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Diagnostic study of Cryptosporidiosis in sheep in Al-Qadisiyah province

Weam Abbas Hamad Mansoor Jadaan Ali Alkhaled Coll. of Vet. Med. / Univ. of Al-Qadisiyah email: Mansoor.Ali@qu.edu.iq
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Abstract

The present study was designed and conducted during the period from September 2015 until April 2016 to evaluate the microscopic features of the *Cryptosporidium* parasite, and using of ocular micrometer for determination of length and width of oocysts. One hundred (100) fecal samples were collected from sheep from different areas in AL-Qadisiyah province. Results of microscopic examination showed that the oocyst of parasite appeared oval or spherical in shape, measured from (4.5-5) µm, with a dark pink or red in color on a blue ground. Fifty one 51(51%) sheep out 100 cases were positive. The highest rate of infection was observed in (1-3) years of age with significant differences at (P<0.05) level. There was no effects of sex on infection rate in sheep, and also observed that the highest rate (89%) was seen in November, while the least rate (11.1%) was in February, so has been showed significant differences at (P<0.05). According to the area the highest rate of infection was (100%) that observed in Al-Shamia while the least rate of infection was (11.1%) that observed in Al-bidder and the different results showed in other regions with a significant difference at (P<0.05).

Key words: Cryptosporidiosis, sheep, diagnosis, intestine protozoa.

دراسة التشخيصيه لداء الابواغ الخبيئه في الاغنام في محافظة القادسية

وئام عباس حمد منصور جدعان علي الخالدي كلية الطب البيطري / جامعة القادسية

الخلاصة

صممت وأجريت هذه الدراسة خلال الفترة الممتدة من شهر أيلول 2015 ولغاية شهر نيسان 2016 لمعرفة الصفات المجهرية للطفيلي ، وقد تم استخدام المقياس العيني المجهري لقياس الطول والعرض للمتكيسة . تم جمع 100عينة براز من الأغنام من مختلف المناطق في محافظة القادسية. اظهرت النتائج المجهرية ان المتكيسة من الطفيلي تبدو بشكل أجسام بيضاوية الشكل أو كروية ، ابعادها (4.5- μ m5) ذات لون وردي داكن أو احمر على أرضية زرقاء. وكانت النتيجه 51 بيضاوية الشكل أو كروية ، الغنام من اصل 100 حالة. و لوحظ أن أعلى معدل للإصابة في الأغنام في سن (1-3) سنوات مع وجود اختلافات كبيرة في مستوى المعنوية (80- μ 0). لم يكن هناك تأثير للجنس في إصابة الأغنام ، ولوحظ ان اعلى نسبه الصابة في شهر شباط حيث بلغت نسبه الاصابة نسبه الاصابة منهر الإصابة حيث بلغت نسبه الاصابة بينما كانت اقل نسبه اصابة مي شهر شباط حيث بلغت نسبه الاصابة بينما كانت اقل المناطق الدراسه ان ناحية الشامية سجلت اعلى اصابة حيث بلغت (80- μ 0) اما ال- بدير بلغت اصابتها (11.1%) حيث كانت اقل المناطق اصابة مع اختلافات معنوية عند مستوى احتمال (μ 0.05) الكلمات المفتاحية: داء الابواغ الخبيئة ، الاغتام ، التشخيص ، الاوالى المعوية.

Introduction

The apicomplexan protozoan parasite *Cryptosporidium* infected a wide range of animals, primarily causing diarrhea, gastritis and/or catarrhal respiratory signs (1, 2) currently there are approximately 25 valid species and more than 50 genotypes of *cryptosporidium* (3, 4). Transmission of Cryptosporidiosis is through ingestion of

oocysts from the infected individuals by contaminated food, water and pasture (5, 6). They should be considered a potential source of infection of *Cryptosporidium* either by direct transmission or by contamination of the environment (7). The species identified in sheep are C_. parvum, C. ubquitum, C. xiaoi, C. hominis, C. fayeri and C. andersoni (8).

Sheep are considered as an important aspect of livestock in the Arab world including Iraq. As a population of sheep in the Mediterranean area there were more than 100,984 million head, while the estimated number of sheep in Iraq was about 10, 50 million head (9). A great wealth requires attention, care and development, as well as sheep constitute the main source of meat in the Mediterranean area, 47.5% of the total meat in developed countries. In addition to be considered as an important source of milk, 21.35%, leather and wool (9). Studies are conducted in Iraq have recorded the infection rate of some intestinal protozoa; like in Baghdad province (85%) in lambs (10); in Al-Qadisiyah province (11) recorded the infection rate of some intestinal protozoa (50.2%) in lambs and children. The parasite is considered one of the basically enteric pathogens comparison with neonatal diarrhea and mortality in lambs and goat kids (12, 13). The most important

protozoa species of sheep reported in Iraq include *Eimeria*, *Cryptosporidium* and *Giardia* (7, 8, 9, 10, 11 and 12).

Materials and methods

One hundred fecal samples were collected randomly from sheep of different age ranged from 1 month to 5 years old of both sexes, during the period beginning from September 2015 to the end of April 2016. The study involved different areas in province of AL-Qadisiyah. Microscopic examination of the oocyst detected by examination each sample via staining the swab by Modified Ziel-Neelsen stain (MZN) (14), and flotation by using Sheather's solution (15), using of ocular micrometer for determination of length and width of oocysts. The data were analyzed by SPSS program (2010), using Chi-square $test(X^2)$ at $(P \le 0.05)$ level of significance.

Results

The *cryptosporidium spp*. oocysts in sheep were seen as an oval or spherical shape object taking dark pink or red color on blue ground, measuring between (4.5-5μm) (by ocular micrometer), when examined by light microscope under high oil emersion lens (Fig.1). Out of hundred (100) examined fecal sample of sheep, 51 (51%) were positive to *cryptosporidium spp*. infection. The highest rate (89.4%) of infection was seen in November, while the least rate (11.1%) was in February (Table 1). Regarding to the areas of the province the highest rates (100%) of infection was that observed in Al-shamia while the least (11.1%) rates of infection was

that observed in Al-bidder, and different rates of infection were seen in other regions and there were significant at (P<0.05) level (Table 2). According to the age of sheep (sheep were divided into four groups; from (1-6) months, (6-12) months, (1-3) years, and from (3-5) years). The results showed that the highest rate of infection (81.2%) that was observed in the ages of (1-3) years, while in the other groups show closely results ranging from (41.1- 46.3%), so there have been a significant differences at (P<0.05) level (Table 3). Our results in sheep showed that there was no significant difference between male and female at (P<0.05) (Table 4).





Fig. (1):-Show *cryptosporidium* oocysts in sheep stained with MZN, its size between (4.5-5µm) measured by ocular micrometer (X100).

Table (1): The rate of cryptosporidiosis infection in sheep according to the months of study

No. of No. of Percentage Month exam. positive % samples samples September 11 7 63.6 % A 2015 10 October 16 62.5 % A November 19 17 89.4 % B 25 10 December 40 % A January 11 5 45.4 % A 2016 2 February 18 11.1 % C 100 51 51 % Total

Similar letters refers to non- significant difference. Different letters denote the significant differences at (P<0.05)

Table (2): The rate of cryptosporidiosis infection in sheep according to the area of the study

| Area | No. of exam. samples | No. of positive samples | Percentage % | |
|--------------|----------------------|-------------------------|--------------|--|
| City center | 11 | 7 | 63.6 % A | |
| Al-Nora | 16 | 11 | 68.7 % A | |
| Al-Daghara | 8 | 2 | 25 % B | |
| Al- Shaffiea | 25 | 12 | 48 % A | |
| Al-Sania | 11 | 6 | 54.5 % A | |
| Al-bidder | 18 | 2 | 11.1 % C | |
| Al-shamia | 11 | 11 | 100% D | |
| Total | 100 | 51 | 51 % | |

Similar letters refers to non-significant difference. Different letters denote the significant differences at (P < 0.05).

Table (3): The rate of cryptosporidiosis infection in sheep according to the age

| Age | No. of exam. samples | No. of positive samples | Percentage % | |
|-------------|----------------------|-------------------------|--------------|--|
| 1-6 months | 41 | 19 | 46.3 % A | |
| 6-12 months | 17 | 7 | 41.1 % A | |
| 1-3 years | 16 | 13 | 81.2 % B | |
| 3-5 years | 26 | 12 | 46.1 % A | |
| Total | 100 | 51 | 51 % | |

Similar letters refers to non- significant difference. Different letters denote the significant differences at (P< 0.05).

Table (4): The rate of cryptosporidiosis infection in sheep according to the sex

| Type | Total | Male | No. of positive samples | Percentage % | Female | No. of positive samples | Percentage % |
|-------|-------|------|-------------------------|--------------|--------|-------------------------|--------------|
| Sheep | 100 | 27 | 12 | 44.4% A | 73 | 39 | 53.4% A |

Similar letters refers to non-significant difference at (P<0.05)

Disscusion

Results are announced that the total infection rate of *Cryptosporidium* in sheep is 51%. This result is according closely with another study in the same area (15) in which the total rate in sheep is 48.8%, while (16, 17, 18) show the rate of infection in sheep is 36% which is less than the present study. Different infected rates are registered (19-31); in Baghdad it found 5.85% (20). Our study not agreed with (20). (11) find the rate in Al-Diwanyah 15.8% in sheep, and 17.7% in Al-Muthana (15), 18% from 50 samples in Basrah (19), 10.1% (27) in Mosul, 36.43% in sheep (7), in Baghdad 74.2 % in ewe and 81.4% in lamb (26) which is higher from our study. The rate of infection in the world is; in brazil 30% which recorded by (31), (22) in Turkey, the rate was 3.8%, and in Nigeria by (23) which is 22.7% in sheep total of 15% in

ZN stain show positive in the study, (25) in Australia, in Bangladesh 11.3% (29), but high infection rate of Cryptosporidium spp. oocysts 64.37% (56/87) were observed in lambs (27). The reason for these differences in infection rates depends on many factors; like size of sample, breeding condition, the method of diagnosis may which has great effect on the detection of the rate of infection specially population density. In Iraq small ruminants breeding in opening farmyard which low number but in world it breed in the flocks in large number in small area lead to infect. The water which is great source of transmition (28), the effected of environment factor is limited because of the oocyst will be sporulated without any special circumstance and have resistant to high temperature and humidity, on the other hand the viability

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of oocyst reduce with high temperature up to 15C° (29). In Iraq the infection will be lower because it is from tropical area compare with world low-temperature areas. According to the months of study, the present study is in agreement with the (19), he recorded high infection rate in November, but we dissimilar with (7) which has the highest infection in February in sheep, The reason of that make the climatic conditions which keep the oocyst in environment still survive, and prepare the condition with the season of birth and milking in this month. The mosquito play important role in transmission Cryptosporidium also in this months will breed deferent number of animals. additionally the study not include all the months of the year. All that factors are

important to make the differences in the rate of infection. Our study pointed that there is differences in the rang of the infection between the areas of study, the highest rate of infection 100% in Shamia but the least rate of infection in Al-bidder 11.1 %, that agreed with (7) which reported no significant difference between the area of study, and diverge with (30,31) those denote a significant difference between the area of study, that because the breeding conditions which rise in area and lower in another and the random way of breeding to defiant animal in the same area in large number lead to contamination of the water, milk, feed, with oocyst and accumulation of feces in the same area which is best media for keeping oocyst a life (31).

Referances

- 1-O'Donoghue PJ (1995) Cryptosporidium and cryptosporidiosis in man and animals. Int. J. Parasitol. 25, 139–195.
- 2-Ryan UM (2010) *Cryptosporidium* in birds, fish and amphibians. Exp.Parasitol.124:113-120.
- 3-Ryan UM, Bath C, Robertson I, Read C, Elliot A, McInnes L, Traub R, Besier B (2005) Sheep may not be an important zoonotic reservoir for *Cryptosporidium* and *Giardia* parasites. Appl. Environ. Microbiol. 71, 4992–499.
- 4-Santin M, Trout JM, Fayer R (2007) Prevalence and molecular characterization of *Cryptosporidium and Giardia* species and genotypes in sheep in Maryland. Vet. Parasitol. 146:17–24.
- 5-Fayer R (2010) Taxonomy and species delimitation in *Cryptosporidium*. Exp. Parasitol. 124: 90-97.
- 6-Rose JB, DE Huffman, A Gennaccaro (2002) Risk and control of waterborne cryptosporidiosis. FEMS Microbiology Review. 26: 113-123.
- 7-Castro-Hermida JA, Gonz lez-Warleta M, Mezo M (2007) Natural infection by *Cryptosporidium* parvum and Giardia duodenalis in sheep and goats in Galacia (NW Spain) Small Rumin. Res.72:96-100
- 8-Xiao L (2010) Molecular epidemiology of cryptosporidiosis: An update. Exp. Parasitol. 124: 80-89.
- 9-Abd Al-Wahab IH (2003) Study in the epidemiology of the intestinal protozoa in the sheep in Baghdad province. M.Sc. Thesis, College of Vet. Medicine, University of Baghdad.
- 10-AL-Gelany BA (2003) Epidemiological and diagnostic study *Cryptosporidium* animal. Ph.D. thesis College of Vet. Medicine, University of Baghdad.
- 11-Dawood KA, Abdullah SR (2007) Identification of some causative agents of diarrhea in children and lambs of Diwaniyh. Al-Qadisiyah J. Vet. Sci.-Supplement of 3rd conference.

- 12-Olson ME, BJ Ralston, R O'Handley, NJ Guselle, AJ Appelbee (2003) What is the clinical and zoonotic significance of cryptosporidiosis in domestic animals and wildlife. p. 51–68.
- 13-De Graaf DC, E Vanopdenbosch, LM Ortega-Mora, H Abbassi, JE Peeters (1999) A review of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol. 29:1269–1287.
- 14-Bearever PC, Jung RC (1985) Animal Agents and Vectors of Human Disease. 5thed –Lea. and Fibiger, Philadelphia –pp:37-41.
- 15-Mohammed MM (2013) Serological study of sheep of coccidiosis in Al-muthana, Kufa J. for Vet. Medical Sci.; 4 (1): 1-9
- 16-Chermette R, Boufassa QS (1988) Cryptosporidiosis a Cosmopital Disease in Animals and Man, 2nd ed. Office International Epizooties. France.
- 17-Causape AC, Quilez J, Sanchez-Acedo C, del Cacho E, Lopez-Bernad F (2002) Prevalence and analysis of potential risk factors for *Cryptosporidium parvum* infection in lambs in Zaragoza (northeastern Spain), Vet Parasitol, 104, 4, 287-98.
- 18-Ruest N, Faubert GM, Coutuer Y (1998) Prevalence and geographical distribution of *Giardia* spp. and *Cryptosporidium* spp. in dairy farms in Quebec.Can.Vet.j.39(11):697-700.
- 19-Ali NH (1998) Prevalence of cryptosporidiosis with zoonotic aspect. M.Sc. Thesis, Collage of Medicine, University of Basrah.
- 20-AL-Zubaidi MTS (2009) Some epidemiological aspects of Cryptosporidiosis in goats and Ultra structural study. Collage Veterinary Medicine Ph.D. thesis, University of Baghdad.133 pp.
- 21-Fadl SR, Kelaf DA, Abbas SM (2011) Prevalence of parasitic infection in sheep from different

- regions in Baghdad, Iraqi J. Vet. Sci; 35(1): 204-209.
- 22-Erman N, Beyazit A, Oz I (2000) The prevalence of cryptosporidiosis in lambs and goat kids in Izmir province. J. Bor. Vet. Cont. Res. Inst. 25:33-38.
- 23-Faleke OO, Sahabi K, Aliyu AB (2006) Prevalence of *Cryptosporidium* in slaughter sheep and goats at Sokoto, Nigeria. Anim. Produ. Res. Adv. 2(3): 179-182.
- 24-Food and Agriculture Organization (FAO) (2009). http://dad.fao.org/cgi-bin/.
- 25-Jenkins DJ (2005) Hydatid control in Australia: Where it began, what we have achieved and where to from here. International J. Parasitology, 35: 733-740.
- 26-Kadhim TA (2009) Histological study of *Cryptosporidiosis* in sheep of Baghdad province. M.Sc. Thesis submitted to the University of Baghdad-College of Veterinary Medicine.

- 27-Khalil Lian Yassin (2000) compared the efficiency in the province Ninoy. rsalh Master College of Veterinary Medicine University of Mosul.
- 28-Sevinc F, Simsek A, Usla U (2005) Massive *Cryptosporidium parvum* infection associated with an outbreak of diarrhea in neonatal goat kids. Turk. J. Vet. Anim. Sci. 29: 1317–1320.
- 29-Siddiki A, Sohana A, Zinat F, Bibi A, Rasel D, Mohammad Alamgir H (2015) Molecular characterization of *Cryptosporidium xiaoi* in goat kids in Bangladesh by nested PCR amplification of 18S rRNA gene. 5(3): 202-207.
- 30-Swadi HA (2008) Study of prevalence of *Giardia* infection in Iraqi sheep of AL-Yusufiya /Baghdad province, Journal of Kerbala. J. University, Vol. 6 No.2 Scientific. P. 128-131.
- 31-Vieira LS, Silva MB, Tolentino AC, Lima JD, Silva AC (1997) Outbreak of Cryptosporidiosis in dairy goats in Brazil. Vet. Rec. 140: 427 428