Shared Pastures and Anthelmintic Resistance in Wildlife and Livestock

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Summary

Parasitic diseases are an important threat to grazing livestock. Until recently, the most accepted control methods were regular, herd-level deworming regime and grazing on "clean" or "safe" pasture. Presence of wild ruminants on pastures was considered as the main risk of parasitic infection. In the last decades, the failure of these conventional attitude was suspected. This study was carried out in Hungary, where springtime, whole-herd deworming is still in practice. Our hypotheses were that the above-mentioned strategy led to high prevalence of anthelmintic resistance; on the other hand, wildlife could not contribute to deleterious parasitosis of livestock. For this, we accomplished an investigation in the close surroundings of typical sheep herds. The aims were to determine the species structure and anthelminthic resistance in the parasite community of the sheep herds and the adjacent roe deer population. As a result, we found that in the roe deer (N=53), a more diverse parasite community exists and the most devastating worm species, *Haemonchus contortus* plays a less important role in it; than in the sheep (N=40). Prevalence of benzimidazole resistance in *H. contortus* was 17.1% and 68.6% in the roe deer and sheep, respectively. Our findings suggest that routine deworming cannot succeed; while presence of roe deer is rather useful, as its parasites attenuate the simplistic, anthelmintic resistant pasture community.

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Introduction

Farming on pastures is a nature friendly, low-input production system; which proposes the least animal welfare issues, if nutrition and animal health is well-managed. In the last decades, the most threatening factors for grazing, especially in small ruminants, are gastrointestinal nematodes (GIN) and their anthelmintic resistance (AR) (Rose et al., 2015). Until recently, the most accepted control methods were regular dose-and-move regime. This meant that, mainly in springtime, the whole herd was treated by an antiparasitic medicine and after a few days awaiting time, the "parasite-free" animals were driven to a "clean" pasture, which has not been grazed for a long time and considered as quasi free from parasites (Michel, 1985; Boa et al, 2001).

Nowadays, the failure of this method is confirmed. After a mass deworming, a part of the parasite community survives; and in this part, genes of AR can occur. If the farmer regularly repeats this practice; the prevalence of AR increases time and time again. Treated animals will excrete a selected, mostly anthelmintic resistant, worm community onto the "clean" pasture; and AR will be general in the close surroundings of a regularly dewormed livestock herd. In these conditions, the presence of wild ruminants could be even advantageous; as they are never treated by anthelmintics, therefore, their less human influenced parasite community can serve a kind of buffer within the parasite pool of the pasture. In this context, the alimentary tract of wild ruminants and the mixed helminth fauna of a natural pasture should be considered as refugia for anthelmintic sensitive worms (van Wyk, 2001; Nagy et al., 2017). On the other hand, wild ruminants can even transmit AR alleles between livestock herds, and further research is needed to assess their exact effect (Chintoan-Uta et al., 2014).

In our study, we investigated the close surroundings of regularly dewormed sheep herds. Based on the examination of abomasa from both sheep and roe deer; we carried out a faunistic analysis and a determination of AR prevalence in the isolated *Haemonchus contortus* population. Our hypothesis were that worm fauna of the two species should be different, and the level of resistance should be lower in roe deer. By confirmation of these facts; we would have liked to support the refugia hypothesis and attempt to assess the role of roe deer in AR transmission or fighting against it.

Materials and methods

Our investigation was conducted in southwestern Hungary between April of 2014 and December of 2016. The study site was characterized by a central, 6000 ha forest monoblock with agricultural lands; mostly pastures around it. We examined abomasa of roe deer (N=53) and sheep (N=40) in order to determine the differences and similarities of abomasal nematode fauna of the two species in the same habitat. The organs of deer were collected from hunting bags, while sheep were sampled at a regional slaughterhouse and on farms. For species identification, we used Lichtenfels et al.'s (1994), Drózdz's (1995) and Rehbein's (2010) works.

For a finer characterization, the genotypic analysis was carried out on codon 200 of β-tubulin gene isotype 1 by Restriction Fragment Length Polymorphism-Polymerase Chain Reaction

described by Tiwari's et al. (2006). We used chi-squere test by GenAlEx software 6.502 version (Peakall and Smouse, 2012) to compare 140 *H. contortus* males (70 from each host) to determine the genotypic and allelic frequencies.

In order to characterize the abomasal nematode fauna of both hosts, we calculated the importance index (I), the Shannon diversity index (H) and the Sørensen coefficient (SC) of similarity (Thul et al., 1985; Legendre and Legendre, 1998) by using ComEcoPac software (Drozd, 2010).

Results

The roe deer had more diverse abomasal fauna (9 species), than the sheep had (2 species). Seven of the worms were proved to be a dominant or codominant species; the rests belonged to subordinate ones (Table 1). We found just two common worm species but just *H. contortus* was considered as a dominant in both ruminant populations. For all parasites, species diversity as reflected by Shannon's diversity index was 1.79 in roe deer, whilst in sheep it was 1. The Sørensen coefficients of similarity between hosts were low (SC=0.36).

Table 1. Importance values (I) of nematode species by hosts

Nematode	Roe deer	Sheep
Ashworthius sidemi	0.13[CD]	0
Haemonchus contortus	21.67[D]	43.99[D]
Teladorsagia circumcincta/T. trifurcata	0.001>	43.94[D]
Spiculopteragia spiculoptera/S. mathevossiani	17.21[D]	0
Spiculopteragia asymmetrica/S. quadrispiculata	0.17[CD]	0
Ostertagia leptospicularis/O. kolchida	34.3[D]	0
Ostertagia ostertagi/O. lyrata	0.009	0
Trichostrongylus axei	0.004	0
Nematodirus oiratianus subsp. *interruptus*	0.004	0

[D] indicate dominant species, while [CD] does codominant ones

The homozygous susceptible (SS) genotype was the more representative in the roe deer (54.3%), than in sheep (2.9%). On the other hand, the homozygous resistant (RR) worms were most prevalent in the sheep (68.6%) and were moderate in the roe deer (17.1%), while the heterozygous (RS) genotype was observed in equal proportion in both hosts (28.6%). Difference of allele frequencies (roe deer: susceptible allele = 68.6%; resistant allele = 31.4%; sheep: susceptible allele = 17.1%; resistant allele = 82.9%) between the host populations was confirmed as significant (p<0.05).

Discussion

In this study, we carried out a faunistic analysis of parasite community in sheep herds and the sympatric roe deer population; and moreover we compared the AR prevalence of the isolated *H. contortus* population. As a general result, we ascertained that roe deer carries a more diverse helminth population, than sheep; and the AR level also differs significantly in the two species.

In the sheep, only two species, *H contortus* and *T. circumcincta/T. trifurcata* were detected, with very similar importance values. Comparing this finding with those obtained during investigation of naturally kept ruminants; the most conspicuous difference is the extinction of competitively superior species (e.g. *T. axei*) for the favour of *H. contortus*. In the lack of regular anthelmintic treatment, *H. contortus* plays an inferior role in a helminth infracommunity (in the abomasum of a host individual). As a competitively inferior parasite species, *H. contortus* has a better capacity to survive in the environment, but cannot invade the host such aggressively as superior ones. It is probable that in the environment of a regularly medicated livestock herd, a lot of surviving larvae accumulate on the pasture, and supersede less environment resistant competitors (Diez-Baños et al., 1981).

This drift in the structure of the helminth fauna is not necessarily due to AR. Most of the anthelmintics have no real ovicide effect; therefore, after deworming, a lot of viable eggs and larvae are excreted to the pasture. In these conditions, the most environment resistant species should reach the most dense population in the environmental pool. Principally; competitively superior species are affected during deworming of the host. This should cause the rising of *H. contortus*, a naturally satellite member of a parasite community.

It is interesting that among the nine parasite species of roe deer, *H. contortus* was confirmed to be dominant. Its importance is not so remarkable as in the sheep, but it is not a satellite species at all. Moreover; the most important, most numerous parasite species of roe deer, *O. leptospicularis/O. kolchida* could not be detected in the studied sheep herds. These findings suggest that rather the sheep farming affects the roe deer habitat, than vice versa.

The comparison of AR prevalence in the two hosts also supports the superiority of human influence. Within the studied habitat, antiparasitic treatment of wildlife has never been in practice; as the authors know. In spite of this; AR is present in the parasite community of the roe deer; though its level is much lower than in the sheep.

Notwithstanding; AR transmitting role of roe deer cannot be excluded by this study, our results support the hypothesis, that habitat overlapping between sheep and roe deer means rather an advantage than a real risk for antiparasitic strategies. Worm community excreted by wild ruminants contains less AR individuals and more competitively superior, non blood-sucking species, which has got a stimulating effect on the host's immune system. These two features behave like a buffer in the environmental pool of parasites; and the effect depends on its portion.

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