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INCIDENCE OF GASTROINTESTINAL NEMATODES IN SMALL RUMINANTS, EFFECT ON GROWTH RATE AND THEIR SENSITIVITY TO DIFFERENT ANTHELMINTICS

Habib Khan[*1], Dr. Ikram Ullah Khan[2], Tunio Muhammad Tarique[3], Zaheer Ahmad[4], Israruddin[5], Khateeb Khan[6]

*[*1]PH.D Scholar, Department of Animal and Poultry Production, Faculty of Veterinary and Animal Science, Gomal University Dera Ismail Khan*
[2]Livestock and Dairy Development Department, Khyber Pakhtunlhwa
[3]Assistant Professor, Department of Agriculture Sciences, Allama Iqbal Open University Islamabad
[4]Faculty of Veterinary and Animal Science, Gomal University DI Khan
[5]Senior Research Officer, Veterinary Research and Disease Investigation Centre, DI Khan
[6]M.Phil Scholar, Department of Zoology, University of Agriculture Dera Ismail Khan

[*1]drhabibsherani@gmail.com, [2]drkhaniu@gmail.com, [3]tarique.tunio@aiou.edu.pk, [4]dr.zaheer86@yahoo.com, [5]israraup@gmail.com, [6]khateebsheranikks@gmail.com

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Corresponding Author: *
Habib Khan

Abstract

The worm infestation has a key impact on the health status and livestock production system, especially small ruminants that are raised on grazing system. In this contribution, we have studied the prevalence of gastrointestinal nematodes and comparative efficacy of most common used anthelmintics (i.e., Levamisole, Oxfendazole, and Ivermectin) in small ruminants in tribal areas of Pakistan. The overall worm prevalence was 74%. Among various types of nematodes, Trichuris Ovis was the most prevalent followed by Heamonchus contortus and Trichostrongylus. Also, the infestation was higher in young and female as compared to adult and male animals, respectively. Furthermore, the efficacy of selected anthelmintics was evaluated based on the reduction of eggs per gram (EPG) of the fecal sample on day 14th, 21st, 28th post-medication. The efficacy of Levamisole was highest (87.74%), followed by Oxfendazole (84.97%) and Ivermectin (82.65%). Likewise, the growth rate was also found higher in the Levamisole treatment group (i.e., 105.71 g/day), followed by Oxfendazole (82.85 g/day) and Ivermectin (70 g/day). Our results not only demonstrate the prevalence of gastrointestinal parasites in small ruminants but also their sensitivity to anthelmintics in vogue that may help in the improvement of the local livestock production system.

INTRODUCTION

Livestock production is directly proportional to their health status (F. U. Rehman, Zhao, Shah, Qureshi, & Wang, 2013). Apart from the endemic and epidemic disease, the worm infestation is one of the serious factors affecting their production in terms of meat, milk, hair and wool yield (Charlier, van der Voort, Kenyon, Skuce, & Vercruysse, 2014) as well as their reproductive performance (F. Rehman,

Qureshi, & Khan, 2014). Small ruminants (sheep and goats) are at higher risk of worm infestation, as they are primarily raised on the grazing system in tribal areas of Pakistan (Hale, 2006).

The nematodes of the gastrointestinal tract are one of the main constraints to goat and sheep production which result in a significant health problem in areas with poor nutrition, substandard management practices, and adverse sanitation condition. In the tropical regions up to 95% of sheep and goats are found positive for the presence of gastrointestinal helminths, but most of the animals infested with parasitic nematodes are subclinical due to the chronic nature of the disease (Bandyopadhyay, Devi, Bera, Bandyopadhyay, & Bhattacharya, 2010).

Gastrointestinal nematodes cause the unproductive use of digested nutrients which results in decreased growth rate (Terefe, Demissie, Beyene, & Haile, 2012), causes anemia due to heavy infestation, death can also occur (Hassan et al., 2011). According to Garedaghi et al., helminths infestation predisposes animals to other pathogenic infection by decreasing the immunity of the host animals resulting in the heavy financial losses (Garedaghi, AP, Naghizadeh, & Nazeri, 2011). The proper control of parasitic infestation in sheep and goat can raise the production to 40%. (Vassiliades, 1984) Practically, it is impossible to eradicate the worms completely and also not desired to keep reasonable immunity level against those parasites in the host. Therefore, especially in a grazing system, keeping lower fecal egg per gram (EPG) count is ideal that is achieved by various anthelmintics including Levamisole, Oxfendazole, Ivermectin, etc.

Given the above merit, we have investigated the gastrointestinal tract nematodes, potential effective anthelmintics and their treatment effect on the body weight gain of the small ruminant population in tribal areas of Pakistan.

1. Materials and Methods

All the chemicals used were laboratory grade and purchased from Sigma Aldrich, otherwise mentioned. The anthelmintics, i.e., Levamisole were purchased with the trade name of El Levanil from Elko pharma Ltd. Pakistan, Oxfendazole with the trade name of Oxafex from Hilton Pharma, Pakistan, and Ivermectin with the trade name of Ivotec from Star laboratories, Pakistan.

1.1. Animals and Area selection

The tribal areas near D. I. Khan were selected for the study. Small ruminants including sheep and goats were selected and the animals dewormed within ten weeks were excluded from the study. A total of 150 small ruminants were included in this study. All included animals were screened for the presence of gastrointestinal nematodes. Out of 150 screened animals, 100 were positively identified by neck ring and randomly distributed into four experimental groups (A, B, C, and D). Group A was treated with 7.5 mg/Kg BW Levamisole; group B was treated with 2.83 mg/Kg BW Oxfendazole, group C was treated with 0.2 mg/Kg BW Ivermectin while group D was kept control (i.e., without treatment).

1.2. Sampling Technique

Sheep and goat were sampled for coprological examination. Approximately 5 g of fecal samples per animal was directly collected from the rectum in sterile a plastic glove. The samples were transported in self-sealed polythene bags at 4 °C to Veterinary Research and Disease Investigation center, Dera Ismail Khan. Each sample was labeled for information like species, sex, age, vaccination, and any co-existing disease.

1.3. Determination of Anthelmintics Efficacy (FECRT)

To determine anthelmintics efficacy, all 100 animals were distributed into four experimental groups. Animals of each group were treated with different anthelmintics. Group A was treated with Levamisole @ 7.5 mg/Kg BW; group B was treated with Oxfendazole @ 2.83mg/Kg BW, Group C was treated with Ivermectin @ 0.2 mg/Kg BW while group D was controlled. All animals were sampled at day zero and EPG in feces were calculated by Mc-Master technique. Similarly, second, third and fourth sampling was performed at day 14th, 21^{st,} and 28th, respectively. EPG was calculated as mentioned previously(Mes, 2003). Percent reduction in EPG was calculated by the formula. $\left[\frac{(\text{1st Sample EPG} - \text{4th Sample EPG})}{\text{1st sample EPG}} \text{ X } 100\right]$.

1.4. Effect of anthelmintics treatment on body weight gain

All the 100 animals were randomly distributed into four groups to determine the effect of anthelmintics treatment on their weight gain. Animals of each group were treated with different anthelmintics as mentioned earlier. Daily weight gain was calculated by dividing the overall weight gain over twenty-eight (number of days) and multiply by 1000 to obtain weight gain in grams.

$$[\frac{(\text{Day 28 Weight (Kgs)} - \text{Day Zero Weight (Kgs)}}{28} \text{ x } 1000)].$$

1.5. Detection and identification of parasitic load

The parasitic load and type identification were performed by the following methods;

1.5.1. Direct floatation method for egg microscopic examination

Approximately 2.0 g of fecal material was added to some amount of the flotation solution. Flotation solution was prepared by dissolving 355g of sugar into 454 ml of water. The specific gravity of the solution was 1.27. After thorough mixing, the suspension was sieved through gauze and poured into a test tube. To fill the tube to the top, more flotation solution was added. A coverslip was placed on the top of the test tube for 15 minutes. The coverslip was vertically removed and placed on a slide and examined under the microscope. Olympus bright field microscope with a built-in inverted camera was used to identify the egg type of particular parasite in fecal samples

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1.5.2. Mc-Master Technique for egg count

The Mc-Master technique was used to count the parasitic eggs as reported earlier(Bosco et al., 2014). Briefly, a 3.0 g of fecal sample was added into a conical flask. Then 42 ml of water was added and soaked for one hour till the feces were soft. The sample was homogenized using a laboratory stirrer until all the pellets were broken up. The sample was sieved through a strainer into a bowl. The sample was swirled, and 15 ml was poured into a centrifuge tube for two 2 minutes at 1500 RPM. The supernatant was poured off gently.The tube was shacked to loosen the sediment. Then, a saturated sugar solution was added to give the same volume as before (15 ml). The tube was upside down for seven to ten times. The sample was drawn immediately with a Pasteur pipette and filled the chamber of McMaster slide. Repeat the same process for the second chamber. All the eggs were counted in both chambers at 40 × magnification. Multiplied the number of eggs by 50 to give the Egg per gram of feces.

1.6. Statistical Analysis

The significance of the study was determined by a simple percentage. The means of different groups were compared using a general linear model (GLM). ANOVA was performed to find a significant difference between different groups. For statistical analysis "Statistics 8.1" was used.

2. Results

2.1. Prevalence of Nematodes

A total of 150 fecal samples (81 sheep and 69 goats) were examined in the research study. The overall point prevalence of nematode in small ruminants in FR Dera Ismail Khan is presented in Table-4. Out of 150 examined fecal samples, 74% (111) were found positive for various eggs of gastrointestinal nematodes. 86.4% of sheep (70/81) and 59.4% (41/69) goats were found positive for various gastrointestinal nematodes. Genera of nematodes identified through the morphology of eggs were Trichuris Ovis, Heamonchus Contortus and Trichostrongylus.

As a whole 78.54% (79/102) percent gastrointestinal nematodes were prevalent in female, while 65.38% (34/52) in male animals. The data shows that animals aged less than one year were more susceptible to gastrointestinal nematodes compared to adult animals. 84.6% (33/39) of small ruminants less than one year of age and 70.1% (80/114) of small ruminants above one year of age were found positive with eggs of gastrointestinal nematodes. **(Figure 1)**

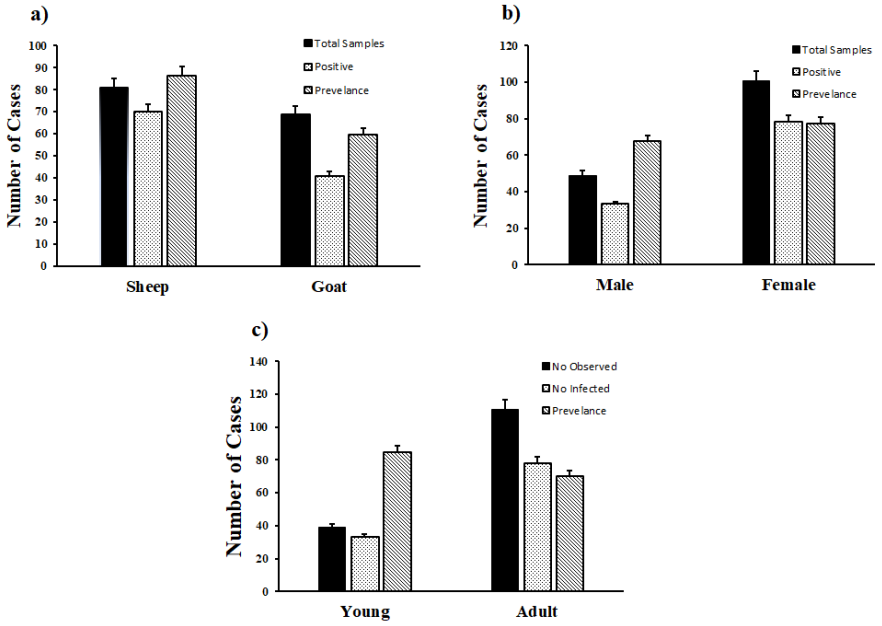

Figure 1: Prevalence of nematodes in the small ruminants**.** Herein, a) is the total prevalence, b) is their revalence based on the animal sex and c) is the prevalence of nematodes in different age groups in small ruminants.

2.2. Nematodes Genera-wise prevalence

The data showed that Trichuris Ovis, Heamonchus Contortus and Trichostrongylus were the major gastrointestinal nematodes found in the study area. Out of the 150 fecal samples screened for gastrointestinal nematodes, 55.55% (85) samples were found positive with the eggs of Trichuris, 41.17% (63) with Heamonchus Contortus and 32.02% (49) with Trichostrongylus. (**Figure 2**) The data also showed that 60% of animals harbored more than one type of nematodes in their digestive tract.

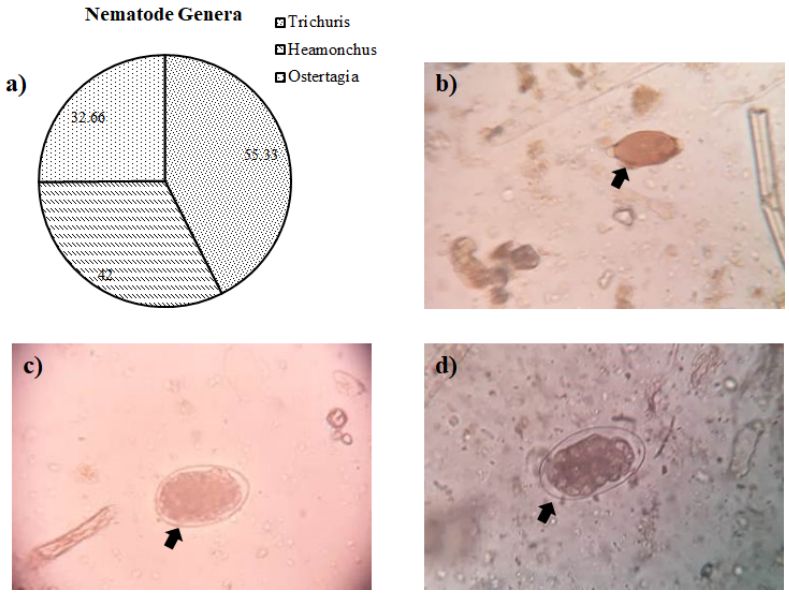

Figure 2: Prevalence of different nematodes genera a) and their identification based on egg types shown as; b) Trichuris Ovis c) Trichostrongylus and d) Heamonchus contortus. The black arrows are showing eggs in micrograph at 40X magnification.

2.3. Comparative Efficacy of selected Anthelmintics

Effect of three selected anthelmintics on the reduction of EPG in feces was evaluated for the treated and control group. The highest reduction in EPG 87.74% was observed after Levamisole treatment, followed by Oxfendazole 84.97% and least reduction in EPG was found with Ivermectin treatment 82.65%, while there was an increase in EPG in control group. **(Figure 3a)** Statistically, there was a significant effect on the reduction of EPG between treated and control groups. Indeed, no significant difference was observed among three trial anthelmintics on reduction in EPG in small ruminants. **(Table 1)**

Independent variables		Mean EPG ± SE	% Decrease in EPG
Levamisole**	Day 0	408 ±108.67	0
	Day 14	92 ±67.20	77.45
	Day 21	72 ±54.16	82.35
	Day 28	50 ±43.30	87.74
Oxfendazole**	Day 0	426 ± 106.65	0
	Day 14	84 ± 57.22	80.28
	Day 21	74 ± 45.91	82.62
	Day 28	64 ± 42.13	84.97
Ivermectin**	Day 0	392 ± 123.05	0
	Day 14	104 ± 51.88	73.46
	Day 21	84 ± 49.41	78.57
	Day 28	68 ± 51.80	82.65

Table 1: Efficacy of various anthelmintics at day 0, 14, 21 and 28 of treatment. In the table "**" is representing the higher significance level, i.e., probability value ≤ 0.01.

2.4. Comparison of weight gain among various treated groups

Effect of three selected anthelmintics on weight gain was evaluated in these experiments. The highest increase in weight was observed after Levamisole treatment (105.71 g/day), then by Oxfendazole (82.85 g/day) and least increase in weight was observed with Ivermectin treatment (70 g/day). Increase in weight gain of the control group was lowest in all (32.85 g/day). **(Figure 3b)**

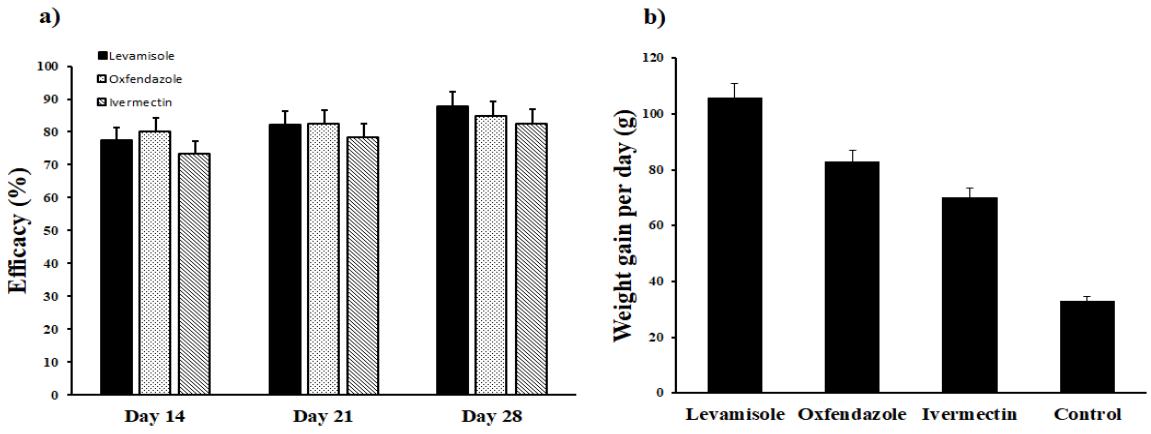

Figure 3: Comparative Efficacy of selected anthelmintics on Day 14, Day 21 and Day 28 post-Medication a) and its effect on small ruminants daily weight gain in grams b).

3. Discussion

This study primarily focused on the parasitic infestation in the livestock population of tribal areas, and associated outcomes on the animal health and production. The study also determined the most efficient anthelmintic with least resistance and higher efficacy.

We have found that 74% of the small ruminants' population had severe nematode parasitic infestation. The higher prevalence in sheep and goat in tropical regions is due to favorable environmental conditions for gastrointestinal nematodes transmission(Mohanta et al., 2007; Zeryehun, 2012), adverse sanitation conditions in rural areas(Badran, Abuamsha, Aref, Alqisi, & Alumor, 2012) and poor nutrition of the host animal(Mbuh, Ndamukong, Ntonifor, & Nforlem, 2008). Similarly, grazing on the common pasture, use of same anthelmintics for an extended period and inappropriate deworming intervals are other promoting factors for gastrointestinal nematodes. Also, most of the small ruminants did not show any clinical sign of helminths infestation due to the chronic nature of the disease.

Sheep and goats in the study area were infested by the same main gastrointestinal nematodes of Trichuris Ovis, Heamonchus contortus, Trichostrongylus. This may be attributed to their mixed farming system and share common pastures, shed and watering places. The helminths species and genera found herein have also been reported from other parts of Pakistan (Ayaz, Raza, Murtaza, & Akhtar, 2013; Gadahi, Arshed, Ali, Javaid, & Shah, 2009; Raza, Iqbal, Jabbar, & Yaseen, 2007)and worldwide(Dagnachew, Amamute, & Temesgen, 2011; Kagira, Mhoma, & Kanyari, 2017; Mohanta et al., 2007).

We found that Trichuris Ovis was the predominant genera in the study area with 55% of overall prevalence. The very same prevalence of the Trichuris was also reported earlier(Gadahi et al., 2009; Khalafalla, Elseify, & Elbahy, 2011). Meanwhile, the Heamonchus contortus was 41.17% prevalent which is in corroboration with other studies (Bendezu et al., 1983; Joshi, 1994; Jurášek, 1986). Likewise, the prevalence of Trichostrongylus was observed around 32.02%, the same as reported by Rahman, i.e., 36.6%(Rahman, 1969).

The prevalence of nematodes concerning host animal also revealed higher incidence in sheep as compared to goats. This can be attributed to the grazing behavior of sheep that expose them to parasitic infestation(Farooq, Mushtaq, Iqbal, & Akhtar, 2012; Khan, Sajid, Khan, Iqbal, & Hussain, 2010). Also, we also found higher infestation rate in young animals as compared to adults. It may be due to their weaker immunological response. The animal becomes immune when they undergo repeated exposure over some time(Dagnachew et al., 2011). Due to the increase in the immunity, older animal recovered from parasitic infection more easily compared to young animals (Tariq, Chishti, & Ahmad, 2010; Zeryehun, 2012). The gender of the host also influences the gastro-intestinal nematodes infestation. The data revealed a higher incidence in female than male animals. Stress during pregnancy and hormonal differences predispose female to more parasitic infection than male animals.

In the current study, Levamisole was found the most effective anthelmintics followed by Oxfendazole and Ivermectin. The pattern of fecal egg count reduction test (FECRT) of the tested anthelmintics was by the results of Varady et al.,(Varady, Praslicka, Corba, & Vesely, 1993). Anthelmintics treatment of small ruminants causes a significant increase in weight gain(Hannan, Mostofa, Haque, & Alim, 2001), and decrease fecal egg counts(Zhong et al., 2017). The increase in weight gain after anthelmintics treatment is due to increase in the dry matter intake, whereas improved weight gain in lambs after anthelmintics treatment of their mothers may be due increased milk production in their mothers(Darvill, Arundel, & Brown, 1978).

The decreased growth rate is due to losses of blood, mucin, plasma, and gastrointestinal tract sloughed epithelium due to gastrointestinal nematodes(Kerr, Bright, Smith, Armstrong, & Higham, 2017). Moreover, gut inflammation and pain, changes in cholecystokinin secretions and gut contents pH, etc. are the additional factor of weight loss(Symons, 1985; Varyani, Fleming, & Maizels, 2017). Additionally, the changes in gastric secretion are due to replacement of functional parietal cell with non-functional parietal cell(Chai, Brown, & Kumarasinghe, 2018), which reduces the concentration of phosphorus in the small intestine

and decrease the feed intake(Mavrot, Hertzberg, & Torgerson, 2015). Complete inappetence has also been observed in heavy and acute parasitic infestation (Candy, Waghorn, Miller, Ganesh, & Leathwick, 2018).

4. Conclusion

In Summary, the gastrointestinal parasitic infestation in small animals (sheep and goat) has a direct impact on their production and health conditions. Also, reporting of these parasites in the small ruminants of the tribal area of Pakistan may help in the improvement of a livestock production system that will directly influence the livelihood on the local dwellers. This study reported a higher incidence of parasites in sheep than goats and more in younger than adults. Moreover, the Trichuris Ovis was the highest prevalent nematode type, and Levamisole was most efficient anthelmintic. These findings will guide the farmers and veterinary practitioner towards better production and improved animal health, respectively.

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