Epidemiology and Multidrug Resistance of Strongyle Nematodes in Ordos Fine-wool Sheep

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Abstract

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 fine-wool sheep and to evaluate the efficacy of currently used anthelmintics. 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 fine-wool 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. are the dominant 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 average percentages of FERC following single administration of drugs were 6.9%, 1.7%, 3.5% and 79.0%, respectively. 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. The prevalence of gastrointestinal nematodes in Ordos fine-wool 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 reasonable dosage regimen of anthelmintics.

Keywords: Ordos fine wool Sheep; Epidemiological Study; Gastrointestinal Nematodes; Anthelmintic Efficacy

Introduction

Ordos fine wool sheep is a kind of merino sheep combination of wool and meat, mainly distributed in the southwestern region of Inner Mongolia, China. 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 parasitized 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 loss directly related to lower milk production, slower growth, and even mortality, or indirectly related to the treatment costs and the associated workload [3,4]. Control of helminths in livestocks becomes especially difficult because deworming is only allowed during the dry period, which occurs consecutively on farms throughout the year. Therefore, treated and untreated livestocks can share the same pastures, and untreated ruminants continue to shed eggs in the feces, increasing the risk of all livestocks ingesting infective stages of helminths while feeding on grass [5].

Today, the livestock industry is increasingly threatened by populations of nematodes resistant to the most commonly used anthelmintic drugs [6]. Resistance to almost every marketed anthelmintic against nematodes in ruminants has developed worldwide, this had elevated the issue of anthelmintic resistance from being a potential problem of the future to being a major threat to small ruminant production in many countries [7,8]. Moreover, concern rises when we consider the fact that levels of resistance can increase abruptly [9].
In addition, the majority of farmers do not have sufficient 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 [10]. Therefore, the deworming program in Ordos fine wool sheep has repeatedly failed, resulting in a serious epidemic of gastrointestinal nematodes in sheep. Few epidemiological investigation of helminthiasis was conducted in current research area of Inner Mongolia.

At the present study, firstly, the epidemiological investigation was performed on gastrointestinal nematodes in Ordos fine wool sheep to understand the current status of nematode diseases. Secondly, the antinematodal effect of several anthelmintics was comparatively studied in naturally infected sheep to screen out the most effective drugs on predominant species of nematodes.

**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**

Selected sheep farms are located in southwest part of Ordos, Inner Mongolia (Nei Mongol), China, named Uxin banner including 6 villages of Wushenzhao, Tuke, Wulantaolegai, Galutu, sulide and Wudinghe (Figure 1). According to different breeding models and different types of grassland, the investigated sheep were pen-fed or grazed on the various pasture types in. Grazed sheep were come from sandy pasture, low-lying pasture and hilly pasture. The majority of sheep farms contain 150 or more sheep, which grazed 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 pen-fed. 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.

![Figure 1: Geographic locations of the sampling sites (▲)](image-url)
Qualitative and quantitative examination

Firstly, 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) [11]. Secondly, 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, as described in Veterinary Clinical Parasitology [12]. 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 [11,12] and typical structures of adult nematodes from autopsy, and this was subsequently further verified on the basis of third-stage larvae (L3) morphology [11].

Anthelmintic Efficacy

For evaluating the efficacy of deworming, 140 severely infected sheep (PGE>2000) were selected, labelled and weighed individually, 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.

\[
\text{FECR(\%)} = \frac{(\text{EPG}_{\text{pre-treatment}} - \text{EPG}_{\text{post-treatment}})}{\text{EPG}_{\text{pre-treatment}}} \times 100\%
\]

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.

![Image](Figures/2.png)

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

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

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 anthelmintics with high efficiency on the predominant species of nematode were screened out, and farmers are courageous to use these drugs clinically in investigated area.

![Image](Figures/3.png)

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

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 *Hemonchus 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 Figures 2 and 3.
The most prevalent eggs seen in feces were from *Hemonchus contortus* (84.3%), followed by *Nematodirus* (65.6%), and 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 most harmful nematodes to Ordos fine wool sheep are *Haemonchus contortus* parasitized in the abomasum and *Nematodirus* parasitized in the small intestine.  

Due to the large differences of pasture types in investigated area, the species and distribution of nematodes are different 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).

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

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

**Figure 2:** Observed images of (A) *Haemonchus contortus* eggs; and (B) *Nematodirus* eggs (10×10)

**Figure 3:** Abomasum mucosa is covered completely with *Haemonchus contortus* in severely infected sheep
The investigation results in 2017 seen in Table 4 showed 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 in 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 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 dominant 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 administered different drugs, as shown in Table 1. On the 14th day after administration, fecal samples were collected and analyzed quantitatively by using modified McMaster technique (Table 5).

The results of anthelmintic efficacy indicated that avermectins, the widely used anthelmintics, have basically no deworming efficacy on predominant species of nematode 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%. However, nitroxynil and closantel injections showed strong anti-nematodal effects, and their FECR reached over 99% after single administration.

**Gastrointestinal nematode drug resistance**

The results of anthelmintic efficacy indicated that avermectins, the widely used anthelmintics, have basically no deworming efficacy on predominant species of nematode 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 and randomly allocated to 6 groups with 10 sheep in each group. And then they 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.

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

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

### Table 6: Comparison of anthelmintic efficacy of Ivermectin with different doses

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

### Table 7: Comparison of anthelmintic effects of Doramectin with different doses

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Pre-treatment EPG (mean)</th>
<th>14th day post-treatment EPG (mean)</th>
<th>FECR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>10</td>
<td>2550</td>
<td>2322</td>
<td>7.0%</td>
</tr>
<tr>
<td>0.3</td>
<td>10</td>
<td>2293</td>
<td>1893</td>
<td>17.4%</td>
</tr>
<tr>
<td>0.4</td>
<td>10</td>
<td>2963</td>
<td>2430</td>
<td>18.0%</td>
</tr>
</tbody>
</table>
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 became 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 gastrointestinal 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**

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 industries as well. However, there are significant differences between the prevailing nematode species and dominant species. Saidi et al. [14] 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 Ostertagia 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% [15]. Baihaqi et al. reported [16] 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 in 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 [17].

Gastrointestinal nematode control programs are mainly based on a combination of both animal management practices, pastures management, and especially the use of anthelmintic drugs [18]. 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 [19-21].

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 [13], 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 do 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 epidemic 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 [10]. Mickiewicz [22] reported, their study shows for the first time that resistance of some species of gastrointestinal nematodes (namely *Haemonchus contortus*) to benzimidazole anthelmintics occurs in goat population in Poland. Bartley [23] emphasized that the anthelmintic resistance of gastrointestinal nematodes of small ruminants has been observed worldwide. Francisca Flávia da Silva [23] 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 [25]. High anthelmintic resistance was also reported by Melo [26], 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 [27]. 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 examine the efficacy of anthelmintic products every time when it be used [28].

**Conclusion**

The comprehensive epidemiological survey and understanding of gastrointestinal nematodes of Ordos fine wool sheep and the nematodes resistance. The findings of this study revealed that grazed sheep was infested severely with gastrointestinal nematodes in research area and most of them became 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.

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**References**


