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Molecular detection of *Haemonchus spp* parasite in sheep in Babylon province

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Abstract
The study involved 100 feces samples of sheep, in Babylon Governorate in Iraq. Used for diagnosis of *Haemonchus spp*, Traditional methods and polymerase chain reaction (PCR), using specific primers and the method was the conventional PCR. Sheep was classified into subgroups according to age, area and sex in this study association of these risk factors with rate of infection. The diagnosis of *Haemonchus spp* in sheep was determined by method Microscopic examination (Traditional methodes). In the present study, the result of the study revealed that infection percent in sheep was (41%) The result of the study revealed that infection percent in sheep by (Traditional methodes) rate according to the geographical areas infections was highest in Western hamza and Al-Musayyab district which were (50%) and the lower prevalence of *Haemonchus* spp was in Al-Qassim district which was (30%), The statistical analysis reveals that there was no significant (p>0.05).The infection rate of *Haemonchus spp* infections by (Traditional methodes) rate was highest in the the (< 1 year) age group which was (44.82%) while the lower prevalence of infection was in the (> 2 years) age group which was (38.70%) no significant (p>0.05) The higher infection rate of *Haemonchus spp* infection by (Traditional methodes) was at females (42.85%) which was and lower prevalence of infection at males which was (36.66%), no significant effect at (p>0.05). In the present study, the result of the study revealed that infection percent in sheep by (PCR) was (68%), 68 feses samples were positive for *Haemonchus worm* visible bands in the PCR product at 454 bp. The result of the study revealed that infection percent in sheep by (PCR) rate according to the geographical areas infections was highest in Al-Qassim and Al-Mahaweel districts which were 80% and the lower prevalence of *Haemonchus* spp was in Al-Musayyab district which was (46.15%), The statistical analysis recorded that there was Significant difference (0.047). The infection rate of *Haemonchus* spp infections by (PCR) rate was highest in the (> 2 years) age group which was (74.19%), while the lower prevalence of infection was in the (< 1 years) age group which was (62.06%) no significant at (p>0.05). The higher infection rate of *Haemonchus* spp infection by (PCR) was at males which was (76.66%) and lower prevalence of infection at females which was (64.28%), no significant at (p>0.05).

Keywords: *Haemonchus Spp*, PCR, sheep, Iraq

1. Introduction
Haemonchus spp. is a blood-sucking *nematode* parasite, which was first described in 1803 by Karl Rudolphi It belongs to the superfamily *Trichostrongylidae* and along they consist the most pathogenic nematode parasites infecting ruminants in the developing world It can infect all species of ruminants (Perry *et al*., 2002) [66], expressing its pathogenicity through the blood feeding activity The genus *Haemonchus* includes over 10 species within which *Haemonchus contortus* and *Haemonchus placei* are the most widespread helminths. According to (Hoberg *et al*. 2004) [43], these two parasites are globally distributed not only in the abomasa of domestic but also in non-domestic ruminants. *H. contortus* infects mainly small grazing ruminants while *H. placei* has been detected in the abomasa of large ruminants (i.e., cattle). Other parasitic species such as *H. longistipes* infect six species of ruminants, focusing on camels, while the rarer species, *H. mitcelli* and *H. vegliai*, infect mainly antelopes and deer, respectively (Scheuerle *et al*., 2012) [74].

2. Materials and Method
The study included collecting feces samples from 100 sheep in Babylon province. These feces samples were subjected to Traditional methodes and molecular detection by PCR assay Rate of *Haemonchus spp* infection in sheep determined by PCR assay

2.1. Collection of blood samples

Fecal samples (5 grams) were collected from 100 sheep, of both sex and different ages, were examined in different areas of the Babylon province, including Mahaweli, AlQasim, Al-Musayyab and Western hamza from September 2023 to January 2024.All samples were transported with ice bags to the laboratory of parasitology in veterinary college/ University AL- Qasim Green for examination.

2.2. Primers That Used in the Present Study

Haemonchus spp isolate ITS fetus's small sub-unit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence. The PCR primers for sheep was designed in this study by using NCBI dbSNP database. GenBank: KJ724525.1 (Table - 1).

2.3. Statistical Analysis

Data were summarized, presented and analyzed using statistical package for social science (SPSS version 16) and Microsoft Office Excel 2007. For the determination of the significant difference among one way analysis ANOVA was used. A p-value

Table 1: The primers names and sequences with product size (NADH dehydrogenase subunit 4 (ND4) gene)

No	(18s rRNA) Primer sequence 5´ to 3´	Amplicon size	Annealing TM	Genbank
PF	GCACATGTAGAAGCTCCCACC	454 bp	54	KJ724525.1
PR	AGAAGGGGGTATACCTCTGTTT			

3. Results and Discussion

3.1. Macroscopic examination of sheep infected with *Haemonchus worm* Infected animals suffered from several clinical signs such as emaciation with pallor of the mucous membrane, anorexia, submandibular oedema (Bottle jaw), diarrhea, anemia, and easy wool shedding (The main clinical signs of sheep infected with digestive parasites) These clinical signs were agreement with researchers (Getachew *et al*., 2007; Williams and Palmer 2012) [38, 83] who founded weight loss, sub-mandibular edema (Bottle jew), diarrhea, sever anemia, anorexia, emaciated, paleness of the mucosal membranes.

2. Microscopic examination

Rate of *Haemonchus worm* infection in sheep determined by (Microscopic examination).

During this study collected of 100 fecal samples of sheep from different areas in Babylon Governorate the infection rate were (41%) to infection with *Haemonchus worm* when examined microscopically after used of methods technique to diagnosis the *Haemonchus* worm eggs (Table 1). the current study agreement with study in Erbil that founded the infection rate of *Haemonchus worm* was (40.46%) Ahmed *et al*., (2015) [18]. Khalaf *et al*., (2023) [48] recorteded (44%), (42%) infection rate with *Haemonchus worm* in mosul and erbil respectively, and nearly similar to results were reported by (Makawi 2000) [57] In Wasit (33.33%) infection rate in haemonchosis. this study agreed with in the world the results of studies like in Bangladesh which prevalence of *Haemonchus contortus* infection in sheep was (40%) Hossain *et al*., (2015) [42]. (Gebresilassie and Tadele, 2015) [37] in Ethiopia was (40.9%). This study disagreement with studies in lraq which recorded differed infection rate was (6.25%) in Al-Diwaniyah, (Jasim and Aaiz 2021) [47]. In AL-Muthanna was (10.53%), (Alani 2023) [7]. While in Baghdad was (22.66%) Al-Dabagh *et al*., (2014) [9].

Table 1: Rate of *Haemonchus worm* infection in sheep determined by (Microscopic examination).

Total No. samples	No. of *Humonchus worm* positive sample	Percentage of positive samples
100	41	41

2.2. Rate of infection of *Haemonchus worm* in sheep determined by (Microscopic examination). According to the geographical areas

The prevalence of *Haemonchus worm* infections in this study was highest in western hamza and Al-musayyab districts which were (50%) (30 out of 15) and (46.15%) (26 out of 12) respectively, although the of *Haemonchus* worm in Al-mahaweel District was (33.33%) (24 out of 8) while the lower prevalence of *Haemonchus worm* Al-Qassim district which was (30%) (20 out of 6) the results of present study were exhibited on high prevalence of *Heamonchus contortus* in sheep some districts in Babylon province. The statistical analysis reveals that there was no significant (p>0.05) difference in the prevalence of parasite among geographical areas in (Table 2). The curreent study agreement with Bangladesh study that recorded (50%) infection rate with *H. contortus* Nahar *et al*., (2015), in Taif, KSA *H. contortus* infection rate was (55.90%) Degheidy *et al*., (2014) [23] and in India (56.38%) Garg *et al*., (2003) [35] also in Pakistan a prevalence rate of (57.8%) for sheep harboring *H. contortus* Gadahi *et al*., (2009) [36]. While these results were higher *Haemonchus worm* than percent that reported in studies lraq like in Salah al-Din infection rate was (8.8%) (Aziz and Mahmoud 2020) [6], in Basrah (8.05%) (Abbas, 2017) [3], Kirkuk (9.09%) Al-Bayati, Jihad *et al*. (2023) [2], Diyala (12.4%) (Hassoon 2018) [40], Baghdad (13%) (Muhaidi, 2016), Mosul (15%) Moosa, Hussien *et al*. (2022) [55]. Also this study disagreement with many studies in Iran which infection rate was (0.22%) (Nazarbeigy *et al*., 2021), (9.3%) (Tehrani *et al*., 2012) [81], (16.2%) (Rahimi *et al*., 2020) [70] and (22%) (Eslami and Nabavi, 1976) [28] respectively in sudan (19.5%) and (26%) (Bibi, *et al*. 2017; Arafa., *et al*. 2011).

Table 2: Rate of the *Humonchus worm* infection in sheep determined by (Microscopic examination) according to the geographical area

Geographic area	Total No.	Positive cases	%
Al-Musayyab	26	12	46.15
Western hamza	30	15	50
Al-Qassim	20	6	30
Al-Mahaweel	24	8	33.33
Total	100	41	41
Calculated X2		2.87	
Calculated P value		0.412(NS)	

NS: No significant difference at p<0.05

2.3. Rate of infection of *Haemonchus worm* in sheep determined by (Microscopic examination). According to

the age group

The infection rate of *haemomchus worm* in this study was highest in the (< 1 year) age group which was (44.82%) (29 out of 13), while the lower prevalence of infection was in the (1-2 year) years age group which was (40%) (40 out of 16) and age group (> 2 year) which was (38.70%) (31 out of 12). The statistical analysis reveals that there was no significant (p>0.05) difference in the prevalence of parasite between age intervals Table (3). These results were agreement with results **(**Silverman and Patterson 1960) [71] whose determined the prevalence of haemonchosis was recorded in older (> 9) months (33.23%) than in younger (< 9) months (39.91%). and infection *Haemonchus* contortus of sheep <12 months was (44.24%) Febretrisiana *et al*., (2021) [29], Agreement with study in Morocco the highest rate is observed in animals less than one year old (43.22%) (Brik, Hassouni *et al*. 2019) [19]. (Abbas 2017; AL-Hasnawy 2014) [11] founded infection rate (27.1%), (32%) respectively, and rate of infection in young was lower than the infection rate in adults, study in Diyala (Younis and Qasim, 2021) [84]. (Mussa 2023) [56] in Ethiopia recorded infection rate of *H. contortus* of the sheep infection according to age in young animal (60%) by adult 44.1% and old 35.5%.

Table 3: Rate of the *Humonchus worm* infection in sheep determined by microscopic examination according to the age group

Age	No. of examined samples	No. of positive samples	Percentage of positive samples
< 1 year	29	13	44.82
1-2 year	40	16	40
> 2 years	31	12	38.70
Total	100	41	41
Calculated X2		0.259	
Calculated P value		0.878(NS)	

NS: No significant difference at p<0.05

2.4. Rate of the *Humonchus worm* infection in sheep determined by microscopic examination according to sex

This current study concluded the rate of infected male sheep with *Haemonchus worm* was (36.66%) (30 out of 11), and females was (42.85%) (70 out of 30) Through the above, we found that the percentage of infection in females was greater than males, as shown in the table (4) when results statistical analyzed showed there are no significant effect on in infected rate at (p>0.05) on sex. These results were compatible with results which recorded from (Jasim, 2021) [47] which he founded that the infection rate in females was high (25.32%) while in males it was low (22.09), Agreed with another study performed by (AL-Hasnawy, 2014) [11] who verified that sheep females appeared with high percentage (48.64%) while in males low (27.63%) in Hilla, *H. contortus* infection rate in females (30.98%) was higher than of males (15.63%) in Morocco Brik *et al*. (2019) [19]. Also Karim *et al*. (2023) recorded the infection rate was low in males (33.333%) while in females was high (48.148%), also (Makawi, 2023) founded in Wasit infection in females (53%) more than in males (42%), In Nepal infection of females sheep was higher (47.1%) than in Males (46.7%) (Shahi, 2023) [75] and nearly similar to results were reported by Qamer *et al*., (2009) in females was (37%) and in males (36.71%). The results of the recent study disagreement with (Tehrani *et al*., 2012; Zelalem *et al*. 2014) [81, 86] who indicated that the infection rate of males is higher than of females.

Table 4: Rate of the *Humonchus worm* infection in sheep determined by microscopic examination according to sex

Sex	No. of examined samples	No. of positive samples	Percentage of positive samples
Male	30	11	36.66
Female	70	30	42.85
Total	100	41	41
Calculated X2		0.333	
Calculated P value		0.564(NS)	

NS: No significant difference at p<0.05

2.4. Examination of fecal samples of the sheep were Traditional methods (microscopic examination)

During this study found that the infection rate for *Haemonchus worm* in sheep by using Direct wet mount, Floatation method, Sedimentation method and Formal ether sedimentation method were (0%, 20%, 10% and 11%) respectively. the infection rate for *Haemonchus worm* was the highest in floatation method in comparison to the rest of mothodss as shown in Table (5) Figure (1). The statistical analysis reveals was highly significant difference at P<0.05 difference in the prevalence of infection in sheep determined by microscopic examination according to methods. The result of the present study is similar to the finding by (Sissay *et al*., 2007; Yagoob *et al.*, 2013; Tehrani *et al*., 2012; Nuruzzaman *et al*., 2012; Irfan *et al*., 2013 and Tasawar *et al*., 2010) [72, 85, 81, 62, 45, 80] using microscopic examination. These study disagreement with many researchers like (Martin *et al*., 1990, Samad 2008; Garretson *et al*. 2009; Shaikh and Naphade, 2021; Shashank *et al*., 2019; Abraham *et al*., 2020; Radavelli *et al*., 2014) [59, 73, 33, 76, 77, 4] whose using direct fecal smear, sedimentation, and flotation techniques were to examine and identify infection The analysis of the samples.

Table 5: Examination of fecal samples of the sheep were Traditional methods (Microscopic examination)

Methods	No. of examined samples	No. of positive samples	Percentage of positive samples
Direct wet mount	100	0	0
Floatation method	100	20	20
Sedimentation method	100	10	10
Formal ether sedimentation method	100	11	11
Calculated X2		21.9	
Calculated P value		<0.0001(HS)	

HS: Highly significant difference at p<0.05

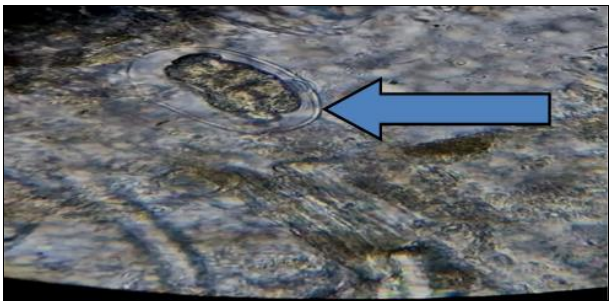

Fig 1: Egg of *Haemonchus contortus* in sample observed under 40x microscope (arrow)

2.5. Morphology Diagnosis

This current study included collecting abomasum samples were collected from sheep and samples were found to be infected of *H. contortus* Initially, when opening the abomasum, the presence of the *H. contortus* was observed in infected animals in different numbers, as the adult worm white ovaries are spirally around red intestine, producing the appearance of a barber's pole diagnosis, as shown in Figure2 (A). The results of the examination showed the presence of male and female worms in abomasum, where the length of females was recorded, which ranged between 28.3 mm and 29.8 mm, while the length in males ranged between 16.2 mm and 18.4 mm as show in figure 2 B (1-2), females were examined under a microscope where they were seen cervical papillae, valvalar flap (Thumb-like) and uterus morphology was also detected as show in figure3 (A). In males, the result of examination under the microscope included posterior end of the male bursa indicates spicules Y shape, left and right spicules. 4 X as shown in figure3 (B).these characteristic features distinguish *H. contortus* adult worm from the other gastric *nematodes*. These results agree with researchers **(**Amana and Alkhaled 2023; Irfan-ur *et al*., 2014; Younis and Qasim, 2021; Boykabulovich 2021; Hade *et al*., 2022; Vlassoff and McKenna 1994; Meshgi *et al*., 2015; Ali *et al*., 2018) [1, 46, 84, 20, 41, 82, 60, 5]. Who observed that females have cervical papillae, valvalar flap (thumb-like) and uterus morphology and males included posterior end of the males bursa indicates spicules Y shape, left and right spicules and disagreement study with study in Saudi Arabia recorded the male's body length were (13.1) mm. and (14.4) mm in both *H. contorts* while the average female's body length was measures (18.5 and 19.6) mm in *H. contorts* (Degheidy *et al*. 2014) [23] in Basrah the measurements of *H. contortus* were (14) mm for male, while, female was (23) mm (Faraj 2012) [30].

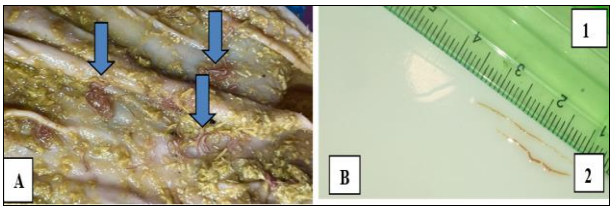

Fig 2A: *H. contortus* (Barber pole) infections within the abomasums (Arrow) B) (1) represents female length. 2) represents male length of *H. controtus*

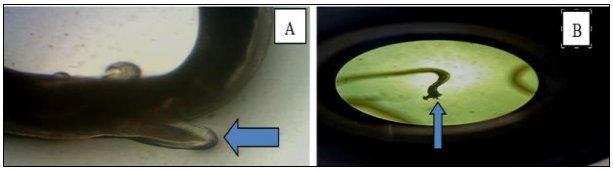

Fig 3A: Represents female *H. controtus* worm, the pointer part indicates valvalar flap (thumb-like) (arrow). B) posterior end of the male bursa indicates spicules Y shape, left and right spicules. 4 X. (arrow)

3. Histopathological changes on mucosal layer of examined abomasums in sheep

The results referred to histopathological changes on mucosal layer of examined abomasums in sheep. The infected abomasum were characterized by necrosis of mucosa with thickening of muscularis layer of mucosa and hyperplasia of parietal cells (glandular epithelium of the mucosa) with edema and infiltration of polymorph inflammatory cells figure 4 (A, B, C, D), in addition, the results were appeared debris of parasite with completely fibrosis in glandular tissue of abomasums figure (), These result agreement with many researchers such as (Saminathan *et al*., 2015; Mravčáková, *et al*., 2021; Mir *et al*., 2007; Tehrani *et al*., 2012; Dutta *et al*. 2017; Ballc *et al*., 2000b; Perez *et al*., 2003; Huntley *et al*., 2004; Bricarello *et al*., 2004; and Amarante *et al*., 2005; Mravčáková *et al*., 2020 and Al-Malki 2017) [78, 52, 54, 81, 24, 21, 64, 39, 16, 13, 53, 12] whose observed thickening of abomasal mucosa due to hyperplasia of mucous and gastric glands thickened muscular layer Abomasum showed necrosis of mucosa and oedema the fibrosis in glandular tissue of abomasums that infiltration in the mucous and gastric glands of abomasum. The of histopathological changes that due infection with of *Haemonchus worm* due to the attachment of the worm with abomasums mucosa and absorption blood amount larger scratching abomasums mucosa and founded worm in part numbers large leading immune responses which occur in infection areas. These result disagreement with (Balic *et al*., 2000; Pathak *et al*., 2014; Abosse *et al*., 2022; Rahman and Collins 1991 and Fávero, Buzzulini *et al*., 2016) [22, 65, 14, 68, 31].

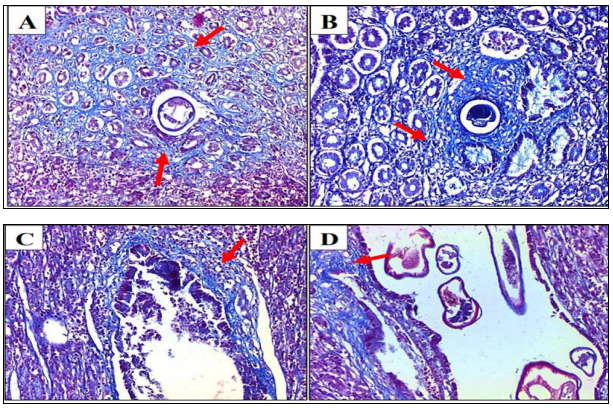

Fig 4: Photomicrograph of abomasum infected with *Haemonchus worm* parasite.A& B/ Section of abomasum characterized by necrosis of mucosa with thickening of muscularis layer of mucosa and hyperplasia of parietal cells (Glandular epithelium of the mucosa) with edema and infiltration of polymorph inflammatory cells (Red arrows) C& D/ and infiltration of polymorph inflammatory cells,appeared debris of parasite with completely fibrosis in glandular tissue of abomasums (Red arrows), Masson trichrome stain. A, B, C, and D: 100x.

Molecular study

Conventional PCR

For confirmation, DNA extraction was performed for samples, including microscopically positive samples. The results showed PCR amplification of the 18S rRNA gene in sheep samples, as shown in Figure (5). The PCR product size was 454 bp.

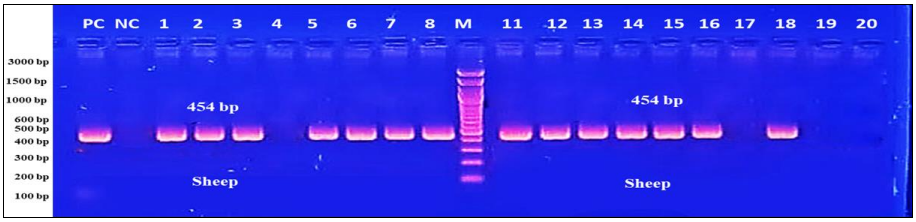

Fig 5: It shows the PCR amplification results of NADH dehydrogenase subunit 4 (ND4) gene of *Haemonchus* spp isolated from Sheep faces. Agarose gel picture appears the PCR product bands with molecular weight of 454 bp. (M) refers to (3000 bp) DNA ladder, (PC) positive control. (NC) Negative control. (1-20) some of PCR results of faces samples

Polymerase Chain Amplification (PCR) results of *Haemonchus worm* infection for sheep

The result of the study revealed that infection percent in sheep by (PCR) was (68%), the 68 feces samples were positive for *Haemonchus worm* Table (6). Among most diagnostic techniques, PCR is more sensitivity and specificity than other tests and less to be affected by autolysis or postmortem changes. In addition, perform egg-based PCR directly from faeces, to reduce processing time has met with success, (Demeler *et al*., 2013; Roeber *et al*., 2012a) [25, 69]. These current study agreement study Indirect-ELISA was found infection rate *Haemonchus* worm was (67.18%) Gowda, A. K. J. (2016) [32], and in Ethiopian study reported infection rate was (67.2%) Badaso and Addis (2015) [17], nearly similar to results PCR was (72%) *Haemonchus* of sheep Höglund *et al*., (2019) [44]. These result disagreement with many studies that reported the infection low rate was (13%) using a PCR assay Elmahalawy *et al*., (2018) [27]. by PCRs was DNA (2%) Sweeny *et al*., (2011). in Tunisia obtained a lower infection rate of (17.00%) Akkari *et al*., (2013) in Bangladesh study Polymerase Chain Reaction (PCR) founded infection rate was (46.5%) were *H. contortus* Mannan *et al*., (2023) [58].

Table 6: Rate of *Haemonchus worm* infection in sheep determined by PCR

Total No. samples	No. of *Humonchus worm* positive sample	Percentage %
100	68	68%

Rate of infection of *Haemonchus worm* in sheep by Polymerase Chain Reaction (PCR) according to the geographical areas

The prevalence of *Haemonchus worm* infections was highest in Al-Qassim and Al-mahaweel districts which were 80% (20 out of 16) and 75% (24 out of 18) respectively, while the prevalence of *Haemonchus worm* in western hamza District was 73.33% (30 out of 22)and the lower prevalence of was in Al-Musayyab district which was 46.15% (26 out of 16) the results of present study were exhibited on high prevalence of *Haemonchus worm* in sheep of some districts in Babylon province. The statistical analysis recorded that there was significant difference (0.047) difference in the prevalence of parasite among geographical areas in (Table 7). The current study agreement with Al-Diwaniyah study that PCR showed that infection rate *H. contortus* was detected was (77.8%) (Alubadi and Al-Fatlawi 2024) [15], and Kashmir /India study that recorded ELISA was (80.0%) infection rate with *H. contortus* Lone *et al*., (2012) [50], also Schallig *et al*. (1995) [79] who founded infection rate *Haemonchus* worm (82.7%) ELISA, These current study disagreement with Duzlu *et al*. (2020) [26] in Turkey recorded was (6.3%) infection rate *Haemonchus contortus* in cattle with qPCR assay.

Table 7: Rate of infection of *Haemonchus worm* in sheep by PCR according to the geographical area

Geographical area	Total examined samples	No. of parasite positive samples	Percentage
Al-Musayyab	26	12	46.15
Western hamza	30	22	73.33
Al-Gassim	20	16	80
Al-mahaweel	24	18	75
Total No.	100	68	68
Chi-square value		7.95	
Calculated P value		0.047 (Significant difference)	

Rate of infection of *Haemonchus worm* in sheep by Polymerase Chain Reaction (PCR) according to the age group

The infection rate of *haemomchus worm* in this study was highest in the (> 2year) age group which was (74.19%) (31 out of 23), while the lower prevalence of infection was in the (1-2 year) years age group which was (67.5%) (40 out of 27) and age group (< 1 year) which was (62.06%) (29 out of 18). The statistical analysis reveals that there was no significant (p>0.05) difference in the prevalence of parasite between age intervals (Table 8)**.** These results in agreement with result of Önder *et al*., (2015) [63] found to be infected with *H. contortus* by qPCR assay were found to be higher in >2 year age group than ≤2 year age group. These result is disagreement with study in Bangladesh infection rate *H. contortus* higher in young (50.8%) age group than adult (42.5%) age group. by PCR technique Mannan *et al*., (2023) [58]. Mohamed *et al*., (2024) [61] recorded sheep under 2 years old exhibited a higher risk, with an infection than their older counterparts

Table 8: Rate of infection of *Haemonchus worm* in sheep by PCR according to the age

Age interval	Total examined samples	No. of parasite positive samples	Percentage
< 1 year	29	18	62.06
1-2 year	40	27	67.5
> 2 years	31	23	74.19
Total No.	100	68	68
Chi-square value		1.02	
Calculated P value		0.601 (No significant)	

Rate of the *Humonchus worm* infection in sheep determined by PCR according to sex

This current study concluded the rate of infected male sheep with *Haemonchus worm* was (76.66%) (30 out of 23), and females was (64.28%) (70 out of 30) Through the above, we found that the percentage of infection in was males greater

than females, as shown in the table (9) when results statistical analyzed showed there are no significant effect on in infected rate at (p>0.05) on sex. These results were compatible with results which recorded from Mannan *et al*., (2023) [58] which he founded that the infection rate in males was high (47.3) while in females it was low (44.3) by PCR, Mohamed *et al*., (2024) [61] recorded infection rates of male 36.70 and 31.40% female sheep, respectively. These result was disagreemeent with (Alani 2023) [7] who verified that sheep females appeared with high percentage (14.24%) while in males were low (7.02%).

Table 9: Rate of infection of *Haemonchus* worm in sheep by PCR according to the sex

Sex	Total examined samples	No. of parasite positive samples	Percentage %
Male	30	23	76.66
Female	70	45	64.28
Total No.	100	68	68
Chi-square value		1.47	
Calculated P value		0.224(No significant)	

4. Conclusions

On the basis of present study, it was concluded that sheep is extremely affected by gastrointestinal parasites sheep were more infected by parasites like, *Haemonchus spp*, Male and female animals in are highly affected by GI parasites. For alleviation and control of these parasites, small ruminants need care, use of anti-helminthic drugs and use of ethnobotanical remedies

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