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Original Article

Epidemiology, Sero-Diagnosis and Therapeutic Studies on Nematodes Infection in Balochi Range-Sheep at District Quetta, Balochistan, Pakistan

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Abstract

Background: Among the infectious organisms of parasitic origin, gastrointestinal nematodes are very important as they have been reported worldwide. The main aim of the present research study to highlight the annual epidemiological contributing factors associated with the prevalence of gastrointestinal nematodes and their control in sheep.

Methods: A total 1200 faecal samples (100 per month) were collected from farmers holding Balochi-sheep (either sexes, 1-5 years old) during January-December 2012 and analyzed to determine the prevalence of nematodes based on microscopy and ELISA based diagnostic assay. Therapeutic efficacies of different synthetic and herbal medicines against these nematodes were assessed by field trials.

Results: Results showed that 23.92% Balochi-sheep were infected with nematodes. Five nematodes infections were recorded with highest prevalence of *Haemonchus* (7.75%) followed by *Nematodirus* (7.58%), *Strongyloides* (4.42%), *Trichostrongylus* (2.33%) and *Trichuris* (1.83%). The younger and older ewes (one and five years) presented higher nematodes prevalence with peak during March/April and August/September. *Haemonchus* and *Trichuris* positive samples based on coprological examination were also showed 92-100% positive sensitivity for these nematodes by the ELISA. Sheep treated with Ivermectin showed higher reduction (97.76%) in nematode egg counts followed by Atreefal deedan (96.42%) and Oxfendazole (95.44%), respectively.

Conclusion: The gastro-intestinal nematodes are prevalent in all age and either sex of Balochi-sheep with peak during summer. The ELISA based diagnosis is more accurte. The synthetic and herbal products are very effective against sheep nematodes.

Introduction

Among the infectious organisms of parasitic origin, gastrointestinal nematodes are very important as they have been reported by various researchers (1-4) for losses in terms of lower milk and meat production in affected animals. The most common feature of gastrointestinal nematodiasis (GIN) is increased loss of endogenous proteins that leads to retarded growth (5); whereas, heavy worm loads had also been reported for high mortality (6). Apart from production losses, the other associated losses related to GIN includes those spent on the purchase of antiparasitic drugs, veterinary services and other control measures (7). Earlier, in Baluchistan province of Pakistan, prevalence rates of 23.75% and 27.9% have been reported for helminthiasis in sheep and goats, respectively (8). Overall prevalence of 93% and 80% for internal parasitic infections in sheep and goats, respectively at Asghara valley of Baluchistan (9); whereas, in other countries such as Spain, a prevalence rate of 100% had been reported for nematodal infections in sheep population of Galicia city (10).

Several factors are known to determine the epidemiological pattern of the associated disease conditions. These include the ecological zone, climatic conditions, husbandry practices and physiological status of the animal (11). For the control and prevention of helminths in sheep, especially the gastrointestinal nematodes, a thorough knowledge of epidemiological relationship of different parasites with their hosts is very important (12).

Mostly these parasites are diagnosed on the basis of conventional methods like direct smear method and flotation/sedimentation techniques with poor sensitivity and specificity. At present, the modern research regarding the diagnosis of parasites is focused on advanced serological and molecular techniques which are highly sensitive and specific; these include different immunoassays and polymerase chain reaction with various modifications (2, 13).

The control of nematodes for the past thirty years relied heavily on the use of chemicals. These compounds have been very successful but the development of anthelmintic resistance in different nematodes in various countries had also been reported (7, 14-16) which is threatening the end of anthelmintic era. To counteract this adverse situation, a lot more 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 (17). However, majority of the evidences reported in ethno-veterinary sources are based on observations, instead of proper experimentation. There are many plants which have been validated scientifically for their anthelmintic properties and are based on their traditional uses (18, 19-21). There are many different kind of indigenous plants have been documented that are in ethno-veterinary medicine system of Baluchistan for the treatment of different ailments including infections caused by endoparasites in livestock (22).

The present study was therefore accomplished to investigate the epidemiological features and ser-diagnosis of major gastrointestinal nematodes infection in sheep. Additionally, comparative efficacies of synthetic and herbal medicines against these nematodes were also assessed in this study.

Materials and Methods

Profile of District Quetta, Balochistan, Pakistan

The Quetta district lies between 30°-03' and 30°-27' north latitude and 66°-44' and 67°-18' east longitudes. The total geographic area of Quetta district is 2653 Km^2. It varies in elevation from 1254 to 3500 meters. The climate is generally dry with very cold (15 to -7 °C) in winter and relatively mild (32 to 35 °C) in

summer season. The district lies out side the range of monsoon currents and the rainfall is scanty and irregular. A variety of range vegetation species (*Artimisia maritime*, *Cymbopogon jawarancusa*, *Crysopogoneri aucheri*, *Nepeta juncea* and *Astragalus stocksic*) are found on the hills and areas surrounding the hills (23).

Experiment 1: Prevalence of sheep nematodes (host, parasite and climate relationship)

a. Prevalence of nematodes in Balochi-sheep

A total nine sheep flocks (comprising 1200 sheep) were randomly selected from district Quetta and its surroundings (with the history of no de-worming for the last 3-4 months) to determine the prevalence of major nematodes. All the sheep were employed ear tags before the initiation of experiment for proper maintenance of individual record of sheep throughout the experimental period. Faecal samples (n=100/month) were collected randomly from these already selected sheep during January 2011 to December 2011. The nematodes eggs were identified on the basis of their morphometric features and in addition, samples were also subjected to faecal-culture for nematodes larval identification (24, 25-26).

b. Meteorological data

The climate data regarding rainfall, relative humidity and temperature during the whole year of 2011 were obtained from Balochistan Agricultural Research and Development Centre (former Arid Zone Research Centre), Quetta, Baluchistan. These climate factors were correlated with the prevalence of major nematodes of sheep.

Experiment 2: Sero-diagnosis of Haemonchus contortus and Trchuris ovis in sheep

a. Collection of sera samples

The blood samples from 1000 sheep were colleted in clean vacationers from randomly selected flocks from district Quetta. These samples after clotting were centrifuged (3500 rpm for 5 minutes) to isolate the sera, followed by proper labeling (sheep tag number, date etc.) and storge at -20 °C till further use. The faecal samples were also collected from the same experimental sheep and analyzed for presence/absence of different nematodes. On the basis of these faecal analyses the same sheep sera samples were categorized as negative and positive with different nematodes. These samples were selected/separated on the basis of negative (n=300), and positive (infected) with *Haemonchus contortus* (n=200) and *Trichuris ovis* (n=200) for further confirmation through ELISA. These two nematodes were only found in slaughtered sheep as per quantity required for preparation of antigen and others were not found; hence these two were selected for ELISA.

b. Negative and positive control sera

The negative control sera was collected on first day of life from three lambs and used in ELISA as uninfected control sera of sheep. The natural positive sera of 30 sheep were collected from Quetta abattoir. Initially sera were collected and then confirmed harboring nematodes, while examination of gastro-intestinal tract of sheep after slaughtering. These sera were labeled accordingly and stored for further usage.

c. Collection and isolation of nematodes

Adult *Haemonchus contortus* were collected from the infected abomasa and *Trichuris ovis* from caecum of infected sheep form Quetta Slaughter-house. The identification of these nematodes was also confirmed using standard keys (26).

d. Preparation of Excretory-secretory antigen

The excretory-secretory antigen was prepared as described previously (27, 28). Briefly, the fresh adult nematodes were washed using 0.01 M phosphate buffer (pH 7.2) and incubated in phosphate buffer saline (PBS) for 5 hours at 37 °C. After incubation nematodes were removed and the suspension containing excretory-secretory products was centrifuge at 15000 rpm for fifteen minutes. The superna-

tant was shifted to dialysis bags and concentrated under moving fan. These samples were stored at -20 °C at AZRC Quetta till further use in ELISA.

e. Preparation of crude somatic antigens

The crude somatic antigen was prepared as described previously (14, 27). The adult 10 nematodes (*Haemonchus* and *Trichuris*) of mixed sexes were collected from infected animals and washed with normal saline solution. Later these worms were transected 2-3 times with a scalpel blade and mixed with PBS (pH 7.4), homogenized in Teflon tissue homogenizer at 4 °C for one hour. The homogenate was kept undisturbed for 24 hours in refrigerator. Supernatant was collected from homogenate and centrifuged in refrigerated centrifuge at 12000 rpm for 15 min. The supernatant was stored as crude solubilized antigen at -20 °C till further use. Protein concentration of crude somatic and excretory-secretory antigens was also determined (29).

f. Enzyme linked immunosorbent assay (ELISA)

The sera samples were analyzed through ELISA as described previously (14, 27). Briefly, microtitration plate was coated with the antigen by adding 100 µl of the antigen (diluted with carbonate-bicarbonate buffer at a final concentration of 2µg/ml) in each well. Followed by incubation at 37 °C for one hour and then at 4 °C overnight. Unbound antigen was removed by washing the plate with wash buffer. Afterward BST (100 µl) was transferred to each well followed by the addition of 100 µl of IgG-HRP to the first well of each row. Two-fold series dilution from wells 1 to 12 was prepared and antigen was added. Immuno-complex was allowed to form by incubating the plate at 37 °C for 1-2 hours followed by thorough washing to remove excess antibodies. Secondary Ab- HRP (100 µl) was added to each well and plates were again incubated for 2 hours at 37 °C. Excessive unbound antibody-enzyme conjugate was washed off by adding washing buffer. Substrate (100 µl) was added to each well and incubated for 2 hours at 37 °C in the dark. The enzymatic reaction was stopped by adding 50 µl of 1M H_2SO_4 to each well. Absorbance of solutions in all the wells was recorded at 450 nm by using an ELISA reader (Biological Diagnostic Supplies Limited, Uk).

Experiment 3: Field/community therapeutic trial

A total of 150 Balochi-ewes (2-5 years old) were used for this therapeutic experiment. These ewes were reared on grazing and grazing pasture was shared by the multiple sheep farmer flocks. The natural nematodes infection was high in these animals being recycled from contaminated range-land. These ewes naturally infected with mix nematode parasites including *T. colubriformis, H. contortus, N. battus,* and *S. papillosus* and *T. ovis* were divided were divided into five treatment groups A, B, C, D and E each containing thirty ewes. Treatments assigned to different groups were as under:

Group A: Orally administered with Deedani at the dose rate of 5 gm/head/day for 3 days/ Group B: Orally administered with Kirmar at the dose rate of 3.5 gm/head/day for 2 days / Group C: Orally administered with Atreefal deedan at the dose rate of 2 gm/kg of body weight as single dose/ Group D: Orally administered with Oxfendazole at the dose rate of 5 mg/kg of body weight /Group E: Subcutaneously administered with Ivermectin @ 20 µg/kg of body weight

The compositions of all the drugs used in this study are mentioned in Table 1.

Drug efficacy

Faecal samples from all these experimental sheep were collected (on day zero) before the administration of different drugs followed by post treatment on day 3rd, 5th, 7th, 10th and 14th to determine the faecal egg counts (26). The efficacies of different anthelmintics used in present study were calculated by using a formula as follows (30).

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

Statistical analysis

The data were analyzed by using Graph Pad Prism-5 computer statistical package at 5% level of probability (31). The prevalence of different nematodes was analyzed through Chi-square test. The data on FEC regarding efficacy of various treatments against nematodes were analyzed by one way analysis of variance in each treatment group individually. In addition, "Dunn's Multiple Comparison Test" was also performed to compare different groups of sheep (infected with different nematodes) with months (January 2011 to December 2011). The sensitivity of ELISA was calculated as True Positive x 100/True Positive + False negative (8).

Table 1: Composition of herbal products and synthetic anthelminics administered to sheep for therapeutic trial

Herbal products	Composition
Deedani	*Mallolus philppinensis, Embelia ribes, Piper longum*
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*)*
Kirmar	*Mallolus philppinensis*
Oxadec	Oxfendazole
Ivectin	Ivermectin 1%

Results

Experiment 1: Epidemiology of nematodes in Balochi-sheep (host, parasite and climate associated factors)

a. Prevalence of nematodes in sheep

The faecal analysis of sheep under study showed overall prevalence of 23.92% for mixed gastrointestinal nematodes. Significant differences ($P<0.05$) were observed between the prevalence rate of different nematodes and their FEC levels. Among these, *H. contortus* showed higher (7.75%) prevalence followed by *N. battus* (7.58%), *S. papillosus* (4.42%), *T. colubriformis* (2.33%) and *T. ovis* (1.83%) during the study period. Similarly, *H. contortus* also showed higher FEC level as compared to the rest of the nematodes (Table 2). The clinical examinations of these sheep flocks showed no any pronounced signs or symptoms of parasitic infection. No mortality was recorded due to parasitic infection in all selected flocks.

b. Age wise prevalence of nematodes

The prevalence of gastrointestinal nematodes infection and their FEC levels showed significant difference ($P< 0.05$) in all five age groups of sheep at Quetta District. Five years old sheep showed higher nematode prevalence followed by 1, 2 and 3-4 year age groups. While, sheep in four year age group showed higher FEC followed by 5, 3, 1 and 2 year of age groups (Table 2).

c. Sex wise prevalence of nematodes

Results showed that the overall prevalence of gastrointestinal nematodes was 25.33% in female-sheep and 20.5% in male-sheep. Although, the rate of prevalence was apparently higher in female but difference between the gender was statistically similar/non-significant ($P>0.05$). However, the annual (2011) prevalence record in male-sheep showed higher prevalence during April, May, July, October, November and December (Annexure 8). The higher mean FEC level ($P<0.05$) was recorded in females than male sheep (Table 2).

d. Month wise prevalence of nematodes and its relationship with metrological data

Results have indicated two peaks of gastrointestinal nematodes prevalence i.e.

March-/April and August/September, 2011 in Balochi-sheep. However, all the encountered nematodes (*S. papillosus*, *T. ovis*, *N. battus, H. contortus* and *T. colubriformis*) showed highest prevalence during August. Similar pattern of FEC level corresponding to nematodes prevalence peaks were also recorded in Balochi-sheep (Table 3). However, the statistically analysis showed significant ($P<0.05$) difference during the different months of the year (2011).

Table 2: Prevalence of sheep nematodes at District Quetta

Nematodes/age/sex wise		Total infected (n=1200)	Prevalence	Mean ± SD of EPG
Nematodes	*Strongyloides*	53	4.42	784± 167 b
	Trichostrongylus	28	2.33	616±176 c
	Haemonchus	93	7.75	1294±243 a
	Trichuris	22	1.83	502±266 d
	Nematodirus	91	7.58	736±139 b
Age	1	63	26.25	882± 201 d
	2	59	24.58	875±121 d
	3	47	19.58	1088± 222 c
	4	47	19.58	1567± 258 a
	5	71	29.58	1181± 73 b
Sex	Male	135	22.5	1474±349 a
	Female	152	25.33	1415±214 a
	Overall	287	23.92	787 ± 95

Table 3: Month-wise prevalence (%) and mean EPG of sheep nematodes

Months	*S. papillosus*	*T. colubriformis*	*H. contortus*	*T. ovis*	*N. battus*
Jan-2011	2 (1500)	-	-	1 (100)	-
Feb-2011	4 (1000)	1(1000)	-	1 (100)	5 (1060)
Mar-2011	11 (1136)	7(843)	1 (200)	4 (875)	3 (1333)
Apr-2011	3 (1033)	5(640)	6 (1550)	2 (550)	4 (800)
May-2011	-	-	12 (1750)	-	1 (200)
Jun-2011	2 (800)	1(1000)	8 (900)	1 (200)	-
July-2011	2 (650)	2(1450)	7 (1171)	-	4 (300)
Aug-2011	20 (1590)	10(1670)	21 (1733)	12 (1000)	26 (1046)
Sept-2011	8 (1300)	2(800)	18 (1794)	1 (3200)	24 (975)
Oct-2011	1 (400)	-	11 (2100)	-	16 (1019)
Nov-2011	-	-	7 (1829)	-	6 (850)
Dec-2011	-	-	2 (2500)	-	2 (1250)

Table 4: Annual metrological records during 2011 (avg. humidity/temperature and total rainfall) of district Quetta

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Humidity (%)	24	33	23	20	12	11	12	16	18	16	22	20
Temp (°C)	6	7	13	18	26	29	29	28	24	20	15	7
Rainfall (mm)	8.9	91.90	49.36	47.20	0.75	2.79	1.01	7.87	6.58	15.74	19.81	0.76

Experiment 2: Sero-diagnosis of sheep nematoes

The comparative sensitivity of coprological examination with ELISA was also determined in present study. The sera samples (n=300) collected from sheep being infected with *H. contortus* and *T. ovis* (based on coprological examination) were found 100% and 92-94% positive with two antigen i.e., excretory-secretory and crude-somatic, respectively (Table 5). The sera of un-infected sheep (based on coprological examination) showed marked difference of ELISA positive based on two antigenic analyses. Based on excretory-secretory antigen, 112 (22.4%) and 141 (28.2%) samples were found positive for *H. contortus* and *T. ovis,* respectively. While, based on crude somatic antigen 131 (26.2%) and 88 (17.6%) samples were found positive for *H. contortus* and *T. ovis,* respectively (Table 5).

Table 5: Comparative sensitivity of ELISA and coprological examination for determination of *H. contortus* and *T. ovis* infection in sheep

Total sample examined	Positive samples		
	Coprological analysis(N)	ELISA	
		ES Antigen N (%)	CS Antigen N (%)
300	300	300 (100)	277 (92.33)
300	-	112 (22.4)	131 (26.2)
300	300	300 (100)	281 (93.67)
300	-	141 (28.2)	88 (17.6)

Experiment 3: Therapeutic trial

All the treated groups of sheep (A, B, C, D and E) showed statistically significant ($P<0.05$) difference of FEC reduction in post-treatment 3-10 days. The sheep treated with Ivermectin showed higher reduction (97.76%) in nematode egg counts followed by Atreefal deedan (96.42%) and Oxfendazole (95.44%). These were found effective against sheep nematdes. Two herbal products i.e., Deedani and Kirmar showed lower percentage of egg counts reduction (42% and 47% respectively) and found not effective against sheep nematdes (Table 6).

Table 6: Mean (±SD) faecal egg counts and percentage reduction in egg counts for different treatments in sheep infected with nematodes

Treatment	0 day	3rd Day	5th Day	7th Day	10th Day	14th Day
Deedani	2167± 165	1787±417 (17.61%)	1417±401 (34.26%)	1243±475 (42.39%)	890±378 (59.09%)	1243±546 (42.44%)
Kirmar	2060 ± 89	1463±457 (29.16%)	1440±467 (29.71%)	1467±501 (28.59%)	1137±642 (44.46%)	1077±669 (47.33%)
Atreefal Deedan	2113± 61.31	1147±390 (45.43%)	800±459 (62.06%)	373.33±473 (81.91%)	193.33±264 (90.99%)	76.66±128 (96.42%)
Oxafax	2127 ± 38.79	1540±368 (27.87%)	653±353 (69.56%)	353±414 (83.11%)	177±385 (91.50%)	97±298 (95.44%)
Ivermectin	2080 ± 132	487±497 (76.62%)	350±468 (83.35%)	147±345 (92.88%)	67±241 (96.86%)	50±187 (97.76%)
Control	2093 ± 48.40	2100±230	2047±378	2107±521	2163±430	2090±679

Discussion

Gastrointestinal nematodes are a major hurdle in the exploitation on livestock sector especially in sheep. There are various reports on the prevalence of nematodes in various regions of Pakistan like Punjab, Pakistan (46.33%), Hyderabad, Pakistan (42%) and Multan, Pakistan (37.18%) (32-34). Our findings are in line with the results of these previous studies. In contrast, as compared to our findings higher prevalence rate (63.50%) of nematodes at Rawalpindi and Islamabad areas of Pakistan has also been reported. Alarmingly higher prevalence (100%) of sheep nematodes has also been reported in some regions like Galicia, NW Spain (10). These variations in prevalence of different nematodes species might be due to the change in climate at different ecologies (36).

Five years old sheep showed higher nematode prevalence followed by those of 1, 2 and 3-4 year age groups. Higher nematode prevalence in adult sheep might be due to grazing on larger area of pastures being contaminated with various flocks and different stress conditions like climate, long daily traveling and gestation (36-37). In contrast, results of some previous studies have also shown higher prevalence (40.31%) in younger sheep (less than nine months) than the older ones (33.08%); that might be due to low immunity in younger sheep than the older ones.

In sex wise prevalence, no significant difference was detected between males and females. Similar findings have been reported previously (6). Conversely, higher prevalence in males than females has also been reported in some previous studies (38). The hormonal difference might be correlated with parasitic susceptibility. It has been reported that males are more susceptible than female due to androgen hormones (26). The females are more resistant to infection that might be due to the stimulatory effects of estrogen on immune response; whereas, the androgen have an opposite effect in males. In contrast, it was clarified that the higher prevalence in females might be due to lower resistance on the part of their reproductive events and insufficient/unbalanced feed against higher need (34). The female animals generally harbored a significantly higher worm burden than male animals due to the enhanced grazing of females during lactation and their low resistance during pregnancy and parturition. The rearing systems also supported the nematodes infectivity it was observed that the goats in the organic system had higher fecal egg counts than the goats in the conventional system (39).

The results of month wise prevalence of nematodes were in agreement with previous studies (33, 40) who reported peak nematode infection during the summer months. The climatic factors are also associated with the development of larval nematode and subsequent infection like optimal temperature ranged from 18-26 ^{o}C and humidity 80-100% (26). Similarly hot, wet and rainfall conditions also favour the higher larval development of nematodes (36, 41). The climate data of district Quetta revealed that, the average humidity was higher (41-77%) during January to March 2011. The average temperature was higher (33-37 ^{o}C) during May to August 2011. A total 272 mm rainfall received during 2011 at district Quetta. Higher rainfall recorded during February and April (Table 4). In present study, the first peak directly showed relationship with favorable climatic condition like rainfall (50-90 mm) during February/March along with raise in temperature and humidity. The second peak of nematodes infection had less correlation with rainfall (less than 10 mm) rather than humidity and temperature that was favorable for nematode egg hatching and subsequently infection to sheep during August/September.

The diagnosis of nematodes mostly depends upon observation of clinical signs and further confirmation with fecal analysis. These diagnostic measures have limitations and clinical signs usually become pronounced when there

is serious infection. The nematode eggs are only observed in faeces after the completion of prepatent period that approximately takes 3–4 weeks. So, reliable serological assay such as ELISA which enables the detection of subclinical or early infection is very important (42). Hence, serological diagnosis should be preferred because *anti-H. contortus* antibodies can be detected as early as one week post infection and thus can facilitate early chemotherapeutic intervention (43). The present study findings are in accordance with Qamar (6) who reported 100% sensitivity of ELISA for anti-*H. contortus* antibodies. The sensitivity differences based on antigen types were also in agreement with Mir et al. (27) who showed that the ELISA sensitivity based on excretory-secretory antigen was significantly higher (87.5%) compared to crude somatic antigen (72.22%).

The sheep that were negative by microscopic examination of faecal material but positive with ELIS indicated that the sheep might have infection but with immature worm stages or arrested form that could be detected after patent period. These results are in agreement with Lone et al. (42) who also compared ELISA with Faceal analysis and detected higher positive samples (77%) with ELISA compared to faecal examination (10.4%). They added that the infection was detected between 18-27 days after the infection with ELISA and it was much earlier than the time required for the infection to reach the patent period (42 days).

In therapeutic trial, the sheep treated with ivermectin showed higher reduction (97.76%) in nematode egg counts followed by Atreefal deedan (96.42%) and Oxfendazole (95.44%), respectively. These results are in agreement with some previous studies (44-45) who found similar FEC reduction with Ivermectin against gastrointestinal nematodes of sheep. Similarly, the lower efficacy of Oxfendazole has also been reported previously (46-47). There are limited references available regarding FEC reduction percentage of nematode in sheep with herbal products. However, such products composed of different botanical plants have been studied by different scientists such as *Citrullus colocynthis* (48), *Emblica officinalis* (49), *Cuscuta reflexa* (50), *Terminalia belerica* (51), *Operculina turpethum (Ipomoea turpethum)* (52), *Embelia ribes* (53), *Terminalia alata* (54) and *Artemisia absinthium* (55).

The combination of these plants as anthelmintics have also been studied by different authors such as *Zingiber officinalis, Piper longum, Trychyspermum ammi, Acorus calamus, Glycyrrhiza glabra, Cuminum cyminum* and *Saussurea lappa* (56); *Citrullus colocynthis, Cuscuta reflexa Mallotus philippinensis* and *Zingiber officinale* (57); *Hagenia abyssinica, Olea europaea var. africana, Annona squamosa, Ananas comosus, Dodonea angustifolia, Hildebrandtia sepalosa* and *Azadirachta inclica* (18); *Ananas comosus* (Bromeliaceae), *Azadirachta indica* (Meliaceae), *Caesalpinia crista* (Caesalpiniaceae) and *Vernonia anthelmintica* (Asteraceae), *Fumaria parviflora* (Papaveraceae) and *Embelia ribes* (58). The efficacy of herbal product Trikatu churna *(Piper nigrum L., Piper longum L. and Zingiber officinale Roscoe)* against GIT parasites have also been reported previously (59-60).

Conclusion

The gastro-intestinal nematodes are prevalent in all age groups and either sex of Balochi-sheep with peak during summer. The ELISA based diagnosis is more accurate. The synthetic (Ivermectin) and herbal (Atreefal deedan) products are equally effective against sheep nematodes.

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