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ISOLATION OF *CRYPTOSPORIDIUM* SPP. FROM RABBITS IN BAGHDAD CITY, IRAQ

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Abstract

Oocysts of *C. cunicunlus* that isolated from rabbits' feces stained with Modified Ziehl Neelsen (MZN) were spherical in shape, red in color surrounded by clear hallow, 3.2± 0.4 X 3.3 ± 0.5µm in diameter. Overall of 180rabbit fecal samples (70 male and 110 female) were collected from December to end of August 2019. Traditional methods (direct smear, flotation and MZN staining), light microscope was used to detect the morphology of parasite oocysts (shape, and diameter). The overall prevalence rate in rabbits was 26.1% NCDC recorded the highest rate of infection 33.1% with significant difference (P≤0.01) .There were significant differences recorded between months (P≤0.01) where May and June recorded the highest rate 40% and the lowest was in December and January10%.
Keywords : *Cryptosporidium spp.*, rabbits, Baghdad city.

Introduction

Cryptosporidiosis is a feco-orally transmitted diarrheal illness (Chalmers and Katzer, 2013). Cryptosporidiosis is a frequent cause of diarrheal disease in humans, and several groups of humans are particularly susceptible to cryptosporidiosis. In developing countries, *Cryptosporidium* infections occur mostly in children younger than 5 years, (Bern *et al.,* 2000). Cryptosporidial oocysts contain four sporozoites, which are not contained within sporocysts (Xiao *et al.,* 2004), and its species are infecting the microvillus border of the gastrointestinal epithelium (Fayer, 2010).With loss of enterocytes and villus atrophy and fusion, accompanied by lamina propria edema and infiltration by inflammatory cells, which can be mixed or primarily mononuclear of a wide range of vertebrate (Tzipori and Ward, 2002). villous atrophy, villous fusion, crypt hyperplasia, and cellular infiltration of the lamina propria have been observed (Vitovec *et al.,* 2006). Intestinal parasites, both protozoa and helminths, are one of the main enteropathogen of rabbits. Some of these parasites are responsible for direct and indirect losses, that are attributed to acute illness and death, premature slaughter and diminution of productive potential such as decreased growth rate, weight loss and late maturity of slaughter stock (Hansen and Perry, 1994).

Materials and Methods

1- Samples collection

A total of 180 local breed rabbits of both sexes and different age were used. Fecal samples (3-5) grams were collected from different ages ranged from 1 month to 3years old, for both sexes during the period from the beginning from 1st December 2018, up to 31th August 2019.

The (180) fecal samples were collected in clean plastic containers, and were tightly closed, given sequential numbers, age, sex, date of sampling also included protective measure was taken such as wearing disposable gloves. The study involved from different location of Baghdad city.

2- Isolation and purification of oocysts for experimental infection by using Sheather's sugar solution (Al-Attar, 1981):

1. Fecal samples (5 – 10 gram) was mixed with 20 ml distilled water in a clean flask then was filtered through four layers of clean gauze to remove the fecal debris. Then the suspension was collected in test tubes and were centrifuged (2700 round/ minute) for 15 minutes.
2. The supernatant was discarded, and few amount of (the suspension was kept with the sediment) and mixed well with Sheather's sugar solution (9ml), then centrifuged with the same procedure mentioned previously.
3. The supernatant was collected on test tube and to prevent the effect of Sheather's sugar solution on the oocysts,it diluted with (distilled water) at a rate of (1:10).
4. By centrifuging (2700 round) for 15 minutesthe diluted solution was sedimented.
5. In a clean test tube the sediment was collected and added an equal volume of (1% Sodium Hypochlorate Solution), mixed well and left for 5 minutes and then added a double volume of distilled water gradually on the wall of the test tube by a syringe of (1 ml) size. Above the solution aclear layer was separated, pulled by a Pasteur pipette and moved to other test tubes.
6. The clear layer washed by centrifugation several times until the disappearance of the chlorine smell from the solution.

3- Oocysts counting

Method was used to count the number of oocysts in one ml of suspension according to the followings:

1. Isolation and purification of the oocysts by using Sheather's sugar solution.
2. The oocysts which isolated by this method from infected cases storage in potassium dichromate solution v/v, and the number of oocysts calculated in 1 ml of suspended oocysts solution calculation in the eight squares of two

chambers of the hemocytometer slid which used for white blood cells calculation.

3. The oocysts count was done by using light microscope ×100then the total numbers of oocysts per 1ml calculate according to the following equation:

Number of oocysts in 1 ml = (1000 x calculated oocysts number) / 8 (Al-Attar, 1981).

4-Measure dimension of oocyst

Dimension of oocyst were measured by using Ocular Micrometer according to: (Chermette and Boufassa ,1988).

5- Preparation of infective dose

T he infective dose prepare by collected and isolate oocysts from infectedanimals feces (Sheather's sugar solution), then calculate of oocysts in 1 mlby hemocytometer method (Al-Zubaidi *et al*., 2018).

6- Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

Results

1. Morphology and measurements of *Cryptosporidium* spp. oocyst using: Modified Ziehl-Neelsen (MZN). Floatation with Sheather's solution

In our study we used Modified Ziehl-Neelsen (MZN) stained smear for examination *C. cuniculus* oocysts which appeared as spherical to oval and almost measurement (4 X 4.5) µm by ocular micrometer. They are acid-fast but oocyst staining within a smear and between specimens, is very variable, and oocysts vary from unstained to partial red staining and complete staining: and fully sporulated forms can be found in which red staining crescentic bodies, the sporozoites, can be seen within an unstained oocyst wall by concentrated wet film(floatation with Sheather's solution)the oocysts appeared rounded bodies with a thin greenish membrane, the four sporozoites looked as black bodies inside the oocysts.

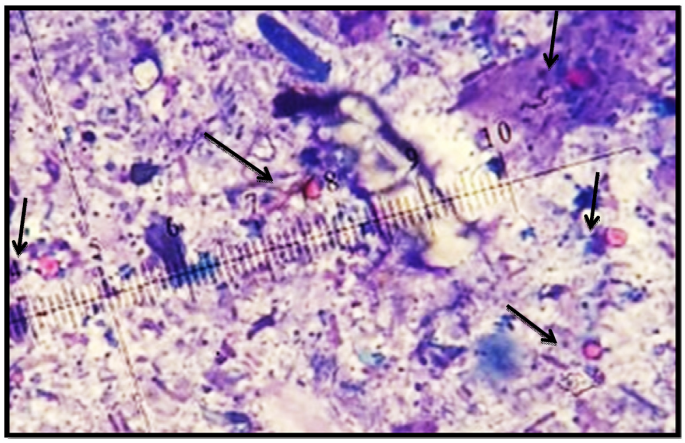

Fig. 1 : *C. cuniculus* oocysts stained with Modified Ziehl Neelsen magnification(X100)

2- Total infection rate of *Cryptosporidium* spp. in rabbit by using traditional methods (microscopic examination)

A total of 180 rabbits fecal samples were examined by traditional methods (Flotation, staining by modified Ziehl-Neelsen (mZN) stain) for detection the prevalence of *Cryptosporidium* spp. and revealed the overall infection rate of *Cryptosporidium* spp. in rabbits in Baghdad province. The results showed that 26.1% (47/180) were positive for *Cryptosporidium* oocysts which it was 26.1% (47/180) of*C.cunculis*and1.7 % (3/180) of *C. parvum* (Table 1).

Table 1 : Total infection rate of *Cryptosporidium* spp. in Rabbits examined microscopically

Host	No. of fecal samples examined	Traditional microscopy	
Rabbits		No. of positive	Percentage (%)
C. cuniculus	180	47	26.1
C. parvum	180	3	1.7
			** (P≤0.01).

2.1- Prevalence rate of *Cryptosporidium* spp. infection in relation to sex

The results of *Cryptosporidium* spp. infection in relation to sex showed there are significant differences (P≤0.05). The male and female recorded 21.4% (15/70) and 29.09% (32/110) respectively rate of infection with *Cryptosporidium* spp. (Table 2).

Table 2 : Prevalence of *Cryptosporidium* infection in rabbits by microscopic examination in relation to sex

Sex of rabbit	No. of samples examined	Positive samples	
		No.	%
Male	70	15	21.43
Female	110	32	29.09
Total	180	47	26.11
Chi-Square (χ^2)	---	---	4.028 *
* (P≤0.05).			

2.2. Prevalence rate of *Cryptosporidium* spp. infection in relation to age groups

Prevalence rates among different age groups showed that the no significant differences, the highest infection rate 26.35% was recorded in rabbits at the age group 6-12 months, while the lowest infection rate 25% was recorded at the age group 1-6 months (Table 3).

Table 3 : Prevalence of *Cryptosporidium* infection in rabbits by microscopic examination in relation to age groups.

Age groups of rabbit	No. of samples examined	Positive samples	
		No.	%
1- 6 Months	32	8	25
6-12 Months	148	39	26.35
Total	180	47	26.1
Chi-Square (χ^2)	---	---	0.944 NS
NS: Non-Significant.			

2.3. Prevalence rate of *Cryptosporidium* spp. in relation to months of study

The results showed that the highest prevalence rate recorded in April and May 40% (8/20), while the results were convergent during the March 35% (7/20). Both June and July 30%(6/20). The lowest prevalence rate recorded during December and January, 10% (2/20), these results showed significant difference (P≤0.01) (Table 4).

Table 4 : Prevalence of *Cryptosporidium* spp.in relation to months

Months	No. of samples examined	No. of positive	Percentage (%)
December	20	2	10.00
January	20	2	10.00
February	20	3	15.00
March	20	7	35.00
April	20	8	40.00
May	20	8	40.00
June	20	6	30.00
July	20	6	30.00
August	20	5	25.00
Total	180	47	26.00
Chi-Square (χ^2)	---	---	10.672 **
** ($P \leq 0.01$).			

Discussion

The size of *Cryptosporidium* rabbit genotype oocysts obtained in this study was4.9 to 5.4 by 4.5 to 5.1 m (Ke Shi *et al.,* 2010). *C. cuniculus* has conclusively been demonstrated to be a human pathogen (Chalmers *et al.*2009 b). The constellation of symptoms is similar to, but with some differences to other *Cryptosporidium spp*., especially the age and sex profile (Chalmers *et al.*2009 a).Investigating the epidemiology of sporadic *C. cuniculus* infection has corroborated these findings (Chalmers *et al*.2011). It is not possible to conclude from this outbreak whether the observed epidemiological characteristics of *C. cuniculus* are unique to this species or artefactual. (Elwin *et al.*2011). Cryptosporidiosis was seen in domestic rabbits (in china). This is higher than rates reported for wild rabbits in previous studies but at the lower end of rates reported for farmed, laboratory, and pet rabbits (Robinson and Chalmers, 2010). Differences in techniques used for the detection of infection and in the management and age of animals probably contributed to the observed variations in the prevalence of cryptosporidiosis in rabbits (Ke Shi *et al.,* 2010). the infection rates of Cryptosporidium sp. in female and male rabbit, with the female animals twice as likely to be at risk as the males. The reason for this disparity is not known, although this outcome could be attributed to the usual practice of having a higher female: Male ratio in a farm and also the retention of female animals for breeding. It could also be related to host intrinsic factors (genetics, physiology and immunology) and extrinsic factors (environment and management practices) (De Graaf *et al*., 1999). This finding supports with the general understanding of helminth infections that female animals are more susceptible to helminthiasis. It is assumed that sex is a determinant factor influencing prevalence of parasitism (Maqsood *et al.,* 1996) and females are more prone to parasitism during pregnancy and peri-parturient period due to stress and decreased immune status (Urquhar *et al*., 1996).

The present study showed that seasons have a significant effect on the prevalence of infection in rabbits, with highest prevalence in summer (24.1%) followed by autumn and spring (15% and 11.6%, respectively) and lowest in winter (8%). Peaks in Cryptosporidium prevalence appear to correspond with warmer seasons in temperate and tropical climates (Bern *et al.,* 2002).

Our findings showed that there was significant difference between the rate of the infection in rabbit and the age, with highest peaks in adult (28.9%) compared to young rabbits (9.1%).(Morgan *et al.,* 2000).

This suggests that maternal antibodies protect animals from developing infections. On the contrary, the present data was not in line with that reported previously, as the age played an important role in the incidence of Cryptosporidium infection as young's more susceptible to infection with Cryptosporidium (Al-Biaty, 2002 and Al Neaimi, 2019).

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