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 use of similar anthelmintics have led to the development of anthelmintic resistance and widespread epidemics of nematodiasis in sheep. This study was performed to understand the prevalence of gastrointestinal nematodes in Ordos Merino sheep and evaluate the efficacy of currently used anthelmintics.

Results: Between March 2017 and April 2019, a total of 4014 fresh fecal samples were collected from sheep, and fecal nematode eggs were qualitatively and quantitatively analyzed to understand the prevalence of sheep nematodiasis. The anthelmintic efficacy of currently used drugs was evaluated in naturally infected sheep. Severe infection by gastrointestinal nematodes was observed in the Ordos Merino sheep. The infection rates of the nematodes in 3 consecutive years were 84.3%, 36.9%, and 42.3%. *Haemonchus contortus* and *Nematodirus* sp. were the predominant nematode species, with infection rates of 84.3% and 65.6%, respectively, in 2017. Moreover, these species had acquired high resistance to ivermectin, doramectin, albendazole, and levamisole; the fecal egg count reduction percentages were 6.9%, 1.7%, 3.5%, and 79.0%, respectively, after a single administration. Nitroxynil and closantel showed strong anthelmintic efficacy against the predominant species *Haemonchus contortus* and other nematodes, but they had 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 evaluated, and the drugs with high efficacy against the predominant species were identified. The findings of this study will provide a good foundation for the appropriate use of anthelmintics.

Background

Ordos fine-wool sheep is a type of Merino sheep mainly distributed in the southwestern region of Ordos, Inner Mongolia, and it plays an important role in animal husbandry in this region because it provides both wool and meat. The total number of Ordos fine-wool sheep in the Inner Mongolia Autonomous Region is 1.57 million [1]. However, the sheep in this area are often infected by helminths, particularly gastrointestinal nematodes (GINs); these epidemics have not been effectively controlled to date and seriously affect the production performance of the sheep and hinder the healthy development of the fine-wool sheep industry. In ruminants, GINs parasitize the abomasum, small intestine, and large intestine. GINs can cause not only chronic diseases [2] but also severe economic consequences directly related to lower milk production, slower growth, and even mortality or indirectly related to treatment cost and associated workload [3].

Because most farmers have insufficient understanding of sheep parasitic diseases, they cannot control these diseases in a scientific and targeted manner. To date, regular whole-flock treatments with anthelmintics are the most commonly used measure to control GINs in sheep, without a correct diagnosis or scientific evaluation of the deworming effects [4]. Therefore, deworming programs have repeatedly

failed in the main distribution areas for Ordos fine-wool sheep, resulting in serious epidemics of GINs in the sheep. Few epidemiological studies of sheep parasitism have been conducted in Inner Mongolia.

In this study, first, an epidemiological investigation of GINs in Ordos Merino sheep was performed to understand the current status of nematode diseases. Second, the anti-nematode efficacies of several anthelmintics in naturally infected sheep were compared to screen for the most effective drugs against predominant nematode species. Third, on the basis of the epidemiological investigation and anthelmintic experiments, scientific and effective prevention and control measures were proposed for early and effective prevention and treatment of GINs in sheep in the study area.

Results

Total infection rate of strongyle nematodes in the sheep

In this study, a total of 4014 sheep from 180 family-owned farms located in the southwestern region of Ordos, Inner Mongolia, China, were investigated between March 2017 and April 2019. The number of sheep investigated in 3 consecutive 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), as shown in Table 1. Therefore, the prevalence of sheep nematodiasis in this area is relatively severe.

In 2017, the epidemic of GINs in the fine-wool sheep in the investigated area was very severe; however, in 2018 and 2019, the survey results showed that the nematode infection rate decreased significantly in the sheep (Table 2). The main reason is that, during the first epidemiological investigation, we performed a comparative study of the deworming effects of several different anthelmintics; 2 types of antihelminthics with high efficacy against the predominant nematode species were screened, and the farmers were encouraged to use these drugs.

Table 1 Total gastrointestinal nematode infection rate in Ordos fine-wool Merino sheep

Investigation period	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 2 Dosing regimen of 6 anthelmintics commonly used for controlling nematodes

Group	n	Drug	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	---	---

On the basis of the egg morphology, typical adult structure, and third-stage larva (L3) morphology, a total of 7 nematodes were initially identified: *Haemonchus contortus*, *Nematodirus* spp., *Oesophagostomum* spp., *Trichostrongylus* spp., *Chabertia* spp., *Ostertagia* spp., and *Trichuris* spp. *Haemonchus contortus* and *Nematodirus* spp. were the predominant species in the infected sheep. The different nematodes and infection rates recorded in 2017 are listed in Table 3. Images of the eggs and adult nematodes observed using a microscope and *Haemonchus contortus* on the surface of the abomasum mucosa after the autopsy are shown in Figs. 1 and 2, respectively.

Table 3 Nematode species and infection rates in Ordos fine-wool Merino sheep in 2017

Nematode species	Total number of samples	Positive samples (total EPG $\geq$ 300)	Infection rate (%)	Parasitic site
<i>Haemonchus contortus</i>	1496	1261	84.3	abomasum
<i>Nematodirus</i> spp.	1496	980	65.6	small intestine
<i>Oesophagostomum</i> spp.	1496	290	19.4	colon
<i>Trichostrongylus</i> spp.	1496	254	17.0	small intestine
<i>Chabertia</i> spp.	1496	196	13.1	large intestine
<i>Ostertagia</i> spp.	1496	106	7.1	abomasum, small intestine
<i>Trichuris</i> spp.	1496	79	5.3	caecum

The eggs of *Haemonchus contortus* (84.3%) were predominantly found, followed by those of *Nematodirus* (65.6%), *Oesophagostomum* (19.4%), and *Trichostrongylus* (17.0%). The eggs of *Ostertagia* (7.1%) and *Trichuris* (5.3%) were the least prevalent. Therefore, nematodes that adversely affect fine-wool sheep in the southwestern region of Ordos are *Haemonchus contortus*, which parasitizes the abomasum, and *Nematodirus*, which parasitizes the small intestine.

GIN infection rate in sheep that graze in different types of pasture

Because of 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 4 different groups: sheep that 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 (Table 4).

The survey results for 2017 showed that the nematode infection rates in the sheep from 4 different investigated areas were quite high, and the highest nematode infection rate (88%) was found in sheep that grazed in low-lying pastures (Table 4). However, the nematode infection rate in the sheep raised in a pen was relatively low. The survey results for 2018 and 2019 showed that the nematode infection rates in all 4 sheep groups decreased significantly, mainly because of the administration of suitable anthelmintics under our guidance.

Table 4 Nematode infection rate in sheep that graze in different types 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)

Anthelmintic efficacy

According to the epidemiological survey for GINs in the sheep, nematode infections were very severe in the study area. Therefore, to examine the deworming effects of extensively used anthelmintic drugs and screen for drugs that effectively kill predominant GIN species in the investigated area, a total of 140 severely infected sheep (PGE > 2000) were selected and randomly allocated to 7 groups and administrated different drugs (Table 1). On the 14th day after administration, fecal samples were collected and quantitatively analyzed using the modified McMaster technique (Table 5).

Table 5 Fecal egg count reduction in each sheep group on the 14th day after drug administration

Group	Drug	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

The fecal egg count reduction (FECR) on the 14th day after administration showed that albendazole, ivermectin, and doramectin had no anthelmintic effects on GINs predominant in the investigated area (Table 5). The average FECR in these 3 sheep groups was less than 10%. Levamisole also had a poor deworming effect on the nematodes, with an FECR of 79% after administration. However, nitroxynil and closantel injections had strong anti-nematodal effects, and the FECR was more than 99% after administration.

GIN drug resistance

The anthelmintic efficacy indicated that avermectins, which are widely used anthelmintics, have no deworming effects on predominant GIN species in the examined area. To verify the resistance of sheep GINs to ivermectin and doramectin, 60 sheep naturally infected with GIN (eggs per gram [EPG] ≥ 2000) were selected and randomly allocated to 6 groups, with 10 sheep in each group. Then, the experimental sheep were subcutaneously injected with 3 different doses of ivermectin and doramectin. Fecal samples were collected on the 14th day after administration for quantitative detection of EPG to evaluate the anthelmintic efficacy.

The results showed that doubling the ivermectin dose had almost no deworming effects on GINs predominant in the sheep of the examined area (Table 6). On the 14th day after ivermectin administration, the FECR in both the therapeutic dose group and increased dose group was less than 15%. Therefore, GINs in this area have become highly resistant to ivermectin.

Table 6 Comparison of the anthelmintic efficacy of different doses of ivermectin

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%

The therapeutic dose and increased dose of doramectin had no deworming effects on the predominant GIN species in the examined area (Table 7). The anthelmintic effect of the therapeutic dose of doramectin was less than 10%, and that of the increased dose of doramectin was less than 20%. This indicates that, although doramectin has no history of clinical use in the area, it also has no anthelmintic effects on digestive tract nematodes because both doramectin and ivermectin belong to the same class of avermectins with the same anti-parasitic mechanism. Thus, the predominant GIN species already possess high cross-resistance to ivermectin and doramectin.

Table 7 Comparison of the anthelmintic efficacy of different doses of doramectin

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%

Discussion

In this study, an epidemiological investigation of sheep helminthiasis showed that epidemics of GINs are very common in Ordos fine-wool sheep, and most of the epidemics are attributable to mixed infections by multiple nematodes in the southwestern region of Ordos, especially infections by *Haemonchus contortus* and *Nematodirus* spp. The overall prevalence of GINs in sheep in 3 consecutive years was 84.4%, 36.9%, and 42.3%. *Haemonchus* spp. was found to be the most prevalent GIN, with a prevalence of 84.3% in 2017. This result is consistent with the epidemiological survey data 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 is relatively serious in southwestern Ordos, and it is a major constraint that affects local sheep farms. We believe that the main reasons for such widespread and severe epidemics of sheep nematodiasis are as follows: (1) Regular whole-flock treatments with anthelmintics are still the most commonly used measure to control endoparasitic infections in small ruminants. (2) Most of the farmers and herdsman have insufficient understanding of sheep nematodiasis, and they use no basic techniques for diagnosing nematodiasis and evaluating the efficacy of anthelmintics. (3) Long-term use of the same anthelmintics has led to the development of high anthelmintic resistance among these nematodes. (4) Most of the farmers and herdsman lack basic pharmacological knowledge and cannot distinguish between the generic and brand names of a drug. Therefore, it is very common for them to repeatedly use the same drug under different brand names.

GIN infection is widespread in small ruminants and serious in most countries worldwide; it is a major factor that affects the healthy development of the sheep industry. However, there are significant differences between the prevailing nematode species and dominant species. Saidi et al. [6] studied the parasitism of GINs in goats in northwest of Algeria for 2 years and reported an overall prevalence of 96%; *Ostertagia* spp. were predominant (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 nematode infections in goats can reach 100% [6]. In Western Pomerania, Poland, the mean extensity of infection with gastrointestinal parasites in both sheep and goats was 100% [7]. Baihaqi et al. reported [8] that 2 types of local sheep are infected with various gastrointestinal worms during the dry and rainy seasons. The highest prevalence of gastrointestinal worms was found in thin-tailed sheep during the rainy season (76.47%), and the predominant GIN in Garole sheep was *Haemonchus contortus* (63.91%), followed by *Oesophagostomum* spp. (16.25%) and *Trichostrongylus* spp. (10.50%). In addition, the most common etiological agents of parasitic diseases in sheep flocks in the UK and elsewhere in Europe, as well as in Canada and the Rocky Mountain States of the USA, are GINs [9].

Control programs for GINs are mainly based on a combination of animal management practices, pasture management, and, especially, anthelmintic drugs [10]. However, intensive use of synthetic anthelmintics

for the treatment and control of GINs in sheep farms has led to the widespread development of resistance to one or more anthelmintic drug classes [11-13].

The deworming experiment results show that the dominant nematode species have developed high resistance to the most extensively used anthelmintics, ivermectin and albendazole, and the average FECR was less than 10%. The dominant species also acquired strong resistance to levamisole (FECR = 79%). However, nitroxynil and closantel, which are not widely used locally, had strong antinematodal effects, and the FECR was more than 99% after a single administration. According to Yong Rong [5], a comparative deworming test of sheep nematodes in the same area showed that the GINs in sheep had developed resistance to albendazole (FECR = 33%), but ivermectin had a strong antinematodal effect (FECR = almost 100%). However, the results of this study showed that an increased dose of ivermectin did not have any deworming effect, and even doramectin, which has not been widely used in this area, had a very poor deworming effect. This indicates GINs endemic to this area have developed high cross-resistance to ivermectin and doramectin.

A wide range of GINs in small ruminants have been proven to be resistant to anthelmintics in a number of countries, such as England, France, Germany, Norway, Sweden, Denmark, Netherlands, Austria, Switzerland, Lithuania, and Slovakia [4]. Mickiewicz [14] published the first report of the resistance of some GIN species (namely, *Haemonchus contortus*) to benzimidazole anthelmintics in a goat population in Poland. Bartley [15] emphasized that the anthelmintic resistance of GINs in small ruminants has been observed worldwide. Francisca Flávia da Silva [16] reported multi-resistance in all evaluated farms, wherein 95% of the farms showed high resistance to albendazole; 85%, ivermectin; 80%, closantel; 40%, levamisole; and 45%, 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, whereas 92% showed resistance to closantel and 53% to levamisole [17]. High anthelmintic resistance has also been reported by Melo [18], who evaluated the efficacy of ivermectin and levamisole in 13 sheep farms in Agreste of the State of Paraíba, the FECR was only 30.9% and 93%, respectively.

The main cause of anthelmintic resistance in nematodes is the prophylactic and repeated use of a limited number of anthelmintics to control GIN infestation without a diagnosis. In most sheep farms, whole-flock treatments are commonly administered once or twice annually, with a very limited rotation of applied anthelmintics and no fecal sampling for efficacy control [19]. To reduce the resistance of the nematodes and achieve effective control of sheep nematodiasis, farmers must use comprehensive measures and a combination of animal management practices, pasture management, and anthelmintic drugs. It is essential to minimize dependency on anthelmintics alone and stop their preventive use as much as possible without prior checking for worm eggs in the feces. In addition, sheep farmers are strongly recommended to check the efficacy of the anthelmintic products every time before use [20].

Conclusion

We conducted a comprehensive survey and anthelmintic study to understand the epidemiology and resistance of GINs in Ordos fine-wool Merino sheep. The findings of this study revealed that free-range sheep are severely infected with GINs in the research area, and most of them have become highly resistant to extensively used anthelmintics such as ivermectin, doramectin, albendazole, and levamisole. The results will provide a good foundation for the development of appropriate anthelmintic regimens.

Methods

Ethical approval

A total of 10 sheep with severe nematode infections were selected and anesthetized with pentobarbital sodium for autopsy to examine the GINs. 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

The investigated sheep were raised in a pen or were free-range sheep southwest of Ordos City, Inner Mongolia, China. The free-range sheep were further classified into sheep that grazed in sandy, low-lying, or hilly grasslands. Most of the sheep farms had 150 or more sheep that grazed year-round on pastures, with variable provision of supplementary feed during winter and early spring.

Sample collection

The study was conducted for 3 consecutive years: from March 2017 to 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 sheep. The samples were placed in cooler and transported to the laboratory and stored at 4°C in the refrigerator until analysis to avoid hatching of the eggs. All the samples were analyzed within 48 h by using the fecal flotation method. The randomly sampled sheep were naturally infected and belonged to both sexes. The sampling was performed in the early morning. For comparative analysis of anthelmintic effects, the sampling was performed pre-treatment and on the 14th day post-treatment.

Qualitative and quantitative analyses

The qualitative method was used to identify the nematode eggs on the basis of morphological observation of different eggs by flotation in a saturated magnesium sulfate solution (specific gravity, 1.32) [21]. The quantitative method was used to count the nematode eggs. Fecal egg counts were performed using the modified McMaster technique with the same solution used for flotation, and it is a relatively simple and cheap method [22]. The minimum detectable limit of the McMaster technique is 25 EPG of feces.

Identification of nematodes

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

Anthelmintic efficacy

To assess deworming efficacy, 140 severely infected sheep (PGE > 2000) were selected, labelled, individually weighed to calculate the correct dosage, randomly divided into 7 groups of 20 animals each, and administered different anthelmintics (Table 2). All the drugs used in this study are commercially available anthelmintics.

Fresh fecal samples were collected directly from the rectum pre-treatment and on the 14th day post-treatment and analyzed using the modified McMaster technique. FECR was calculated according to the following formula:

$$\text{FECR (\%)} = (\text{EPG}_{\text{pre-treatment}} - \text{EPG}_{\text{post-treatment}}) / \text{EPG}_{\text{pre-treatment}} \times 100\%$$

List Of Abbreviations

GINs: Gastrointestinal nematodes; L3: Third-stage larvae; EPG: Eggs per gram; FECR: Fecal egg count reduction.

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 conducted the field trials. LE, JW and SH performed the statistical analysis and revised the manuscript. All authors revised the manuscript and read and approved the final draft for publication.

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Availability of data and materials

All relevant information has been included in the manuscript. The data analyzed 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 each farmer, 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.

References

1. Pan ZF, Wang ZQ, Liu XD, Xia RH, Shi RL, Li SQ. Inner Mongolia Statistical Yearbook 2018. Beijing: China Statistics Press; 2018, p580.
2. Zajac AM, Garza J. Biology, Epidemiology, and Control of Gastrointestinal Nematodes of Small Ruminants. *Vet Clin North Am Food Anim Pract.* 2020; 36(1): 73–87.
3. Charlier J, Van der Voort M, Kenyon F, et al. Chasing helminths and their economic impact on farmed ruminants. *Trends Parasitol.* 2014; 30(7):361–367.
4. Lambertz C, Pouloupoulou I, Wuthijaree K, et al Anthelmintic resistance in gastrointestinal nematodes in sheep raised under mountain farming conditions in Northern Italy. *Vet Rec Open.* 2019; 6(1): 1-6.
5. Yong R, Gali B, Hasi S. Epidemiological investigation of sheep helminth infections and comparative anthelmintic studies in Wushen area. *Journal of Inner Mongolia Agricultural University (Natural Science Edition).* 2009; 30(4):20-24 (in Chinese).
6. Saidi M, Stear MJ, Elouissi A, et al. Epidemiological Study of Goat's Gastrointestinal Nematodes in the North West of Algeria. *Trop Anim Health Prod.* 2020, 2020;10.1007/s11250-019-02193-6. doi:10.1007/s11250-019-02193-6
7. Juszczak M, Sadowska N, Udała J. Parasites of the digestive tract of sheep and goats from organic farms in Western Pomerania, Poland. *Ann Parasitol.* 2019; 65(3):245-250.
8. Baihaqi ZA, Widiyono I, Nurcahyo W. Prevalence of gastrointestinal worms in Wonosobo and thin-tailed sheep on the slope of Mount Sumbing, Central Java, Indonesia. *Vet World.* 2019; 12(11):1866-1871.
9. McMahon C, Edgar HWJ, Barley JP, et al Control of Nematodirus spp. infection by sheep flock owners in Northern Ireland. *Ir Vet J.* 2017; 70, 31:1-11.

10. Castagna F, Palma E, Cringoli G, et Use of Complementary Natural Feed for Gastrointestinal Nematodes Control in Sheep: Effectiveness and Benefits for Animals. *Animals*. 2019; 9(12). [10.3390/ani9121037](#).
11. Geurden T, Hoste H, Jacquiet P, et Anthelmintic resistance and multidrug resistance in sheep gastrointestinal nematodes in France, Greece and Italy. *Vet Parasitol*. 2014; 201(1-2):59–66.
12. Baltrušis P, Halvarsson P, Höglund J. Exploring benzimidazole resistance in *Haemonchus contortus* by next generation sequencing and droplet digital PCR. *Int J Parasitol Drugs Drug Resist*. 2018; 8(3):411–419.
13. Mondragón-Ancelmo J, Olmedo-Juárez A, Reyes-Guerrero DE, et Detection of Gastrointestinal Nematode Populations Resistant to Albendazole and Ivermectin in Sheep. *Animals*. 2019; 9(10): [10.3390/ani9100775](#).
14. Mickiewicz M, Czopowicz M, Górski P, et The first reported case of resistance of gastrointestinal nematodes to benzimidazole anthelmintic in goats in Poland. *Ann Parasitol*. 2017; 63(4):317–322
15. Bartley DJ, Jackson F, Jackson E, et Characterisation of two triple resistant field isolates of *Teladorsagia* from Scottish lowland sheep farms. *Vet Parasitol*. 2004; 123(3-4):189-199
16. Flávia da Silva F, Bezerra HMFF, Feitosa TF, et Nematode resistance to five anthelmintic classes in naturally infected sheep herds in Northeastern Brazil. *Rev Bras Parasitol Vet*. 2018; 27(4):423-429.
17. Veríssimo CJ, Niciura SCM, Alberti ALA, et Multidrug and multispecies resistance in sheep flocks from Sao Paulo state, Brazil. *Vet Parasitol*. 2012; 187(1-2):209-216.
18. Melo LRB, Vilela VLR, Feitosa TF, et Anthelmintic resistance in small ruminants from the Semiarid of Paraíba state. *Brazil Ars Vet*. 2013; 29(2):104-108.
19. Lambertz C, Pouloupoulou I, Wuthijaree K, et Anthelmintic efficacy against gastrointestinal nematodes in goats raised under mountain farming conditions in northern Italy. *BMC Vet Res*. 2019; 15:216 (2019). [/doi.org/10.1186/s12917-019-1968-8](#).
20. Ploegera HW, Everts RR. Alarming levels of anthelmintic resistance against gastrointestinal nematodes in sheep in the Netherlands. *Vet Parasitol*. 2018; 262:11–15.
21. Kong F. *Veterinary Parasitology*. Beijing: China Agricultural University Press; 2011 p391-394 (in Chinese).
22. Anne M. Zajac, Gary A. Conboy. *Veterinary Clinical Parasitology*, 8th Edition, John Wiley & Sons, Inc; 2012, p3-12.

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