

Survey and Risk Factors of *Cryptosporidium* spp. Infection in Sheep

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Abstract

The aim of this research is to detect the prevalence rate of infection with *Cryptosporidium* spp. in sheep in different regions of Nineveh Province, Iraq. 100 sheep fecal samples were collected. That are younger than a month to more than 6 months. All samples have been tested by using direct methods 1% Lugol's iodine, flotation method by using sugar solution, and modified Ziehl–Neelsen staining to investigate for oocysts of *Cryptosporidium* spp. The study showed the total prevalence rate of infection with *Cryptosporidium* spp. in sheep was 52%. There are no significant differences ($p < 0.05$) in prevalence rates among animals aged less than 6 months and animals more than 6 months. The results showed A difference significantly between the infection rates in males and females, 81.2% (45\55), 15.6 % (7\45) respectively. As well as, Infection with *Cryptosporidium* in sheep was significantly influenced by several potential risk components such as breeding of animals, consistency of feces, herd size, as well as keeping with other animals, bedding type, and occurring of pregnancy in ewes.

Keywords: *Cryptosporidium* spp., Prevalence, Risk factors, Sheep.

Introduction

The protozoan parasite *Cryptosporidium* is an intracellular extra cytoplasm that belongs to the phylum Apicomplexa (1). Its causative agent of cryptosporidiosis which is, a global infection with medical and veterinary significance that

can infect a variety of vertebrate animals, and humans, infection of this parasites causes acute gastroenteritis abdominal pain, and diarrhea (2,3). First identified cryptosporidiosis in Australian lambs that had diarrhea, but due to coincidental infections with pathogenic bacteria, the organism could not be held

responsible. Early in the 1980s, Research on experimental and natural infections showed that the primary cause of lamb diarrhea is *Cryptosporidium* (4, 5). Goats and sheep are chief hosts for *Cryptosporidium* species and are principally spreading the infection through the fecal–oral pathway. This can lead to significant economic losses by causing symptoms including diarrhea, weight loss in the lamb, and even death (6). Therefore, *Cryptosporidium* was identified by the World Health Organization as a serious zoonotic parasite (7). Oocysts of *Cryptosporidium* that are expelled together with a host's feces are instantly infective. One can become infected with *Cryptosporidium* oocysts via direct contact with infected animals as well as animal coughing leads to food or water contaminated with oocysts of the parasite (8). Because they may infect a variety of hosts, certain species, like *Cryptosporidium parvum*, seem to lack host specificity. Because of their tremendous genetic variety, the later species really considerable adaptation abilities (9).

The reason behind the problem by the fact that a valuable supply of organic materials and nutrients is animal manure, such as phosphate, potassium, and nitrogen. Techniques that improve the sustainability of industrial processes are being promoted globally as a result of global initiatives to adopt environmentally friendly practices (10). The aim of this study was to find out how common *Cryptosporidium* in sheep in Nineveh province and examine the impact of several risk factors such as age of animal, sex, consistency of feces, herd size, breeding type, bedding type, and occurring of pregnancy in animals.

Materials and Methods

Samples collection

Fecal samples of about (8-10 gm) were gathered from 100 sheep of different ages from month August to November 2024. from different regions in Nineveh province, Iraq (Hammam Alalleel, Kaeyara, Alhamdania, Talkeef, center and regions of Mosul city). Feces were taken from the rectum placed in sterile clean plastic containers and Recording information was completed for each animal, it comprised: age, sex, breeding, fecal consistency (normal, diarrheal), herd size, raised with other animals, bedding type, and consumption of colostrum. After that, these samples were transported to the laboratory and kept at 4 °C in a refrigerator for analysis.

Fecal Examination

For fecal analysis to determine the consistency of the fecal sample, a macroscopical inspection was conducted, which was categorized as a microscopical examination. by direct methods, 1% Lugol 's iodine, and flotation method by using sugar solution for examined the oocysts of parasites in feces using the light microscope at 40X (11, 12). Modified Ziehl–Neelsen staining consisted of smearing feces on sterile glass slides which is can be summarized as follows: the smears were fixed with absolute methanol for 3 minutes, stained with basic Carbol fuchsin for 10 minutes, and decolorized with 2.5% acid alcohol for 1 minute. After that used counterstain with 1% methylene blue for 5 min. Then wash the slides and leaving to dry. Later all Smears were examined under a light microscope at 40X and 100X magnification to investigate the oocysts of *Cryptosporidium* and it were measured by an ocular micrometer (13,14).

Statistical Analysis

Calculate the rates of parasitic infection among different variables. The prevalence percentage is related to the risk factors. The association

between risk factors and infection status was determined using the chi-square test (χ^2). When the P-value was less than 0.05, the values were deemed statistically significant in every instance (15).

Results

Among 100 fecal samples were examined microscopically by different methods the

general predominance of *Cryptosporidium* spp. was observed to be 52%. The prevalence rate was 100% in sheep which is aged less than a month to 6 months statistically there were significant differences between sheep aged less than 6 months and sheep aged more than (Table 1).

Table 1. Infection rate of *Cryptosporidium* spp. in sheep based on age.

Age	No. of sample examination	No. of sample positive	Percentage%
1 month <	10	10 a	100%
1-3 months	20	20 a	100%
3-6 months	20	20 a	100%
> 6 months	50	2 b	4%
Total	100	52	52%

At P<0.05, same letters indicate no significant differences, whereas different letters indicate

significant differences

Cryptosporidium oocysts were round and brilliant red, on a blue backdrop. At least one *Cryptosporidium* oocyst could be easily identified in a fecal sample, it was deemed positive. In direct smear, staining with Lugol's iodine and flotation method the oocysts appeared spherical in shape (Fig.1).

In Modified Ziehl–Neelsen staining oocysts of parasite appeared as red spherical in shape on a blue backdrop, with a diameter ranging from 4 to 6 μ m (Fig. 2). A higher infection rate significantly was 81.2% in males while in females was 15% (Table 2).

A higher infection rate significantly was 81.2% in males while in females was 15.6% (Table 2).

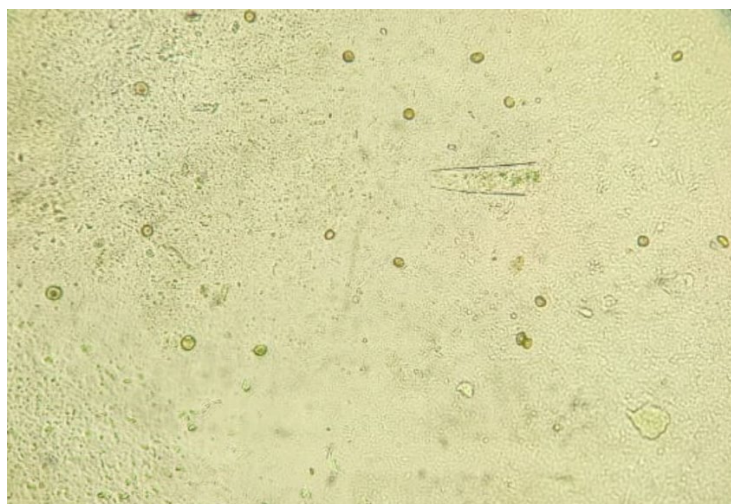


Fig.1. Oocyst of *Cryptosporidium* spp. by Lugol 's iodine

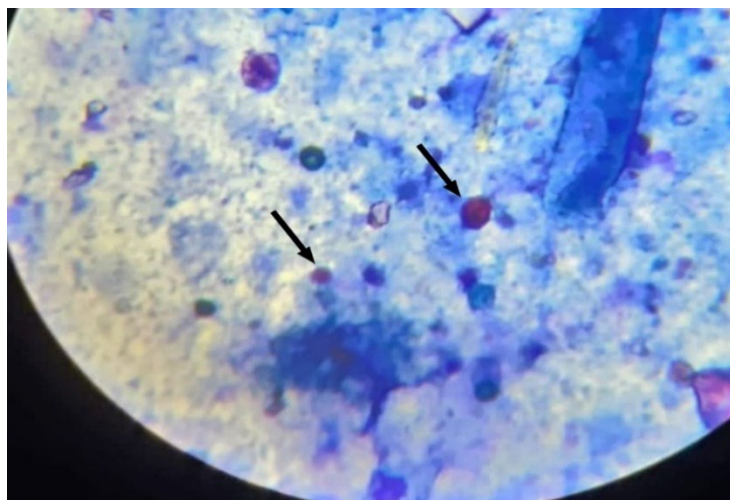


Fig.2. Oocyst of *Cryptosporidium* spp. staining by Modified Ziehl–Neelsen stain.

Table 2. Infection rate of *Cryptosporidium* spp. in sheep based on Gender.

Gender	No. of sample examined	No. of sample positive	Percentage %
Males	55	45 a	81.2
Females	45	7 b	15.6
Total	100	52	52

At $P < 0.05$, different letters indicate significant differences.

This study shows there is no significant relationship between *Cryptosporidium* prevalence and fecal consistency; the prevalence of *Cryptosporidium* associated with diarrhea was 57.1% and 48.6% in normal fecal samples (Table 3). *Cryptosporidium* infection, with sheep in breeding sheep only having a higher infection rate (61.5%) than sheep

keeping with other animals was (34.3%) (Table 4). The herd's size was another significant factor, prevalence rate of *Cryptosporidium* infection in sheep which is in herd size less than 100 animals having a higher infection rate (80%) than sheep in herd size more than 200 animals (13.3% ($p < 0.05$)) (Table 5).

Table 3. Infection rate of *Cryptosporidium* spp. in sheep based on Fecal consistency.

Fecal consistency	No. of sample examined	No. of sample positive	Percentage %
Normal	35	17 a	48.6
Soft	30	15 a	50
Diarrhea	35	20 a	57.1
Total	100	52	52

At $P < 0.05$, same letters indicate no significant differences.

Table 4. Infection rate of *Cryptosporidium* spp. in sheep based on Type of breeding.

Type of breeding	No. of sample examined	No. of sample positive	Percentage %
Sheep only	65	40 a	61.5
Keeping with other animals	35	12 a	34.3
Total	100	52	52

At P<0.05, same letters indicate no significant differences

Table 5. Infection rate of *Cryptosporidium* spp. in sheep based on Herd size.

Herd size	No. of sample examined	No. of sample positive	Percentage %
<100	50	40 a	80
101-200	35	10 b	28.6
>200	15	2 b	13.3
Total	100	52	52

At P<0.05, same letters indicate no significant differences, whereas different letters indicate significant differences.

The type of bedding also influenced the prevalence of *Cryptosporidium* infection significantly, with sheep on cementum bedding having a higher infection rate (100%) than sheep on soil bedding (36%) ($p<0.05$) (Table 6). In pregnancy ewes the infection rate was 16%, while in non-pregnancy ewes the infection rate was 15% (Table 7).

Discussion

This investigation showed that 52% (52/100) of sheep were infected with *Cryptosporidium* spp. The result of this research which greater than the result obtained by earlier studies carried out in Iraq (16), they indicated that sheep in Duhok City, the area closest to Mosul City with comparable breeding circumstances, had an 11.7% rate., and Babylon city recorded 31% in sheep (16,17). And 24.19% was recorded in sheep in Sulaymaniyah city (18). The findings of this study are consistent with earlier research conducted in Tikrit city, which found that 56.25

% of sheep were infected with *Cryptosporidium* (19). As well as numerous researches have examined the prevalence rates of *Cryptosporidium* spp. infection in sheep in neighboring countries like Turkey, Kuwait, Iran, and Egypt, Various levels of reporting have been made on these studies. (20-23). The effect of variables including study design, sample size, diagnostic techniques, local weather, breed variety, animal immunological state, and environmental circumstances might help to explain why prevalence rates of cryptosporidiosis vary between studies (24).

Additionally, the research demonstrates a significant disparity between male and female infection rates, 81.2% and (15.6 %), respectively. This may be due to the most males are raised in closed housing for the purpose of meat production therefore, the chance of exposure to infection is greater in this type of breeding. The results were in agreement with previous study by (25) who found that 6.02% in

male and 4.22% in female in Kurdistan, Iran and (26) found that 14.3%, 10.3% in male and female respectively in Iran. Since, the statistical analysis showed that the differences significant between animals younger than six months and those older than six months.

This result is in line with earlier research (27,28,29). This might be a result of the newborn lambs weakened immunity and the oocysts shedding from diseased dam's ewes because of hormonal imbalances (30). There is no correlation between sheep fecal consistency and the prevalence of cryptosporidiosis, this finding is consistent with previous studies (31, 32). Although other studies demonstrate that the incidence of cryptosporidiosis is closely linked to animal diarrhea (33, 34). Furthermore, *Cryptosporidium* spp. recoding prevalence in sheep breeding alone and in combination with other animal species, it has been demonstrated that *Cryptosporidium* is an apicomplexan protozoon that can infect many different species of animals, such as cattle, sheep, goats causing potentially fatal diarrhea, particularly in young animals (22). Also sheep breeding with other animals such as goats which are characteristic very activity led to increase chances for food and water contamination.

In current study indicated that animal's owners in Mosul regions prefer sheep in smaller groups than 100 animals, hence prevalence rate of infection with *Cryptosporidium* spp. was greater in smaller flocks than in larger ones.

Additionally, a strong correlation was found between the prevalence rate of cryptosporidiosis in animals and their homes.

This study shows prevalence of cryptosporidiosis was higher rate in animals

where bedding is cementum in animal houses, this might be because constant cleaning and washing with water lead to found high humidity in animal pens that could likely provide a microclimate that is conducive to the existence or survival of *Cryptosporidium* oocysts in animal shelters or farms. This raises the level of contamination in feed and water, which may increase the chances of sheep being infected with the parasite *Cryptosporidium* (35). Females showed infected with cryptosporidiosis both in pregnant 16 % and non-pregnant animals 15% however there were no notable variations, this outcome was caused by stressors that suppressed the immune system of females, especially during pregnancy and lactation (36,37).

Conclusions:

The study conducts the existence of protozoan parasites in sheep from different regions in Ninavah province, Iraq. Traditional methods were used for diagnosis oocysts of *Cryptosporidium* spp. In fecal samples of sheep. Results were confirmed, the infection rate with *Cryptosporidium* spp. In sheep was greatly impacted by some potential risk factors, such as, age, sex, herd size, animal state, type of bedding, fecal consistency, and type of breeding.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

Table 6. Infection rate of *Cryptosporidium* spp. in sheep based on Type of bedding.

Type of bedding	No. of sample examined	No. of sample positive	Percentage %
Cementum	25	25 a	100
Soil	75	27 b	36
Total	100	52	52

At P<0.05, same letters indicate no significant differences, whereas different letters indicate significant differences

Table 7 -Infection rate of *Cryptosporidium* spp. in sheep based on animal state.

Animal state	No. of sample examined	No. of sample positive	Percentage %
Pregnancy	25	4 a	16
Non -pregnancy	20	3 a	15
Total	45	7	15.6

At P<0.05, same letters indicate no significant differences,

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انتشار وعوامل خطر الإصابة بطفيلي الكريبتوسبورديوم في الأغنام

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فرع الاحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

يهدف هذا البحث إلى تحديد معدل انتشار الإصابة بطفيلي الكريبتوسبورديوم في الأغنام في مناطق مختلفة من محافظة نينوى/ العراق. تم جمع 100 عينة براز من الأغنام التي تتراوح أعمارها بين شهر إلى أكثر من 6 أشهر. تم فحص جميع العينات باستخدام الطريقة المباشرة، محلل اليود 1٪، طريقة التطويق باستخدام محلل السكر وطريقة زيل نيلسن المعدلة للبحث عن أكياس طفيلي الكريبتوسبورديوم. أظهرت الدراسة أن معدل انتشار الإصابة بطفيلي الكريبتوسبورديوم في الأغنام كان 52٪. لا توجد فروقات ذات دلالة إحصائية ($p < 0.05$) في معدلات الانتشار بين الحيوانات التي تقل أعمارها عن 6 أشهر والحيوانات التي تزيد أعمارها عن 6 أشهر. وأظهرت النتائج وجود فروق معنوية بين معدل الإصابة في الذكور والإناث، 81.2% (55\45)، 15.6% (45\7) على التوالي. كما تأثرت الإصابة بالكريبتوسبورديوم بشكل معنوي بعدة عوامل خطورة مثل تربية الحيوانات، وقوام البراز، وحجم القطيع، والعيش مع حيوانات أخرى، ونوع الفرشة، وحدوث الحمل في الحيوانات.

الكلمات المفتاحية: الكريبتوسبورديوم، الانتشار، عوامل الخطر، الأغنام.