IDENTIFICATION OF *Strongyloides papillosus* AND OTHER GASTROINTESTINAL PARASITES OF CATTLE IN BASRAH PROVINCE

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ABSTRACT

The present study has been conducted for detection of *Strongyloides papillosus* parasite isolated from cattle in Basrah province from November 2018 to June 2019. 255 and 300 samples were collected from fecal and serum of bovine, respectively. Samples were collected from slaughterhouse and animals from regions in Basrah province. Ten serum samples were from Mosul province. 255 fecal samples submitted to the parasites lab. for routine microscopic examination and 7.54% of those samples were detected positive for S.*papillosus*. Serum samples tested by ELISA (SS-IgM) and the results confirmed that 34.7% of samples were infected with *S.papillosus.* ELISA showed a high infection rate in comparison with microscopic examination. Also, this study has detected other types of gastrointestinal parasite of cattle. Parasites identified in this study included: nematode, *Toxocara vitulorum* (13.2%),*Capillaria bovis* (1.88 %), *Gongylonemia spp*. (3.77%) (first report in Basrah city),*Oesophagostomum spp.*(3.77%), *Trichuris spp*. (3.77 %), *Trichostrongylus spp*. (20.75%),*Ostertagia spp.* (1.88%); Cestoda ,*Moniezia expansa*(1.88 %); Protozoan, *Eimeria spp.* (3.77 %),*Balantidium spp.* (1.88 %),*Isospora spp*. (1.88%), *Giardia lamblia* (3.77%), *Cryptosporidium spp*. (5.66%), *Entamoeba histolytica*(11.32%) and Trematode , *Paramphistomum spp*. (7.54%),*Fasciola spp*. (5.66%).

INTRODUCTION

Nematode infections of cattle are a constraint on effective breeding of cattle in pastures all over the world [(1). (2)] Noted that predominant Strongyloide species have been observed in sheep and sheep. For many years *Strongyloides papillosus* in

particular has been considered to be of doubtful pathogenicity in livestock [3]*S.papillosus* can infect cattle and sheep through ingestion, skin penetration and lactating ewes milk. Only female worms happen in the small intestine as parasites [4]The Pathogenic influence of the parasite on host is a result of the presence of migrating larvae, which damage the host's tissues mechanically [5] *Strongyloides* within the Rhabditoidea superfamily are small, only the female worms are parasitic and adult worms produce eggs by the process of parthenogenesis first stage larvae (L1) of parasite are excreted in the hosts' feces [6] Gastrointestinal parasitism caused a disease of different genera of parasites that inhabit the digestive tract of livestock , causing economic losses [7]

S. papillosus rate was observed in the cattle 10.7% in Kirkuk province [8], 11.49% in Mosul city [9] and 2.8% in Basrah city [10]. Diagnosis of *Strongyloides spp.* larvae in the fecal remains the standard by wet direct smear, sedimentation and flotation techniques have a low sensitivity [11] .There is no generic gold diagnostic method available for *Strongyloides*. Serologic method works by detecting IgM serum against a crude extract of *S.stercoralis* filariform larvae. Many studies evaluating ELISA are conducted in proven cases of *Strongyloides* following parasitological detection of larvae in stool samples. Animal studies showed that IgM was positive one week after experimental infection of dogs with *S.stercoralis* and IgM showed an increase soon after experimental infection [12]. The aim of this study is to identify the current infections of the *Strongyloides papillosus* with direct and serological ELISA (SS-IgM) examination to compare between two procedures and identification another gastrointestinal parasites from cattle in Basrah province.

MATERIALS AND METHODS

Samples Collection: Total of 300 serum samples and 255 fecal samples were collected from cattle collection from slaughterhouse, and animals from AlQurna farms and different regions in Basrah province and 10 serum sample from Mosul province, in a period from November 2018 to June 2019.

Identification methods

Laboratory examination: Fecal samples were subjected to macroscopical; color, diarrhea, softy and semi-solid feces, and microscopical examination. Detection and identification of parasite eggs and larvae were carried by applying direct microscopic examination, and concentration method: floatation and sedimentation method

according to techniques and morphological characteristics suggested by (13,14,15,16) .

Serological methods: The Kit was used sandwich enzyme-linked immunosorbent assay (ELISA) to qualitatively analyze Bovine *Strongyloides stercoralis* Antibody (SS-IgM) in Bovine serum (MyBioSource in San Diego) USA ,88 out of 310 serum samples were selected for ELISA, the first group (Ss&i) samples of the expected area of infection *Strongyloides papillosus* from slaughterhouse and ALQurna farms in Basrah province, the second group (pi) samples of cattle infected with other parasite , the third group (PiM) samples from Mosul city, the fourth group (N) negative samples from Basrah province as in (Table 1).

Table1: Groups of serum samples of *S.papillosus* examined by the ELISA.

GR,No.	samples	Area of collection	N. of serum samples
1	(Ss&i)	AlQurna farms and slaughterhouse in Basrah province	23
2	(pi)	Different area in Basrah province	18
3	(PiM)	Mosul province	10
4	(N)	Basrah province	37

RESULTS

In this study, the direct microscopic examination and Concentrated methods showed the presence of 4 cases of *Strongyloides papillosus* eggs and larvae in the fecal samples and 8 cases identifiction by serologic examination as in (Table 2). The overall gastrointestinal parasites in cattle were (20.78%) .They are identified by direct and concentration methodes, they included Nematode: *Trichostrongylus spp*. (20.75%), *Toxocara vitulorum* (13.2%), *Strongyloides papillosus* (7.54%), *Oesophagostomum spp*. (3.77%), *Gongylonemia spp*. (3.77%) (First reported in Basrah), *Trichuris spp*. (3.77%), *Ostertagia spp*. (1.88%), *Capillaria bovis* (1.88%). Cestode:*Moniezia expansa*(1.88%).Trematode: *Paramphistomum spp*. (7.54%),*Fasciola spp*.(5.66%). Protozoa: *Entamoeba histolytica*(11.32%), *Cryptosporidium spp*. (5.66%),*Giardia lamblia*(3.77%),*Eimeria spp*. (3.77%).*Balantidium spp*. (1.88%), *Isospor spp*. (1.88%) (Table 3, figure 1).According to data, gastrointestinal parasite infection was (27.27%) in December, (15.90%) in February and (31.57%) in April (Table 4) with significant differences (P <0.001). Data analysis revealed that the percentage of gastrointestinal parasites in female was (30.66%) and in male was (16.66%) without significant difference (P<0.012) (Table 5). The percentage of gastrointestinal parasites in cattle among different regions of

Basrah province without significant differences (P<0.037) (Table 6). The present study demonstrated the percentage of the gastrointestinal parasites species depend on sex (Table 7).

Table 2: The percentage direct, concentration and serologic examination to diagnosis of *S.papillosus*.

S.papillosus	Direct examine N.I %	Floatation method N.I %	Sedimentation method N.I %	ELISA assay N.I %
	2(3.7%)	1(1.88%)	1(1.88%)	8(34.7%)
Total	7.54%			(34.7%)

N.I=Number infected , (%)=Percentage.

Table 3 : percentage of gastrointestinal parasite species in Cattle.

Gastrointestinal parasite	N.E	N.I	N.P.	(%)
(Nematode)				
Strongyloides papillosus	255	53	4	7.54
Toxocara vitulorum	255	53	7	13.2
Capillaria bovis	255	53	1	1.88
Gongylonemia spp.	255	53	2	3.77
Oesophagostomum spp.	255	53	2	3.77
Trichuris spp.	255	53	2	3.77
Trichostrongylus spp.	255	53	11	20.75
Ostertagia spp.	255	53	1	1.88
)Cestodes(				
Moniezia expansa	255	53	1	1.88
(Protozoan)				
Eimeria spp. Balantidium spp. Isospora spp.	255	53	2	3.77
Giardia lamblia	255	53	1	1.88
Cryptosporidium spp.	255	53	1	1.88
Entamoeba histolytica	255	53	2	3.77
	255	53	3	5.66
	255	53	6	11.32
(Trematoda)				
Paramphistomum spp.	255	53	4	7.54
Fasciola spp.	255	53	3	5.66

N.E = Number examined, N.I.=Number infected , N.P. Number positive, (%)=Percentage.

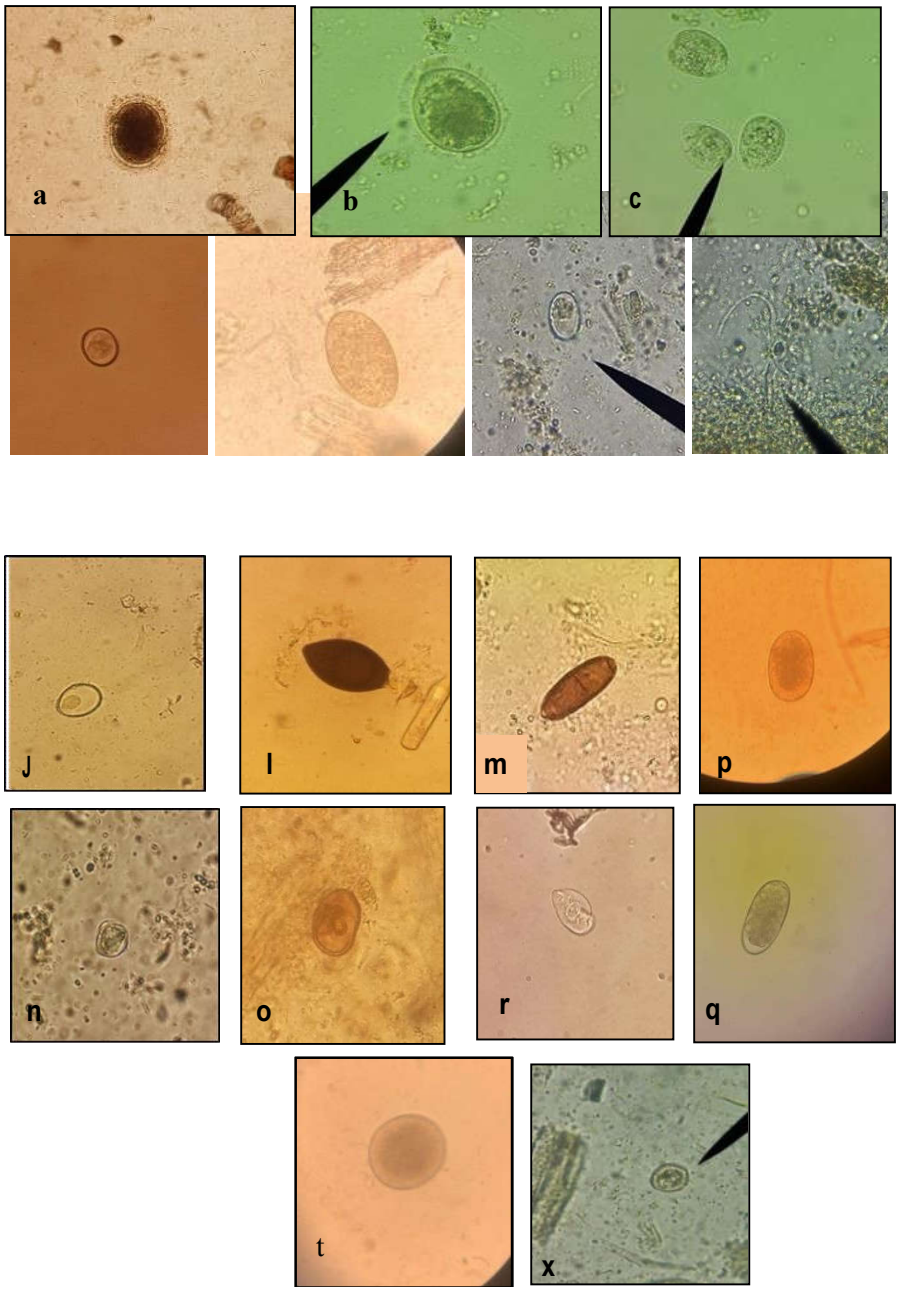

Figure 1: Direct and sedimentation method of fecal samples of animals :
a-*Toxocara vitulorum*(40x) b-*Balantidium spp.*(40x) c-*Strongyloides papillosus*(40x) e-*Isospora spp.*(100x) f- *Fasciola spp.*(40x)) g-*Cryptosporidium spp.* (100x) i-Larva *S.papillosus* (100x) J-*Eimeria spp.* (100x) l-*Trichuris spp.*(100x) m-*Capillaria bovis* (100x) n-*Moniezia expansa* (40x)o-*Gongylonemia spp.*(100x) p-*Oesophagostomum spp.(*40x*)* q-*Trichostrongylus spp.* (40x) r- *Giardia lamblia* (100x)t- *Ostertagia spp.* (40x) x- *Entamoeba histolytica*(100x).

Table 4 : Relation of gastrointestinal parasite infection with months of year

Month	N.E	N.I	%
November	10	0	0
December	22	6	27.27
January	24	10	41.66
February	44	7	15.90
March	54	7	12.96
April	38	12	31.57
May	35	11	31.42
June	28	0	0
Total	255	53	20.78
X^2= 24.62 , P <0.001			

N.E = Number examined, N.I.=Number infected , N.P. Number positive, (%)=Percentage.

Table 5: Relation of gastrointestinal parasite infection with sex of animals.

Sex	N.E	N.I	(%)
Male	180	30	16.66
Female	75	23	30.66
Total	255	53	20.78
X^2= 6.302 , P <0.012			

N.E = Number examined, N.I.=Number infected , N.P. Number positive, (%)=Percentage.

Table 6 : Relation of gastrointestinal parasite infection with area of study.

Region	N.E	N.I	(%)
Slaughtered	200	36	18
ALQurna	55	17	30.90
Total	255	53	20.78
X^2= 4.36 , P <0.037			

N.E = Number examined, N.I.=Number infected , N.P. Number positive, (%)=Percentage.

Table 7 : Percentage gastrointestinal parasite species in male and female infected cattle.

Gastrointestinal parasite	Male				Female			
	N.E	N.I	N.P.	(%)	N.E	N.I	N.p.	(%)
(Nematode)								
Strongyloides papillosus	180	30	4	13.3	75	23	0	0
Toxocara vitulorum	180	30	4	13.3	75	23	3	13.04
Capillaria bovis	180	30	1	3.33	75	23	0	0
Gongylonemia spp.	180	30	2	6.66	75	23	0	0
Oesophagostomum spp.	180	30	2	6.66	75	23	0	0
Trichuris spp.	180	30	0	0	75	23	2	8.69
Trichostrongylus spp.	180	30	1	3.33	75	23	10	43.37
Ostertagia spp.	180	30	1	3.33	75	23	0	0
(Cestodes)								
Moniezia expansa	180	30	1	3.33	75	23	0	0
(Protozoan)								
Emeria spp.	180	30	1	3.33	75	23	1	4.34
Balantidium spp.	180	30	1	3.33	75	23	0	0
Isospora spp.	180	30	1	3.33	75	23	0	0
Giardia lamblia	180	30	1	3.33	75	23	1	4.34
Cryptosporidium spp.	180	30	2	6.66	75	23	1	4.34
Entamoeba histolytica	180	30	3	10	75	23	3	13.04
(Trematoda)								
Paramphistomum spp.	180	30	3	10	75	23	1	4.34
Fasciola spp.	180	30	2	6.66	75	23	1	4.34

N.E = Number examined, N.I.=Number infected , N.P. Number positive, (%)=Percentag

In the present study ELISA results demonstrated that cut-off values > 0.25 .The infection of *S.papillosus* in (Sss&i) group were 8 serum samples from total 23 samples, 2/18 serum samples of (pi) group were identified as infected with this parasite, but there is no cross reaction 0/10 in (pim) group, while the (N) group demonsted 3/37 serum samples positive to *S.papillosus* parasites (Fig 2).On the other hand, the comparison between study group by ELISA showed that the existed of IgM not only in the suspected infection group(Sss&i) but also in (pi) and(N) groups. Which showed Significant differences at ($P \leq 0.05$)(Fig2).

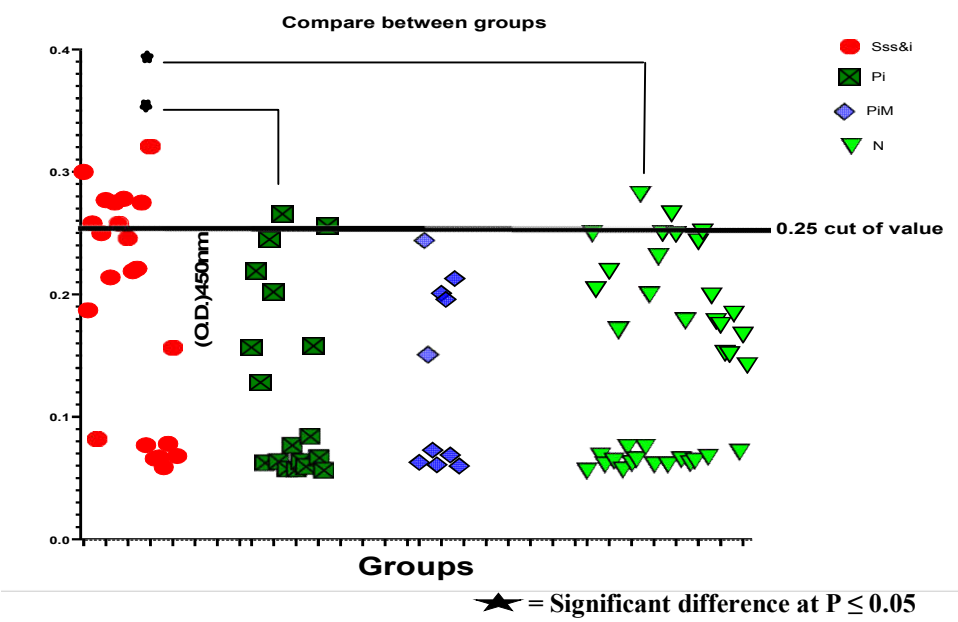

Fig 2 : Results of ELISA to qualitatively analysis bovine *S. stercoralis* antibody (SS-IgM) in Bovine serum, between all groups.

Sss&i = *S. papillosus* group.No=23 , Pi= Infection with another parasites .No.=18, PiM= Infection with another parasites in Mosul. No.=10, N=negative serum=37.

The percentage of sensitivity and Specifity in ELISA(SS-IgM) kit which estimated as in table (8).

Table 8 : Number of serum infection with *S.papillosus* and Another type of gastrointestinal parasites

Serum of infection cattle	No.	+Ve	-Ve
S. papillosus	4	4(a)	0(b)
Another gastrointestinal parasites	18	2(c)	16(d)

(a)= True positive ,(b)= False negative, (c)= False positive, (d)= True negative.

Sensitivity % = $\frac{a}{a+c}$=100% Specifity % = $\frac{d}{d+c}$= 88.8%

The data of ELISA results show that there were significant differences between suspected infection area (Ss&i) group and (N) group while there is no significant differences between (Pi) group and (PiM) group with negative group at (P ≤0.05) Fig (3,4,5).

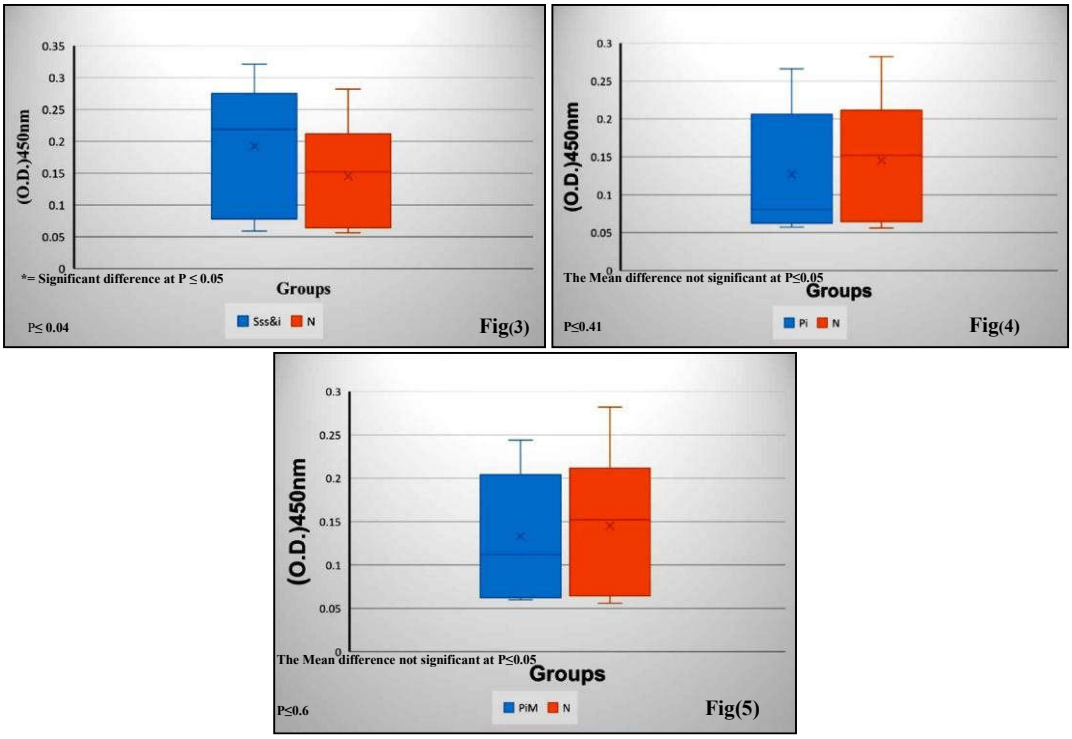

Fig 3,4,5: The ELISA results to qualitatively analysis bovine *S. stercoralis* antibody (SS-IgM) in bovine serum, compared the Negative group(N) with *S. papillosus* group(Ss&i) , the Infection with another parasites group (pi) and the infection with another parasite in Mosul (PiM).

DISCUSSION

The overall prevalence of gastrointestinal parasite infection in the areas of this study could be attributed to poor immunity of hosts as a result of malnutrition, grazing of young and adult animals together in poorly drained land provide an ideal condition for the eggs of end parasites to build up clinical infestation of the host [17,18] . The percentage of gastrointestinal parasites in this study (20.78%) while[19] was reported (21.50%) of gastrointestinal parasite in mosul (9) were reported (53.53%) in Mosul province and [20] reported 69.60 % in Ethiopia.

Species of gastrointestinal parasite recovered in the present study was 7.54%, also reported in some countries [10] 2.8% in Basrah and [21] 3.76% in India, the results also

showed that the cattle are infected with parasite *Gongylonemia spp.* (3.77%).On the

other hand, the high percentage of infection cattle in April and January with significant difference

may be explained by the increase in rain, humidity and decrease temperature [22].

However the female showed a high percentage infection as compared with male of cattle this finding is in an agreement with [23]. The high percentage of gastrointestinal parasites infection in different regions of Basrah province infection without significant difference may be attributed to open grazing in pasture. Through laboratory examination of 255 fecal samples by using direct examination and concentration methods to diagnosis *S.papillosus* we could detect (7.54 %) while serological examination detect(34.7%) that agrees with [23] reported (35.29%) in Diyala, (9) (11.49%) in mosul province, [8] were (10.7%) in Kirkuk provinc and [24] reported (28.45%) in India while disagree with [10] reported (2.8%) in Basrah province, [24] were observed (3.4%) in India and [25] were observed (0.49%) in India.

The percentage of *S. papillosus* infection by using microscopic examination was (7.54%) low than serological examination because of low sensitivity of this examination [27] Also, may be of temperature and salinity in basrah province, according to [28] the most favorable conditions for the development of the parasite are a temperature above 20°C and a rain extent ensuring level of humidity. [29]) referred that infective larvae of *S. stercoralis* are attracted to low concentrations of salt and avoid of high concentrations of salty.

The ELISA assay may be the best diagnosis, the ELISA test is one of the most effective and reliable immunological tests in all research and medical centers, because of its accuracy and specificity. Data in this study showed that Specify at 88.8% and Sensitivity at 100% ,on the other hand, the comparing among study group by ELISA showed that the existed of IgM not only in the suspected infection group(Sss&i) but also in (pi) and (N) groups. Which showed Significant difference at ($P \leq 0.05$) (Fig 2). In this study the result of ELISA, the infected cattle showed positive result in comparison with negative control uninfected cattle (N) group but this uninfected cattle may had previous infection and also (pi) group of cattle infectad another parasites which this groups showed a positive result of the infected *S.papillosus* that agreed with [30,31,32] who indicated that the ELISA assay has a high sensitivity, yet

serological antibody tests can have cross-reactivity if other helminth infections are present or these animals may have a previous infection which means that IgM antibody appeared in the serum during few weeks of infection that agreed with [12] who indicated that animal studies showed that IgM was positive one week after experimental infection of dogs with *S.stercoralis*.

تشخيص طفيلي *Strongyloides papillosus* والطفيليات المعوية الأخرى التي تصيب الابقار في مدينة البصرة

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الخلاصة

أجريت هذه الدراسة لتشخيص طفيلي*Strongyloides papillosus* المعزول من الأبقار في مدينة البصرة للفترة من الاول من كانون الثاني ٢٠١٨ لغاية نهاية حزيران ٢٠١٩. تم جمع (٢٥٥) عينة براز ومصل الأبقار ، من المجزرة المركزية ومناطق مختلفة في مدينة البصرة. بالإضافة الى (١٠) عينة مصل من محافظة الموصل. فحصت عينات البراز في مختبر الطفيليات بالفحص المجهري الروتيني حيث تم الكشف عن ٧.٥٤ ٪ من تلك العينات تم الكشف عن *S.papillosus*عينات المصل التي تم اختبارها بواسطة ELISA (SS-IgM) وأكدت النتائج أن ٣٤.٧ ٪ من العينات كانت مصابة *S.papillosus* أظهرت ELISA نسبة إصابة عالية مقارنة بالفحص المجهري. أيضا ، كشفت هذه الدراسة عن أنواع أخرى من الطفيليات المعوية المعوية للماشية. وشملت الطفيليات التي تم تحديدها في هذه الدراسة الديدان: nematode ,*Capillaria bovis* (1.88 %), *Ostertagia spp.* (1.88%), *Gongylonemia spp*. (3.77%) (first report in Basrah city),*Oesophagostomum spp.*(3.77%), *Trichuris spp*. (3.77 %), *Toxocara vitulorum* (13.2%), *Trichostrongylus spp*. (20.75%),; Cestoda ,*Moniezia expansa*(1.88 %); Protozoan, %),*Balantidium spp.* (1.88 %),*Isospora spp*. (1.88%), *Giardia lamblia* (3.77%), *Eimeria spp.* (3.77) *Cryptosporidium spp*. (5.66%), *Entamoeba histolytica*(11.32%) and .*Fasciola spp*. (5.66%), *Paramphistomum spp*. (7.54%) ,Trematode

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