ≤ 18 شهرًا بنسبة 23.40% (OR = 0.8، 95% CI: 0.6-1.4). يعد الموسم أحد العوامل الوبائية، وفي الدراسة الحالية، وجد معدل إصابة أعلى بين الحيوانات خلال مواسم الأمطار بنسبة 23.61٪ (OR = 0.9، 95٪ CI: 0.6-1.4). علاوة على ذلك، كان معدل الإصابة أعلى بين الحيوانات التي عاشت مع الأبقار بنسبة 25.97٪ (OR = 0.7، 95٪ CI: 0.5-1.2). تؤكد الدراسة حدوث عدوى الكريبتوسبوريديوم بين الأغنام والماعز في منطقة الدراسة. يجب إثبات إمكانية مساهمة الماشية في داء خفيات الأبواغ البشري.

الكلمات الدالة: الكريبتوسبوريديوم، حيواني المنشأ، الأغنام، الماعز، السليمانية.