

ALTERNATIVE METHODS FOR CONTROL OF PARASITIC DISEASES IN ANIMALS

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Traditional methods of control of parasitic diseases in animals with large dosages of chemotherapeutic drugs (anthelmintics) are losing popularity due to helminths developing resistance to them, while public becomes more sensitive to chemical residues in animal products. Recent studies show, however, that many helminth-caused diseases can be effectively controlled through use of less conventional methods such as plant extracts, natural enemies of helminths and other ecological features.

Keywords: biological control, helminthosis, plant extracts, natural enemies of helminths.

Parasitic diseases in animals may develop in different ways. On the one hand there are massive infections with certain dangerous species that can directly cause death of animals affected and on the other there are parasites less dangerous by themselves, that could appear as a predisposing factor for the development of secondary deficiency and infectious diseases. The impaired health condition of the animals in a great number of cases exerts a negative impact on their productivity and reproduction and as a result economic losses arise.

Conventional methods of controlling parasites use synthetic chemotherapeutic drugs (anthelmintics). Largely because of the remarkable developments in these products in terms of efficacy, safety, spectrum of activity while remaining relatively inexpensive, livestock producers have relied almost exclusively on their use [37].

During the last 10–15 years, however, an increasing need of development of new alternative methods for control of parasitic worms in animals has been observed. The major reason is the serious development of anthelmintic resistance in parasites' populations [24, 34, 36]. The global tempo of development and extent of anthelmintic resistance in helminths indicates that the numerous anthelmintics and their usage strategies developed and implemented over the period of last 40–50 years may have been incorrectly applied. On the other hand, the rapidly increasing number of organic farms in many countries over the last ten years and the consumer pressure for reduced chemical residues in products require finding of new, safe for the animals and environment, methods for control of parasitic diseases [15].

Biological control of animal parasites could become a strong factor for integrated parasite control in near future. Biological control is defined as the usage

of parasites' natural enemies which maintain a parasitic population at levels lower than would occur in their absence. This not only includes classical natural organisms but also those that have been genetically modified to enhance these properties [37]. Biological control can be divided into two major categories – natural and applied [15]. Natural biological control uses native or co-evolved natural enemies in the environment without human intervention. A number of organisms have been identified to exploit the free-living stages of parasites as food source. Those organisms are micro-arthropods, protozoa, predacious nematodes, viruses, bacteria and fungi. Another method for biological control of helminths uses plant extracts [2, 11, 26, 27].

In the present work a literature review of some investigations carried out in the field of alternative methods for control of parasitic diseases in animals, helminthosis in particular, is presented in hope that the obtained data would be of use both to researchers in the field of parasitology and to practicing veterinarians.

1. Biological control of helminths using plant extracts

a) Usage of plant tanines.

Paolini et al. [29] have assessed the possible impact of condensed tannins on goats infected with adult *Haemonchus contortus*. They have found that administration of tannins has been associated with a significant decrease in egg excretion which persisted until the end of experiment. This reduction has not been associated with any difference in worm numbers, but with a significant decrease in female fecundity. No significant changes in the mucosal density of three inflammatory cell types have been detected between the two groups. These results indicate that the major consequence of tannin consumption in goats was a reduction in worm fecundity and egg output.

In other study Paolini et al. [30] have investigated the effects of condensed tannins (CT) on adult populations of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* and on the establishment of infective larvae of these two species. Two groups of kids have been infected with L3 of *T. colubriformis* and *T. circumcincta*. After 7 weeks, quebracho extracts have been administered *per os* for 8 days to one group. The results have shown that tannin administration was associated with a decrease in egg excretion and a decrease in female fecundity, but with no changes in worm numbers. These changes have been associated with an increased number of intestinal mast cells.

Paolini et al. [31] have investigated *in vitro* effects of extracts from 3 woody plants (*Rubus fruticosus*, *Quercus robur*, *Corylus avellana*) on trichostrongyles and have compared them to that of sainfoin, a legume plant. The effects have been measured on 3rd-stage larvae and adult worms of *Teladorsagia circumcincta*, *H. contortus* and *Trichostrongylus colubriformis*. The effects of plant extracts have varied according to the plant sources, the parasite species and stages. For the woody plants, significant inhibitory effects have been obtained on both stages of abomasal species. Results for *T. colubriformis* have been more variable. Effects of sainfoin extracts have been significant on 3rd-stage larvae of *T. colubriformis* and *H. contortus* and on abomasal adult worms. In order to assess the effects of tannins, polyethylene glycol (PEG), an inhibitor of tannins, has been added to hazel tree, oak and sainfoin extracts. Without PEG, significant inhibitory effects on 3rd-stage larvae and adult worms have been confirmed. After addition of PEG, the larval migration and motility of adult worms have been restored in most cases. These results have confirmed variations in effects depending on factors related to plants or parasites and suggest that tannins are partly responsible for the effects.

Brunet et al. [8] have studied the role of tannin-rich extract concentration on the exsheathment of *H. contortus* and *T. colubriformis*. The authors have incorporated in their experiments sainfoin (*Onobrychis viciaefolia*) as the bioactive plant. A set of *in vitro* assays has been performed, measuring the changes observed, after 3 h of contact with increasing concentrations of sainfoin, on the rate of

artificial exsheathment. The results have indicated that sainfoin extracts interfered with exsheathment in a dose-dependent manner and the process overall has been similar for both nematodes. The restoration of control values observed after adding PEG to extracts has confirmed a major role of tannins. A second study has been performed *in vivo* on rumen-cannulated sheep fed with different proportions of sainfoin in the diet to verify these *in vitro* results. The consumption of a higher proportion of sainfoin has been indeed associated with significant delays in *Haemonchus* exsheathment. According to the authors the interference with the early step of nematode infection might be one of the modes of action that contributes to the anthelmintic properties of tanniniferous plants.

Minho et al. [24] have investigated the anthelmintic effect of condensed tannin extracts (CTE) from *Acacia molissima* on lambs naturally infected with *H. contortus* and *T. colubriformis*. Twenty Santa Inês sheep have been divided into four groups and allocated to four paddocks with five animals each in a 60-day trial. Two groups had CTE (1,6 g/kg LW) administered for two consecutive days at the beginning of the trial and 30 days later and two groups have been maintained control throughout the trial. The animals have been weighed every 14 days; blood has been collected once a week and faecal egg counts have been measured twice a week. Twenty-eight days after the final CTE administration, all lambs have been slaughtered and worm burden counts have been determined. Mean body weight changes were not different among treatments. Globular volume for the treated group was higher or showed tendency to be higher than the control group at days 14 ($P = 0,043$), 21 ($P = 0,074$), 28 ($P = 0,026$), 42 ($P = 0,007$) and 48 ($P = 0,089$). No differences were observed in haemoglobin values between treatments. The CTE administration was associated with a reduction in FEC ($P = 0,003$) and worm burden in the abomasum ($P < 0,003$), but not in the small intestine. The results have confirmed the anthelmintic effects of CTE on gastrointestinal nematodes in lambs and demonstrated the potential use of CTE as an alternative endoparasite control in livestock.

b) Other plant products.

Administration of plant-based preparation Loshtak *per os* as tablets made from standardized dust of bryony (*Bryonia alba*) roots was studied in experimental dictyocaulosis of lambs [26, 27], in experimental ascaridosis of chickens [11] and experimental hymenolepidosis of white outbred mice. It has been shown that the preparation increases the animals' natural resistance to these infections through activation of immunocompetent cells and phagocytes [27].

Hördegen et al. [17] have investigated the anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. Forty-eight helminth-free lambs have been divided into eight groups (A–H) of six animals. Groups A–G have been infected artificially with 10 000 third stage larvae of *H. contortus* and 20 000 third stage larvae of *T. colubriformis*, whereas group H has remained uninfected. Thirty days post infection the lambs have been treated orally with a single dosage of one of the following products: group A with 3 mg/kg body weight (BW) of an aqueous ethanol extract (70 %, v/v) of the seeds of *Azadirachta indica* A. Juss syn. *Melia azedarach* L. (Meliaceae); group B with 1 g/kg BW of a raw powder of the leaves of *Ananas comosus* (L.) Merr. (Bromeliaceae); group C with 0,3 mg/kg BW of an aqueous ethanol extract of a 1 : 1 mixture (g/g) of *Vernonia anthelmintica* (L.) Willd. (Asteraceae) seeds and *Embelia ribes* Burm (Myrsinaceae) fruits; group D with 183 mg/kg BW of an aqueous ethanol extract of the whole plants of *Fumaria parviflora* Lam. (Fumariaceae); group E with 28 mg/kg BW of an aqueous ethanol extract of the seeds of *Caesalpinia crista* L. (Caesalpinaceae); group F with 25 mg/kg BW of pyrantel tartrate and group G with 50 % ethanol. The trials have shown that only the ethanol extract of *F. parviflora* has caused a strong reduction of the faecal egg counts (100 %) and a 78,2 and 88,8 % reduction of adult *H. contortus* and *T. colubriformis* on day 13 post treatment. The extract has been as effective as the reference compound

pyrantel tartrate. The authors suggest that the ethanol extract itself or single constituents of *F. parviflora* could be a promising alternative source of anthelmintic for the treatment of gastrointestinal trichostrongylids in small ruminants.

The *in vitro* effects of extracts of four tropical plants (*Zanthoxylum zanthoxyloides*, *Newbouldia laevis*, *Morinda lucida* and *Carica papaya*) on the egg, infective larvae and adult worms of *Trichostrongylus colubriformis* have been screened for potential anthelmintic properties [19]. Significant effects have been observed with the four plants on *T. colubriformis* but they differed depending on the life cycle stage of the parasite. Extracts of each plant have induced a dose-dependent inhibition of egg hatching. Using a larval inhibition migration test, the effects on the infective larvae have been also detected with the four plant extracts. In contrast, for adult worms the effects have been statistically significant only for *N. laevis* and *C. papaya*. No significant activity has been shown for *M. lucida* and *Z. zanthoxyloides*. The results suggest the presence of some anthelmintic properties associated with these four plants, which are traditionally used by small farmers in western Africa.

Extracts or ingredients of six different plant species have been tested against exsheathed infective larvae of *H. contortus* using a modified methyl-thiazolyl-tetrazolium (MTT) reduction assay [18]. Pyrantel tartrate has been used as reference anthelmintic. Bromelain, the enzyme complex of the stem of *Ananas comosus* (Bromeliaceae), the ethanolic extracts of seeds of *Azadirachta indica* (Meliaceae), *Caesalpinia crista* (Caesalpinaceae) and *Vernonia anthelmintica* (Asteraceae), and the ethanolic extracts of the whole plant of *Fumaria parviflora* (Papaveraceae) and of the fruit of *Embelia ribes* (Myrsinaceae) have shown an anthelmintic efficacy of up to 93 %, relative to pyrantel tartrate. The authors suggest that based on these results obtained with larval *H. contortus*, the modified MTT reduction assay could be a possible method for testing plant products with anthelmintic properties.

Bizimenyera et al. [7] have tested *in vitro* activity of *Peltophorum africanum* (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *T. colubriformis*. Eggs and larvae of *T. colubriformis* have been incubated at 23 °C in the extracts of the leaf, bark and root of *P. africanum* at concentrations of 0,008–25 mg/ml for 2 and 5 days, respectively. Thiabendazole and water have been used as positive and negative controls, respectively. Inhibition of egg hatching and larval development has been increased significantly ($P < 0,05$) with increasing concentrations of the extracts. Concentrations of 0,2–1,0 mg/ml of the extracts of leaf, stem bark, and root bark of *P. africanum* have completely inhibited the hatching of eggs and development of larvae. No eggs and larvae of *T. colubriformis* could be observed in wells incubated with all the three extracts at concentrations of 5 and 25 mg/ml. Those results of the authors have supported the traditional use of *P. africanum* against nematode parasites.

Maphosa and Masika [22] have collected and documented information from farmers and herbalists on medicinal plants used by farmers in the control of internal parasites in goats in the Eastern Cape Province, South Africa. According to their data the plant family Asphodelaceae has been frequent in usage, comprising 21,4 % of the plants, and the *Aloe ferox* has been the most utilized species (50 %). Leaves have been the most frequently used plant parts (45,9 %). Medicinal plants have been generally used in combination with other plants, and/or non-plant substances, but a few plants have been used on their own. These medicinal plant remedies have been administered orally, mainly using bottles and this has been done twice in summer at intervals of one month, only once in winter and when need arises thereafter. The authors consider that if safety and efficacy of discussed plants could be confirmed, they could form an alternative cost effective strategy in managing helminthiasis in the province.

Since *A. ferox*, *L. leonurus* and *E. elephantina* have been plants frequently used by resource-limited farmers in the Eastern Cape Province of South Africa to

control gastrointestinal parasites in goats, a study has been conducted to validate their anthelmintic activities *in vitro* on the egg and larvae of the nematode parasite *H. contortus* [23]. The crude aqueous extracts of leaves of *A. ferox* and *L. leonurus*; and roots of *E. elephantina* have been used. Eggs and larvae of the parasite have been incubated at 25 °C in aqueous extracts at concentrations of 0,625–20 mg/ml for 48 h and 7 days for the egg hatch and larval development assays respectively. Albendazole and water have been the positive and negative controls respectively. Inhibition of egg hatching and larval development increased significantly with increasing concentrations of the extracts. *E. elephantina* and *L. leonurus* extracts have had 100 % egg hatch inhibition at concentration as low as 2,5 mg/ml and 1,25 mg/ml respectively, whereas *A. ferox* extracts has had 100 % inhibition at concentrations of 20 mg/ml. At the lowest concentration tested (0,625 mg/ml), *E. elephantina* has inhibited egg hatching >96 % and this has been comparable to albendazole at the same concentration. *E. elephantina* and *L. leonurus* also have totally inhibited larval development at concentrations of 1,25 mg/ml. The study has provided evidence that *A. ferox*, *E. elephantina* and *L. leonurus* extracts possess anthelmintic activity, thus justifying their use in the treatment of gastrointestinal helminthosis.

Bashtar et al. (2011) have tested plant *Artemisia cina* for biological control of cestode *Moniezia expansa*. They have used different concentrations of its crude extract *in vitro* and *in vivo*. *In vitro* results have indicated that the plant extract has been efficacious at all concentrations tested. Electron microscopic examination has showed that many structures of the treated worms have been affected. The most affected sites have been the scolex and the microtriches of the outer tegumental surface. *In vivo*, treatment of heavily infected animals has showed an anthelmintic effect, since the complete absence of eggs has been recorded 9 days after treatment when fecal investigations have been done.

Shaziya and Goyal [35] have studied the anthelmintic activity of *Carica papaya* extract against *Ancylostoma caninum* in infection in mice. Two experiments have been set up. In experiment 1, two groups (A and B) and in experiment 2, three groups (A, B and C) of mice have been taken for larval recovery and mast cell and eosinophil counts respectively. Group A mice have been treated with plant extract (*Carica papaya*) 0,2 ml/mouse, on days 14 and 7 before challenge infection, and on day 0 mice have been challenged with 500 *A. caninum* larvae. Group B mice have been challenged only with dose of 500 *A. caninum* larvae. Group C have served as a non-treated control. Results of plant extract treated mice has clearly demonstrated a reduction of larvae in group (A) when compared with group (B) of mice. Large number of mucosal mast cell have been observed on day 16 in all groups. Eosinophil levels have been markedly reduced in 24 days after challenge infection in all groups. The authors suggest a potential role of *C. papaya* extract as an anthelmintic activity against intestinal nematodes infection.

2. Natural enemies in the environment

a) Usage of various fungi species.

Waller and Faedo [37] have tested ninety-four fungi species for their ability to reduce the number of infective larvae of sheep nematodes in faecal cultures, and also for their ability to produce nematode-attractant and nematocidal substances against these free-living stages under *in vitro* conditions. Reductions of infective larval numbers exceeding 80 % have been consistently recorded when 100–250 conidia per 1 g faeces of various species from the genera *Arthrobotrys*, *Geniculifera* and *Monacrosporium* have been used. Even concentrations as low as 10 conidia/g faeces have resulted in a significant reduction in infective larval numbers compared to control cultures. The study has demonstrated that whilst many fungal species exhibit nematophagous activity against a variety of free-living nematodes, few show efficient activity against the free-living stages of parasitic nematodes in the

sheep faecal environment. The most active among the latter category were six species of *Arthrobotrys*, two species of *Geniculifera* and two species of *Monacrosporium*.

The anthelmintic effect of the nematophagous fungus *Duddingtonia flagrans* has been tested by a number of authors [9, 10, 21].

Larsen et al. [21] have investigated the effect of the *D. flagrans* on calves infected by trichostrongyles under natural grazing conditions. Their study has been conducted in the grazing season with yearling calves exposed to a pasture with a natural mixed trichostrongyle larval infection. The results of the authors had shown that daily feeding with the microfungus *D. flagrans* during the first 2 months of the season has led to a lowered herbage infectivity and a reduced acquisition of *Ostertagia* sp. and *Cooperia* sp. later in the season. In addition, the procedure has delayed the onset of clinical disease. This was due to the nematode-destroying effects of the fungi in the dung excreted by the fungus-treated calves, as evidenced by results from a parallel *in vitro* assay on faecal larval cultures.

Trials aimed at control of nematode *H. contortus* in small ruminants in a wet, tropical environment using *D. flagrans* have been performed by Chandrawathani et al. [9]. Initially, pen trials have been conducted with individually penned groups of sheep and goats at dose rates of 125 000 spores and 250 000 spores/kg live weight per day. At the lower dosage larval reduction has been between 80 and 90 % compared with the pre-treatment levels. At the higher dose rate, there has been virtually complete suppression (>99 % reduction) of larval recovery. Trials using the fungal feed blocks have shown that when animals have been individually penned, they have consumed only small amounts of the block (particularly goats), hence little effect on larval recovery in faecal cultures has been observed. Grouping animals according to species and dose rate has induced satisfactory block consumption and subsequent high levels of larval reduction in faecal cultures. These larval reductions have been mirrored by the presence of fungus in faecal cultures. These trials have been followed by a small paddock trial, whereby three groups of sheep have been fed either a feed supplement without fungal spores, supplement with spores, or offered fungal blocks. The dose rate of spores in the latter two groups has been 500,000 spores/kg live weight per day. Egg counts have been significantly reduced in the two fungal groups, compared with the control group and the latter has required two salvage anthelmintic treatments to prevent mortality due to haemonchosis. Pasture larval numbers on the two fungal group plots have been also much lower than on the control plot.

Long-term field studies have been conducted on two government managed small ruminant research farms, located in different geo-climatic regions and approximately 300 km separate from each other, on Peninsula Malaysia [10]. As a result of these studies the authors have concluded that the deployment of the nematophagous fungus, *D. flagrans*, can improve the level of parasite control of sheep in the tropics above that which can be achieved by the short-term rotational grazing strategy alone.

Paraud and Chartier [32] have examined the effect of daily feeding of goats with spores of *D. flagrans* on third-stage larvae (L3) of *T. circumcincta* as well as its effect on the survival of first-stage larvae (L1) of *Muellerius capillaris*. Twenty-two culled dairy goats previously raised in a zero-grazing system have been twice infected at monthly intervals with 5 000 and then 7 500 *T. circumcincta* L3. Eight animals have been infected with a benzimidazole-susceptible (BZs) strain while the remainder have received a benzimidazole-resistant one (BZr). Six culled goats naturally infected with *M. capillaris* have been purchased from private farms. All the goats have been divided in two groups, one group receiving daily 5×10^5 chlamydospores of *D. flagrans*/kg body weight per goat for seven consecutive days in the food, the other group acting as control. For *T. circumcincta*-infected goats, individual egg counts and coprocultures (13 days, 25 °C) followed by L3 extraction with the Baermann method have been performed. For *M. capillaris*-infected goats,

extraction of L1 with the Baermann apparatus has been individually performed on day 0 and after coprocultures on days 7, 10 and 14. Reductions in percentage development of *T. circumcincta* L3 in fungus groups compared with control groups has ranged from 84 % (BZs strain) to 90 % (BZr strain). A decrease in *M. capillaris* L1 recovery has been noted on days 7 and 10 (a reduction of 70 % compared with day 0) and on day 14 (85 %), but this pattern has been similar in both groups, whether receiving the fungus or not. At the dosage of 5×10^5 spores/kg body weight, *D. flagrans* has been highly effective in reducing the larval development of *T. circumcincta* in goats faeces. In contrast, the fungus has not reduced *M. capillaris* L1 survival in faeces at above mentioned tries.

Impact of the nematophagous fungus *D. flagrans* on *M. capillaris* larvae has been also studied in other experiments [33]. Two goats naturally infected with *M. capillaris* have received *D. flagrans* chlamydospores at the daily dose rate of 5×10^5 spores/kg BW for 8 days. Faeces have been collected individually before, during and 11 days after spore administration. On each day of harvest, the initial larval output has been determined. The remaining faeces have been subjected to coproculture at 21 °C for 7 days. At the end of this period, L1 have been collected and used to infect snails (30 snails per goat isolate each snail given 40 L1 by direct deposit of the larvae on the foot of the snail). These snails have been artificially challenged in contrast to others that have been exposed to natural infection by exposure to faeces carrying first-stage *M. capillaris* larvae. The natural infection has used the same number of snails, i.e. 30 snails deposited on the faeces of each goat. After 3 weeks at room temperature, the infective larvae present in the snail foot have been counted. The authors have not determined difference in the survival of the L1 in faeces after coproculture whether the faeces contained *D. flagrans* or not. The infectivity of the extracted larvae from the two goats before and after fungal administration has been the same. The number of infective larvae per snail obtained after «natural» infection has showed variations that have not been related to the presence of *D. flagrans* mycelium in faeces. These trials clearly indicate that *D. flagrans* has been unable to alter the infectivity of *M. capillaris* first-stage larvae and thus cannot be considered as a non-chemotherapeutic alternative approach to the control of this lungworm in goats.

b) Usage of other natural enemies of helminths.

Some features of family Echinostomatidae trematodes allow to use them as agents of biocontrol of other trematodes, mainly schistosomes. Such features include mollusk sterilization with domination of Echinostomatidae partenites over those of other trematodes [16, 20]. Studies of using Echinostomatidae for biocontrol of bird schistosomes were performed in New Zealand [13, 14] with some success, though they did not reach a stage of immediate practical applicability.

3. Affecting environment or intermediate hosts

a) Control tactics.

This is, according to Be'er, Voronin [5] usage of environmental factors (including those affected by human activity such as sanitary and veterinary work) so that they lead to situation where decrease in numbers of the parasites' hosts (or decrease in their infection level) decreases risk of human or domesticated animals infection. It must be based, on the one hand, on prognosis (based on results of monitoring in this respect) of the specific situation on the particular parasitosis and, on the other hand, on expert assessment of «control tunnels», which are defined as reaching such range of levels numbers of specific hosts/carriers which minimize risk of infection for hosts which are next along the parasite life cycle.

Our studies have shown that decrease in numbers of mollusk intermediate hosts (g. *Lymnaea* in case of human cercariasis) for less than 1 order of magnitude dramatically decreases risk of next host (humans) infection. Adding a decrease of

numbers of definitive hosts (city populations of mallard duck *Anas platyrhynchos* in this example) we can reach a practical absence of the risk.

Note that if we reach the «control tunnel» range in respect to numbers of hosts in one of links in the parasite life cycle, it would be not necessary for numbers of its other life stage hosts to reach minimal levels necessary for infection risk decrease.

Note also that while it is often easier to assess general numbers of a parasite's hosts, numbers of infected hosts are what actually affect numbers of parasites at the next life stage and risks of infection. So, there are possibilities to decrease them through dehelminthization of host populations. While in cases of mollusk intermediate hosts this is quite difficult (though some possibilities are mentioned above and below), there were works studying possibilities of dehelminthization of populations of city ducks to reduce risks of human cercariasis [28].

b) Effects of ultra-low dosage of some molluscicidal substances on trematode parasites.

Fight with intermediate agents such as mollusks for schistosomes and other trematodes has been shown to lower infection level more than drug administration campaign does [3]. At the same time, usage of normal molluscicidal substances adversely affect normal water biocenosis, causing death of not only mollusks but many other water invertebrates, so it can't be recommended as normal method for control of parasitic diseases in animals, though usage of artificial ponds for watering, etc. with regular cleaning of their sides can be recommended.

During studies of some molluscicides (Bayer-73, phenasal) [4] an effect of high lethality on *Opisthorchis*-infected mollusks (*Codiella*) had been noted at agent concentrations much lower than usual lethal dosage (0,001 and 0,01 mg/l while LD₅₀ was 0,1–0,5 mg/l). Further studies had shown that about 90 % mollusks dying at such sublethal concentrations were infected with *Opisthorchis*. Possible explanations of this effect are either through the mollusks' organisms being weakened by the infection, so that dosage non-lethal for healthy mollusks killed them, or through one-time death of the parasites themselves (as even concentrations of the drugs in question as low as 0,00001 mg/l had been proved to be lethal to *Opisthorchis* cercariae both in water and in hosts) and the hosts' intoxication with products of their decay. Similar effects had been proved for natrium salt of 2,3,5-trichlorine-4-nitrosalicilanilide (USSR patent 677286 2558912 /23-04, 1979) and some antibiotics such as phyto bacteriomyicine, metabolite of *Act. laundulae* (strain 696), which was lethal for *Opisthorchis* cercariae at concentrations as low as 0,1 mg/l (after 60 min), while being lethal for hydrobionts (*Lymnaea*, *Viviparus*, water insects, fish fries) only after 48-hour exposition at concentration of 25 mg/l.

These effects could be useful for reducing risks of infection with mollusk-transmitted parasites such as trematodes without adversely affecting normal water fauna as molluscicides are prone to do (Be'er, 2005).

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References

1. Abdel-Rahman B., Mohey Hassanein, Abdel-Ghaffar F. et al. Studies on monieziasis of sheep I. Prevalence and antihelminthic effects of some plant extracts, a light and electron microscopic study. // Parasitol. Res. – 2011. – V. 108, № 1. – P. 177–186.
2. Anthony J., Fyfe L., Smith H. Plant active components – a resource for antiparasitic agents? // Trends in Parasitol. – 2005. – V. 21, № 10. – P. 462–468.
3. Be'er S.A. An assessment of epidemiological and economical efficacy of molluscicides usage in foci of schistosomiasis (a literature review) // Med. Parasitol. – 1980. – № 4. – P. 56–62
4. Be'er S.A. Biology of the agent of opisthorchiasis. – Moscow, 2005. – 336 p.

5. Be'er S.A., Voronin M.V. Cercarioses in urbanized ecosystems. – Moscow: Nauka, 2006. – 240 p.
6. Be'er S.A., Voronin M.V. Biology of the agents of schistosomiasis. – Moscow, 2011. – 202 p.
7. Bizimenyeraa E.S., Githioria J.B., Eloffa J.N., Swana G.E. *In vitro* activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis* // Vet. Parasitol. – 2006. – V. 142, № 3–4. – P. 336–343.
8. Brunet S., Aufrere J., El Babili F. et al. The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of a tannin-rich plant extract (sainfoin) both *in vitro* and *in vivo* // Parasitology. – 2007. – V. 134, № 09. – P. 1253–1262.
9. Chandrawathania P., Jamnaha O., Waller P.J. et al. Biological control of nematode parasites of small ruminants in Malaysia using the nematophagous fungus *Duddingtonia flagrans* // Vet. Parasitol. – 2003. – V. 117, № 3. – P. 173–183.
10. Chandrawathani P., Jamnah O., Adnan M. et al. Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans* // Vet. Parasitol. – 2004. – V. 120, № 3. – P. 177–187.
11. Chubarian F.A., Petrosyan R.A., Movsesyan S.O., Boyakhchyan G.A. Effects of Loshtak on natural resistance of chickens at experimental *Ascaridia galli* infection // Konf. «Theory and practice of fighting parasitic diseases». – Moscow, 2002. – V. 3. – P. 374–375.
12. Coles G.C. Anthelmintic resistance – looking to the future: a UK perspective // Vet. Sci. – 2005. – V. 78. – P. 99–108.
13. Davis N.E. Population dynamics and larval trematode interactions with *Lymnaea tomentosa* and the potential for biological control of schistosome dermatitis in Bremner Bay, Lake Wanaka, New Zealand // J. of Helminthol. – 1998. – V. 72. – P. 319–324.
14. Davis N.E. Cercarial dermatitis and the possibility of biological control in lake Wanaka, New Zealand // A thesis submitted for the degree of Doctor of Philosophy at the University of Otago, Dunedin, New Zealand. – 2000. – 100 p.
15. De S., Sanyal P.K. Biological Control of Helminth Parasites by Predatory Fungi // VetScan. – 2009. – V. 4, № 1. – P. 1–8.
16. Fashuyi S.A. Field observations on the possibility of using echinostomes to control schistosome infections in snails // Helminthologia. – 1986. – V. 23, № 1. – P. 37–41.
17. Hördegen P., Hertzberg H., Heilmann J. et al. The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs // Vet. Parasitol. – 2003. – V. 117, № 1–2. – P. 51–60.
18. Hördegen P., Cabaret J., Hertzberg H. et al. *In vitro* screening of six anthelmintic plant products against larval *Haemonchus contortus* with a modified methyl-thiazolyl-tetrazolium reduction assay // J. of Ethnopharmacol. – 2006. – V. 108, № 1. – P. 85–89.
19. Hounzangbe-Adote S., Fouraste I., Moutairou K., Hoste H. *In vitro* effects of four tropical plants on the activity and development of the parasitic nematode, *Trichostrongylus colubriformis* // J. of Helminthol. – 2005. – V. 79, № 01. – P. 29–33.
20. Jourdane J., Kulo S.D. Perspective d'utilisation de *Echinostoma togoensis* dans le control biologique intestinale en Afrique // Ann. Parasitol. hum. et comp. – 1982. – V. 57, № 5. – P. 443–451.
21. Larsen M., Nansen P., Wolstrup J. et al. Biological control of trichostrongyles in calves by the fungus *Duddingtonia flagrans* fed to animals under natural grazing conditions // Vet. Parasitol. – 1995. – V. 60, № 3–4. – P. 321–330.
22. Maphosa V., Masika P.J. Ethnoveterinary uses of medicinal plants: A survey of plants used in the ethnoveterinary control of gastro-intestinal parasites of

goats in the Eastern Cape Province, South Africa // *Pharmaceutical Biology*. – 2010. – V. 48, № 6. – P. 697–702.

23. Maphosa V., Masika P.J., Bizimenyera E.S., Eloff J.N. In-vitro anthelmintic activity of crude aqueous extracts of *Aloe ferox*, *Leonotis leonurus* and *Elephantorrhiza elephantina* against *Haemonchus contortus* // *Trop. Animal Health and Producty*. – 2010. – V. 42, № 2. – P. 301–307.

24. Minho A.P., Bueno I.C.S., H. Louvandini H. et al. Effect of *Acacia molissima* tannin extract on the control of gastrointestinal parasites in sheep // *Animal Feed Sci. and Technol.* – 2008. – V. 147, № 1–3. – P. 172–181.

25. Molento M.B. Parasite control in the age of drug resistance and changing agricultural practices // *Vet. Parasitol.* – 2009. – V. 163. – P. 229–234.

26. Movsesyan S.O., Boyakhchyan G.A., Chubaryan F.A. et al. Effects of plant preparation Loshtak on immune status of lambs in case of experimental dictyocaulosis // In: *Differential diagnostics of parasites and aspects of parasite-host relationships*. – Moscow: Institute of Parasitology, 1994. – P. 13–19.

27. Movsesyan S.O., Chubaryan F.A., Petrosyan R.A. et al. Animal non-specific resistance and ways of its correction in some helminthoses // *Transac. of the Institute of Parasitol. RAS «Progress in General Parasitology»*. – Moscow, 2004. – V. 44. – P. 221–230.

28. Müller V., Kimmig P., Frank W. Die Wirkung von Praziquantel auf *Trichobilharzia* (Digenea, Schistosomatidae), einen Verursacher von Badermatitiden beim Menschen // *Appl. Parasitol.* – 1993. – V. 34, № 3. – P. 187–201.

29. Paolini V., Bergeaud J.P., C Grisez C. et al. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus* // *Vet. Parasitol.* – 2003. – V. 113, № 3–4. – P. 253–261.

30. Paolini V., Frayssines A., De La Farge F. et al. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats // *Vet. Res.* – 2003. – V. 34. – P. 331–339.

31. Paolini V., Fouraste I., Hoste H. In vitro effects of three woody plant and sainfoin extracts on 3rd-stage larvae and adult worms of three gastrointestinal nematodes // *Parasitology*. – 2004. – V. 129, № 01. – P. 69–77.

32. Paraud C., Chartier C. Biological control of infective larvae of a gastrointestinal nematode (*Teladorsagia circumcincta*) and a small lungworm (*Muellerius capillaris*) by *Duddingtonia flagrans* in goat faeces // *Parasitol. Res.* – 2003. – V. 89, № 2. – P. 102–106.

33. Paraud C., Cabaret J., Pors I., Chartier C. Effect of the nematophagous fungus *Duddingtonia flagrans* on *Muellerius capillaris* larvae in goat faeces // *Vet. Parasitol.* – 2005. – V. 131, № 1–2. – P. 71–78.

34. Seed M., Iqbal Z., Jabbar A. Oxfendazole resistance in gastrointestinal nematodes of beetal goats at livestock farms of Punjab (Pakistan) // *Acta Veterinaria Brno*. – 2007. – P. 79–85.

35. Shaziya Bi., Goyal P.K. Anthelmintic effect of Natural Plant (*Carica papaya*) extract against the gastrointestinal nematode, *Ancylostoma caninum* in Mice // *ISCA J. Biological Sci.* – 2012. – V. 1, № 1. – P. 2–6.

36. Van Wyk J.A. Refugia- overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance // *Onderstepoort J. of Vet. Res.* – 2001. – V. 68. – P. 55–67.

37. Waller P.J., Faedo M. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: screening studies // *Vet. Parasitol.* – 1993. – V. 49, № 2–4. – P. 285–297.

Альтернативные методы борьбы с паразитарными болезнями у животных

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Традиционные методы борьбы с паразитарными болезнями животных путем использования химиотерапевтических препаратов (антигельминтиков) теряют популярность из-за развития у гельминтов резистентности к ним и возможного попадания остаточных количеств химических препаратов в продукцию животноводства. Настоящие исследования показали, что со многими гельминтозами можно эффективно бороться путем использования экстрактов растений, естественных врагов гельминтов и других экологических приемов.

Ключевые слова: биологический контроль, гельминтозы, экстракты растений, естественные враги гельминтов.