Epidemiology and Multidrug Resistance of Strongyle Nematodes in Ordos Merino Sheep

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Research article

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Abstract

Background: Regular whole-flock treatments and long-term repeated use of similar anthelmintics has led to the development of anthelmintic resistance and widespread epidemic of sheep nematodiasis. The current work was carried out to understand the prevalence of gastrointestinal nematodes in Ordos merino sheep and to evaluate the efficacy of currently used anthelmintics.

Results: A total of 4014 fresh fecal samples were collected from sheep from March 2017 to April 2019 and fecal nematode eggs were qualitatively and quantitatively analyzed for understanding the prevalence of sheep nematodiasis. The anthelmintic efficacy of currently used drugs was evaluated in naturally infected sheep. The gastrointestinal nematode infection was serious in Ordos merino sheep. The infection rates of nematodes in three consecutive years were 84.3%, 36.9%, and 42.3%, respectively. The *Hemonchus contortus* and *Nematodirus sp*. were the dominantly infected species with the infection rates of 84.3% and 65.6% in 2017. Moreover, these species were acquired highly resistance to the Ivermectin, Doramectin, Albendazole and Levamisole. The percentages of FERC were 6.9%, 1.7%, 3.5% and 79.0%, respectively, following single administration. However, Nitroxynil and Closantel showed a strong anthelmintic efficacy on popular dominant species *Haemonchus contortus* and some other nematodes, while almost no effect on *Nematodirus sp*.

Conclusions: The prevalence of gastrointestinal nematodes in Ordos merino sheep and their resistance to commonly used anthelmintics were comprehensively understood, and the high efficacy drugs on predominant species were screened out. The results will pay a good foundation for developing of reasonable drug use.

Background

Ordos fine wool sheep is a kind of merino sheep combination of wool and meat, mainly distributed in the southwestern region of Ordos, Inner Mongolia, and plays an important role in animal husbandry production in this region. The total number of Ordos fine wool sheep was 1.57 million, which is one of the most important fine wool sheep in Inner Mongolia Autonomous Region[1]. However, the epidemic of helminths in sheep, particularly gastrointestinal nematode parasitism in this area is very common, which has not been effectively controlled for a long time, seriously affects the production performance of sheep and hinders the healthy development of the local fine wool sheep industry. Gastrointestinal nematodes (GIN) of ruminants parasitize in abomasum, small intestine, and in large intestine. These nematode gastrointestinal infections not only can cause chronic disease [2], but also can cause severe economic consequences directly related to lower milk production, slower growth, and even mortality, or indirectly related to the treatment costs and the associated workload [3].

In addition, the majority of farmers have insufficient understanding of the sheep parasitic diseases, lack scientific and targeted control of parasitic disease. Regular whole-flock treatments with anthelmintics is still the most commonly used measure to control gastrointestinal nematode infections in sheep with

neither have a correct diagnosis nor scientific evaluation of the deworming effect [4]. Therefore, the deworming program in the main distribution areas of Ordos fine wool sheep has repeatedly failed, resulting in a serious epidemic of gastrointestinal nematodes in sheep. Few epidemiological study have been conducted in current research region of Inner Mongolia concerning sheep parasitism.

At the present study, firstly, a epidemiological investigation was performed on gastrointestinal nematodes in Ordos merino sheep to understand the current status of nematode diseases. Secondly, the anti-nematode efficacy of several anthelmintics was comparatively studied in naturally infected sheep to screen out the most effective drugs on predominant species of nematodes. Thirdly, based on the results of epidemiological investigations and anthelmintic experiments, the scientific and effective comprehensive prevention and control measures are proposed to provide a reliable scientific evidence for early and effective prevention and treatment of sheep gastrointestinal nematodes in research area.

Results

Total infection rate of strongyle nematode in sheep

Total of 4014 sheep from 180 family owner farms located in southwestern region of Ordos in Inner Mongolia, China, were investigated in this study during March 2017 to April 2019. The number of sheep investigated consecutive three years was 1496, 1256 and 1262, and the overall prevalence of nematode infection in each year was 84.4%(1262/1496), 36.9%(464/1256), and 42.3%(534/1262), respectively, as shown in table 2. Therefore, the prevalence of sheep nematodiasis in this area is relatively serious.

It is known from table 2 that the epidemic of gastrointestinal nematodes in fine wool sheep in the investigated area in 2017 was very serious, however the survey results in 2018 and 2019 showed that the nematodes infection rate of sheep decreased significantly. The main reason is that during the first epidemiological investigation, we carried out a comparative study on deworming effects of several different anthelmintics, and two kinds of antihelminthics with high efficiency on the predominant species of nematode were screened out, and farmers are courage to use these drugs clinically in investigated area.

Based on morphological characteristics of the eggs and the typical structure of adults, combined with third-stage larvae (L3) morphology, a total of 7 nematodes were initially identified during the study. They are Hemonchus contortus, Nematodirus spp., Oesophagostomum spp., Trichostrongylus spp., Chabertia spp., Ostertagia spp. and Trichuris spp. Among them, the Haemonchus contortus and Nematodirus spp are the predominant species in infected sheep. The different nematodes and infection rate was shown in table 3 (2017). Some of egg images and adult nematodes observed through a microscope, and the Hemonchus contortus on the surface of the abomasum mucosa following autopsy are shown in Fig 1 and Fig 2.

The most prevalent eggs were from Hemonchus contortus (84.3%), followed by Nematodirus (65.6%), then followed by Oesophagostomum (19.4%) and Trichostrongylus (17.0%). The least prevalent eggs

were from Ostertagia (7.1%) and Trichuris (5.3%). Therefore, the nematodes that are most harmful to fine wool sheep in the southwestern region of Ordos are Haemonchus contortus parasitized in the abomasum and Nematodirus parasitized in the small intestine.

Gastrointestinal nematode infection rate of sheep grazing in different type of pastures

Due to the large differences in pasture characteristics and landform types in the southwestern region of Ordos, the species and distribution of nematodes in this region are affected to some extent. Therefore, the experimental sheep were divided into four different groups: sheep grazed freely in sandy pasture, low-lying pasture and hilly pasture, as well as, sheep raised in a pen. Then the epidemiology of sheep nematodiasis was investigated and analyzed, respectively (Table 4).

As can be seen from Table 4, the survey results in 2017 showed that the nematode infection rates of sheep from the four different investigated areas are quite high, and the highest nematode infection rate (88.0%) was found in sheep grazed low-lying pasture. However, the nematode infection rate of sheep raised in a pen is relatively low. The survey results in 2018 and 2019 showed that the nematode infection rates of all four groups of sheep have decreased significantly, mainly due to the administration of suitable anthelmintics under our guidance.

Anthelmintic efficacy

According to the epidemiological survey of gastrointestinal nematodes in sheep, sheep nematode infection was very serious in research area. Therefore, in order to further examine the deworming effect of extensively used anthelmintic drugs and to screen out the drugs with prominent killing effect on predominant species of gastrointestinal nematodes in investigated area, a total of 140 severely infected sheep (PGE>2000) were selected and randomly allocated to 7 groups, and administrated different drugs, as shown in table 1. On the 14th day after administration, fecal samples were collected and quantitatively analyzed by means of modified McMaster technique (Table 5).

It is known from Table 5 that the fecal egg count reduction result on the 14th day after administration shows that albendazole, ivermectin and doramectin basically no anthelmintic effect on gastrointestinal nematodes prevalent in investigated area. The average FECR in these three groups of sheep was less than 10%. Levamisole also had a poor deworming effect on nematodes, with a FECR of 79.0% after administration. However, nitroxynil and closantel injections showed strong anti-nematodal effects, and their FECR reached over 99% after administration.

Gastrointestinal nematode drug resistance

The results of anthelmintic efficacy indicated that avermectins, the widely used anthelmintics at present, have basically no deworming efficacy on predominant species of nematode in examined area. In order to further verify the resistance of sheep gastrointestinal nematodes to ivermectin and doramectin, 60 sheep naturally infected with gastrointestinal nematodes (EPG≥2000) were selected again and randomly allocated to 6 groups with 10 sheep in each group. And then the experimental sheep were injected

subcutaneously with three different doses of ivermectin and doramectin, respectively. Fecal samples were collected on the 14th day after administration for quantitative detection of EPG to evaluate the anthelmintic efficacy. The grouping, dosing schedule and results are shown in Tables 6 and 7.

The results in Table 6 show that doubling the dose of ivermectin also almost no deworming effect on gastrointestinal nematodes in sheep prevalent in the examined area. On the 14th day after ivermectin administration, the FECR of sheep in both therapeutic dose group and in increased dose group was less than 15%. Therefore, the epidemic gastrointestinal nematodes in this area have become highly resistant to ivermectin.

It can be known from Table 7 that the therapeutic dose and the increased dose of doramectin still have no deworming effect on the predominant species of gastrointestinal nematodes that are prevalent in the examined area. Anthelmintic effect of the therapeutic dose of doramectin is less than 10%, and the anthelmintic effect of increased dose of doramectin is still less than 20%. This indicates although doramectin has no history of clinical use in this area, but it also has no anthelmintic effect on the digestive tract nematodes due to both doramectin and ivermectin belong to the same class of avermectins with same anti-parasitic mechanism. In other words, the prevailing dominant species of gastrointestinal nematodes already possess high cross-resistance to ivermectin and doramectin.

Discussion

The epidemiological investigation of sheep helminthiasis found that the epidemic of gastrointestinal nematodes in Ordos fine wool sheep is very common and serious, and most of they are mixed infection with multiple nematodes in the southwestern region of Ordos, especially the infections of Haemonchus contortus and Nematodirus spp. The overall prevalence of nematode infection of sheep in three consecutive years was 84.4%, 36.9% and 42.3%, respectively. Haemonchus spp. was found to be the most prevalent gastrointestinal nematode with prevalence of 84.3% in 2017. This result is much similar to data of epidemiological survey of sheep helminthiasis obtained by Yong Rong et al [5] from the same region, the total nematode infection rate was 80.7%, and the dominant species were Haemonchus contortus and Nematodirus spp. Therefore, sheep nematodiasis has been relatively serious in southwestern Ordos, which is one of the main constraint factors affecting the local sheep farms. We believe that the main reasons for such a widespread and severe epidemic of sheep nematodiasis are as follows: (1) Regular whole-flock treatments with anthelmintics is still the most commonly used measure to control endoparasitic infections in small ruminants. (2) Majority of farmers and herdsmen have insufficient understanding of sheep nematodiasis, and they have no basic techniques of diagnosing nematodiasis and evaluating the efficacy of anthelmintics. (3) Long-term repeated use of the same anthelmintics has led to the development of high anthelmintic resistance among these worms. (4) Most of the farmers and herdsmen lack the basic pharmacological knowledge, and do not distinguish between the generic name and brand name of the drug. When encountering the same drug with different brand names, it is considered to be different drugs. Therefore, it is very common to repeatedly use the same drug without knowing it.

Gastrointestinal nematode infection in small ruminants is widespread and much serious in most of countries around the world, and is one of the main factors affecting the healthy development of the sheep industry as well. However, there are significant differences between the prevailing nematode species and dominant species. Saidi et al [6] studied the parasitism of gastrointestinal nematodes of goat in the North West of Algeria for 2 years showed an overall prevalence of 96% with dominance of the Oatertagia spp. (56%) followed by Trichostrongylus spp. (20%), and the epidemiology was affected by season, age, type of grazing, and area. In some other parts of Africa, the prevalence of goat nematode infection can reach 100% [6]. In Western Pomerania of Poland, the mean extensity of infection with gastrointestinal parasites in both sheep and goats was 100% [7]. Baihaqi et al reported [8] that two types of local sheep are infected with various gastrointestinal worms during the dry and rainy seasons. The highest prevalence of GI worms was found in thin-tailed sheep during the rainy season (76.47%), and the dominant GI nematode infection in Garole sheep was H. contortus (63.91%), followed by Oesophagostomum spp. (16.25%)and Trichostrongylus spp. (10.50%). In addition, sheep flocks the UK and elsewhere in Europe, also in Canada and the Rocky Mountain States of the USA, the most common aetiological agent of parasitic diseases is gastrointestinal nematodes [9].

Gastrointestinal nematode control programs are mainly based on a combination of both animal management practices, pastures management, and especially the use of anthelmintic drugs [10]. However, the intensive use of synthetic anthelmintics for treating and control of gastrointestinal nematodes in the sheep farms has led to the widespread development of resistance to one or more anthelmintic drug classes at the same time [11-13].

From the results of our deworming experiments, we can see that the dominant nematodes species infected in sheep have developed high resistance to the current most extensively used ivermectin and albendazole, and the average FECR egg is less than 10%. As well as, this dominant species acquired strong resistance to levamisole, its FECR is 79%. However, the nitroxynil and closantel, which are not widely used locally, show strong antinematodal effects, and their FECR is more than 99% following a single administration. According to report by Yong Rong [5], the comparative deworming test of sheep nematodes in the same area showed that the gastrointestinal nematodes of sheep had developed resistance to albendazole with FECR of 33%, but ivermectin showed a strong antinematodal effect, and the FECR is almost 100%. However, the results of current study on resistance to gastrointestinal nematodes in sheep showed that the increased dose of ivermectin did not have any deworming effect, even the doramectin, which has not been widely used in this area before, also has a very poor deworming effect. This indicates that the gastrointestinal nematodes endemic in this area have developed high cross-resistance to ivermectin and doramectin.

Anthelmintic resistance to a wide range of gastrointestinal nematodes in small ruminants were proven in a growing number of countries, such as England, France, Germany, Norway, Sweden, Denmark, Netherlands, Austria, Switzerland, Lithuania and Slovakia [4]. Mickiewicz [14] reported, their study shows for the first time that resistance of some species of gastrointestinal nematodes (namely Heamonchus contortus) to benzimidazole anthelmintics occurs in goat population in Poland. Bartley [15] emphasized

that the anthelmintic resistance of gastrointestinal nematodes of small ruminants has been observed worldwide. Francisca Flávia da Silva [16] reported, multi resistance was observed in all evaluated farms, wherein 95% of farms had high resistance to albendazole, 85% to ivermectin, 80% to closantel, 40% to levamisole, and 45% to monepantel. In another study involving 35 sheep farms in the state of São Paulo, resistance to albendazole and ivermectin was observed in 100% of the farms studied, while 92% showed resistance to closantel and 53% to levamisole [17]. High anthelmintic resistance was also reported by Melo [18], when evaluating the efficacy of ivermectin and levamisole in 13 sheep farms in the Agreste of the State of Paraíba, with a reduction of only 30.9% and 93%, respectively.

The main cause of anthelmintic resistance of nematodes is the prophylactic and repeated use of limited anthelmintics as a means to control gastrointestinal nematode infestation without a diagnosis. If treatments are conducted in most sheep farms, commonly whole-flock treatments once or twice annually with a very limited rotation of applied anthelmintics and without fecal sampling for efficacy control are applied [19]. In order to reduce the resistance of nematodes and to achieve effective control of sheep nematodiasis, farmers must take comprehensive measures with combination of both animal management practices, pastures management, and the use of anthelmintic drugs. It is a great urgency to minimize dependency on anthelmintics only and stop using these preventively as much as possible and without prior checking for worm eggs in feces. It is also strongly recommend that sheep farmers should check the efficacy of anthelmintic products every time when it be used [20].

Conclusion

We undertook a comprehensive survey and anthelmintic study to understand the epidemiology of gastrointestinal nematodes of Ordos fine wool merino sheep and the nematodes resistance. The findings of this study revealed that free-range sheep infected with gastrointestinal nematodes very severely in research area and most of them become highly resistant to extensively used anthelmintics such as invermectin, doramectin, albendazole and levamisole. The experimental results will pay a good foundation for the subsequent development of reasonable anthelmintic regimen.

Methods

Ethical approval

A total of 10 sheep with severe nematode infections were selected and anesthetized with pentobarbital sodium for autopsy to examine gastrointestinal nematodes. All study procedures and animal care activities were conducted in accordance with the Bioethics Committee of the College of Veterinary Medicine, Inner Mongolia Agricultural University (12150000460029509N), Hohhot, China.

Selection of farms

According to different breeding models and different types of grassland, the investigated sheep are from sheep raised in a pen and free-range sheep in southwest of Ordos city, Inner Mongolia, China. Free-range

sheep are further divided into sheep grazing in sandy grassland, low-lying grassland and hilly grassland. The majority of sheep farms contain 150 or more sheep, which graze year-round on pastures with a variable provision of supplementary feed during winter and early spring.

Sample collection

The study was conducted for three consecutive years, from March 2017 till April 2019. A total of 4014 sheep from 180 family farms were examined: 1496 in 2017, 1256 in 2018 and 1262 in 2019. Fresh fecal samples were directly collected from the rectum of the individual sheep. After collection, the samples were stored cool during transportation to the laboratory and then stored at 4°C in the refrigerator until analysis to avoid hatching of the eggs. All of samples were analyzed within 48 h by fecal flotation method. The randomly sampled sheep were naturally infected and belonged to both sexes, and were came from sandy pasture, low-lying pasture, hilly pasture and raised in a pen. Sampling was carried out at early morning during March to April in consecutive three years. For comparative study of anthelmintic effects, sampling was carried out pre-treatment and 14th day post-treatment.

Qualitative and quantitative examination

The first was the qualitative method employed to find and identify the nematode eggs based on the morphological observation of different eggs by flotation using a saturated magnesium sulfate solution (specific gravity is 1.32) [21]. The second was the quantitative method used to count nematode eggs. Fecal egg counts (FEC) were undertaken using the modified McMaster technique with a same solution as the flotation fluid, a method relatively simple and cheap procedure to carry out, as described in Veterinary Clinical Parasitology [22]. The minimum detectable limit of the McMaster technique was 25 eggs per gram (EPG) of feces.

Identification of nematodes

The identification of nematode species were based on morphological observation of eggs by microscope [21,22] and typical structures of adult nematodes those came from autopsy, and this was subsequently further verified on the basis of third-stage larvae (L3) morphology [21].

Anthelmintic Efficacy

With the objective of assessing the efficacy of deworming, 140 severely infected sheep (PGE>2000) were selected, labelled and weighed individually to calculate the correct dosage, and then randomly divided into 7 groups comprising of 20 animals each, and given different anthelmintics, as shown in table 1. All of drugs used in this study are commercially available anthelmintics.

Fresh fecal samples were collected from the rectum directly at a day of pre-treatment and 14th day post-treatment, and analyzed by means of modified McMaster technique. The reductions in fecal egg counts (FECR) was calculated according to the following formula.

FECR(%) = (EPGpre-treatment - EPGpost-treatment) / EPGpre-treatment×100%

Abbreviations

FECRT: Fecal egg count reduction test; GINs: Gastrointestinal nematodes; L3: Third stage larvae; EPG: Eggs per gram; FEC: Fecal egg counts.

Declarations

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Authors' contributions

LE and JW performed the experiments and collected the raw data. LE, XH and SH designed the study and wrote the initial draft of the manuscript. XH and SH supervised the study. BB and YH participated in conduction of field trials. LE, JW and SH performed statistical analysis and revised the manuscript. All authors revised the manuscript, read and approved the final manuscript for publication.

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Availability of data and materials

All relevant information has been included in the manuscript. Data analysed for this manuscript are available from the corresponding author on request.

Ethics approval and consent to participate

The research involved obtaining information from farmers and treating sheep naturally infected with strongyle nematodes. The study was conducted in accordance with the Bioethics Committee of the College of Veterinary Medicine, Inner Mongolia Agricultural University (12150000460029509N), China. For the field trials, informed verbal consent was obtained from the each farmers, and the proceeding was approved by the Ethics Committee.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Tables

Table 1 Grouping and dosing regimen of 6 commonly used anthelmintics for controlling nematodes

Groups	n	Drugs	Dose (mg/kg)	Route of administration
1	20	Albendazole tablets	15	po
2	20	Ivermectin injection	0.2	sc
3	20	Doramectin injection	0.2	sc
4	20	Levamisole tablets	7.5	po
5	20	Nitroxynil injection	0.3	sc
6	20	Closantel injection	5.0	sc
7	20	Negative control	——	——

Table 2 Total gastrointestinal nematode infection rate of Ordos fine wool merino sheep

Investigation time	Number of investigated sheep	Number of infected sheep (EPG≥300)	Infection rate (%)
March to April 2017	1496	1262	84.4
March to April 2018	1256	464	36.9
March to April 2019	1262	534	42.3

Table 3 Nematode species and infection rates in Ordos fine wool merino sheep (2017)

Nematode species	Total samples	Positive samples (total EPG≥300)	Infection rate (%)	Parasitic site
Hemonchus contortus	1496	1261	84.3	abomasum
Nematodirus spp.	1496	980	65.6	small intestine
Oesophagostomum spp.	1496	290	19.4	colon
Trichostrongylus spp.	1496	254	17.0	small intestine
Chabertia spp.	1496	196	13.1	large intestine
Ostertagia spp.	1496	106	7.1	abomasum, small intestine
Trichuris spp.	1496	79	5.3	caecum

Table 4 Nematode infection rate of sheep grazing in different type of pastures

Pasture type	Infection rate（%）		
	March to April 2017	March to April 2018	March to April 2019
Sandy pasture	82.7 (311/376)	35.0 (112/320)	38.4 (123/320)
Low lying pasture	88.0 (330/375)	43.9 (137/312)	51.0 (158/310)
Hilly pasture	85.0 (323/380)	36.4 (113/310)	42.9 (133/310)
Raised in a pen	76.3 (286/375)	32.5 (102/314)	36.9 (118/320)

Table 5 Fecal egg count reduction rate for each group of sheep at 14th day following drug administration

Groups	Drugs	n	Pre-treatment EPG (mean)	14th day post-treatment EPG (mean)	FECR (%)
1	Albendazole	20	2513	2272	9.6
2	Ivermectin	20	3523	3396	3.6
3	Doramectin	20	3212	3026	5.8
4	Levamisole	20	3135	2476	79.0
5	Nitroxynil	20	2359	23	99.0
6	Closantel	20	2705	22	99.2
7	Untreated	20	3330	3753	-12.7

Table 6 Comparison of anthelmintic efficacy of Ivermectin with different doses

Dose (mg/kg)	n	Pre-treatment EPG (mean)	14th day post-treatment EPG (mean)	FECR (%)
0.2	10	2007	1907	5.0%
0.3	10	3075	2690	12.5%
0.4	10	3420	2948	13.8%

Table 7 Comparison of anthelmintic effects of Doramectin with different doses

Dose (mg/kg)	n	Pre-treatment EPG (mean)	14th day post-treatment EPG (mean)	FECR (%)
0.2	10	2550	2322	7.0%
0.3	10	2293	1893	17.4%
0.4	10	2963	2430	18.0%

Figures

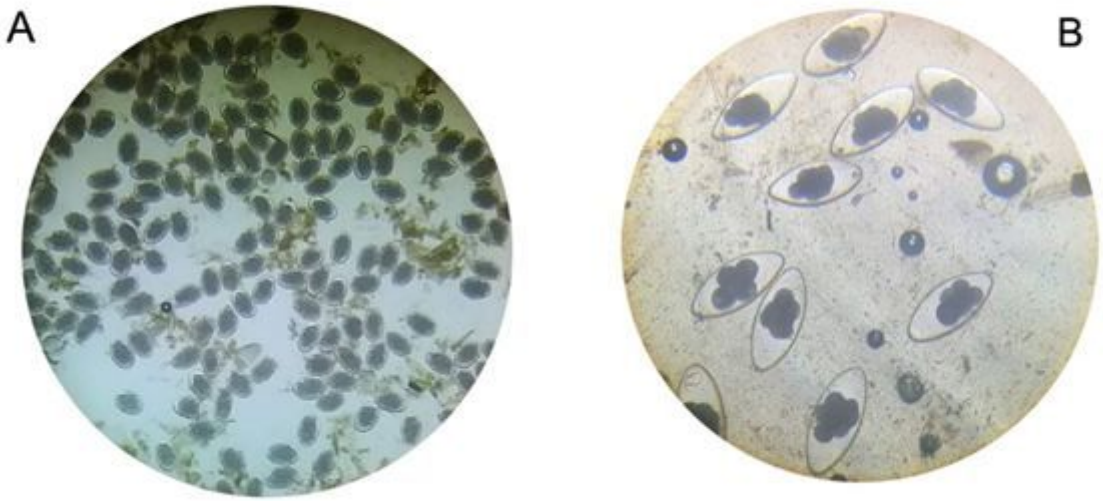

Figure 1

Observed images of Haemonchus contortus eggs (A) and Nematodirus eggs (B) (10×10)

Figure 2

Abomasum mucosa is covered completely with Haemonchus contortus in severely infected sheep