CALCULATION OF THE SHEDDING RATE OF CRYPTOSPORIDIUM OOCYSTS FROM THE NATURAL INFECTED SHEEP

M. H.Kawan
Assist.Prof
Department of Parasitology, Faculty of Veterinary Medicine, University of Baghdad
email: MMKvet@yahoo.com

ABSTRACT

The objective of this study to calculate means of oocysts in feces natural infected sheep. A total of 150 sheep fecal samples collected from different regions of Baghdad province(AL-Sholla , AL-Horia, AL-Taji, and Abo-Grab)-Iraq, during the period from the beginning of January to the end ofMay 2016. Three laboratory methods(modified acid fast stain, sheather's sugar solutionand calculation of oocysts in feces of infected animals by haemocytometer) were used for diagnosis of Cryptosporidial oocysts and study the shedding rate of oocysts from infected male and female(pregnant and non pregnant)animals. The total infection rate was 44.66%(67/150), and the highest infection rate was detected in March, while the lowest infection rate was recorded in January 63%(19/30), 20%(6/30) respectively. Theresults were revealed significance difference between male and female infection rate, 31.74%(20/63), 54.02%(47/87) respectively. The average number of shed oocysts per gram of feces from infected non pregnant ewes was 1440 oocysts per gram while in infected pregnant ewes was 2082 oocysts per gram. The shedding rate of oocysts in pregnant ewes show the highest rate in the end period of pregnancy, while the lowest rate of shedding in the beginning period of pregnancy.

Keywords: *Cryptosporidium*,oocysts, shedding, pregnant, sheep

حساب معدل طرح اكياس بيض البوغ الخبيء من الاغنام المصابة طبيعيا

مي حميد كوان
أستاذ مساعد
فرع الطفيليات /كلية الطب البيطري /جامعة بغداد

المستخلص

كان الهدف من الدراسة حساب معدل طرح اكياس بيض البوغ الخبيء من الاغنام المصابة طبيعياز حيث تم جمع 150 عينة براز أغنام تضمنت مناطق مختلفة من محافظة بغداد \ العراق (الشعلة، الحرية، التاجي، وابو غريب) أعتبارا من شهر كانون الاول الى شهر مايس 2016. أستعملت ثلاث طرق مختبرية (صبغة زيل نلسون المحورة) محلول شيذر السكري و حساب اعداد اكياس البيض في البراز بواسطة الهيموسايتوميتر) لتشخيص أكياس بيض الطفيلي ودراسة معدل طرح اكياس البيض من الذكور والاناث (الحوامل وغير الحوامل) المصابة. اعلى نسبة للاصابة الكلية بلغت(150/67) 44.66% ،وسجلت اعلى نسبة للاصابة في شهر أذار واوطأها في كانون الثاني وبلغت(30/19) 63% و(30/6) 20% على التوالي، سجلت الدراسة فرقا احصائيا في نسب الاصابة بين الذكور والاناث، (63/20) 31.74% و(87/47) % 54.02 على التوالي كان معدل طرح أكياس البيض لكل غرام براز من النعاج غير الحوامل المصابة 1440 كيس بيضة لكل غرام. في حين بلغ 2082 كيس بيضة لكل غرام في النعاج الحوامل . سجل اعلى معدل لطرح اكياس البيض للنعاج الحوامل في الفترة الاخيرة من الحمل، في حين سجل اقل معدل للطرح في بداية فترة الحمل.

الكلمات المفتاحية: البوغ الخبيء،اكياس البيض،طرح، حمل، الاغنام

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INTRODUCTION

Cryptosporidium is an intestinal protozoan parasite that allegedly infects alimentary canal of human and animals, it considered as oneof important zoonotic parasite(21). *Cryptosporidium* was first described impacting in rats by Tyzzer in 1907 (40). Cryptosporidiosis was first described in lamb with diarrhea in Australia in 1974 by Barker and Carbonell and has consequently been revealed in 12 other nations (21). Younger animals (calves, lambs, goat kids) appear to be more delicate to sickness,in neonatal ruminants, cryptosporidiosis considered a main cause of diarrhea and death, with important economic loss (2, 14.19,23, 28, 38,40). The parasite have monoxenous life cycle which mean asexual and sexual stages happen within one host, within 1-3days, the prepatent period varies from 1-3 weeks, whereas the patent periodwhich mean duration of oocysts shedding, can be differs from several days to month, indicating the possibility of this infection continue to persist. (31).Cryptosporidiosis transmitted via the fecal-oral transmission from infected animal to the another or from infected animals to human through contaminated food and water with mature oocysts(21,32). The prevalence of *Cryptosporidium* infection in differentreports reach to 85% in lambs, some of these report, inEthiopia, 2.6%, in Australia 3.7%-47%, in Brazil 13.6%-46.5%, in Turkey 25.7% in Mexico, 29% , and 42.1% in Serbia (32,37). In Iraq the parasite recorded by Al-Zubaidi,(8) and Al-Azzui, (4) in cattle, while in sheep and lambs the parasite recorded by Abd-Al-wahab,(3),Kadhim, (24), Al-Seady, (7) and Al-Zubaidi, (10).*Cryptosporidium* considered as the most important parasite causing diarrhea in sukling lambs, and adult ewes act one of the main sources of infection of these lambs. This study aimed to investigate the shedding rate of *Cryptosporidium* oocysts from natural infected sheep.

MATERIALSAND METHODS

A total of 150 sheepfecal samples were collected from both sex(87 females and 63 males) from different regions of Baghdad province(AL-Sholla, AL-Horia, AL-Taji, and Abo-Grab**)** -Iraq, during the period from January to May 2016. Fecal samples were collected directly from the rectum of animals, in a clean plastic container and given sequential numbers, and all information, age, sex and date of sampling were recorded on it. The samples were transported in a cooling box to the department of parasitology in the College of Veterinary Medicine-University of Baghdad for laboratory diagnosis.

Examination of samples: Three laboratory methods were used to diagnosedCryptosporidial oocysts, and study the shedding rate of oocysts from infected male and female (pregnant and non pregnant), Sheather's sugar solution (Fig: 1) Modified Zeihl-Neelsen Stain (MZN) (Fig: 2) and calculation of *Cryptosporidium* oocysts in feces of infected sheep by using haemocytometer. (1, 10, 12, 13, 16, 41).

Isolation and Calculation of the *Cryptosporidium* oocysts

After isolating and purifying the parasite oocysts which found in the feces of infected sheep by using flotation with Sheather's sugar solution (4, 9, and 16). The number of oocysts calculated in 1 ml of suspended oocysts solution by using haemocytometer slid which used for white blood cells calculation in the eight squares of the two chamber of this slid, then the total number of oocysts per 1ml calculate according to the following equation: Al-Attar, (1) (Fig: 1)

Number of oocysts in 1 ml = (1000 x calculated oocysts number) / 8

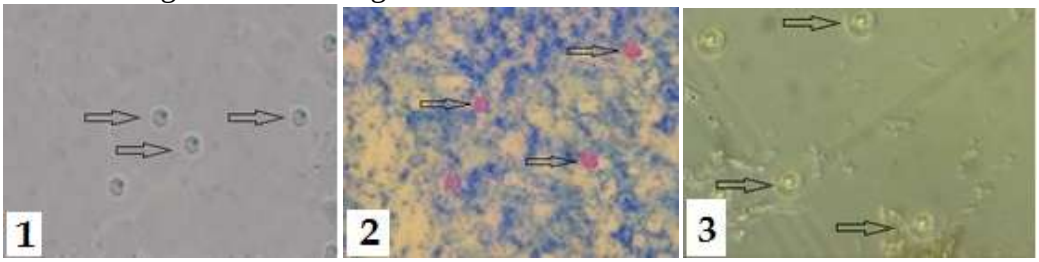

Fig 1.*Cryptosporidium*oocysts in Flotation by sheather's sugar solutionx10
Fig 2.*Cryptosporidium*oocysts by modified acid fast stain x100
Fig 3. Calculation of *Cryptosporidium* oocysts by haemocytometer slid 40x15

Statistical analysis: The Chi-square test was used for the comparison between the results. One way ANOVA was also performed and comparisons were done using LSD. Differences were considered statistically significant at P<0.05 (38).

RESULTS AND DISCUSSION

The results of this study recorded 44.66 % (67/150) of sheep infected with Cryptosporidiosis in different areas of Baghdad city, the highest infection rate in AL-Taji60 % (21/39), while the lowest rate of infection in Abo-Grab 32.5% (13/36) (Table:1).This results agreed with Khalil, (26)in Mosul and Al-Gilani, (5) in Baghdad who recorded infection rate in sheep 36.43%, 48.8%respectively, also agreed with Al-Kaabi, (6) in Diwaniyah province who found 27.5% of sheep shed *Cryptosporidium* oocysts, also the result agreed with Falek*et al*, (18) in Nigeria who recorded 22.7 of sheep infect with Cryptosporidiosis. while the result disagreed with Abd-Al-Wahab, (3) who found higher infection rate 74.2% and Khadim, (24) who show lower infection rate 15.8 in Baghdad city, also disagree with Nouri and Karami, (30) and Harandiand Ardakani,(23) in Iran who recorded 17.2%, 13.3% of sheep respectively infected with cryptosporidiosis, and also disagreed with Rayan *et al*, (34) in Australia and Tembue *et al*, (39) in Brazil who found 2.6% , 3.7% of sheep respectively infected with the parasite. The differences in infection rates in different regions in the world and in Iraq may be due to differences in the number of animals checked, sample size, climatic conditions, and as wall as different diagnostic methods used in laboratory.(21)

Table 1: Prevalence of *Cryptosporidium* spp according to areas

Areas	No.of Examined fecal Samples	No. of positive fecal samples	percentage %	Chi square value	P
AL-Sholla	**35**	**17**	**43.58**	**2.96**	**0.39**
AL-Horia	**40**	**16**	**44.44**		
AL-Taji	**39**	**21**	**60**		
Abo-Grab	**36**	**13**	**32.5**		
Total	**150**	**67**	**44.66**		

The result of study indicated that the animals age effect on infection rates with the highest rate of infection showed in age group 6-12 months 71.42% (21/35) while the lowest infection rate recorded in age group < 36 months 30% (12/40), with significant differences at p<0.05 (Table: 2).

Table 2. Prevalence of *Cryptosporidium* spp according to the age groups

Age (months)	No. of Examined fecal Samples	No. of positive fecal samples	percentage %	Chi square value	P
6-12	**35**	**21**	**71.42**	**7.22**	**0.06**
13-24	**36**	**15**	**41.66**		
25-36	**39**	**15**	**38.46**		
36<	**40**	**12**	**30**		
Total	**150**	**67**	**44.66**		

This results agreed with Abd-Alwahab,(3), Kadhim, (24) and Al-Zubaidi, (10) in Baghdad who found the highest infection rate of cryptosporidiosis in age group less than six month which reach 81.46%, 34.95% and 70% respectively. Also the result agreed with Xiao *et al*, (42), Causpe*et al*, (15) who recorded high infection rate in neonatal lambs 78.3% and 66.4% respectively. This finding agreed with EI-Wahed (17) in Egypt, Sari *et al*. (35) in Turkey who reported high prevalence rate of parasite in small lambs. The highest infection rate in small animal may be due to high shedding rate of *Cryptosporidium* oocysts from dam which contaminate the food and water in farm and increase the chance to infect lambs (21). The result of this study showed significance difference (p<0.05) in infection rate according to the months of study, the

highest rateof infection recorded in March 63.33% (19/30), while the lowest rate of infection recorded in January 20% (6/30) (Table: 3). This result agrees with Abd Al-Wahab (3) and Kadhim (24) and Al-Zubaidi, (10) who recorded high infection rate of cryptosporidiosis among lambs in March and April. This result may be duo to good environmental condition (temperature and humidity) for the parasite and large number of *Cryptosporidium* oocysts, that shed from pregnant and lactating ewes in the farm which considered as a source of infection to the lambs (21,22,27,35)

Table 3. Prevalence of *Cryptosporidium* spp according to the Months

Examination methods	No.of fecal Examined Samples	No. of positive fecal samples	percentage %	Chi square value	P
January	**30**	**6**	**20**	**13.64**	**0.008**
February	**30**	**11**	**36.66**		
March	**30**	**19**	**63.33**		
April	**30**	**15**	**50**		
May	**30**	**16**	**53.33**		
Total	**150**	**67**	**44.66**		

The study shows significance difference between male and female infection rates, 31.74% (20/63) and 50.02% (47/87), respectively (Table 4). This result agreed with Akinkuotu and Fagbemi, (2014) in Nigeriawho found the high infection rate in females than males, 48.4%, 30.4% respectively, while this result disagreed with Abd Al-Wahab (3), Kadhim (24)in small lambs in Baghdad city and Rasheed (33) in goat kids in Iraq, who found no significance differences in the infection rate between male and female due to equal possibility of exposure to the contaminated environment (20). Also Table (4) shows the highest infection rate recorded in pregnant ewes while the lowest rate in non pregnant ewes,72.5%(29/40), 38.29% (18/47) respectively, with significant differences($p<0.05$) this result may occur due to the hormonal changes in pregnant animals and its effects on the immune status of the animal body(21,28,43).

Table 4. Prevalence of *Cryptosporidium* spp according to the sex and status

Sex	No.of Examined fecal Samples	No. of positive fecal samples	percentage %	Chi square value	P
Male	**63**	**20**	**31.74**	**7.33**	**0.006**
Female	**87**	**47**	**54.02**		
Pregnant	**40**	**29**	**72.5**	**10.17**	**0.001**
Non pregnant	**47**	**18**	**38.29**		

The study Calculate the shedding rate of *Cryptosporidium* oocysts from natural infected sheep, male and females (pregnant and non pregnant)(Table: 5).The increase and decrease of oocysts shedding in feces of infected animals occurring due to the hormonal changes and its effects on the immune status of the animals (11,21,28). The results of study recorded the highest mean numbers of oocysts shedding per gram of feces seen in pregnant ewes than non pregnant, 2082 oocysts per/gm 1440 oocysts per/gm respectively **with** significant differnces, and also the highest mean numbers of oocysts shedding per gram was shown in the end period of pregnancy while the lowest number of oocysts shedding per/gm recorded in infected males, 2460 oocysts per/gm, 1020 oocysts per/gm respectively with significant differnces. This results agreed with Kehrli *et al*,(25) and Nckerson *et al*,(29) who recorded a decreasing in efficiency of neutrophil cells during pregnancy, perparturition, parturition and post parturition periods, also Yang *et al*,(44) who recorded a decreasing in B and T lymphocyte cells 60% and 40% respectively in same period, which leads to reduced the immunoglobulin's especially the major types, IgG , IgM and IgA , synchronized with the pe-

riod of parturition and lactation, that lead to increase of oocysts shedding per/gm from infected animals.

Table 5. Total No and mean of oocysts shedding from infected animals

Sex of animals	Infected animals	Total No of shed oocysts per/gm feces	Mean No of shed oocysts per/gm feces
Male	**20**	**28560**	**1020±88.67^{e}**
Non pregnant female	**18**	**20160**	**1440±98.65^{d}**
Pregnant Female	**29**	**52050**	**2082±211.43^{b}**
First period pre.	**15**	**27450**	**1830±187.66^{c}**
End period pre.	**10**	**24600**	**2460±223.21^{a}**
Total	**67**	**100770**	**1504±114.21**

Different superscript refers to significant differences at p<0.05=

In conclusion, the increase of mean number of oocysts shedding in feces of infected pregnant ewes than males which occurring due to the hormonal changes and its effects on the immune status of the animals.

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