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Anthelminthic resistance of *Haemonchus contortus* in small ruminants in Switzerland and Southern Germany

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Anthelminthika Resistenz von *Haemonchus contortus* bei kleinen Wiederkäuern in der Schweiz und in Süddeutschland

Zusammenfassung. In Süddeutschland und in der Schweiz wurden zwei Ziegen- und zwei Schafbestände ermittelt, bei denen aufgrund post-therapeutisch fortbestehender klinischer Anzeichen wie gastrointestinale Störungen, Inappetenz und Abmagerung, eine verminderte Anthelminthika-Wirksamkeit vermutet wurde. Der bei den untersuchten Ziegen beider Herden gezielt durchgeführte Eizahlreduktionstest zeigte, dass die Eprinomectin-Behandlung (1 mg/kg KG, Pour-on) lediglich zu einer EpG-Reduktion von 17,4 % bzw. 27,5 % führte. Diese Werte deuten auf das Vorkommen einer Eprinomectin-Resistenz in diesen Herden hin. Die anschließende Moxidectin-Behandlung (1 mg/kg KG, Pour-on) einer der beiden Herden führte zu einer Eizahlreduktion von 99,1 %.

In den beiden Schafherden wurden jeweils 30 zufällig ausgewählte Tiere in drei Gruppen eingeteilt, die jede mit einem anderen Anthelminthikum behandelt wurde. Der Eizahlreduktionstest erbrachte EpG-Reduktionen von 70,8 % bzw. 55,3 % (Albendazol-Gruppen), 52,4 % (Fenbendazol-Gruppe) bzw. 47,3 % (Oxfendazol-Gruppe). In den beiden Moxidectin-Gruppen (0.2 mg/kg BW, oral) betrug die Reduktion 100% bzw. 44,3 %. Vor und nach der Behandlung durchgeführte Koprokulturen zeigten, dass *Haemonchus contortus* die vorherrschende Helminthenspezies ist.

Summary. Two goat and two sheep flocks have been found to be suspicious of a clinically evident reduced anthelmintic efficacy, i.e. lacking improvement of gastrointestinal disorders, insufficient weight gain and continuing inappetence after anthelmintic treatments. In order to conduct an appropriate evaluation of the efficacy the following trials were performed: the faecal egg count reduction test on the studied goats of the two herds revealed a reduction of the egg-excretion after the eprinomectin-treatment (1 mg/kg BW, pour-on) of 17.4% and 27.5%, respectively, which clearly confirms the occurrence of anthelmintic resistance against eprinomectin in these two herds. The alternatively administered moxidectin-treatment (1 mg/kg BW, pour-on) of one flock resulted in a 99.1% faecal egg count reduction.

In both sheep flocks, 30 randomly selected sheep were divided in three groups and each group was treated with a different anthelmintic, according to the instructions for use.

The faecal egg count reductions for the various groups treated orally with benzimidazoles were 70.8% and 55.3% (albendazole), 52.4% (fenbendazole) and 47.3% (oxfendazole). The two moxidectin-treated groups (0.2 mg/kg BW, oral) showed an EpG-reduction of 100% and 44.3%, respectively, thus also demonstrating resistance against macrocyclic lactones. Pre- and post-treatment faecal larval cultures revealed *Haemonchus contortus* as the predominant resistant species.

Key words: Anthelminthic resistance, *Haemonchus contortus*, FECRT, goat, sheep.

Introduction

Infections with gastrointestinal nematodes (GIN) represent a major constraint in small ruminant husbandry. On many farms, the continuous anthelmintic treatments appear to be the only possible way of control.

In Germany no anthelmintics are registered for the administration in goats. Therefore, the anthelmintics have to be rededicated by a veterinarian. In Switzerland albendazole, fenbendazole and eprinomectin are registered anthelmintics for goats [1]. Due to the long withdrawal times for benzimidazoles, most organic dairy farmers administer eprinomectin for the control of GIN-infections. Similarly, because of the well known benzimidazole resistance of GINs, most sheep farmers mainly use macrocylic lactones for the treatment of GIN-infections. Although, many farmers in Southern Germany and Switzerland are concerned about the reduced efficacy of anthelmintics in small ruminants, especially in goats, very limited information is available on the recent spread of anthelmintic resistance (AR) [2, 3]. However, the knowledge of the latter is most important to change the habits of treatment. To delay the

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development of AR and its further spread, affected farms should continuously be identified as a basis for a more selective treatment according to prior coproscopic analysis.

Therefore, in the context of a coproscopic survey on alternative treatment schemes in Southern Germany and in Switzerland, faecal samples were collected from 29 sheep flocks and 10 goat flocks and analysed for GIN prevalence and a possible occurrence of AR. The objective of the present paper is to describe the identified flocks with AR and its association with *H. contortus*.

Materials and methods

Faecal egg counts

Faecal samples, taken from the rectum, were analysed by the faecal egg count reduction test (FECRT) according to Coles et al. [4]. The samples were processed using a modified McMaster-method with a sensitivity of 30 eggs per gram of faeces (epg). The efficacy of the treatment, i.e. the faecal egg count reduction (FECR) was calculated according to WAAVP-guidelines [4–6].

Larval culture

The detectable GIN species were determined by larval cultures. Therefore, pooled faecal samples of each treatment group of all four flocks were mixed with vermiculite and incubated for 12 days at 22°C. The collection of the larvae was performed according to Roberts and O'Sullivan [7]. From each culture, at least 100 third-stage larvae were morphologically differentiated and identified accordingly [8, 9].

Animals

The present investigation was carried out in two goat and two sheep flocks. All four flocks took part in a preliminary coproscopic survey. During this survey flock owners sent pooled faecal samples of their animals before and 12 days after the regular treatments to our institute. The FECRTs of the pooled samples revealed insufficient reductions and therefore the anthelmintic treatments were repeated in the four flocks by checking individual animals. Goat flock 1, consisting of 16 goats of various breeds (Chamoisee, Anglo-Nubian, Saanen and mixed breeds) in the Swiss Emmental and will be referred to as "Swiss-flock". The "Black-Forest-flock" consisting of 90 "Deutsche Weiße Edelziegen" is located in the Black Forest, Germany. Of this flock, 21 randomly chosen goats were included in this study. Both goat farmers administered eprinomectin (1 mg/kg BW, pour-on) for the last four treatments, due to short withdrawal-times.

The two sheep flocks are situated in Southern Germany, one in Bavaria and one in Baden-Wuerttemberg. The Bavarian flock consists of 60 Suffolk sheep and will be referred to as "Allgaeu-flock". After a clinically observed lack of efficacy after an albendazole treatment, i.e. no weight gain, continuing inappetence and gastrointestinal disorders, 30 randomly selected sheep were divided into three groups of 10 sheep each, which were sampled on the day of treatment and 12 days later. The sheep flock located in Baden-Wuerttemberg is called "Alb-flock" and consists of 45 Dorper lambs which showed a reduced efficacy of a previously performed moxidectin treatment. Out of them three groups of 10 sheep each were formed and sampled on the day of treatment and 13 days later.

Treatment

All goats were first treated with 1 mg/kg BW eprinomectin (Eprinex®-PourOn, Merial/Biokema SA) according to Swiss regulations. The subsequent treatment of the "Black-Forest-flock" was performed using moxidectin (Cydectin®-PourOn, 1 mg/kg BW, FortDodge) according to the previously indicated eprinomectin-dosage.

The sheep were orally treated with albendazole (Valbazen®-1.9%, 3.8 mg/kg BW, Pfizer), fenbendazole (Panacur®-2.5%, 5 mg/kg BW, Intervet), oxfendazole (Oxfenil®, 5 mg/kg BW, Virbac) and moxidectin (Cydectin®-0.1%, 0.2 mg/kg BW, Fort Dodge) according to the instructions of the manufacturer and WAAVP recommendations [5, 6].

Statistical analysis

Statistical analysis was run using SPSS 15.0 and 16.0, and Microsoft-Excel-2003 software. Small flock sizes and health status did not allow leaving animals untreated as a control group. Resistance was considered present if the FECR was less than 95% and the lower 95% confidence limit (Cl) for the reduction was less than 90% [4]. In some cases, faecal egg counts (FEC) were higher after treatment than before, due to a large biological variability of the egg production and faecal egg excretion. This resulted in "unrealistic" negative FECR-values which were defined as 0% reduction for reasons of calculation of the mean FECR of each treatment group.

In addition the non-parametric Wilcoxon-rank-sum-test was calculated to evaluate potential significant differences between the paired FEC-values before and after treatment.

Results

The statistical analysis of the FECRTs performed in the 4 flocks revealed the occurrence of AR of GINs against several anthelmintic drugs. In seven of the nine treatment-groups mean FECR was <95% and the 95% confidence limit of the reductions was <90% and thus proved resistance. In both goat flocks AR against eprinomectin was detected. However, the subsequent treatment with moxidectin of the "Black-Forest-flock" was effective. The analysis of the treatment efficacy in both sheep flocks revealed resistance against albendazole, fenbendazole, oxfendazole and in the "Alb-flock" also against moxidectin. The mean FECs, the range of the FECs, the FECR and the 95% confidence limit of the reductions of the treatment-groups are presented in Table 1. The results of the Wilcoxon-test (Table 1) show that there are no significant changes between the paired FEC-values, when FECRs are very low as in both eprinomectin-treated goat flocks. Moreover, in the "Swiss-flock" the ranks are mostly positive, i.e. in most paired samples the FEC after treatment is higher than before.

The predominant species found in the post-treatment larval culture of all treatment-groups was *H. contortus* (Table 2).

Discussion

The presented results on the reduced FECR clearly indicate the occurrence of anthelmintic resistance of *H. contortus* against eprinomectin in both goat flocks and against albendazole, oxfendazole and fenbendazole in the sheep flocks and in the "Alb-flock" also against moxidectin. In the 1990s, benzimidazole-resistant GINs in Switzerland, Germany and other European countries have been reported by Hertzberg et al. [10, 11].

It is well known that the pharmacokinetics and the efficacy of anthelmintics vary significantly between sheep and goats [12, 13]. In goats, the metabolism of drugs is accelerated and thus leads to a reduced drug availability, which may contribute to the failure of treatment. As a consequence, the treat-

Table 1. Mean faecal egg count pre- and post-treatment (FEC-pre; FEC-post), minimum and maximum FEC, comparison of the paired samples (Wilcoxon-rank-sum-test) and mean faecal egg count reduction percentages (FECR) with standard error (S.E.), standard deviation (S.D.) and lower 95% confidence limit (CI) in two goat and two sheep flocks in Southern Germany and Switzerland treated with four different active agents. Negative FECR values were classified as 0% reduction

Livestock	Drug	Mean FEC-pre [epg] (min-max)	Mean FEC-post [epg] (min-max)	Wilcoxon: exact significance *P* (2-sided)*	Wilcoxon: paired sample ranks**	Mean FECR (S.E./S.D.)	Lower 95% CI
Goats							
"Swiss-flock"	EPR	2608 (0–11160)	3630 (0–18510)	0.397	positive	17.4% (11/44)	–6.8
"Blackforest-flock"	EPR	1553 (270–4590)	1184 (300–4560)	0.074	negative	27.5% (7/30)	13.7
	MOX	1426 (120–8220)	3 (0–30)	0.000	negative	99.1% (–/–)	–
Sheep							
"Allgaeu-flock"	ABZ	783 (90–2130)	237 (0–660)	0.025	negative	70.8% (12/37)	44.5
	FBZ	1490 (90–3360)	531 (0–3060)	0.028	negative	52.4% (12/38)	25.0
	MOX	693 (60–2250)	0	0.002	negative	100% (–/–)	–
	MOX	1647 (120–3690)	865 (0–1920)	0.389	negative	44.3% (12/39)	16.6
"Alb-flock"	ABZ	2490 (210–5580)	746 (0–1680)	0.004	negative	55.3% (10/33)	31.7
	OXF	1476 (90–2850)	870 (0–3660)	0.84	negative	47.3% (13/40)	18.8

EPR Eprinomectin; *MOX* moxidectin; *ABZ* albendazole; *FBZ* fenbendazole; *OXF* oxfendazole; * Wilcoxon-test significant: $P<0.05$; **Rank positive: FEC-post > FEC-pre; Rank negative: FEC-post < FEC-pre.

Table 2. Genus and percentage of infective larvae found in coprocultures of the nine treatment groups

Treatment group	Larvae pre-treatment [No. %] (*n*=200)	Larvae post-treatment [No. %] (*n*=…)
Goats		
"Swiss"-Eprinomectin	Ha: 84.5; TT: 15.5	Ha: 98; TT: 2; *n*=200
"Black Forest"-Eprinomectin	Ha: 87.3; TT: 12.7	Ha: 100.0; *n*=200
"Black Forest"-Moxidectin	Ha: 100.0	NL
Sheep		
"Allgaeu"- Albendazole	Collective sample:	Ha: 92; TT: 3.5; Oe: 4.5; *n*=200
"Allgaeu"- Fenbendazole	Ha: 73.5; TT: 15.5	Ha: 98; TT: 2; *n*=200
"Allgaeu"- Moxidectin	Oe: 11	NL
"Alb"-Moxidectin	n.d.	Ha: 86; TT: 9; Str: 5; *n*=100
"Alb"-Albendazole	n.d.	Ha: 96; TT: 4; *n*=100
"Alb"-Oxfendazole	n.d.	Ha: 91; TT: 5; Str: 4; *n*=100

NL No larvae found; *n.d.* not done; *Ha Haemonchus* spp.; *Oe Oesophagostomum* spp.; *TT Trichostrongylus-Teladorsagia*-complex; *Str Strongyloides papillosus.*

ment-dose for goats has to be adapted to their particular metabolism by increasing the dose (double cattle dose) in order to reach higher plasma levels [14]. This was realized in the new Swiss regulations for eprinomectin [15]. In both goat flocks prior to this analysis the cattle-dose was used and this continuous under-dosing of eprinomectin could have contributed to the development of resistance.

Recent information of the GIN spectrum of sheep and especially goats in Germany and Switzerland is limited. Former studies mostly reported high prevalence levels of *Trichostrongylus* spp. and *Teladorsagia* spp., whereas *H. contortus*-infections have not been specified [16, 17]. However, in 1999 the first Swiss benzimidazole-resistant *H. contortus*-strain was isolated [11]. In the present flocks the prevalence of *H. contortus* with more than 74% was high. This could be due to the selection of the GIN-population as a consequence of the inefficacious anthelmintic treatments prior to this survey, i.e. only few GIN species survived, including *H. contortus*. Coles et al. [18] showed that *H. contortus* is able to rapidly develop resistance against anthelmintic drugs, if larvae which survived anthelmintic treatment can reinfect the animals. Consequently, this ability of *H. contortus* may lead to a gradual increase of its prevalence and therefore to a predominance of this species in affected areas of Europe. *H. contortus* is known as the predominant resistant GIN species in Southern USA, in Africa and in Australia, where a lot of imported small ruminant breeds come from [19]. In agreement with this it was speculated that *H. contortus*-carrying Boer goats, imported from Africa, introduced benzimidazole- and ivermectin-resistant *H. contortus*-strains into Switzerland [3]. The "Alb-flock" with its Dorper sheep is a typical trading farm and similarly, sheep of the flock could easily have caught up resistant GINs from newly imported sheep. Comparatively, the "Allgaeu-flock" consists mostly of Suffolk and dairy sheep and generally grazed on its home pasture without any trading. It is not known whether this is the reason why moxidectin was still efficacious. It is most likely that the introduction of sheep carrying resistant worms into flocks with non-resistant worm populations might add to the spreading of resistance to previously unaffected farms. Imported animals and their parasite burden are being traded throughout Europe and thus potentially spread their worm infections, both susceptible and resistant ones. In all four flocks, newly introduced animals were anthelmintically treated against GIN-infections and held in quarantine, before integrating them into the flock. However, neither in these cases nor after routine anthelmintic treatment, a coproscopic analysis was performed to verify the efficacy of treatment. In addition, only one surface-limited pasture grazed without interruption, was available on all four farms. It is thus likely that the long-term use of the same anthelmintics and pastures without verifying anthelmintic efficacy, led to an increase of the proportion of resistant *H. contortus* larvae in refugia.

The presently found multiple resistance of *H. contortus* on the same farms and its predominance in middle European GIN-populations of small ruminants, is alarming, mainly because of the lack of an alternative anthelmintic drug for the farmers and of the killing capacity of *H. contortus*. Therefore, it is important to establish new regimens of treatment with regard to the prevention of a further spreading of resistance or hopefully even the restoration of anthelmintic efficacy. Strategies including refugia, alternate grazing or targeted treatment have to be put into practice and appropriate recommendations for the antiparasitic management should be communicated to the farmers and veterinarians.

Conflict of interest

The authors declare that there is no conflict of interest.

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