Effectiveness of the new Iverlong prolonged-release drug for gastrointestinal nematodoses in sheep

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Abstract. The article presents the results of experiment on testing a prolonged form of the Iverlong drug against gastrointestinal nematodes in sheep. The work was carried out on 30 lambs. The lambs of the first group were subcutaneously injected with the Iverlong preparation at a dose of 1 ml/50 kg of live weight (in 1 ml of 50 mg of Ivermectin plus PLGA prolongator), the lambs of the second group were treated with the same preparation, but at a dose of 0.5 ml/50 kg of live weight. The lambs of the third group served as a control, which were subcutaneously injected with the well-known Ivomek drug in the recommended dose (1 ml/50 kg of live weight). The effectiveness from the prolonged form of Iverlong in 2 doses against nematodes of the gastrointestinal tract was taken into account by means of coprological studies before drug administration and every 15 days for three months. According to the results of coprological studies, it was found that the Iverlong drug in a dose of 1ml/50 kg of the animal's live weight showed a high prolonged anthelmintic effect and for 60 days protected animals from infection by nematodes of the gastrointestinal tract.

1 Introduction

An important problem in veterinary medicine remains the prevention and treatment of parasitic diseases in farm animals, which cause significant economic damage to animal breeding. Ivermectin (IVM), a broad-spectrum anthelmintic drug, is widely used in veterinary medicine of Russia. The composition of the pharmaceutical preparation affects the absorption of the IVM and its systemic availability. After the first approved preparation (IVM) (propylene glycol/glycerin, 60:40) used at a dose of 200 mg/kg of live weight was put into practice, various pharmaceutical modifications were analyzed to increase its activity [1].

Anthelmintic drugs require achieving effective concentrations at the location of the

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parasite for a certain period of time to ensure their effectiveness. The processes of absorption, distribution, metabolism and excretion (pharmacokinetic phase) directly affect the concentration of the drug achieved at the site of action, and the effective therapeutic effect. Physical and chemical properties, such as solubility in water and the rate of dissolution, determine the ability of anthelmintic compounds to accumulate in parasites and, therefore, the ultimate therapeutic efficacy. The process of transcuticular absorption is the main route of various drugs penetration into the body of nematodes and cestodes. In addition to other factors, the route of administration can significantly affect the pharmacokinetic behavior of anthelmintic molecules and change their effectiveness. Oral administration increases the effectiveness of the drug against nematodes located in the gastrointestinal tract [1].

A number of authors note from macro cyclic lactones the high efficacy of the Moxidectin drug, it is a highly lipophilic macro cyclic lactone endoctocide. It is many times more effective than Ivermectin against gastrointestinal nematodes, being effective against Ivermectin-resistant strains, such as Haemonchus contortus and Trichostrongylus colubriformis, and also has a long therapeutic effect [2-4].

The effect of prolonged-release anthelmintics on nematodes is of interest, because pasture contamination is reduced after treatment, which can lead to a reduction in infection and an improvement in the growth of lambs, a decrease in the number of anthelmintic treatments [5].

A number of authors express concerns that the use of prolonged-release drugs may change the species balance in the nematode population of the gastrointestinal tract, and some species will have a selective advantage over others, taking into account alleles that contribute to anthelmintic resistance [6-9].

Alternative treatment strategies, including vaccines, biological control and breeding of parasitic-resistance animals, are unlikely to be widely available in the near future, and even then they will be integrated with chemotherapy.

To continue effective control of parasites, it is necessary to use available resources more efficiently. With an appropriate approach, the combined use of metabolic inhibitors can prolong the effect of the drug, expand the availability and enhance the effect of existing anthelmintic drugs.

All this predetermined the need to study the effectiveness from the new dosage form of the Iverlong prolonged-release anti-parasitic drug developed in the Agrovetzashchita Research and Introduction Center (Moscow). In previous experiments, we established the high therapeutic and prophylactic efficacy of the Iverlong preparation several series for psoroptosis and nematodoses of sheep, horned cattle hypodermatosis and revealed its residual amount in the organs and tissues of animals [10-13].

The aim of this work was to determine the effective period of the Iverlong drug against nematodes in the gastrointestinal tract of sheep.

2 Materials and methods

Commercial experiment to study the anti-parasitic efficacy of the new Iverlong prolonged-release preparation was carried out at the experimental station of the North Caucasus Federal Agricultural Research Center on ewes (250 heads) and lambs (240 heads). All ewes stock in the winter-stall period (January) was prophylactically treated with Ivermectin preparation at a dose of 1 ml/50 kg of body weight. It should be noted that during the whole grazing period, the lambs were together with the ewes.

According to the principle of analogues, 30 lambs were selected at the age of 1.5-2 months, which were divided into 3 groups of 10 animals. In all experimental lambs, ear numbers were recorded in the journal and each group of animals was labeled with paint of

different colors. The lambs of the first group were subcutaneously injected with the Iverlong drug at a dose of 1 ml/50 kg of live weight (in 1 ml of 50 mg of Ivermectin plus PLGA prolongator); the lambs of the second group were treated with the same preparation, but in a dose of 0.5 ml/50 kg of live weight. The lambs of the third group served as a control, which were subcutaneously injected with the well-known Ivomek drug in the recommended dose of 1ml/50 kg of live weight. In the lambs of all three experimental groups, feces were taken directly from the rectum into individually labeled containers. Feces were sampled before drug administration and every 15 days for three months (15, 30, 45, 60, 75 and 90 days), right up to the lambs' weaning from the ewes. The test samples were cooled at 4º C, and on the same day in the laboratory by flotation method with a saturated solution of ammonium nitrate, eggs of trichostrongylidis were counted in 1 g of each feces sample.

To predict the intensity of the invasion (II), we used a polynomial of the 2nd order in the form:

$$\gamma(x) = p_1 x^2 + p_2 x + p_3.$$

Computer modeling was carried out using the integrated mathematical package Matlab.

The data of experimental drug efficacy for the amount of helminthes eggs secreted in 1 g of feces were subjected to statistical analysis using the Plokhinsky method (1978). In all cases, the value of T (Student test) was taken to indicate statistical significance, $P<0.05$.

Results

The results of coprological studies conducted every 15 days are presented in Table 1.

Table 1. The effectiveness of the prolonged Iverlong drug form with nematodoses of sheep (n=30) according to the results of coprological studies

No. group	Dose ml/50 kg	II / IE	The number of nematode eggs in 1 g of feces						
			Before ad-ministration	After administration in (days):					
				15d.	30d.	45d.	60d.	75d.	90d.
1	1,0 ml	II, (count)	112.2± 24.4	0	0	11.0± 3.92*	46.4± 10.63	265.7± 57.87	652.9± 113.33*
		IE, (%)	100	0	0	70	80	90	90
2	0,5 ml	II, (count)	98.5± 15.39	0	7.2± 1.96*	27.6± 4.61*	163.2± 17.53	1058.7± 208.46*	1328.0± 162.13*
		IE, (%)	100	0	60	90	100	100	100
3	Ivomek 1,0 ml.	II, (count)	104.9± 15.04	0	39.0± 8.11	72.8± 9.93	359.4± 43.8*	729.2± 88.12*	889.4± 95.23*
		IE, (%)	100	0	80	100	100	100	100

Note: **II** – invasion intensity, the number of helminth eggs on average per animal;
IE – invasion extensity, the number of infected animals in %;
* - T, test of Student, P<0.05

According to the results of coprological studies, it was found that after treatment in 15 days in all groups of lambs, the extensity and intensity of nematode invasion were 0, but on the 30th day we noted an increase in the number of secreted nematode eggs in animals in the second and third groups. On the 60th day, in the lambs of the first group we recorded nematode eggs, but with a low invasion intensity (46.4 ± 10.63 in 1 g of feces) compared to lambs of the 2nd and 3rd groups (II = 163.2 ± 17.53 and 359.4 ± 43.8, respectively).

The values of the invasion intensity for 3 groups of animals, presented in Table 1, can be approximated by a polynomial of the 2nd order. This allows you to predict the intensity

of invasion for the next 30 days.

The polynomial coefficients for the three groups of animals are as follows:

The first group: $p_1 = 0,1927$; $p_2 = -12,1064$; $p_3 = 132,5571$.

The second group: $p_1 = 0,3445$; $p_2 = -16,8093$; $p_3 = 132,0929$.

The third group: $p_1 = 0,1844$; $p_2 = -6,7569$; $p_3 = 78,2286$.

It should be noted that the approximation error for the second group of animals was slightly higher than for the first and the third.

In Fig. 1, markers in the form of circles represent the measured values of the invasion intensity (II) in the 1st group, asterisks indicate the measured values of the II in the 2nd group, and squares mark the values of the II in the 3rd group. For a better perception, these markers are interconnected by thin continuous lines.

To predict the intensity of invasion (II), a second-order polynomial was used. Regression dependencies illustrating the prognosis of the invasion intensity development (II) are shown in Figure 1: for the first group, this is a dash-dotted line; for the second group, this is a dotted line; for the third group, this is a dashed line.

Despite the fact that the approximation error for the second group of animals is slightly higher than for the rest, it is safe to say that the lowest value of the invasion intensity (II) on the 120th day after administration of the drug will be observed in animals of the first group. The highest value of the II is in animals of the second group.
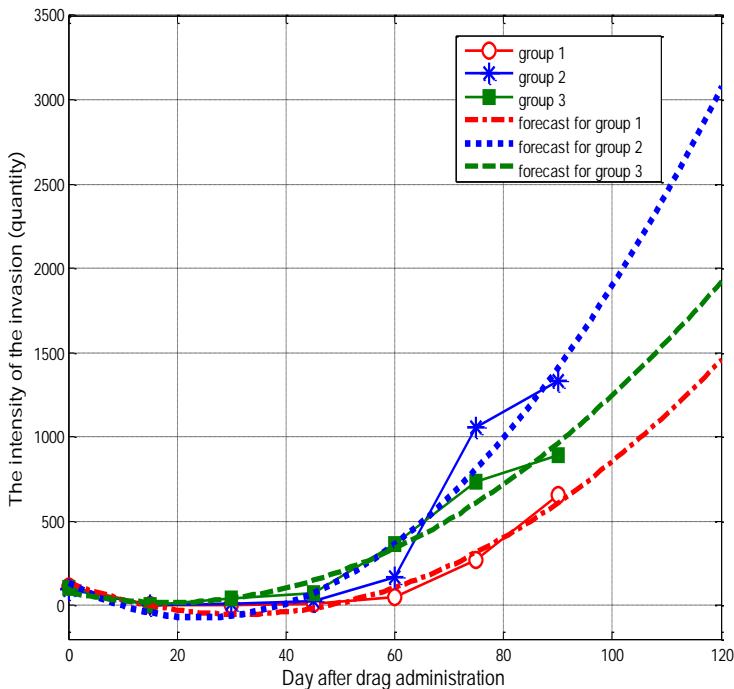
Fig.1. Prediction of the invasion intensity up to 120 days after drug administration.

3 Discussion

An analysis of the coprological studies' results presented in Table 1 shows that before treatment, the number of helminth eggs in lambs of all groups was low (98-112 specimens),

since lambs used pasture for about 25 days and not all helminthes developed to a mature stage and began to secrete their eggs into the environment.

After treatment, in15 days in all groups of lambs, the extensity and intensity of nematode invasion were 0, which indicates the high efficiency of the drugs used. On the 30th day, we note an increase in the number of secreted helminth eggs in lambs of the second group, which can be explained by an insufficient dose of the active substance for a prolonged action. Lambs of the third group began to secrete helminth eggs, which is quite natural, since they were treated with the Ivomek drug, which has a short therapeutic period [14, 3].

On the 60th day, the lambs of the first group were also invaded by nematodes, but with a low invasion intensity (46.4 ± 10.63 in 1 g of feces) compared with lambs of the 2nd and 3rd groups (II = 163.2 ± 17.53 and 359.6 ± 43.8, respectively).

Figure 1 presents a forecast picture of an increase in the intensity of invasion, where it can be confidently stated that the lowest value of the invasion intensity (II) on the 120th day after the administration of the drug is observed in animals of the first group. Prolonged-release drugs are widely used in Europe and New Zealand; there are Moxidectin, Doramectin (1.0 and 3.5%). The first clinical trials of prolonged-release Moxidectin (Cydectin® 2% LA for sheep) were conducted in Europe [15], which established continuous efficacy (>90%) for 140 days for Teladorsagia, 119 days for Haemonchus and 115 days for Trichostrongylus. The prolonged effectiveness of the preparations may provide new opportunities in the development of anthelmintic programs for sheep.

It should be noted that in the available literature we did not find works on testing prolonged preparations against Moniezia in sheep, which cause huge economic damage to sheep breeding.

4 Conclusion

Thus, the Iverlong preparation at a dose of 1 ml/50 kg of the animal's live weight showed a high prolonged anthelmintic effect and for 60 days protected the animals from infection by gastrointestinal nematodes.

We did not note any deviations from physiological norm in animals in the first and the following days after administration of the drug.

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