Use of Salt lick Briquettes «Ivirsalt» on Natural Areas of Preferential Protection in Russia for the Prevention of Parasitic Diseases in Wild Hoofed Animals

Abstract
As test objects served elks and dappled deer infested with helminths. The purpose of this work is to study the efficacy of a new antiparasitic drug «Ivirsalt» used for prevention of helminth infection in wild hoofed animals. The therapeutic efficacy of new anthelmintic drug «Ivirsalt» for nematodosis of wild hoofed animals was tested on 120 dappled deer, 7 elks and 1 roe deer on the territory of Losiny Ostrov National Park (Elk Island), Moscow.

«Ivirsalt» is provided in form of salt lick briquettes with a mass of 5 kg containing the active ingredient ivermectin and sodium chloride in the proportion 0,1: 99, and 9. We put salt lick briquettes to the feed-troughs on feeding places and saline soils based on the average daily salt consumption. The preparation was given within 14 days taking into account that animals approach salt lick briquettes not less than once a week. To determine the efficacy of preparation by the method of coproscopy the fecal samples were collected before and every 10 days after distribution of salt lick briquettes.

Materials and Methods: Standard lifetime and post-mortem helminthological examinations of animals (coproscopy, K.I. Skryabin method of full post-mortem helminthological examination, 1928). The research results showed that the intensity of gastrointestinal nematode infestations of dappled deer aged 1,5 to 3 years has decreased by 5,4 times, in deer older than 3 years-by 3,1 times.

Keywords: Natural areas of preferential protection in Russia; Elks; Dappled deer; Roe deer; Helminthosis; Salt lick briquettes; Anthelmintic drug - «Ivirsalt»; Treatment; Efficacy

Introduction
Based on the long-term research conducted since 2006 we have determined that wild hoofed animals of Losiny Ostrov National Park are infected with nematodes. The extensity of infection is 100% [1-5].

Regardless of the fact that the infection rate in adult animals is less than in young, the adult animals are an important source of infection distribution and a contributing factor to epizootics. In case of wide spread of parasitic diseases the threat of infection transmission from wild to domestic animals and human is possible [6-9].

In the fight against parasitic diseases in farm and wild animals various struggle measures are suggested including monitoring of livestock number, removal of animal corpses, change of pastures, rational distribution of biotechnical objects, and other veterinary-sanitary and common household measures.

Nowadays there are no pharmaceutical formulations of antiparasitic drugs which would be comfortable for free feeding of farm and wild animals, and moreover, could help avoid high intoxication of animals as well as overdose during the treatment.

Salt lick briquettes with ivermectin can be used as preventive and remedy against endo- and ectoparasites for wild animals in hunting farms, natural reserves and other natural areas of preferential protection as well as for farm animals.

Materials and Methods
Salt lick briquette samples containing ivermectin for nematodosis have been tested by methods of helminthological examinations of wild hoofed animals (elk, deer; roe deer) living in of Losiny Ostrov National Park, Moscow. Before giving salt lick briquettes to wild animals (as preventive and remedy) the fecal samples were collected from different places of feeding grounds to detect their helminth infestation by various coprological methods: Kotelnikov GA [6] (testing for extensity and intensity of infestation); number of nematode eggs/larvae was calculated in counting chamber of Migachova & Kotelnikov GA [7]. Fecal samples were collected every 10 days after giving Ivirsalt. Fecal samples from animals of all groups were examined during the whole time period when the salt lick briquettes with ivermectin were given.

The standard life-time and the post-mortem helminthological examinations of animals by K.I. Skryabin method, 1928 were conducted. Organs and tissues from animals underwent an autopsy have been sent to the Laboratory of Pharmacology, Toxicology and Therapeutics at the All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants named after K.I. Skryabin, Russia.
Institute of Fundamental and Applied Parasitology of Animals and Plants named after K.I. Skryabin to make the pharmacological and toxicological evaluation of the briquettes. The briquette mass was determined every 10 days during the preventive treatment of animals. Besides, we observed the animals approaching salt lick briquettes. Our observations were registered with the camera.

Helminthological examinations of wild ruminants in Losiny Ostrov National Park were conducted within a year. The drugs containing the active ingredient ivermectin which has been produced using fermentation of fungi Streptomyces avermectilis have a pronounced antiparasitic effect on larval and adult nematodes of gastrointestinal tract and lungs, larvae of subdermal and gastric gadflies, lice, bloodsuckers, sarcoptic ticks (Sarcoptidae).

Mechanism of ivermectin effect was described in details by many researchers. In nematodes it stimulates the release of gamma-aminobutyric (GABA) acid by nerve-endings, strengthens the connection to post synaptic GABA receptors blocking transfer of nerve impulses that results in paralysis and further death of helminths. In ticks and insects the transfer of nerve impulses between nerve-endings and muscle cells is blocked that also may cause paralysis and death of parasites. Ivermectin has no effect on acetylcholine which is the main mediator of nervous system in mammals [10].

According to literature sources ivermectin used at the therapeutic dose 0.2 mg/kg has no embryotrophic effect [11]. Khera KS [8], Brent RL [9] while investigating the embryotrophic properties on mice determined the negative effect of drug used at the dose of 0.4 mg/kg. In embryos of mice from experimental groups the malformations “fau lupine” were registered. In rats the disturbance of emryogenesis was observed when using the drug at the dose of 10 mg/kg. Malformations in embryos of rabbits were determined while applying the dose of 3 mg/kg [9-14].

Pharmacological properties of ivermectin were studied and described by many authors [9-20]. However, different production technologies of the same compound may affect its biological and toxicological properties.

The use of various antiparasitic drugs is often harmful for the offspring; therefore, it is necessary to detect the eventual teratogenic effect of the drug before use it in practice. The effect of different chemical compound during pregnancy may cause disturbance of physiological functions and lead to pathological changes of embryonic development.

Results and Discussion

The formulation of salt lick briquettes «Ivirsalt» was created and the pilot batch of briquettes for medical and preventive treatment of farm and wild animals against endo- and ectoparasites produced.

The anthelmintic drug «Ivirsalt» consists of the active ingredient ivermectin and sodium chloride mixed and pressed in proportion 0.1:99.9. The anthelmintic drug is available in form of beige-colored salt lick briquettes with ivermectin (with the mass of 5 and 10 kg) which are produced by homogeneous mixing of sodium chloride with the drug and then pressed under pressure not less than 1,020 atm. Ivermectin contained in briquettes assures high anthelmintic effectiveness, good acceptability of drug; sodium chloride boosts high bioavailability and the effectiveness of drug, encourages free feeding of briquettes by animals that helps avoid stress of animals, reduces costs for treatment measures. For antiparasitic treatments in Losiny Ostrov National Park the salt lick briquettes with ivermectin with the mass of 5 kg were used. Animal groups were put together on 16.01.2013 on the territory of Mytischi forest park-40th and 49th compartments of the ranger’s district.

- **Group No1:** Feeding ground no. 1 (in ranger’s district) - up to 50 dappled deer - 11 briquettes
- **Group No2:** Elk biostation - up to 50 dappled deer - control group (did not get briquettes with the drug).
- **Group No3:** Feeding ground no. 2 (middle-aged broadleaf forest) - up to 20 dappled deer - 4 briquettes.

The same ground is available for wild boars (up to 2-3 families). On the territory of elk biostation were kept elks (4 females and 3 males, 1.5 - 9 years of age) and one roe deer (3 year old female).

For the purpose of experimental variability 4 salt lick briquettes were placed on the feeding ground for elk and one briquette - on the feeding ground for roe deer.

The experiment was finished on 26.02.2013, after that each salt briquette was weighted. The experimental results are shown in Table 1.

**Results of experiments on animals**

Along with the distribution of salt lick briquettes the fecal samples from all groups of tested animals were collected. Within the whole experiment’s period the fecal samples were collected every 10 days and examined by the method of coproovoscopy; the preparations were microscoped, pictures were taken; statistical processing of intensity of helmith reproductive output per 1 g of feces was performed.

Helminthological examinations by K.I. Skrybin method were conducted on 5 head (2 elk and 5 dappled deer): elks - 1 male 4-5 year of age; hit by a car in Uralskaya street of Moscow (Yauzsky forest-park); 1 elk male 1.5 year of age, called Abyolka (Elk biostation) and dappled deer - 4 females 1.5-3 years of age, teared to pieces by dogs, one year old male tested for EI and II.

The results of coprological examinations of tested animals (dappled deer; deer; elk and roe deer) which have been conducted to determine the effectiveness of salt lick briquettes with ivermectin show that the nematode infection in animals fall below the upper limits of average values of intensity of larval release:

- **Group No1:** (approx. 50 head) dappled deer aged 1.5 - 3 years - from 98,52 larvae examples per 1 g of feces to 18,15 g and dappled deer aged 3 - 6 years: from 68,37 to 21,86 g.
- **Group No2:** (control) (approx. 50 head) dappled deer aged 1.5 - 6 years - from 100,12 larvae examples per 1 g of feces up to 80,98.
- **Group No3:** (approx. 20 head) dappled deer aged 1.5 - 6 years - from 80,98 larvae examples per 1 g of feces up to 61,74.

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Group No: 4 (7 elk aged 1 - 9 years and 1 roe deer - 3 years), elk from 111, 08 larvae examples per 1 g of feces up to 37, 06, and roe deer: from 139 larvae examples per 1 g of feces up to 33, 3.

The average daily consumption of briquettes Ivirsalt per 1 animal head is shown in Table 2.

Group No: 1 (approx. 50 head) dappled deer aged 1.5 - 3 years - 0.0074;

Group No: 3 (approx. 20 head) dappled deer aged 1.5 - 6 years - 0.0016;

Group No: 4 7 elk aged 1 - 9 years - 0.023 and 1 roe deer aged 3 years - 0.003.

It is necessary to indicate significant differences in the average daily consumption of briquettes between group no. 1 and group no. 2. Animals of group No. 2 slightly licked and eat of briquettes in comparison to the animals from group no. 1 (0.0016 kg and 0.0074 kg, respectively).

We explain that by the disturbing factor. Animals of group No. 2 come to the feeding ground along with wild boars. Together with environment protection inspectors we often observed how the wild boar families frightened away dappled deer harems from this feeding ground.

However, due to the limited territory of the national park the workers cannot afford to organize a feeding ground for wild boars in any other place to isolate animals from each other during the feeding period. The received result of carpological examination show that the deer of group No. 2 have consumed noticeably less preparation than the animals of group no. 1. By the end of experiment the average intensity of nematode larvae release in this group was 80, 98 examples per 1 g of feces vs 18, 15 (age of 1, 5-3 years) and 21,86 (3-6 years), respectively (Table 2).

Table 1: Results of experiments on animals.

| No | Groups | District | Number of Briquettes | Mass, kg Total (16.01.13) | Number of Fecal Samples (16.01.13) |
|----|--------|----------|----------------------|---------------------------|-----------------------------------|
| 1  | f.g.1 (r.d.) (d.d. - Approx. 50 head) 1,5 - 6 Year | 11 | 55 | 43,8 | 30 |
| 2  | f.g. 2 (r.d.) - Control (d.d.-Approx. 50 head) 1,5 - 6 year | 4 | 20 | 19 | 30 |
| 3  | f.g.3 (wood) (f.g. - approx. 20 head) 1,5 - 6 year | 4 | 20 | 15,1 | 21 |
| 4  | e.b. (open-air cage ) (elks - 7 head, 4 ♀ и 3 ♂, 1,5-9 year) | 1 | 5 | 4,9 | 3 |
| 5  | e.b. (Pennage) (Roedeer - 1 head, ♀, 3 year.) | --- | --- | --- | --- |

f.g.: feeding ground; r.d.: ranger’s district; d.d.: dappled deer; e.b.: elk biostation

Table 2: Comparison table of coprological examinations of animals in experiment.

| No. of Group | Tested Animals | 16.01.2013 (Number of Nematode Larvae In 1 G of Feces) | 06.02.2013 (Number of Nematode Larvae In 1 G of Feces) | 16.02.2013 (Number of Nematode Larvae In 1 G of Feces) | 26.02.2013 Number of Nematode Larvae In 1 G of Feces | Average Daily Consumpt of Ivirsalt Per 1 Head Within 30 Days of Experiment(Kg) |
|--------------|----------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-----------------------------------------------|---------------------------------|
| 1 (approx.50 head) | Dappleddeer aged 1,5 - 3 years | 98,52 | 53,29 | 37,04 | 18,15 | 0,0074 |
|                | Dappled deer Aged 3 - 6 years | 68,37 | 50,91 | 39,97 | 21,86 |            |
| 2 control (approx.50 roa.) | Dappled Deer aged 1,5 - 6 years | 100,12 | 89,78 | 86,19 | 80,98 |            |
| 3 (approx.20 head) | Dappled deer aged 1,5 - 6 year | 80,98 | 83,13 | 81,2 | 61,74 | 0,0016 |
| 4 (7 head) | Elk aged 1,5 - 9 year old (4 ♀ and 3 ♂) Roe deer (3 year old ♂) | 139 | 104,6 | 81,6 | 33,3 | 0,003 |

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Conclusion

High efficacy of the drug «Ivirsalt» used for treatment and prevention purposes as salt lick briquettes with ivermectin and a wide spectrum of effect of the new anthelmintic drug for intestinal nematodes of wild hoofed animals are determined. Salt lick briquettes for wild animals are put into feeder son feeding grounds or on soils with high alkalinity taking into account the average daily consumption of salt.

The preparation can be given once in 14 days if the animals approach salt lick briquettes not less than once a week. In the pasture period the salt lick briquettes with ivermectin can be used for prevention of nematode infection in animals. Experimental investigations of the embryotrophic effect of ivermectin at the therapeutic dose 0,2 mg/kg have shown that ivermectine at the tested dose does not have embryotoxic and teratogenic effects.

Based on the scientific research works the Instruction for the use of «Ivirsalt» (project) from 08.10.2014 approved by the Board of Academics of All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants named after K.I. Skryabin, has been created.

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