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Detection of gastrointestinal nematodes resistance against Ivermectin in sheep

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Abstract To date, Ivermectin is one of the advisable medications widely used in the therapy, domination effort of internal and external parasites in ruminants, but its efficacy and resistance developing during the last years remain questionable. The efficacy of Ivermectin and the resistance state of gastrointestinal; round worms in sheep farms was the goal of the current work survey. 300 fecal samples collected from male sheep (≥7 months) represented 6 sheep flocks (n=730) from different regions in Mosul city, Iraq. The efficacy of the Ivermectin in one farm with EPG ≥200 was evaluated using a fecal egg count reduction test on day zero (0) and day 14 post-treatment by subcutaneously injecting Tectin® (each 1 ml contains 10 mg Ivermectin) 0.2 mg/kg B.W. Moreover, the World Association of Advancement for Veterinary Parasitology (WAAVP) guidelines were referenced to detect the resistance. The overall prevalence of gastrointestinal nematodes was 47%, comprising Haemonchus spp. 6.66%, Ostertagia spp. 8.66%, Nematodirus spp. 14%, and Trichostrongylus spp. 17.66%. The efficacy of Ivermectin (71%) and the lower limit confidence interval (95%) was 46%, indicating AR to Ivermectin. As documented above, resistance of nematodes to anthelmintic classes, including Ivermectin, could potentially serve as a risk role in propagation, consequence, and maintaining parasitic burden to the productivity of ruminant farms, in addition to solidification set for hindering, monitoring and awareness of the sources of resistance being recommended.

Keywords: *Ivermectin, Sheep, Nematodes, Resistance, Efficacy.*

Introduction In equatorial and subtropical territories, gastrointestinal nematode (GIN) have significant health influence in small ruminants, inducing significant production losses (1,2). Clinical and economic risks of these nematodes are usually due to sustained weight, death, decreased food intake, anemia, and retarded weight gain are considerably the most clinical signs (3,4,5). Moreover, diminutive feeding capacity affects absorption of nitrogen, energy, and mortality in heavily infested animals (2). The ruminal-intestinal nematodes genera described as important parasites in small ruminants (6). Notably, hit with further one species occur commonly, and consequently, complexity of the outcomes and treatment procedures elevated (7).

In human and veterinary clinic, Ivermectin is a confirmed medication for domination parasitic attacks (8). The drug belongs to the avermectins group, which that comprises of 16 macrocyclic lactone

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member compounds found in the Institute of Kitasato in japan in 1967 extracted from Streptomyces avermitilis, and thereafter registered in the World Health Organization (9,10,11). Ivermectin is a positive synergistic modulator that eclectically opens inhibitory glutamate-gated chloride ion channels and induces suppression of pharyngeal pumping, motility, and egg release in the reproductive system. (8,12,13,14). Worldwide, it was announced that the extreme and inconsequential use of antiparasetic for abundant periods participated in developing parasites resistant phenomena, which could constrain global food security (15,16). Negatively, documented literatures around the world increased (17,18). Available medication for gastrointestinal nematodes basically relies on repellent drugs, but repeated administration faces multiple resistant parasites. Awareness about anthelmintic resistance and its genetic mechanisms requires more and continual detection for different medications (19).

The fecal egg count reduction test (FECRT) one of the frequent modes utilized to reveal of anthelmintic impedance performed in conjunction with coprocultures to investigate the encompassed parasites (20,21). Recently molecular technique were used for detection gastrointestinal nematodes such as Haemonchus spp. (22,23).

Small ruminants, including sheep, have a decisive service in promoting the socio-economic revival in abundant countries (24). On account of the productivity regimens, they can be hindered by multiple influences, including infestations of external and internal parasites. So, this work attempted to find out the resistance issue of Ivermectin using the reduction test observed in sheep in Mosul city, Iraq.

Materials and Methods

Ethical approve

This research approved by UM.VET.2024.098

Animal of study

A 730 local male sheep from six private flocks (A, B, C, D, E, and F) raised for fattening purposes participated in this study from various locations in Mosul between October 2024 and February 2025. The animals were ≥ seven months, the farm size was between 75 and 150, and there was no history of deworming. The most significant clinical indications, history, and epidemiological data were documented in a clinical card provided by the producers during the sampling procedure.

Fecal samples collection and handling

Three hundred (300) individual fresh fecal samples under septic conditions were obtained and spotted in containers, carried to the laboratory for examination. For detection of ruminal-intestinal nematodes, flotation, sedimentation, and Mac master techniques were used according to Sabatini *et al.* (25). The distinguishing of the nematode eggs done according to Mahmood *et al.* (26).

Fecal egg count and fecal egg count reduction test (FECRT)

Fecal egg counts (FEC) were employed by flotation method protocol (27). McMaster egg counting technique, degree of infestation, and eggs per gram (EPG) were categorized according to Soulsby and Helminths, (28). EPG = (eggs of 2 pads) x50 (29,30).

To investigate the efficiency of Ivermectin, through surveying a flock of sheep (n=75 and EPG ≥200 was selected. 50 sheep were marked, and further, the selected animals were given Ivermectin (Tectin® 1%) (31). Fecal egg count and EPG were ranked in day Zero (control group T1) prior to injection and on day 14 (treated group T2) for the next medication (32). The equation FECRT%=(1-T2/T1) ×100 was utilized (20,21,33).

Statistical analysis

The status of ruminal-intestinal nematodes in sheep was analyzed in Excel sheath software for Windows 7. Significant divergence between T1 and T2 models, FECRT, and lower limit for confidence intervals of 95% were dialed in SPSS software. The definitive decision for the presence of resistance relies on WAAVP directions in which the farm was assessed as resistant if the egg reduction rate was <95%, the lower limit of the 95% confidence interval was <90%. Moreover, the resistance was doubtful when the scores didn't meet (34,35).

Results

Fecal analysis of the sheep samples for the current survey revealed that the overall prevalence of ruminal-intestinal nematodes was 47% (141/300), comprising Haemonchus spp. 6.66% (20/300), Ostertagia spp. 8.66% (26/300), Nematodirus spp. 42/300 (14%), and Trichostrongylus spp. 17.66% (53/300) (Table 1) (Figure 1).

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Table 1: The status of ruminal-intestinal nematodes in sheep by McMaster method

Type of nematodes	No. of samples examined	No. positive samples	Prevalence %
Haemonchus spp.	300	20	6.66
Ostertagia spp.		26	8.66
Nematodirus spp.		42	14
Trichostrongylus spp.		53	17.66
Overall		141	47

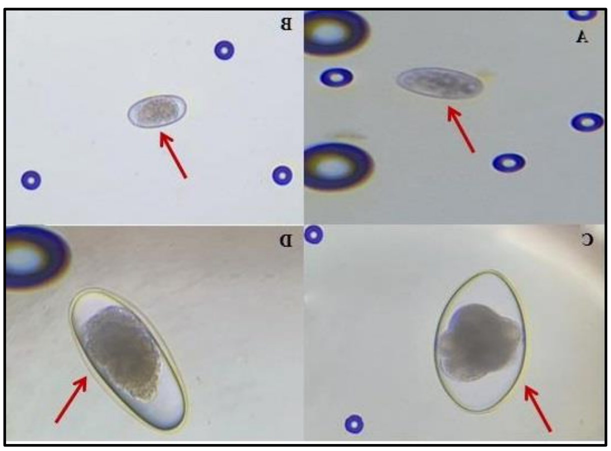

Figure 1: Ruminal-intestinal nematode eggs: A. Haemonchus spp., B. Ostertagia spp., C. Nematodirus spp. D. Trichostrongylus spp. under microscopic examination X400.

Considerably, Ivermectin lowers the fecal eggs on treated animals parallel to the day 0 (Table 2). The outcome also counted a 71% rate of FERCT, and the lower limit of 95% was 46% (Table 2). This finding indicates that ruminal-intestinal nematodes are resistant to Ivermectin.

Table 2: Limit confidence level of 95% in sheep, n=50

Drug	No. animals	± EPG (T1)	± EPG (T2)	FECRT %	CI 95 %
Ivermectin	50	211±201.38[a]	62± 39.82[b]	71%	46%

± mean and standard error of mean, CI confidence interval, letter a and b significant difference at $P<0.05$

Discussion

The gastro-intestinal nematodes have a direct and indirect economic impact on farms productivity worldwide, especially in poorly managed areas, through interference with weight, milk, expense fees for medications, and mortality in heavily infected animals (36-38). In the current study, the overall prevalence of ruminal-intestinal nematodes was 47%. This finding was nearer than the total infection rate reported in the cities of Mosul and Erbil which was 46.49% (39). In the literature, the infection rate of was 42.85% (40), 38% (41), and 30.73% (42). Further, in Sulaymaniyah province was 78.15% (43). There are several works that have reported the infection rate of nematodes in sheep in different countries, such as in Turkey, which was 76% (44), in Iran, which was 94% (45), in China, which was 55.7% (46), and in Pakistan, which was 72.92% (47). The prevalence of nematodes in sheep differs among countries due to different management practices, sample size, efficacy of laboratory tests used, and seasonal climatic variations between countries (48-50). Numerous nematode species were detected in sheep in this study, such as Haemonchus spp., Ostertagia spp., Nematodirus spp., and Trichostrongylus spp. These results agree with Khalaf et al. (39), Moosa et al. (41), and Jwher et al. (42), who reported all these nematode species in Nineveh province, Iraq.

Results showed that the Ivermectin significantly lowered the rate of eggs in the feces in the post-treatment group in contrast with egg excretion to the pre-injection time with reduction rates (71%). These results match the earliest literature vision (31,32). It has been known that Ivermectin has a broad parasiticidal vigor versus round alimentary parasites and skin mange and lice (14,51). Burgess et al., (52) revealed that Ivermectin still the major available antiparastic preference in the livestock population, mainly for deworming of roundworms and macrocyclic lactone members, as yet is the generally an anthelmintic purpose in the Great British sheep. Cattle farms of the United States of America (53), as well as in Iraq (31). Outcome also points out that the rate of egg sliding is 71%, the lower limit confidence level of 95% was 46%, and based on the WAAVP reference, this view indicates resistance to Ivermectin. Our finding is parallel to previous investigations (6,18,54,55). Presence of anthelmintics resistance could be due to widespread, frequent, uncontrolled use and incorrect doses of common anthelmintic. It has been clarified that the promiscuous application, readily obtainable, short and frequent period application, re-administration of multiple drug classes under one-year time, and mass treatment (56-58). Moreover, gene expression alternation (59,60). In contrast to the current result, Shihab and Hassan, (31) indicate that the ruminal-intestinal nematodes resistant to Ivermectin were suspected. Generally speaking, mass planning could participate in the development of resistance, which is currently a global issue of concern (61,62).

Conclusions

Current article is premier report consider the resistance feature of Ivermectin in naturally infected sheep with ruminal-intestinal nematodes in Mosul city, Iraq. Ivermectin known to be frequently choice therapy used by veterinarians and farmers against gastrointestinal and ecto-parasites of small and large ruminants in Mosul. Resistance of ruminal-intestinal nematodes to anthelmintic classes, including Ivermectin, could potentially serve as a risk role in consequence and maintaining parasitic burden to the productivity of ruminant farms. In addition, establishing rational measures for prevention, control, and awareness of the sources of resistance is recommended.

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Conflict of Interest

This article's authors are all contented, and there are no conflicts.

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