Research Article

Monitoring Anthelmintics Resistance and Assessing Effectiveness of Herbal Anthelmintics against Small Ruminants' Nematodes Infection in Pakistan

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Abstract | The gastrointestinal nematodes are common pathogens in grazing sheep/goats throughout the world which impairs productivity and leads to high economic losses. In most part of the world, drug resistance against anthelmintic is now very common. In this context, various alternative control programs including herbal use against these worms is also an important option. To explore the problem, three available synthetic anthelmintic (Oxfendazole alone, oxfendazole-Levamisole combination and Ivermectin) were administered to natural major nematodes (*Haemonchus*, *Trichuris*, *Strongyloides* and *Trichostrongylus*) infected sheep and goats. Overall results revealed susceptibility of these anthelmintics (97-99% Confidence Interval of Faecal Egg Count Reduction-FECR) against four nematodes and no evidence of resistance recorded. However, three herbal anthelmintics (Atreefal Deedan, Deedani and Kirmar) available in Pakistan were tested against nematodes as an option of alternate remedy. Among these Atreefal Deedan showed highest (90-96%) FECR, followed by Deedani (80-83%) and Kirmar (32-60%). It is concluded that, on small scale assessment no drug resistance observed against few worms in sheep and goats in Pakistan. A broader study is recommended for assessment of drug resistance and also evaluation of available or practiced anthelmintic.

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Introduction

The livestock sector occupies a unique position in the National Agenda of the economic development of Pakistan. This sector plays a vital role in revenue generation of the country and is presumed to play an important role in poverty alleviation to uplift the socioeconomic condition of Pakistan's rural masses. The animal production operations provide 35% income directly or indirectly to nearly 8 million rural families in Pakistan (Anonymous, 2015-16). In country prospective, livestock contributes approximately 58.6 percent to the agriculture value added and 11.6 percent to the overall GDP during

2015-16. Gross value addition of livestock at constant cost factor of 2005-06 has increased from Rs. 1247 billion (2014-15) to Rs. 1292 billion (2015-16), showing an increase of 3.63 percent over the same period last year (2015). There are about 29.8 and 70.3million heads of sheep and goats, respectively in Pakistan (Anonymous, 2015-16).

Sheep and goats are excellent source for meat production in Pakistan, producing 37,000 tons of meat per annum and provide good source of animal protein. There are certain constraints of physiological and infectious origin (Raziq et al., 2010) which limited the growth of this industry in terms of poor production performance and reproductive efficacy; thus affecting the GDP value along with socio-economic status of livestock farmers. Poor animal health and diseases are usually associated to the cause of low farm productivity and indirectly to low profit margin. The other factors which effect their productivity is persistent drought and shortage of range vegetation due to overgrazing/ utilization of trees/shrubs as fuel wood.

Among the infectious organisms of parasitic origin, gastrointestinal nematodes are the main cause for production losses in terms of lower milk and meat production in affected animals (Nasreen et al., 2008; Souza et al., 2012; Tasawar et al., 2012; Zeryehun, 2012). The most common feature of gastrointestinal nematodiasis (GIN) is to increase loss of endogenous proteins that leads to retarded growth (Jackson et al., 2009); whereas, heavy worm loads have also been reported for high mortality (Qamar, 2009). Apart from production losses, the other associated losses related to GIN - is financial losses on the purchase of anti-parasitic drugs, veterinary services and other control measures (Thomas et al., 2011).

In last three decades the control of nematodes are relying on chemical treatments and many compounds were very effective but the development of anthelmintic resistance against nematodes in various countries had - been reported (Thomas et al., 2011; Arunkumar, 2012) which is threatening the end of anthelmintic era. To counteract this adverse situation, many studies have been conducted in different parts of the world which showed that there are many medicinal plants that have the potential to be used as anthelmintic (Jabbar et al., 2006). However, majority of the evidences reported in ethno-veterinary sources are based on old people observations, instead of proper experimentation. There are many plants which have been scientifically validated for their anthelmintic properties - based on their traditional uses (Iqbal et al., 2004; Iqbal et al., 2005; Githiori et al., 2006; Iqbal et al., 2006). Ihsanullah (2005) documented that 21 different kind of indigenous plants are being used in ethno-veterinary medicine system of Balochistan for the treatment of different ailments including infections caused by endo-parasites in livestock since last many decades.

Keeping in view the above mentioned scenario, present study was accomplished to investigate the resistance against synthetic anthelmintics and herbal control option of nematodes infections in small ruminants in different ecologies of Pakistan.

Materials and Methods

Selection of site, animals and farmers

The study area was situated in two ecologies of Pakistan i.e. Ziarat valley (30° 22' 47 N and 67° 43' 38 E) in Balochistan and second site was Dhulli (32°51'31.02" N 72°11'28.22E) in district Talagang, Punjab Province. At each location four farmers of small ruminant based on range grazing practices were selected during January 2015. Each farmer has mix sheep and goats with a flock size of 60-80 heads. As these animals were expected by having higher nematodes infectivity and according to our knowledge, they were not given any anthelmintics since last 3-4 months.

Experimental design and parasitological techniques

All the female animals (ewes/does) as per higher number of availability between 2-4 year divided in four groups and ear tagged for proper record keeping. The ewes and does at Ziarat were of Harnai and Khurasani breed respectively while at Dhulli, they were White-Shinwar (Afghani cross) and Beetal, respectively. The faecal samples were collected directly from the rectum of these selected animals and examined during January to August 2015 for nematodes infection which was determined through observation of parasitic eggs in faecal sample analysis followed by Faecal Egg count or parasitic larvae through faecal culture (Thienpont et al., 1979; Soulsby, 1982; Urquhart et al., 1996). A total 80 animals (40 ewes and 40 does) were further selected during August 2015 from these flocks at each site based on peak major four nematodes infection i.e., *Haemonchus*, *Trichuris*, *Strongyloides*

and *Trichostrongylus* with infectivity level higher than 2000 FEC. The laboratory results indicated abundance of these four major nematodes equally found in animals at both sites. Other than these many internal parasites were also observed but neglected due to low infection rate. These infected animals were divided in to four equal groups i.e., A, B, C and D. The animals in first three groups were treated with three synthetic anthelmintics comprised of Oxfendazole, Oxfendazole-Levamisole and Ivermectin respectively while last group kept as control of infected animals for comparison (Table 1). These anthelmintics were selected to assess the resistance as most of the farmers being administered to their flocks twice a year since last one or two decades.

Table 1: *Experimental groups of ewes/does at two sites treated with synthetic anthelmintics.*

Groups	Ziarat site		Dhulli site		Treatment	Dose per kg body wt.
	Ewes	Does	Ewes	Does		
A	10	10	10	10	Oxfendazole	5 mg
B	10	10	10	10	Oxfendazole-Levamisole	7.5 mg
C	10	10	10	10	Ivermectin	200ug
D	10	10	10	10	Control	-

Similarly, 48 animals (24 ewes and 24 does) were also selected separately from each site based on same major nematodes infection i.e., *Haemonchus*, *Trichuris*, *Strongyloides* and *Trichostrongylus* with infectivity level higher than 2000 FEC. These infected animals were divided in to four equal groups i.e., A, B, C and D. The animals in first three groups were treated with three herbal anthelmintics comprised of Atreefal Deedan, Deedani and Kirmar respectively while last group kept as infected control for comparison (Table 2).

Faecal samples from all these experimental animals were collected post treatment on day 3rd, 5th, 7th, 10th and 14th for counting the FEC as described by Urquhart et al. (1996). The efficacies of different anthelmintics used in present study were calculated by using the formula as described by Ali (2001).

$$\text{Efficacy (\%)} = \frac{\text{FEC before treatment} - \text{FEC post treatment}}{\text{FEC before treatment}} \times 100$$

Statistical analysis

The Faecal Egg Reduction percentage was calculated by the formula R%= 100 (1-Xt/Xc) where Xt was treated group and Xc was control group (Coles et al., 1992, 2006). A reduction in eggs per gram of faeces less than 95% with confidence interval between 90% to 95% were taken as an indication (Coles et al., 1992) for the existence of anthelmintic resistant regarding nematodes in treated animals. Calculations were made according to (Coles et al., 1992) using a spreadsheet that was created by Angus Cameron, Aus. Vet. Animal Health Services for the University of Sydney. Their calculations are based on those of the Reso FECR4 test analysis program (Version 2.0).

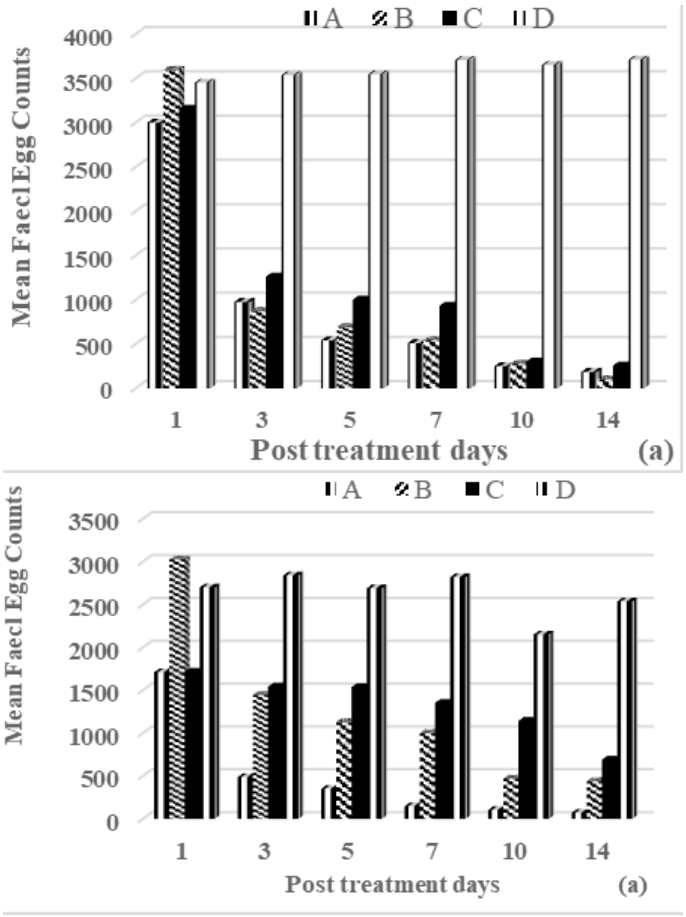

Figure 1: *Mean FECR% in sheep and goats treated with synthetic anthelmintics against nematodes infection at Ziarat (a) and Dhulli (b).*

Results and Discussion

Comparative efficacy and resistance of synthetic anthelmintics against sheep/goats nematodes

All the treated groups of animals showed higher (97-99%) Confidential Interval (CI) in FEC reduction on post-treatment day 14 days (Table 3 and Figure 1). These results revealed there was no resistance of three synthetic anthelmintics against four nematodes of sheep and goats at two target sites. At Ziarat site, the animals in Oxfendazole-levamisol treated group B and Ivermectin group C showed higher CI (99%)

Table 2: *Experimental groups of ewes/does at two sites treated with synthetic anthelmintics.*

Groups	Ziarat site		Dhulli site		Treatment	Composition	Dose rate
	Sheep	Goat	Sheep	Goat			
A	6	6	6	6	Atreefal Deedan	*Emblica officianalis, Terminalia bellerica, Terminalia chebula, Embelia robusta, Ipomoea turpethum, Saussurea lappa, Mallotus philippinensis, Lupinus albus, Artemisia absinthium, Darmina turki, Cascuta reflexa, Black salt, Brassica cernua, Citrullus colocynthis, Cyprus scariosus, Zingiber officinale*, liquid glucose and sugar	2 gms/kg body weight once
B	6	6	6	6	Deedani	*Mallolus philppinensis, Embelia ribes, Piper longum*	5 gms/head/day for 3 days
C	6	6	6	6	Kirmar	*Mallolus philppinensis*	3.5 gms/head/day for two days
D	6	6	6	6	Control	-	-

Table 3: *Mean FECR% and CI in sheep and goats treated with synthetic anthelmintics against nematodes infection at two sites.*

Site	Groups	Post treatment Days						
		1st	3rd	5th	7th	10th	14th	CI
Ziarat	A	3000±19	975±17(59%)	540±15(77%)	510±12(80%)	250±71(92%)	185±61(94%)	98a
	B	3595±15	870±12(73%)	690±11(80%)	540±13(85%)	275±11(92%)	100±68(97.5%)	99a
	C	3155±10	1260±82(59%)	1000±15(68%)	930±62(70%)	300±68(91%)	255±86(92%)	99a
	D	3450±24	3535±23(-5%)	3545±27(-3%)	3705±31(-6%)	3650±29(-6%)	3710±30(-8%)	-
Dhulli	A	2865±28	730±13(60%)	580±11(71%)	540±13(74%)	340±12(86%)	250±99(92.5%)	97a
	B	2955±15	1205±10(59%)	960±16(76%)	884±81(73%)	270±68(92%)	88±48(98%)	99a
	C	3105±97	1155±79(62%)	915±15(70%)	890±77(71%)	267±72(92%)	216±81(93%)	98a
	D	3100±24	3285±27(-7%)	3340±28(-10%)	3405±34(-7%)	3420±33(-8%)	3510±32(-13%)	-

CI: *Confidence Interval; a susceptible or effective.*

Table 4: *Efficacy of herbal anthelmintics (Mean FECR% and CI) against nematodes infection in sheep and goats at two sites.*

Site	Groups	Post treatment Days						
		1st	3rd	5th	7th	10th	14th	CI
Ziarat	A	1710 ± 53	487 ± 91 (72%)	350 ± 85(80%)	148 ± 63 (91%)	103 ± 58(94%)	70 ± 46 (96%)	99a
	B	3025±17	1445±14(50%)	1130±14(59%)	1000±12(64%)	470±13(82%)	440±11(83%)	96a
	C	1717 ± 55	1543 ± 85(10%)	1537 ± 81(10%)	1353 ± 90(21%)	1140 ± 11(34%)	691 ± 12 (60%)	90b
	D	2700±28	2840±33(-1%)	2690±35(-4%)	2820±38(-4%)	2510±41(-7%)	2535±36(-10%)	-
Dhulli	A	2655±13	1455±16(41%)	1050±16(57%)	970±10(61%)	290±76(86%)	175±75(90%)	97a
	B	2775±21	1455±12(42%)	1140±13(53%)	1010±12(58%)	440±13(81%)	440±12(80%)	96a
	C	1660 ± 71	1373 ± 85(17%)	1247 ± 81(25%)	1113 ± 10 (33%)	1020 ± 87(39%)	1137 ± 11(32%)	83b
	D	2750±21	2936±23(-1%)	2989±24(-1.4%)	3021±22(2%)	3173±23(-11%)	3323±23(-5%)	-

CI: Confidence Interval, a susceptible or effective, b resistance or low effective.

than Oxfendazole treated group A (98%). However, the animals in group B present higher Faecal Egg Reduction (97%) followed by A (94%) and C (92%). Similarly, in Dhulli site, animals in Oxfendazole-levamisol treated group B showed higher CI (99%) followed by Ivermectin treated group C (98%) than Oxfendazole treated group A (97%). However, the animals in group B present higher Faecal Egg Reduction (98%) followed by C (934%) and A (92%).

Comparative efficacy of herbal anthelmintics against sheep/goats nematodes

The animals in groups A and B treated with Atreefal Deedan and Deedani showed higher Confidential Interval (96-99%) in FECR while animals in group C treated with Kirmar showed lower (83-90%)

reduction after post-treatment of 14 days (Table 4 and Figure 2). The result revealed that, the former two herbal anthelmintics are effective against the sheep/ goats nematode infection. However, on the basis of Faecal Egg Reduction percentage only Atreefal Deedan performed above 90% at each target site.

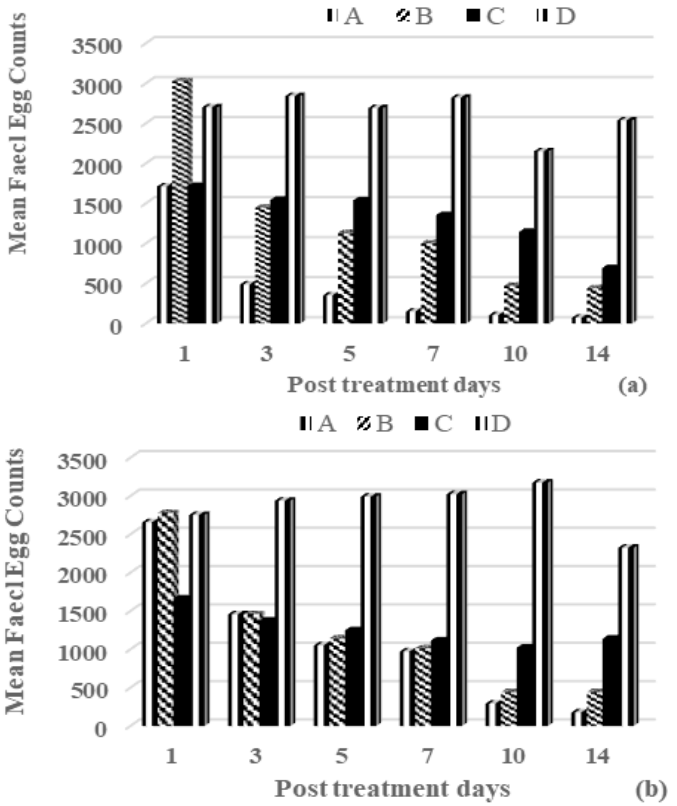

Figure 2: *Efficacy of herbal anthelmintics (Mean FECR%) against nematodes infection in sheep and goats at Ziarat (a) and Dhulli (b).*

Table 5: *Comparative economic values of anthelmintics.*

S. No.	Anthelmintics	Price (Rs.)	Cost/animal (Rs.)
1	Oxfendazole 1000 ml	650	5.85
2	Oxfendazole-Levamisole 1000 ml	1400	28
3	Ivermectin 50 ml	900	17
4	Ateefal Deedan 100 g	100	2
5	Deedani 15 g	57	57
6	Kirmar 7 g	40	40

Comparative economic values of anthelmintics

The Table 5 presents an economic comparison of the five anthelmintics used in present study. The present study results showed that, all three synthetic and two herbal anthelmintics like Atreefal Deedan and Deedani are effective against four nematodes infection in small ruminants. Among the synthetic anthelmintics "Oxfendazole" is cheaper. However, as the Atreefal Deedan markedly effective and economical among all the anthelmintics. At this movement it is early to recommend this remedy for the control of nematodes in small ruminants in Pakistan. Because there are many other nematodes and even cestodes/ trematodes infecting as mix multiple infection at a same time. In the light of these results, further research is proposed on wider scale to address the entire parasitic range for assessing the efficacy of these available herbal products.

The target area farmers were administered three synthetic anthelmintics twice a year since last 5-8 years. The results of present study reveals that these three anthelmintics i.e. Oxfendazole, Oxfendazole-Levamisole and Ivermectin are effective rather resistance against four nematodes of sheep and goats at two ecologies of Pakistan. The reason might be that the area farmers used these anthelmintics in rotation basis. Similar findings regarding these anthelmintics efficacies were also experience in different parts of the world (Han-Bo et al., 1997; Swarnkar et al., 1999; Sheferaw and Asha, 2010). In contrast to these few researchers also experienced resistance with some species of nematodes to these anthelmintics (Bartley et al., 2006; Menkir et al., 2006; Mirhadi et al., 2011). These resistances in particular areas might be due to extensive use of anthelmintics. Therefore, it is recommended that the anthelmintics may be administered on rotational basis.

There are limited references available regarding herbal anthelmintics against parasitic infection in sheep and goats. However herbal products tested under this study composed of different botanical plants were reviewed/surveyed on individual plants basis as anthelmintic properties by different researchers such as *Artemisia absinthium* by Tariq et al. (2009), *Embelia ribes* by Asadulla et al. (2011). *Cuscuta reflexa* by Pavan et al. (2012), *Terminalia belerica* by Manohar et al. (2012), *Operculina turpethum (Ipomoea turpethum)* by Nitin et al. (2012), *Citrullus colocynthis* by Borhade et al. (2013) and *Emblica officinalis* by Satyajit et al. (2013). The combinations of these plants as anthelmintics were also reviewed by different researchers (Githiori et al., 2004; Hussain, 2008; Hordegen et al., 2006; Reddy et al., 2011; Chalapathi et al., 2011; Malvankar, 2012). Keeping in view the situation, it is recommended that a wider study may be launched for further evaluation of efficacy of herbal products based on different parasites and various ecologies of Pakistan.

Conclusions and Recommendations

In small ruminant farming based on rangelands, parasitic infections are generally most prevalent and important health problems. In most part of the world, resistance of worms in sheep and goats against anthelmintics is now very common. The present study results showed no resistance against three anthelmintics. However, the alarming situation needs to assess the resistance of synthetic anthelmintics on wider scale for the future safety and to receive healthy animal production in Pakistan. To overcome this issue, the herbal remedy is very important choice as evident from the present study that Atreefal Deedan markedly effective and cheapest among all the anthelmintics. At this movement it is early or difficult to recommend this remedyfor the control of nematodes in small ruminants. In the light of these results, it is recommended that, further research needed on wider scale, while considering entire parasitic range for assessing the efficacy of these products. Beside these it is clear that, some actions are urgently needed in these areas by providing proper nutrition according to animal physiological conditions and clean hygienic climate control housing to boost up their immunity against the diseases like internal parasites and also for the achievement of higher productivity.

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Author's Contribution

Abdul Razzaq: Developed research plan, implement in the field and lab diagnosis, wrote results and discussion.
Muhammad Islam: Wrote abstract, editing whole manuscript.
Zahra Islam: Wrote introduction and methodology.
Zahid Fatima: Data collection from the field and compilation.
Farmanullah: Statiscal analysics and graphs.

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