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ANTHELMINTIC RESISTANCE AS AN ENVIRONMENTAL ISSUE

Abstract

Anthelmintic resistance (AR) represents the reduced sensitivity of parasites to substances used for the treatment of parasitosis of animals, ie, the possibility of their survival, to the recommended doses of used antiparasitics used for certain types of parasites. The appearance of this undesirable characteristic occurs as a consequence of inadequate use of antiparasitics during the treatment of diseased animals as well as the need for better economic results in modern livestock production. Available data indicate that AR has been recorded in many countries, and preliminary studies show that it is also present in our country. Pasture farming allows the spread of AR in nature and adverse effects on wildlife.

Key words: *anthelmintic resistance, nature, wild animal*

UVOD

Parasitosis in domestic animals often leads to health and economic problems (Sutherland A. Ian, Dave M. Leathvick; 2011). The finding of different types of parasites in domestic animals is a sign that the production characteristics of the infested individuals are reduced, but also that their health status has changed (Sutherland A. Ian, Dave M. Leathvick; 2011). Often, just confirming the presence of parasites is not enough to determine the degree of health endangerment of the animal, but it is important to determine how much, the parasites are burdened with the organism, ie to determine the number of parasites in a gram of feces. For the control of the parasitofauna of the infested individuals, in addition to the appropriate diagnostics, the knowledge of anthelmintics is also important. This primarily implies that the therapy is carried

out in an adequate manner, ie, first of all, to determine the required dose in relation to the body weight of the individual being treated and to administer the drug in an adequate manner (Falzon CL et al.; 2013). As with antibiotics and the development of antimicrobial resistance, long-term use of anthelmintics may lead to the development of anthelmintic resistance. Anthelmintic resistance represents a reduced sensitivity of parasites, ie the possibility of survival, to the recommended doses of used antiparasitics used for certain types of parasites (Gasbarre C. Louis; 2014). This characteristic is acquired during long-term use of anthelmintics and is transmitted through genes to the next generations of parasites. Animals that are in inadequate production conditions on farms are exposed to long-term anthelmintic therapy, which leads to the development of resistance of parasites and their persistence in the herd (Falzon C.L. et al.; 2013). Such circumstances, in the absence of expert laboratory control, lead to the survival of resistant species of parasites on farms with the possibility of their spread inside and outside the farm.

A special problem is represented by animals that are raised in a free way during the year, ie they are allowed to graze. In such circumstances, there is a possibility that resistant species of parasites can be transmitted to nature. In this way, resistant parasites can endanger deer and roe deer and spread through them in a larger area.

In our paper, we will present data related to the mechanisms of action of anthelmintic drugs in relation to leeks, mechanisms of resistance development, data on the presence of resistance in the world and preliminary data from our studies. We will also point out the problem of spreading anthelmintic resistance and what are the possibilities for alternative ways to prevent parasitosis

MATERIAL AND METHODS

The study was limited to examining the presence of gastrointestinal parasites in small ruminants. Two flocks of sheep with 30 individuals each were examined. Herds are kept on pastures during warmer days (spring – autumn). The test was performed by determining the number of hatched eggs of *Strongyloides spp*. in grams of feces before and after administration of Ivermectin. Feces were taken from the rectal ampoule so that each individual was controlled. Control of the presence of parasites was done before the application of ivermectin and 2 weeks after the application of the drug.

The effect of ivermectin is monitored by the method of determining the reduction of faecal egg count reduction (FECRT) (Sutherland A. Ian, Dave M. Leathwick; 2011, Falzon C.L. et al.; 2013). For testing, we used a mini flotation system and a hypertonic NaCl solution. Weigh 5 grams of feces, put in a glass of mini flots, add 45 ml of NaCl solution and emulsify well. Feces must be completely dissolved. A pestle and mortar should be used if the faeces cannot be dissolved in the mini float. Pour the obtained suspension from the mini floats into the graduated chambers (part of the equipment floats) and wait for 10 minutes. After 10 minutes, the chambers are examined with a microscope (x 200) (two chambers are poured) and the observed eggs are counted. The obtained number of eggs is multiplied by 5 and the number of eggs in 1 g of feces is obtained. To determine the effectiveness of ivermectin, it uses a percentage reduction

in the number of eggs in the feces. A reduction in the number of eggs of 99.9% is expected if there is no resistance, egg reduction >98% indicates susceptibility of the parasite to the drug, 95-98% indicates a possible suspicion of the presence of AR, while ≤95% egg reduction can be said to be anthelmintic resistance.

RESULTS AND DISCUSSION

The mode of action of anthelmintics depends on the type of preparation used. Levamisole / morantel act as acetylcholine agonists, leading to depolarization of the nematode muscle sac membrane with loss (outflow) of Na +. The development of levamisole resistance occurs when a mutation occurs and acetyncholine receptor deficiency occurs and there is no reaction to cholinergic agonists (such as acetincholine nicotine, carbamyl chloride and levamisole). The binding of cholinergic agonists varies between levamisole-sensitive and resistant parasites. These differences have been found to affect the laying of parasite eggs. The difference between sensitive and resistant parasites is related to the reduction in the number and / or sensitivity of the cholinergic receptor. (Prichard Roger; 1994). Ivermectin works by opening glutamate-dependent chloride channels in the neuromuscular membranes of nematodes and arthropods. Opening the chloride door leads to paralysis of the parasite. (Prichard Roger; 1994; Prichard Roger; 2007). The onset of ivermectin resistance is not yet clear but it is thought that changes at subunit receptors bound to ligand ion channels play an important role (Prichard Roger; 2007). In addition to this mechanism, there is a possibility that changes in ATP-bound cassettes that are important for ivermectin action contribute to resistance (Prichard Roger; 2007). The action of benzimidazole is based on its binding to helminthic tubules. Resistance to this preparation occurs as a consequence of a decrease in the number of receptors to which drug molecules bind. The number of receptors in susceptible strains remains the same. Resistance in *Caenorhabditis elegans* is linked to the ben-1 gene encoding beta tubulin. This gene also encodes phenylalanine amino acid production. Besimidazole resistance occurs when ben-1 gene deletion (which is not lethal) or certain mutations occur. It has also been confirmed that changes in gamma tubules are associated with benzimidazole resistance in nematodes (Prichard Roger; 1994).

Table 1 shows the results of preliminary tests that we conducted in our laboratory.

Table 1. Results of changes in the number of eggs in sheep feces before and after ivermectin administration

	Before application of ivermectin		After application of ivermectin	
	No. examined	No. positive	No. examined	No. positive
Heard 1	23	23	23	4*
Heard 2	19	19	19	4*

* number of sheep with ≤95% reduction in the number of eggs/g of feces

Resistance occurs as a consequence of the use of anthelmintics and represents the adaptive power of parasites caused by gene mutations, deletions or amplifications of specific genes or epigenetically (by methylation of genes or altering gene expression). Animals carrying parasites after treatment with anthelmintics begin to secrete eggs of resistant parasite, so that strains that have acquired resistance appear on pastures, as a consequence of hereditary changes in genes. The appearance of resistance in parasites to anthelmintics is influenced by various factors. One of the important factors is the frequency of treatment with parasite preparations. The data indicate that the development of resistance can be expected in the circumstances of regular cleaning from parasites. Studies have confirmed that the development of anthelmintic resistance in *H. contortus* can be expected in humid climates after 10-15 treatments over a year in small ruminants (Dorny P.; 1994). When the same preparation is used for a long period of time, resistance can occur after three or four treatments. A treatment procedure based on the use of only one drug in a longer period leads to the appearance of resistance.

In South Africa and New Zealand, treatment with ivermectin for a longer period without replacement with another drug has led to the emergence of resistant strains of *H. contortus* (VanWyk JA et al.; 1989). One of the important factors that lead to the appearance of resistance is the incorrect way of treatment, ie subdosing. Treatment with inadequate doses of anti-parasite drugs can lead to tolerance and later to parasite resistance. In the circumstances of subdose treatment, heterozygous strains of parasites can survive and later move from a tolerant to a resistant relationship to the preparations (Hoekstra R et al.; 1997). On the other hand, it is good to know that for all animals, the recommended doses of treatment (recommended doses by the manufacturer) are not equally effective. In an experiment when sheep and goats were treated with the same anthelmintic for a longer period of time, resistance in goats developed more rapidly, which is explained by a different drug metabolism (Hennessy D.R. et al.; 1994). The application of anthelmintics in prophylactic cases can contribute to the appearance of resistance. Nowadays, animal trade has become one of the important factors in the spread of resistant strains of parasites among countries as well as in nature.

What is interesting when it comes to anthelmintic resistance is the fact related to the percentage of resistant strains of parasites in the population. Namely, in order to determine the presence of resistant strains of parasites by standard phenotypic tests (egg excretion or FECR – reduction of the number of eggs in the feces), it is necessary for 25% of the parasite population to become resistant to some of the drugs used (eg benzimidazole). The population accumulates genes encoding resistance (Anderson C. et al.; 1998). Therefore, AR prevention must be based on preventing the accumulation of resistance genes in parasites in certain areas. For these reasons, it is important to ensure the presence of susceptible parasite strains that will dilute the genes of resistant strains. Prevention of resistance is also related to different approaches to animal therapy. The opinion that only animals that have symptoms of the disease or show a significant delay in production should be treated, has its justification, because in that way the population of parasites that were not in contact with antiparasitics is left. Studies have shown that due to aggregation, 20-30% of animals carry 80% of parasites. Treatment of these animals alone contributes to the survival of a small number of

parasites that have not come into contact with anthelmintics and they, in perspective, represent a population that will dilute the resistance genes (Sreter T. et al.; 1994).

To combat against the development and spread of resistance to antiparasitics, it is important to prevent the introduction of resistant strains of parasites on the farm, ie in the herd. The most important thing for this type of control is the implementation of strict quarantine measures. Separate keeping of animals on dry surfaces, with permanent removal of feces and anthelmintic treatment with various antiparasitics allows to reduce the number of eggs excreted in the feces. After 14 days of therapy, perform a parasitological examination and determine the absence of parasites. The herd should then be released to pasture contaminated with parasites. This procedure is important due to the fact that a possibly resistant strain of the parasite left after triple treatment (moxidectin, levamisole, albendazole) would have nothing to dilute its genetically acquired resistance on pasture that is not contaminated with susceptible strains of the parasite (Fleming SA et al.; 2006).

Synergistic application of two anthelmintics gives good results when there is the absence of resistance. In such circumstances, it takes 20 years to develop resistance. Synergism between albendazole and ivermectin or diethylcarbamazine, as well as mebendazole and levamisole or pyrantel when it comes to the treatment of soil-borne parasites, gives good results (Utzinger J., Keiser J.; 2004). In small ruminants, the purpose of administering two preparations with a synergistic effect is important on the one hand to successfully remove the parasite in animals and when more than two different species of parasites are present, and on the other hand to prolong the period for resistance (Leathwick DM; 2012).

The natural resistance of certain breeds (eg sheep) also plays an important role in the fight against the presence of parasites and the later appearance of resistance to antiparasitics. By crossing them, satisfactory natural resistance of sheep to parasites can be achieved. This method of raising natural resistance in sheep can take a long time (8-10 years) and has been implemented in some regions of New Zealand and Australia (Bisset S.A. et Al. 2001).

Diet also plays an important role in preventing parasitosis. Protein needs are growing when it comes to the presence of nematodes. The reaction of the immune system and its functioning depends on the amount of protein present in food, ie in the body. The immune system requires an increased amount of amino acids because it opposes the negative influence of parasites. Insufficiently clear data related to the reduction of food intake during the process of entry of gastrointestinal nematodes in sheep is explained by the change in the structure of intake of certain nutrients. An increase or decrease in phosphorus, cobalt, copper, or molybdenum intake may affect the immune response of infected sheep or a general decrease in parasite load (Coop R.L., Kyriazakis I.; 2001). Pasture nutrition requires special attention. It is very important that the pastures used for food are "rested" during use. After grazing, which is during the warm period of the year (spring, summer and autumn), the winter period (the period of colder weather) is important for cleaning the pastures. Distillation grazing is also beneficial because it reduces pasture contamination by parasites. The number of sheep and goats on a certain pasture area should correspond to the ratio of 1 cow: 5-7 sheep per hectare. Sheep prefer to graze just above the ground goats prefer to graze near trees and shrubs.

This is important to know because most parasite larvae are found at a height of 50 mm (Niezen J.H. et al.; 2002).

Over the last two decades, the importance of various active substances produced by plants has been examined. It has been observed that some of these substances may have antimicrobial, antiparasitic or insecticidal effects. For these reasons, a number of herbal substances are used to treat humans and animals against parasitic infections. (Satyavati GV et al.; 1976) (Garlic – *Allium sativum*; Black cumin – *Nigella sativa*; Pelin – *Artemisia spp*., *Balanites aegyptiaca*, *Acacia spp*. (Mimosa), *Pumpkin* (seed), *Commiphora molmol* (Desert shrub), *Calendula micrantha officinalis* (Neven), *Peganum harmala* and *Tumeric* (curcumina) (Akhtar MS et al .; 2000, Massoud A.M. et al.; 2012).) Plants that contain tannins can affect various developmental forms of parasites. Their effect has not yet been fully elucidated, it is believed that on the one hand it enables better protein absorption, which indirectly enhances the immune system of hosts, while on the other hand, tannins have a direct effect on the viability of parasites.

Parasite control in sheep and goats can also be done with the use of biological agents, such as fungi (*Duddingtonia flagrans*), which by their parasitism on nematode larvae lead to a reduction in their number. Their influence cannot completely eliminate larvae from pastures but can reduce them significantly (Waller P.J. et. al.; 2001). In addition to this preventive measure, vaccination against parasites can contribute to their reduction in animals and nature.

CONCLUSION

According to our tests, the presence of anthelmintic resistance was registered in two tested herds. The number of animals with ivermectin-resistant strongylids is about 20% in herds. This figure may not be a significant problem given that 25% of the herd alone should be positive in order to speak of "true resistance". These data certainly warn and indicate that it is necessary to establish monitoring for anthelmintic resistance and monitor its development. Since AR has been observed in a large number of countries, it is necessary to apply their experience and try to apply some of the possible, alternative, procedures to reduce the use of antiparasitics.

Impact on nature is possible, because animals that are bred on pastures for a part of the year, represent a possible incubator for the spread of resistant species of parasites in nature and its spread to a wider population through wild animals. Certainly, the crossing of resistant strains with susceptible parasites from nature reduces the danger associated with AR. However, it is important to practice the phenomenon and treat alternative solutions.

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ANTIHELMINTSKA REZISTENCIJA KAO EKOLOŠKI PROBLEM

Abstract

Antihelmintska rezistencija (AR) predstavlja smanjenu osetljivost parazita na preparate koji se koriste za lečenje parazitoza životinja, odnosno, mogućnost njihovog preživljavanja, na preporučene doze upotrebeljenih antiparazitika koji se koriste za pojedine vrste parazita. Pojava ove neželjene karakteristike nastaje kao posledica neadekvatne upotrebe antiparazitika tokom lečenja obolelih životinja kao i potrebe za što boljim ekonomskim rezultatima u savremenoj stočarskoj proizvodnji. Dostupni podaci ukazuju da je AR zabeležena u mnogim zemljama, a preliminarna ispitivanja pokazuju da je prisutna i našaj zemlji. Pašnjački način uzgoja životinja omogućava širenje AR u prirodi i neželjeni uticaj na divlje životinje.

Ključne reči: *antihelmintska rezistencija, priroda, divlje životinje*