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# ISOLATION AND IDENTIFICATION OF *CRYPTOSPORIDIUM* SPECIES INFECTION IN LAMBS IN BABYLON PROVINCE, IRAQ

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**ABSTRACT**

The purpose study was to determine the prevalence of infection with Cryptosporidium in lambs in Babylon Province, Iraq. One hundred fecal samples were collected from lambs aged 1 to 12 months in 3 areas of the province of Babylon, in the center of Iraq. The samples were screened for *Cryptosporidium* spp. Oocysts using Sheather 's concentration solution and updated Ziehl–Neelsen staining process. *Cryptosporidium* spp. oocysts were present in 31% of lambs. The results showed no significant differences ($p > 0.05$) in prevalence rates among different area of study. The infection rates in Al-Hilla, Al-Musaib and Al-Qasim were 27.5%, 30% and 36.66% respectively. The sex of lambs had no effect on the infection ratio with *Cryptosporidium*. The infection rate was 26.3 % for males and 37.2 % for females respectively. The results showed significant ($p < 0.05$) differences in prevalence rates among different age groups of lambs infected with *Cryptosporidium*, with the highest infection rate in age group <1 – 3 months (51.3%) and the lowest rate in age group 7–9 months (15.3%). The parasite presence. In lambs in the province of Babylon, this *Cryptosporidium* spp. may also be included in the etiology of diarrhea-exhibiting lambs.

**Keywords:** *Cryptosporidium*, Oocysts, Prevalence, Lambs.

## Introduction

*Cryptosporidium* species are zoonosis can infected human and a wide range of animals hosts worldwide its obligate intracellular (extra-cytoplasmic) protozoan parasites (Ryan *et al.,* 2014).

In Australia, the diseases was first described in lambs with diarrhea; the causative function of coincidental infections with pathogenic bacteria could be attributed to the organism. Its function as the key etiological agent of diarrhea in lambs was verified in natural and experimental infection studies in the early 1980s. (Snodgrass *et al*., 1984).

The diseases is primarily spread via the fecal-oral pathway, primarily via oocyst-contaminated food or drink, through contact with infected animals or unintentionally in laboratories (Bouzid *et al*., 2013). Cryptosporidiosis not only zoonotic given its source of infection and its means of dissemination, but it also attracts the interest of researchers in the veterinary community due to fears about the harmfulness of the disease, its potential to spread quickly and to be difficult to monitor, as well as the major economic impacts and losses of the disease. (Zhang *et al*., 2017).

The studies conducted in Iraq recorded the prevalence of *Cryptosporidium* in Ninevah Province 26.66% (Abdullah, 2005), while Yakoob. and Kathim (2009) recorded the prevalence rate of *Cryptosporidium parvum* in Baghdad province 15.8%.

## Materials and Methods

The study included one hundred fecal samples of lambs from different ages of both sexes in different regions of Babylon province. From the beginning of January 2019 to the end of June 2019, 15-20 grams of fecal samples were taken from each lambs, fecal smears were prepared from each sample, and stained as the primary diagnosis of *Cryptosporidium* oocysts using the modified acid-fast staining technique (Beaver and Jung, 1985).

A sample was considered positive for parasites oocyst was detected bearing the correct morphology, optical properties, internal structures, size, and shape (Fayer *et al*., 2000).

**Statistical Analysis:** Data has been converted into a computerized database structure. There was a call for expert statistical guidance. Computer aided statistical tests using SPSS version 17, variables were evaluated by Yat's Chi-square test (Development, 2013).

## Results

### 1. Rate of infection of *Cryptosporidium parvum* in relation to areas of study:

The total infection rates of *C. parvum* in lambs in Babylon province was 31.5%. The infection rates in Al-Hilla, Al-Musaib and Al-Qasim were 27.5%, 30% and 36.66% respectively, without significant differences (Table 1).

**Table 1 :** Infection rate with *Cryptosporidium* spp. relation toareas of study:

| Area | No. of Samples examined | No. Positive | Infection Rate % |
|---|---|---|---|
| Al-Hilla | 40 | 11 | 27.5 |
| Al-Musaib | 30 | 9 | 30 |
| Al-Qasim | 30 | 11 | 36.66 |
| Total | 100 | 31 | 31 |

$X^2 = 1.1$

**2. Rate of infection of *Cryptosporidium* spp. in relation to sex:**

The rates of infection in male and female were not differing statistically 26.2% and 37.2% respectively (Table 2).

**Table** 2 **:** Infection rate with *Cryptosporidium* spp. relation to sex:

| Sex | No. of Samples examined | No. Positive | Infection Rate % |
|---|---|---|---|
| Male | 57 | 15 | 26.3 |
| Female | 43 | 16 | 37.2 |
| Total | 100 | 31 | 31 |

$X^2$= 2.4

**3. Rate of infection of *Cryptosporidium* spp. in relation to age groups:**

The results showed significant difference ($p < 0.05$) in the prevalence rates among different age groups. The highest rate of infection in lambs was 51.3% recorded at age group 1 – 3 months and the lowest 15.3% at age group 7 – 9 months (Table 3).

**Table 3 :** Rate of infection with *Cryptosporidium*s pp. relation to age groups:

| Age groups (Months) | No. of Samples examined | No. Positive | Infection Rate % |
|---|---|---|---|
| 1- 3 | 37 | 19 | 51.3 |
| 4 – 6 | 21 | 5 | 23.8 |
| 7– 9 | 26 | 4 | 15.3 |
| 10- 12 | 16 | 3 | 18.7 |
| Total | 100 | 31 | 31 |

$X^2$= 18.7

**4.6.5. Oocysts of *C. parvum*:**

The oocysts of *C. parvum* like oocyst in Modified Ziehl Neelsen stain appeared as spherical densely stained pink to red bodies with a clear halo around the oocyst, against a dark blue background of the methylene blue stain (Fig. 1). Morphologically in Sheather's sugar floatation, the oocysts appeared as round or oval refractile bodies with a thin greenish membrane, the four sporozoites looked as black bodies inside the oocysts (Fig. 2).

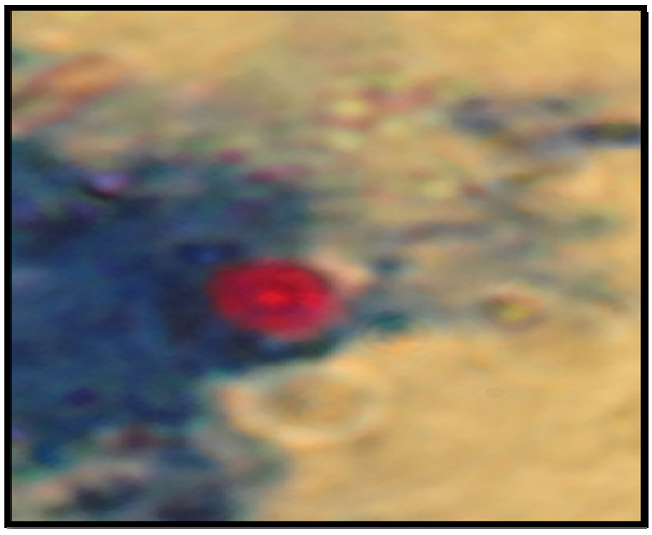

**Fig. 1 :** *C. parvum*like oocysts stained with Modified Ziehl Neelsen magnification (100x).

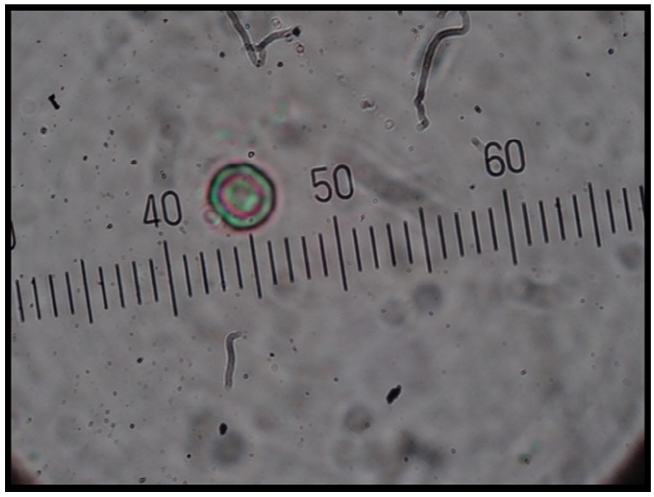


**Fig. 1 :** *C. parvum*like oocysts, floatation with Sheather's sugar (100x).

## Discussion

Several studies recorded different rates of *C. parvum* infection due to different areas of samples collection. In Iraq (Abd Al-Wahab, 2003) showed that the prevalence of *C. parvum* in Baghdad province was 81.46%, (Abdullah, 2005) recorded 26.66% in Nineveh Province, Dawood and Abdullah. (2007) showed that the prevalence of *C. parvum* in Diwaniya province was 27.5% and (Yakoob and Kathim, 2009) showed that the prevalence of *C. parvum* in Baghdad province was 15.8%.

The differences in rate of infection due to difference in areas of samples collection, shorter period of study, in addition to other factors affecting the prevalence such as number of samplings, methods of rearing and methods of diagnosis used which have highly effective on determination such ratios particularly the density of the flocks.

The results were in accordance with (Wahed, 1999) in Egypt, (Vahedi *et al.,* 2009) in Iran, whom recorded the prevalence rates of *C. parvum* were 30%and 28.6% respectively. In the meantime the prevalence rate of *C. parvum* in current study was lower than those established by Silva-Fiuza *et al.* (2011) in Brazil (47%) and Connelly *et al.* (2013) in Scotland (37%), this differences were due to most of these studies were carried on rearing farms with high number of density which facilitate the contamination of soil and drinking water and considered the main sources of infection transmission.

In Iran Khezri *et al.* (2013) and Gharekhani *et al.* (2014) recorded lower rate of infection (10.24%, 11.3% respectively). The most important reason of those lower rates of infection was age of animals that used in studies (more than one year old) which were more resistant to infection (Sari *et al.,* 2009).

Females showed numerically higher rate of infection than males but no significant differences was existed, this result was due to stress factor faced the females immuno suppression, particularly pregnancy and lactation (Khalil, 2010).

The results were in agreement with those found by (Yakoob and Kathim, 2009) recorded 12.9% in male and 16.72% in female in Baghdad province (Khezri and Khezri,2013) recorded 6.02% in male and 4.22% in female in

Kurdistan, Iran and (Gharekhani *et al.,*2014) recorded 14.3% in male and 10.3% in female in Iran.

In Al-Diwaniya province, (Dawood and Abdullah, 2007) showed that cryptosporidiosis morbidity higher in male 14.9% than in female 12.6% due to number samples collection, environmental condition and areas of study.

The results were in accordance with previous studies whom showed that the highest rate of infection appeared at age group of <1- 3 months up to 1 year then the infection decline (Abdullah, 2005; Sari *et al.,* 2009; Yakoob and Kathim, 2009 and Gharekhani *et al.,* 2014).

Such high rate of infection was due to an increase in the oocysts shedding from ewes during pre-parturient and suckling period, difference of hormonal excretion which caused decline in immunity level, mixed rearing and bad management as well as incomplete immune system in these young animals lead to highest prevalence in age group 1- 3 months.

The lowest rate of infection in animals aged between (7– 9) months was due to many factors: such as low number of samples collected, and separation of these animals from their dams.

In Baghdad province (Yakoob and Kathim, 2009) showed that the rate of infection was decline during infection recurrence due to acquired immunity of these animals. But other studies found that *Cryptosporidium* infection occur in age group more than 1 year with significant difference (Al-zubaidi, 2012 and Budu-Amoako *et al.*, 2012).

In contrast (Al-Tamimi, 2014 ) in Babylon recorded the highest infection rate (22,44%) of *C. andersoni* in cattle at age group 1 – 2 years old, while (Gharekhani *et al.,* 2014) in Iran recognized high infection rate (16.7%) of *Cryptosporidium* at age <12 months old in sheep. These differences were due to the different managemental regimes and season of samples collection. Some factors may be related to the overcrowding and the unhygienic conditions under which the animals are housed. *Cryptosporidium* oocyst excretion by ewes during parturition may play a role in the initiation of cryptosporidiosis in lambs (Causape *et al*., 2002).

The results were compatible to the results of (Kadhim, 2009) in Iraq, who recorded the measurements of *C. parvum* oocysts (4.3 x 4.8) µm, while (Khalil, 2010) recorded that the measurements of *C. parvum* oocysts (4.0 - 4.4 x 5.2 – 5.5) µm. Fayer and Xiao 2008) measured the oocyst of *C. parvum* with average (4.5 x 5) µm.

## Conclusions

Our research suggests that the frequency of Cryptosporidium spp. Lamb infections are higher in areas of Babylon than in other rural areas of Iraq. To minimize the risk of lamb infection and mortality, morbidity rate and loss of income in sheep farms, instigation of control programs is suggested.

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