

RESEARCH ON ANTHELMINTIC RESISTANCE IN TWO FLOCKS OF SHEEP FROM ALBA AND HUNEDOARA COUNTIES

CERCETĂRI PRIVIND REZISTENȚA LA ANTIHELMINTICE ÎN DOUĂ TURME DE OVINE DIN JUDEȚELE ALBA ȘI HUNEDOARA

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ABSTRACT | REZUMAT

Anthelmintic resistance is a serious challenge throughout the world in sheep flocks. While in Europe there is a lot of data supporting the presence of the phenomenon, in Romania it is often controversial. This paper aims to identify this phenomenon of anthelmintic resistance in two sheep flocks from Hunedoara and Alba counties. Two of the most widely used anthelmintics were tested: albendazole (ABZ) and ivermectin (IVM). To determine the efficacy of each anthelmintic, the faecal egg count reduction test (FECRT), which remains the gold standard in this regard, and two additional methods, described by Dash and Kochapakdee, respectively, were used. The results obtained by the three formulas were similar, justifying their use. Thus, the FECRT for ABZ in Alba County was 93.18% and for IVM 97.67%, implying suspected resistance for ABZ and no resistance for IVM. In Hunedoara County, the FECRT results for ABZ were 86.44% and 94.73% for IVM, representing the presence of resistance to ABZ and suspicion of resistance to IVM.

Keywords: sheep, resistance, albendazole, ivermectin

Rezistența la antihelmintice reprezintă o serioasă provocare peste tot în lume în efectivele de ovine. Dacă în Europa există numeroase date care să ateste prezența fenomenului, în România acestea sunt adesea controversate. Această lucrare are ca scop identificarea acestui fenomen de rezistență la antihelmintice în două efective de ovine din județele Hunedoara și Alba. Au fost testate două dintre cele mai folosite antihelmintice: albendazolul (ABZ) și ivermectina (IVM). Pentru stabilirea eficacității fiecărui antihelmintic s-a recurs la testul de reducere a numărului de ouă din fecale (FECRT), care rămâne gold-standardul în acest sens, precum și la două metode suplimentare, descrise de Dash, respectiv Kochapakdee. Rezultatele obținute prin cele trei formule de calcul au fost similare, justificând utilizarea lor. Astfel, FECRT pentru ABZ în județul Alba a fost de 93,18%, iar pentru IVM de 97,67%, ceea ce semnifică suspiciunea rezistenței pentru ABZ, respectiv lipsa rezistenței pentru IVM. La efectivul din județul Hunedoara rezultatele FECRT pentru ABZ au fost de 86,44%, respectiv 94,73% pentru IVM, ceea ce reprezintă prezența rezistenței la ABZ și suspiciunea acesteia pentru IVM.

Cuvinte cheie: ovine, rezistență, albendazol, ivermectină

In the case of small ruminants, parasitism with helminths, especially gastrointestinal nematodes, is considered extremely harmful. This is due to both intrinsic and extrinsic factors such as the possibility of harbouring an impressive number of worms, the cumbersome installation of immunity, the rapid acquisition of resistance to anthelmintic drugs, the way of grazing close to the ground with the probability of ingesting a large number of parasitic elements, the possibility of implementing control programmes and so on (23). For these reasons, the losses recorded in the small ruminant breeding sector can be very high. Thus, in the USA, losses

were estimated between 42 and 222 million dollars, with year-related variations (15, 27), while in Australia, the reported estimated losses were almost 500 million dollars (8).

Another important problem is represented by the phenomenon of resistance to anthelmintic drugs, both within the flock and outside of it. This aspect of resistance raises serious questions regarding its distribution. Resistance has been frequently reported throughout European countries (1, 2, 16, 20, 25); however, data regarding the status of this phenomenon in Romania is scarce and sporadically reported (3, 4, 5, 6, 18).

This paper aimed to highlight the presence of this phenomenon in two flocks of sheep from two counties located in central Romania using three different methods.

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METHODS AND MATERIALS

Faecal samples were collected from two flocks of sheep from Alba and Hunedoara Counties during the year 2022. Faecal samples coming from fifteen sheep from each flock, aged between one and seven years, were randomly selected and subjected to coproscopic examinations. The animals grazed in the sub-mountain areas of the counties. The faecal sample examination was performed using the Willis, polyvalent, and McMaster methods, the latter being used to quantify the parasite burden. The FECRT (faecal egg count reduction test) was used (11) to establish the effectiveness of some anthelmintic drugs by comparing the number of eggs expelled from faeces before and after treatment. FECRT still remains the "gold standard" for identifying the resistance phenomenon (17, 26, 11). In addition to the classic equation, two other equations were used, described by Dash et al. (7) in 1988 and Kochapakdee et al. (13) in 1995:

$$\% \text{ FECRT} = \frac{\text{EPG before treatment (day 0)} - \text{EPG after treatment (day 14)}}{\text{EPG day 0}} \times 100$$

$$\% \text{ FECR} = 100 \times (1 - [T2/T1][C1/C2])$$

where T1 and T2 stand for the arithmetic average of the treated group before and after treatment, and C1 and C2 similarly represent the arithmetic average of the control group, the same as the treated group, respectively:

$$\% \text{ FECR} = 100 \times (1 - [T2/T1])$$

where T1 and T2 represent the arithmetic average of the treated group before and after treatment, excluding the control group.

Albendazole (ABZ) and ivermectin (IVM) were used for deworming. Five of the most intensively parasitized individuals in the group were treated with one of the two substances mentioned.

RESULTS AND DISCUSSIONS

The results obtained following the coproscopic examination of samples collected from the two studied flocks are presented in Tables 1 and 2.

The average age of the population in Alba County subjected to examination was quite high- close to 4 years. Gender-wise, specimens originating from female animals represented the majority of examined samples - 3/15 (20%).

According to Table 1, parasites belonging to the classes Protozoa, Trematoda, and Nematoda were noticed. Thus, all examined sheep were parasitized with digestive strongyles (100%), with a parasitic load ranging between 100 EPG and 700 EPG with an average of 356.67 EPG. They were followed, in descending order, by trematodes - 9/15 (60%), *Eimeria* spp. - 8/15 (53.33%), with a low parasitic load, respectively *Strongyloides* spp. - 3/15 (20%), in which EPG varied within very close limits: 50 and 100.

Analysing data obtained from the processing of samples from sheep in Hunedoara County, it can be observed that, compared to Alba County, a new form of parasitism was noticed, namely the presence of *Moniezia* spp., adding a new class of parasites to the list - Cestoda. The age average was more compact, around 2 years, practically half of the value recorded for the studied population from Alba County.

Table 1

Results of a coproscopic examination of sheep faecal samples collected from Alba County

Crt. no.	Fas/Param	Dig. strong.	<i>Eimeria</i> spp.	<i>Strongyloides</i> spp.	Age	Sex
1	-	300	0	50	7	M
2	-	100	0	0	4	F
3	+	350	0	0	7	F
4	+	300	50	0	7	M
5	++	450	0	100	7	M
6	-	150	0	0	5	F
7	+	400	100	0	5	F
8	++	700	0	0	2	F
9	+	350	0	0	2	F
10	-	200	100	0	2	F
11	+	350	150	0	2	F
12	++	500	100	0	1	F
13	-	250	450	0	1	F
14	-	300	50	0	1	F
15	+	650	100	100	1	F
Sum	nd	5350	1100	250	54	-
Average	nd	356.666667	73.33333333	31.25	3.6	-

Note: + - light infection; ++ - medium infection; +++ - heavy infection; nd - not determined

Table 2
Results of a coproscopic examination of sheep from Hunedoara County

Crt. no.	Fas/Param	Dig. strong.	Eimeria spp.	Strongyloides spp.	Moniezia spp.	Age	Sex
1	+	500	100	50	0	2	F
2	++	800	0	0	100	2	F
3	++	850	350	100	0	2	F
4	++	1050	400	250	300	2	F
5	+	450	50	100	0	2	F
6	+	750	100	50	400	1	F
7	+	700	50	50	0	1	F
8	++	850	50	350	50	2	M
9	-	400	50	50	0	2	F
10	-	100	0	50	0	2	F
11	-	150	100	50	0	3	F
12	+	600	50	100	0	2	F
13	-	100	50	450	350	1	F
14	+	550	100	100	0	1	F
15	++	700	50	150	0	1	F
Sum	nd	8550	1500	1900	1200	26	
Average	nd	570	100	126.666667	80	1.733333	

Note: + - light infection; ++ - medium infection; +++ - heavy infection; nd - not determined

In this case as well, the female population was predominant among the study subjects, with only one male selected for the study (6.67%).

Similar to results obtained from the population in Alba County, all the sheep were infected with digestive strongyles (100%), and the parasitic load varied between 100 EPG and 1050 EPG, with an average of 570 EPG. *Strongyloides* spp. ranked second, with 14 out of 15 infected animals (93.33%), with a parasitic load ranging between 50 and 450 EPG with an average of 126.67 EPG. Third place was occupied by *Eimeria* spp., with 13 infected individuals (86.67%), followed by trematodes - 11/15 (73.33) and *Moniezia* spp. - 5/15 (33.33%) with a parasitic load between 50 and 400 EPG and an average of 80 EPG (Fig. 1).

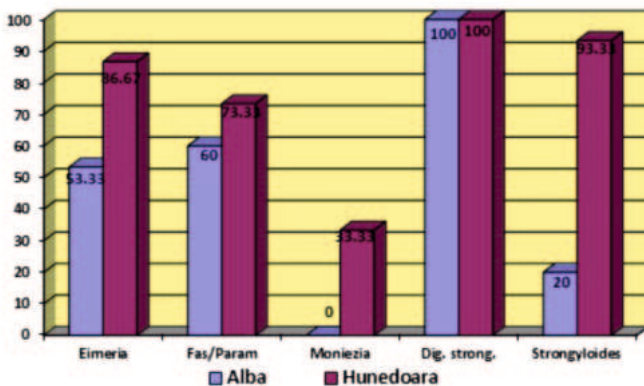


Fig. 1. The prevalence of digestive parasites in the two studied flocks

The results obtained when testing the effectiveness of ABZ and IVM in the two flocks are shown in tables 3 - 6.

Table 3

The effectiveness of albendazole (ABZ) in livestock from Alba County

	Day 0	Day 7	Day 14	Day 28
Sheep 3	350	0	0	50
Sheep 4	300	0	0	50
Sheep 8	700	50	50	50
Sheep 9	350	0	50	50
Sheep 12	500	0	50	100
Sum	2200	50	150	300
Average	440	10	30	60

Obviously, only the parasitic loads of digestive strongyles, which are mainly reported as having anthelmintic resistance, were taken into account.

The application of the classic FECRT equation for ABZ in the case of the flock from Alba County revealed an effectiveness of 93.18%, a value classified as being right below the lower limit of the safety interval, which apparently suggests susceptibility for the phenomenon of resistance to this substance.

$\% \text{ FECRT} = [(EPG \text{ d}0 - EPG \text{ d}14)/EPG \text{ d}0] \times 100$, where EPG d0 = 440; EPG d14 = 30. Thus, the equation becomes: $\% \text{ FECRT} = [(440 - 30)/440] \times 100 = 410/440 \times 100 = 0.9318 \times 100 = 93.18\%$.

An identical result was obtained while calculating the effectiveness of albendazole according to the third equation, i.e. 93.20%.

$\% \text{ FECR} = 100 \times (1 - [T2/T1])$, where T1 = 440 and T2 = 30, the equation becomes $\% \text{ FECR} = 100 \times (1 - 0.068) = 100 \times 0.932 = 93.20\%$.

Practically, very slight differences appear among

the three calculation methods, which do not influence the result; therefore, only the classical "golden standard" method of FECRT will be used for further calculations.

Table 4

The effectiveness of ivermectine (IVM) in livestock from Alba County

	Day 0	Day 7	Day 14	Day 28
Sheep 5	450	0	0	0
Sheep 7	400	0	0	50
Sheep 11	350	0	0	50
Sheep 14	300	0	0	0
Sheep 15	650	0	50	50
Sum	2150	0	50	150
Average	430	0	10	30

The use of the classic equation for FECRT in the case of IVM demonstrated an efficacy of **97.67%**, placing the value above the lower limit of the safety interval and confirming the absence of the resistance phenomenon to this substance.

The results obtained for the flock from Alba County raise the suspicion that resistance to benzimidazole substances might be present, while for ivermectin, this phenomenon is absent. The main favouring factor of the resistance phenomenon to benzimidazoles is based on the excessive use of these substances in comparison to ivermectin, which is less used.

In the case of the five specimens collected from Hunedoara County, the average EPG on day 0 was 590, decreasing to 80 on day 14.

By calculating the effectiveness of albendazole according to the classic FECRT equation, we obtained a value equal to 86.44%, highlighting the presence of re-sistance to benzimidazoles in this flock.

$\% \text{ FECRT} = [(EPG \text{ d0} - EPG \text{ d14})/EPG \text{ d0}] \times 100$, where $EPG \text{ d0} = 590$; $EPG \text{ d14} = 80$. Thus, the equation becomes: $\% \text{ FECRT} = [(590 - 80)/590] \times 100 = 510/590 \times 100 = 0.8644 \times 100 = \mathbf{86.44\%}$.

Table 5

The effectiveness of albendazole (ABZ) in livestock from Hunedoara County

	Day 0	Day 7	Day 14	Day 28
Sheep 2	800	0	50	100
Sheep 3	850	50	100	150
Sheep 4	1050	100	250	300
Sheep 10	100	0	0	0
Sheep 11	150	0	0	50
Sum	2950	150	400	500
Average	590	30	80	100

For ivermectin, the FECRT calculation provided a value of 94.73%, ranking just below the lower borderline for resistance, practically inducing the suspicion that resistance might occur to the action of macrocyclic lactones.

$\% \text{ FECRT} = [(EPG \text{ d0} - EPG \text{ d14})/EPG \text{ d0}] \times 100$, where $EPG \text{ d0} = 570$; $EPG \text{ d14} = 30$. Thus, the equation becomes: $\% \text{ FECRT} = [(570 - 30)/570] \times 100 = 540/570 \times 100 = 0.9473 \times 100 = \mathbf{94.73\%}$.

Table 6

The effectiveness of ivermectine (IVM) in livestock from Hunedoara County

	Day 0	Day 7	Day 14	Day 28
Sheep 5	450	0	0	0
Sheep 6	750	0	50	150
Sheep 7	700	0	0	50
Sheep 8	850	0	100	150
Sheep 13	100	0	0	0
Sum	2850	0	150	350
Average	570	0	30	70

Thus, albendazole resistance was noted in the flock from Hunedoara County, while in the flock from Alba County, there was only a suspicion regarding albendazole resistance. As for ivermectin, there was only suspicion of resistance in the flock from Hunedoara County. Digestive strongyles proved to be the most numerous, affecting all examined individuals. In Romania's neighbouring countries, the prevalence of these infections varied between 100% (identical to the results in this study) in Bulgaria (19) and 74.5% in Serbia (14). Studies carried out in the western area of Romania confirmed prevalence rates between 75% (10) and 92.6% (24). Resistance to anthelmintic drugs has proved to be more and more widespread throughout the world. In the United States, *Haemonchus contortus* was the first species to acquire resistance to several substances, such as benzimidazoles, levamisole, ivermectin, and moxidectin (12). Thus, in 17% of the investigated farms, resistance to all mentioned anthelmintic drugs was recorded (9). Studies carried out in Brazil demonstrated multiple resistances present in several species of digestive strongyles (*H. contortus*, *Trichostrongylus colubriformis*, *Cooperia* spp., and *Oesophagostomum columbianum*) to trichlorfon, albendazole, ivermectin, levamisole, moxidectin, and closantel. For none of these substances, the effectiveness exceeded 65% (22). In Europe, the most widespread is the resistance to benzimidazoles. That one for levamisole and ivermectin is kept within relatively low limits, and the resistance for moxidectin is just at the beginning (12). However, cases of multiple resistances have been reported in *Teladorsagia circumcincta* (21). In Europe, the dominant species in terms of resistance to anthelmintic drugs remain *H. contortus* and *Trichostrongylus* spp.

CONCLUSIONS

Polyparasitism was present in both examined flocks. The most widespread parasites were the digestive

strongyles (100%), and the least reported species was *Moniezia* spp. (33.33%), present only in the flock from Hunedoara County. The FECR method remains effective in detecting the phenomenon of anthelmintic resistance. Considering the results obtained by using three different equations to calculate anthelmintic efficiency, we recommend the FECR method be used in field trials.

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