The Monitoring of Some Anthelmintic Results in Control of Fasciolosis in Extensive System Dairy Sheep in Albania

Veterinary Medicine

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Abstract

The infestation from Fasciola hepatica in Albania was prescribed at a very high level in extensive system dairy sheep herd of flat area. To compare the efficacy and results of some anthelmintics-active drugs in F. hepatica control we organized a trial during year 2012 in a dairy sheep farm in central Albania. We established 5 experimental groups with 50 sheep on each.

All faeces from all sheep were controlled on different dates according the methodology with fast flotation and sedimentation method for fasciola eggs and the PCV level (Hematocrit).

Laboratory tests before treatment, reported an average of 115-130 eggs of Fasciola, per gram of faeces, which represented a serious invasion and hemotocrit or PCV was 26,4%-27,1%.

The first and second group were treated with 10mg/kg.b.w of Triclabendazol per os (PO), one of the benzimidazolics that was introduced with delay in Albania. Third group was administered a combination of 10 mg/kg of Triclabendazole + 7.5 mg/kg.b.w PO of Levamisole. Fourth group was administered 10 mg /kg/b.w PO of albendazole. The fifth group was not treated (control group). Three weeks later from the treatment the trial was repeated under the same methodology.

In autumn a coprology control was organized for all groups before and after treatment. The same treatment was repeated as in spring except the first and fifth group which were not treated.

The conclusion from the experimental results is that the combined treatment of Triclabendazole + Levamisole, twice a year, in spring and autumn, shows a better effect against F. Hepatica than the treatment with Triclabendazole or Albendazole only.

Introduction

Fasciolosis is a helminthoses of a wide territorial extension causing significantly adverse economic effects. All animals are susceptible to it and affected but the most serious damages are reported among sheep. In Albania, it may be prevalent alongside the entire coastal area from Saranda to Shkodra, but also in the remote areas of the country and the valley of Devoll, Shkumbin, Mat, Drin, Vjosa, Shushica and of other smaller rivers.

The maintenance of hydrovores, embankments and irrigation canals from 1990 onwards has not been adequate. Consequently, a lot of floods have occurred and the water has returned in some

part of the flat land territory and former swamps. In this way the habitat is ideally expanded to favor the development of the biological cycle of fasciolas. Hence, it has been increasingly prevalent mainly in sheep and cattle.

According to the data and researches conducted after 1990 (Almuca et al,2008) 85.5 % to 100 % of the sheep herds raised in Albania are infested with fasciolosis.

Different anthelminthics are used to cure this disease but dehelminthisation with albendazole is mostly used. The use for a long time (over 25 years) and sometimes without observing the scientific dosing criteria has consequently led to a lower effectiveness of this preparation in the fight against fasciolosis.

The purpose of the study was to reiterate the importance of the effective use of anthelminthics and prevention of the phenomenon of chemoresistance to keep under the control the infestation of animals with fasciolosis.

Materials and methodology

The trial was conducted in two stages during 2012. The sheep herd used in the trial were administered albendazole each year (in spring) against fasciolosis.

Initially, the sheep were randomly divided into 5 groups with 50 heads each (the sheep of almost the same age group, i.e 3-4 years of age, were selected). They were matriculated but to distinguish the individuals of each group more quickly they were marked on the back by using five different sprinkled colors.

First stage

On 15 March 2012 the sheep involved in the trial (250 heads) were checked for the presence of fasciola by controlling their faeces in order to detect the eggs of *F. hepatica*. The faeces were individually taken in the rectum (over 10 grams from each sheep). Their tests were carried out in the parasitology laboratory of the Institute of Food Safety and Veterinarian Service of Tirana (ISUV), through the method of rapid quantified sedimentation.

Also, on 15 March 2012 the blood was preleaved by all sheep of the trial groups and was sent to the laboratory to calculate the value of the PCV in percentage (%) (The blood content was put in capillary tubes or microhematocrit tubes which were filled lengthwise up to ¾ and were placed in the microhematocrit centrifuge for five minutes).

Also, on the same date, 15 March 2012, the first group of sheep was orally administered triclabendazole PO at a dose of 10 mg/kg/live weight. The weight of the heaviest sheep was referred to as the average weight of the cattle. Also, the second group was equally treated with triclabendazole as the first group did.

The third group was administered a combination of 10 mg/kg/ weight of triclabendazole + 7.5 mg/kg/ live weight PO of levamisole. The fourth group was administered 10 mg /kg/ b.w PO of albendazole. The fifth group (of control) was not subject to dehelminthisation.

Three weeks after the date of treatment (5/4/2012) the sheep of all groups were again checked for the presence of eggs of *F.hepatica* under the same methodology and also blood was taken and the value of the hematocrit-PCV was calculated (blood content was taken in the capillary tubes by anticoagulants which were filled up to ¾ and were centrifuged for five minutes).

Second stage

On 15/10/2012 the sheep of all groups were checked for the presence of eggs of *F.hepatica* (n/v/g/f was calculated), blood was taken and the value of the PCV was calculated in percentage. On the same date the second treatment by fasciolicids was carried out in accordance with the following scheme: the first group was not treated, the second group was administered Triclabendazole at the dose of 10 mg/kg/ live weight, the third group was administered 10 mg/kg of Triclabendazole + 7.5 mg/kg/ live weight Levamisole, the fourth group was again administered 10 mg/kg of albendazole. The fifth group (control) was not treated. Three weeks later, on 5/11/2012, the sheep of all groups were subject to tests to calculate the n/v/g/f and the value of the PCV for each group. The period falling three weeks after the treatment is confirmed by the literature sources as the most suitable time to assess the fasciolicid effect of anthelminthics (Michael A et al 2005, George B 2003, Tremacid 120-2003). All data on the values of n/v/g/f and of the PCV for the five groups and for both stages of the trial were processed by statistical methods.

Results of the trial

The data of the trial are presented in the following tables.

Table No 1. Number of eggs of *F. hepatica* in the trial groups prior and after treatments.

Group	Heads	First stage 2012			Second stage 2012		
		15 March		5 April	15 October		5 November
		n/v/g/f	Dehelminthization	n/v/g/f	n/v/g/f	Dehelminthization	n/v/g/f
1	50	122	Triclabendazole 10 mg/b.w	25	70	Not treated	80
2	50	130	Triclabendazole 10 mg/b.w	28	72	Triclabendazole 10 mg/b.w	20
3	50	118	Triclabendazole 10 mg/b.w+ Levamisole 7.5 mg/b.w	15	45	Triclabendazole 10 mg/b.w+ Levamisole 7.5 mg/b.w	4
4	50	115	Albendazole 10 mg/b.w	50	90	Albendazole 10 mg/b.w	60
5	50	120	Not treated	132	160	Not treated	180

Table No 2. Values of hematocrit (PCV) prior and after the treatments by fasciolicids

Group	Heads	First stage 2012			Second stage 2012		
		15 March		5 April	15 October		5 November
		PCV %	Dehelminthization	PCV %	PCV %	Dehelminthization	PCV %
1	50	27.1	Triclabendazole 10 mg/b.w	31.5	30.6	Not treated	31
2	50	26.4	Triclabendazole 10 mg/b.w	32.3	31.5	Triclabendazole 10 mg/b.w	34.8
3	50	26.6	Triclabendazole 10 mg/kg. p +Levamisole 7.5 mg/b.w	32.1	30.3	Triclabendazole 10 mg/b.w +Levamisole 7.5 mg/b.w	37
4	50	26.8	Albendazole 10 mg/b.w	30.9	30.1	Albendazole 10 mg/b.w	30.3
5	50	27	Not treated	28.3	28	Not treated	27.5

Discussion

Animals hosting parasites are a source of infestation of the fasciolosis. Together with their faeces they eliminate the eggs of fasciola that contaminate the environment (stables, paddocks, yards, pastures, locations where they drink water etc). Due to the high level of the fertility of fasciola and asexual reproduction of their larvae's in the organism of the intermediate host (Lymnaea truncatula), a small number of animals hosting parasites is sufficient to transmit the disease within a wide territory (Huber H 1980, Georgi J.R 1990).

In Albania, as a Mediterranean country, the most suitable period for the development of the biological cycle of F. hepatica (monthly average temperature varies from 10-11°c to 24-25°c) is from March to November and the metacercariaes appear in the pastures since March (Allmuca H 2008, Zanaj S 2003). This is one of the reasons why the researchers periodically turn to the therapy against fasciolosis and why in our trial we have started to treat the sheep herds since March.

A strategy of treatment against fasciolosis must aim to:

a) help animals get rid of helminthic fauna (not only from mature populations but also from the pre-imaginal ones) at the moment when it has the highest colonizing capacity and when the external environmental conditions favor the development of exogenous stage of the biological cycle of the fasciola but possibly of other parasites simultaneously colonizing the sheep;

b) create a repeated and effective obstacle in order to interrupt the biological cycle of parasites in the main points of the demographic development of their populations in order to periodically reduce the level of contamination of animals and the environment (Ambrosi M 1991, George B 2003).

We believe that we observe the high level criteria cited by the literature sources in applying the scheme we propose for the enhancement of the effectiveness of sheep treatment against fascioliosis in a known fasciolous area.

The treatment of sheep has started in March in order to liberate the animals from the growing stains of fasciola present in the liver (the fasciola in sheep live for a period of more than 5 years) and on the other hand, we may prevent in this way the contamination of pastures with eggs of fasciola, which after (at least) three months will significantly increase the level of re-infection of those animals. We should bear in mind that in March the pastures have started to be contaminated with metacercariae (cercariae) that have were developed during winter in L.truncatula snails (Huber H 1980; Georgi J.R 1990; Pucini V 1992; Smyth J.D 1994).

The first group of sheep (table No.1) is treated by triclabendazole one of the benzimidazolics that was introduced with delay in Albania and which is highly effective both for mature stains and for the larvaes of fasciola (Michael A et al 2005, NADIS 2007, George B 2003; Tremacid 120-2003). In early spring, the effect on the larvae's of fasciola is certainly of minor importance because in March there are practically no larvae of fasciola in the livers of sheep (or a very small number of them may be found as the metacercariae may live even for a period of five months on the dry grass).

Further, the second group of sheep is treated by triclabendazole like the first group but with the difference that this group will be treated one more time with this preparation in autumn to help them get rid of infections during spring, summer and autumn.

The third group is treated by the combination of triclabendazole + levamisole. The purpose was to liberate the sheep from fasciola and from gastrointestinal and pulmonary nematodes through the effect of levamisole which as already known, is quite effective against them but on the other hand, it has immunostimulating effects directly influencing the resistance of animals against the larvae of fasciola (Susan K et al 2003).

The fourth group was subject to treatment with albendazole in order to check the effects of the long use of this preparation in the fight against fasciolosis. The fifth group was not treated it was as a control group. The milk for public consumption in all treated groups was not allowed as long as it was recommended in the label of the respective medicament.

According to the coproscopic tests carried out on 15 March 2012 (Table No 1) an average of more than 80 eggs was found for a gram of faeces (v/g/f) in the five trial groups. The consulted literature asserts that when 80-150 /v/g/f are found in the faeces of sheep, infection is considered serious and of adverse consequences to the health and production of animals (Ambrosi M 1981, Zanaj S 1998). This fact highlights the necessity of the treatment of animals with fasciolids in early spring.

The data furnished by the coproscopic tests carried out three weeks after the dehelminthization (5/4/2012) highlight the fact that in the first two trial groups treated with triclabendazole and in the third group treated with a combination of triclabendazole + levamisole n/v/g/f/, the number of eggs per gram of faeces is significantly reduced, which means that the animals are liberated from fasciola. This is not the case for the group treated with albendazole where 50 v/g/f were found. Such infection is considered as an alert signal (Ambrosi M 1981, Zanaj S 1998). Hence, the

effectiveness of albendazole against fasciola is compromised. An increase from 120 to 132 was marked in the n/v/g/f control group.

In statistically processing the results according to the Chi square test, we observe statistically confirmed differences between the coproscopic results prior and following the treatment and between the groups treated with different anthelminthics. This fact shows that the result of the effect of anthelmenthics is statistically confirmed in comparison to the group of control and the difference of the effect between different antihelmenthics used in the trial groups is statistically confirmed.

As for the coproscopic control conducted during the second stage of the experiment, 6 months later, namely on 15/10/2012, on the day of the second treatment with anthelmenthics, there is an increase of n/v/g/f in all trial groups. This is expected and is a consequence of the re-infections occurred during the period from March to October. Two months (from the moment of infection) are sufficient for the larvae of the fasciola to pass onto the bile ducts of the liver and to start to hatch the eggs.(Cox .F.E.G 1982, Euzeby J 1982, Georgi J.R 1990, Smyth J.D 1994).

Evidently, in the first three groups the infection level has not reached to 80 v/g/f. Hence, this infection level is likely to influence the health of animals and it is clearly required the dehelminthalization we have undertaken during this period. The synergism of triclabendazole with levamisole is noticed in the third group where 25-27 eggs per 1 gram of faeces were reported less than in the first two groups that were administered only triclabendazole. An average of 90 v/g/f was reported in the group treated with albendazole. In this amount of eggs there is clearly a share of fasciola not killed by this antihelmenthic during the first treatment. Similarly, the differences between the first three groups and the control group are clearly distinguished. The level of 160 v/g/f is considered as a very serious infection.

The last column of the first table reflects the results of the coproscopic tests dated 5/11/2012, namely three weeks after the second treatment. We observe that in the first group that was not treated against fasciola in autumn, an average of 80 v/g/f was reported. Thus, irrespective of the preparation used in spring, the autumn treatment against fasciola is also necessary in the fasciolous areas so as the animals may hibernate being free from trematodes. This conclusion is also reinforced by the results of the coproscopic tests of the sheep of the second group in which 20 v/g/f were reported.

In the third group (treated both in spring and in autumn by the combination of triclabendazole+ levamisole) only 4 v/g/f of fasciola and also a small number of eggs of gastrointestinal and pulmonary nematodes (about 85 v/g/f) were reported. We believe that this result establishes our proposal to treat the sheep in spring and in autumn with the combination of triclabendazole + levamisole at the highest rate of estimation, hence, we recommend it as the best scheme to keep under control the infection of sheep from the fasciolosis but also from strongilates.

In the fourth group treated with albendazole in autumn an average of 60 v/g/f was reported. This result reinforces the opinion articulated as above that the effect of this preparation against trematodes is compromised by the phenomenon of chemoresistance.

On 5/11/2012, an average of 180 v/g/f was reported in the control group compared to 120 v/g/f reported in spring, so we have to do with a very serious invasion which will be further deteriorated during winter.

In the statistical processing of the results of the second stage according to the Chi square test, again we observe statistically confirmed differences between the coproscopic results prior and after the treatment as well as between the groups treated with different antihelmenthics.

This result is incompatible with the literature data confirming that the level of infection of animals from the fasciolosis increasingly grows by the end of autumn and during winter

(Cox F.E.G 1982,Georgi J.R 1990, Pucini V 1992, Smyth J.D 1994).

The results of the group of control(180 n/e/g/f in the control group), confirm once more the effectiveness of the strategy we propose in the fight against fasciolosis.

The second table gives indication of laboratory data on the average values of the PCV, Packed Cell Volume-Hematocrit of the groups of sheep during both trial stages prior and after the treatment with antihelminthics as we discussed above.

Average values of the PCV for the sheep vary from 31.7 % at 1 year of age to 36.1% at 5-6 years of age. The sheep involved in the trial were 4 years old.

Our data indicated an anemia of the animals on 15/3/2012, also expressed by the abnormal values of the PCV with an increase from 26.6 % to 27.7 %. We have already documented that during this period the entire herd was under the pressure of a serious infection from fasciolosis.

This disease is always characterized by a state of weakness and anemia of different levels. This parasite seriously damages the hemopoesis due to serious disorders of the metabolic processes in the liver and on the other hand the fasciola consume the liquids of the organism to feed themselves (Boray J.C 1971, Cox F.e.g 1982, Smyth J.D 1994).

Three weeks after the dehelminthisation we see that the values of PCV have been significantly increased in the four dehelminthised groups, whereas in the control group the values of PCV are almost equal (the differences are not confirmed).

From 5/4/2012 to 15/10/2012 the table shows a decreasing trend of the values of the PCV in the five groups of sheep. The coproscopic tests have also proven that during that period the infection level from the fasciola has increased. Thus, the increase of the infection level leads to the fall of the rates of the PCV.

A trend of the fall of rates of the PCV is reported in the control group, reaching to 27.5% in the middle of November. The highest level of the PCV (37 %) is reported in the third group of sheep that is treated twice with the combination of Triclabendazole + Levamisole.

In PCV results only in third group (treated both in spring and in autumn by the combination of triclabendazole+ levamisole) observe statistically confirmed differences between the treated and control group.

As stated above, the simultaneous treatment of sheep with triclabendazole and levamisole twice a year, in spring and in autumn, maintains the infection from helminthic fauna at economically acceptable levels. This is proven by both the coproscopic test and the hematological one.

Conclusions and Recommendations

1) As regards the control of fasciolosis, the treatment of sheep once a year with triclabendazole (in spring) does not protect them from the re-infestation during summer and autumn.
2) The biannual treatment against the fasciolosis with triclabendazole, in spring and in autumn may keep under control only the infestation with fasciolosis but not with other gastrointestinal and pulmonary parasites.
3) The biannual treatment of sheep with the combination of triclabendazole + levamisole, in spring and autumn guarantees the control of infestation from the hepatic, gastro-intestinal and pulmonary fauna and a better health state than in the treatments only with triclabendazole.
4) The use of albendazole twice a year (in spring and autumn) does not prove successful in the control of fasciolosis.
5) To ensure the control of the epidemiological situation of the fasciolosis in the fasciolous areas and to maintain at economically acceptable levels the infestation of sheep from it and from the gastrointestinal and pulmonary fauna, we recommend their dehelminthization twice a year with the combination of Triclabendazole + Levamisole, once in early spring and once in the middle of autumn.
6) The further use of albendazole in the treatment of sheep against fasciolosis is not recommended due to the consequential resistance.

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