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Avermectin-resistance in gastrointestinal nematodes of Boer goats and Dorper sheep in Switzerland

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Abstract: Zusammenfassung Anthelmintika-Resistenz von Magendarmstrongyliden bei kleinen Wiederkäuern ist in Südafrika weit verbreitet und sowohl Dorperschafe als auch Burenziegen wurden von dort in einigen Fällen in die Schweiz importiert. Diese Studie hatte zum Ziel, das Auftreten von Avermectin-resistenten Magendarmstrongyliden bei diesen Rassen in der Schweiz zu untersuchen. Gesamthaft nahmen 24 respective 12 Betriebe mit Burenziegen und Dorperschafen an der Studie teil. Stützend auf den Eizahlreduktionstest wurden Avermectin- resistente Magendarmstrongyliden in 46% der Burenziegen- und 58% der Dorperschafbetriebe gefunden. Haemonchus contortus und Trichostrongylus spp. waren die dominierenden resistenten Spezies gemäss Anzüchtung in Larvenkulturen. In den Betrieben mit nachgewiesener Avermectin- Resistenz wurden die Tiere nach natürlicher Reinfektion mit Levamisol behandelt. Mit Ausnahme eines Betriebes mit fraglichem Resultat gab der Eizahlreduktionstest keinen Hinweis auf Levamisol-Resistenz. Die Resultate lassen vermuten, dass Avermectin- Resistenz in der Schweiz bei Betrieben mit Burenziegen und Dorperschafen weit verbreitet ist. Die häufige Haltungsform von Weidetieren aus verschiedenen Betrieben auf voralpinen und alpinen Weiden begünstigt die Weiterverbreitung der resistenten Populationen innerhalb des Landes. Abstract Anthelmintic resistance in gastrointestinal nematodes among small ruminants is widespread in South Africa and Dorper sheep and Boer goats have been imported into Switzerland from this country on a number of occasions. Therefore, this study aimed to investigate the occurrence of avermectin (AVM) resistant gastrointestinal nematodes (GIN) in these breeds in Switzerland. A total of 24 and 12 Boer goat and Dorper sheep enterprises respectively participated in the study. According to the faecal egg count reduction test (FECRT) AVM- resistant GIN populations were confirmed in 7 of the 24 Boar goat enterprises and suspected in a further 8 farms. Likewise AVM-resistance was confirmed in 2 of 12 Dorper sheep enterprises and suspected in a further 6 enterprises. Haemonchus contortus and Trichostrongylus spp. were the dominant resistant species according to larval cultures. In the farms with detected AVM-resistance the animals were additionally treated with levamisole after natural reinfection. With the exception of one farm with a 'close-to cutoff- result' the FECRT gave no indication for resistance against levamisole. The results indicate that AVM-resistance is widespread in Swiss small ruminant farms keeping Boer goats and Dorper sheep. The common tradition of grazing animals from different farms on prealpine and alpine pastures could favour the spread of resistant populations within the country.

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Avermectin-resistance in gastrointestinal nematodes of Boer goats and Dorper sheep in Switzerland

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Summary

Anthelmintic resistance in gastrointestinal nematodes among small ruminants is widespread in South Africa and Dorper sheep and Boer goats have been imported into Switzerland from this country on a number of occasions. Therefore, this study aimed to investigate the occurrence of avermectin (AVM) resistant gastrointestinal nematodes (GIN) in these breeds in Switzerland. A total of 24 and 12 Boer goat and Dorper sheep enterprises respectively participated in the study. According to the faecal egg count reduction test (FECRT) AVM-resistant GIN populations were found in 46% and 58% of the farms with Boer goats and Dorper sheep respectively. *Haemonchus contortus* and *Trichostrongylus* spp. were the dominant resistant species according to larval cultures. In the farms with detected AVM-resistance the animals were additionally treated with levamisole after natural reinfection. With the exception of one farm with a 'close-to cutoff-result' the FECRT gave no indication for resistance against levamisole. The results indicate that AVM-resistance is widespread in Swiss small ruminant farms keeping Boer goats and Dorper sheep. The common tradition of grazing animals from different farms on prealpine and alpine pastures could favour the spread of resistant populations within the country.

Key words: small ruminants – gastrointestinal nematodes - avermectins - anthelminthic resistance – nematodes – Switzerland

Introduction

Anthelmintic resistance of gastrointestinal nematodes (GIN) has become a global problem for the sheep and goat industry (Mwamachi et al., 1995; Waller, 1997; Gopal et al., 1999; van Wyk et al., 1999; Hertzberg and Bauer, 2000; Zajac and Gipson, 2000; Veale, 2002; Chandrawathani et al., 2003). A particular threat arises from GIN populations which have developed multiple resistance against more than one anthelmintic class (van Wyk and Malan, 1988; Watson and Hosking, 1990; Waller, 1994).

In Europe the situation is characterized by a widespread occurrence of benzimidazole resistance (Hertzberg and Bauer, 2000). Levamisole resistance has been reported from France (Chartier et al., 2001), Denmark (Mortensen et al., 2003), Great Britain (Bartley et al., 2004), Slovakia (Várady et al., 1994) and Germany (Harder, 2002). Single cases of AVM-resistance in goat nematodes are documented from Scotland (Jackson et al., 1992) and Denmark (Maingi et al., 1996). The first case of AVM-resistance in Switzerland was recently reported from a small farm in the area of Zurich keeping a flock of Boer goats imported from South Africa (Schnyder et al., 2005). The isolated population of *H. contortus* exhibited resistance against mebendazole and ivermectin.

After this initial finding it was the aim of the present study to investigate the occurrence of AVM-resistant GIN populations in Boer goats and Dorper sheep, which are the major two small ruminants breeds of South African origin, imported into Switzerland.

Animals, materials and methods

A total of 24 out of 60 South African Boer goat farms and 12 out of 21 Dorper sheep farms distributed over the country and contacted by the respective breeding organisations, were examined during the year 2004. In addition, 11 farms from eastern Switzerland with local sheep breeds were randomly chosen. Owners were asked to respond to a questionnaire including general farm management, animal movements, worm control practices, and anthelmintic usage. Conditions for inclusion in the study were the lack of anthelmintic treatment during the previous weeks and a mean faecal egg count (FEC) ≥ 200 per g faeces. This was determined by a pooled faecal sample sent by the owners. Animals in the last two months of gestation and animals < 4 months were excluded. Pre- and 10-14 days post-treatment samples were individually collected directly from the rectum. Animals were treated with the recommended dose of doramectin (0.2 mg per estimated kg body weight, Dectomax®, Pfizer). Strongyle nematode FECs were determined using the modified McMaster technique according to Schmidt, (1971), with a sensitivity of 50 eggs per gram.

Efficacy of avermectins was tested with the faecal egg count reduction test (FECRT), based on the recommendations of the World Association for the Advancement of Veterinary Parasitology (Coles et al., 1992). The mean egg excretion before and 10-14 days after the anthelmintic treatment were compared. Calculation of the FECR was performed using maximum likelihood mathematical techniques with a negative binomial statistic model (Torgerson et al., 2005). Resistance was considered present if the FECR was less than 95% and the lower 95% confidence limit (C.I.) for the reduction was less than 90%. If only one of the two criteria is met, anthelmintic resistance was suspected (Coles et al., 1992), but where the animal number is too small, C.I. was not possible to calculate.

If resistance against doramectin was present, the respective strongyle population was also tested for resistance against levamisole under the same conditions, using a combined product with triclabendazole (Endex®, 8.75%, Novartis) which is the only registered formulation including levamisole in Switzerland. Sheep received the recommended dose of 7.5 mg levamisole/kg body weight, while goats received 12 mg levamisole/kg body weight (Chartier and Hoste, 1997). In cases of detected or suspected anthelmintic resistance, faecal cultures were performed (Eckert, 1960) and the infectious third stage larvae (L3) differentiated according to MAFF (1986) and Levine (1968) at 400 magnification. Relations between the farm specific parameters and the resistance situation (based on the FECR values) were tested for significance using Chi-square test and the significance level was determined at $p=0.005$.

Results

A total of 174 Boer goats, 61 Dorper sheep and 82 meat sheep from domestic breeds were included in the study (Table 1). Two farms held both Boer goats and Dorper sheep (B16 identical with D2; B17 identical with D6) and one farm held Dorper sheep together with domestic White Alpine breed sheep (D3 identical with S8). In total, 47 farms located mainly in the eastern and the central parts of Switzerland participated in the study. Flock size ranged between 1 and 22 animals, with a mean of 7 animals. Seventy-three percent of the farms participated with less than 10 animals.

AVM-resistant GIN populations were found in 11 Boer goat flocks (46%), in 7 Dorper sheep flocks (58%) and in 2 flocks with domestic meat sheep (18%, Table 1) (n.s.). In one of these farms (S8), animals were held together with few Dorper sheep, which also showed AVM-resistance. In the other farm (S9) only the ram, temporarily moved from another farm, housed resistant nematodes. There was no correlation between the history of direct import from South Africa or animal transfer in Switzerland and the presence of AVM-resistance ($p>0.05$).

Larval differentiation after doramectin treatment was possible in 12 of 20 flocks with resistant populations. With one exception, *H. contortus* was the dominating resistant species (Table 3). In 13 out of 20 flocks a FECRT for levamisole resistance was undertaken. Twelve parasite populations (92%) were sensitive to levamisole, whilst in one population the result was doubtful (FECR = 95%, C.I = 78-97%). In this flock the surviving strongyle population consisted of 80% *Trichostrongylus* spp. and 20% *Ostertagia* spp.

In 24 out of 36 farms (67%) Boer goats and Dorper sheep were held for meat production and in 23 farms (64%) for breeding purposes. Seven out of 24 (29%) Boer goat farms and 4 out of 12 Dorper sheep farms (33%) held directly imported animals in their flocks. All 24 Boer goat and 10 Dorper sheep farmers (83%) had purchased animals from other Swiss farms. Except for one Boer goat farm, goats and Dorper sheep were allowed to graze regularly. In 14 (58%) Boer goat und 11 (92%) Dorper sheep farms other small ruminants or South American camelids were kept. On nearly all of these farms Dorper sheep and Boer goats had direct contact with the other ruminants. Four (17%) Boer goat and 1 (8%) Dorper sheep farm shared pastures with small ruminants from other farms. Twenty-five percent of the Boer goat owners and a third of the Dorper sheep owners grazed their animals on alpine pastures distant from the home farm during the summer. On these pastures animals from 4 Boer goat and all Dorper sheep farms grazed together with animals from 10 to 30 other farms. The question, if parasites represent a problem on their farm, was negated by 15 (63%) of the Boer goat, half of the Dorper sheep and by 5 of the 11 owners of domestic sheep. For nematode control the majority of farmers within the 3 groups relied on both, macrocyclic lactones and benzimidazoles (Table 2). In 11 out of 36 Boer goat and Dorper sheep farms (31%), macrocyclic lactones were used regularly against ectoparasites, often due to the cantonal veterinary legislation. The frequency of anthelmintic treatments averaged at approximately 4 per year in all 3 types of farms. Nineteen (53%) Boer goat and Dorper sheep owners, respectively 6 (55%) owners of other sheep breeds stated not to have problems with anthelmintic resistance so far.

Discussion

After the initial finding of a GIN population in Boer goats, resistant against ivermectin and benzimidazoles (Schnyder et al., 2005), the aim of the present study was to investigate the prevalence of AVM-resistance in the Swiss population of this breed. Boer goats were initially introduced from South Africa into Switzerland some years ago without any precautions to prevent introduction of anthelmintic resistant GIN. The study was extended to Dorper sheep to investigate a second type of animal imported from South Africa.

The detected prevalences of 46% and 58% positive Boer goat and Dorper sheep farms respectively show that AVM-resistant GIN have already successful established in these breeds. Levamisole was found to be still fully effective, confirming observations from the farmers using this compound. With a participation rate of 40% and 58% of Boer goat and Dorper sheep farms respectively representative numbers of animals of these breeds were included in the study. In comparison with other studies the low number of animals per farm is obvious. Based on the average of seven animals it was not appropriate to establish an experimental design including untreated control groups in the single farms. An untreated control group may serve as an indicator for documenting natural variation in the faecal egg counts, occurring independently of the applied drug (Coles et al., 1992), but according to the international standards such control groups are not mandatory for the documentation of anthelmintic resistance based on the FECRT. In accordance with the first documented case (Schnyder et al., 2005), *H. contortus* was found to be the dominant AVM-resistant species on the basis of coprocultures, a pattern which is unique for European conditions. Previous findings of AVM-resistant trichostrongylid populations were reported from Scotland and Denmark (Jackson et al., 1992; Maingi et al., 1996). In both cases *Ostertagia* spp. seemed to be the dominant resistant nematode. In the case of Denmark there was multiple resistance including BZ's and levamisole (Maingi et al., 1996). In the Southern hemisphere *H. contortus* is the dominant nematode species and has the most widespread resistance problem in Australia (Le Jambre, 1993; Waller et al., 1995) and Malaysia (Chandrawathani et al., 2003). Triple resistant *H. contortus* have been detected from the Southern United States (Zajac and Gipson, 2000). In Africa, multiple resistant *H. contortus* are locally a severe problem in Kenya (Mwamachi et al., 1995) and South Africa (van Wyk et al., 1997), from where Dorper sheep and Boer goats were imported into Switzerland.

With one exception AVM's had been used in all farms which were affected by AVM-resistance. The average treatment frequency of 4 anthelmintic applications per year is slightly higher compared with data from a recent nationwide study, indicating an average treatment frequency of 3.7 and 3.2 for sheep and goats, respectively (Meyer, 2001). Taken into account that AVM were not the only anthelmintics used, the risk for *de novo* genesis of AVM-resistance in the investigated farms is probably relatively low. Because AVM-resistance in Switzerland was previously absent, it is likely that the resistant GIN populations were imported with their hosts. However a confirmation of this hypothesis was beyond the scope of the present study. In general, international and national animal movements result in a considerable risk for disseminating resistant GIN populations. In one of the few documented cases benzimidazole-resistant GIN were imported with sheep from Great Britain and France into Greece (Himonas and Papadopoulus, 1994). In a second case Angora sheep exported from New Zealand harboured an AVM-resistant trichostrongyle population which was successfully detected in Slovakia before being released (Várady et al., 1993). Until 2004 small ruminants could legally be imported from South Africa into Switzerland or into the European Union without any quarantine measures regarding resistant helminth populations. Now regulations have changed so that only embryos can be directly or indirectly imported.

Farmers imported Boer goats and Dorper sheep into Switzerland to use these robust and fairly unpretentious animals to improve, both quantitatively and qualitatively the meat production of some of the local breeds. Although only a minority of farmers had directly imported animals of South African origin, about 50% of them were trading with the animals, establishing new flocks with imported animals or with their offspring. This situation probably explains the absence of a statistical correlation between the presence of directly imported animals and the occurrence of AVM-resistance.

Sheep and goats grazed throughout the year. Thus, the conditions for persistent contamination of herbage and subsequent reinfections were present. Co-grazing or alternate usage of pasture of Boer goats and Dorper sheep with animals of local breeds was frequently observed. This serves as an epidemiologically important interface for the dissemination of resistant helminth populations. One documented case from the present study is a farm keeping mainly sheep of the White Alpine breed. The farmer purchased some Dorper sheep, which were found to harbour AVM-resistant *H. contortus*. Subsequently the White Alpine sheep were found positive for AVM-resistant GIN, probably derived from the Dorper sheep. In a second case, only the ram, which was present temporarily on the farm, was harbouring resistant nematodes; in this case transmission to the other animals has yet to occur. The current situation is characterized by a persistent dissemination of resistant helminth populations into areas which lack any direct contact to Boer goats and Dorper sheep. This trend will be potentiated by temporary common grazing of animals of different farms during summer, which is a typical feature of the prealpine and alpine grazing management. The risk is even enhanced by the common and in most cases mandatory practise to treat sheep with AVM's prior to transfer to alpine pastures as a prophylactic measure against sheep scab (Jacober et al., in press).

This illustrates the conflicting aims of avoiding resistance and concurrent prophylaxis of other diseases. So far, the continuous monitoring of several hundred farms on the basis of quantitative faecal egg counts, has not indicated a noticeable prevalence of AVM-resistant GIN populations in the domestic sheep and goat population (M. Schönmann, personal communication). However, despite the cessation of imports from South Africa, it is likely that the established population of resistant GIN will be sufficient to survive and possibly expand. Farmers are therefore encouraged to perform coprologically based quarantine measures on the basis of the remaining effective anthelmintics. Due to the lack of efficacy of the benzimidazoles in 80 - 90% of the Swiss farms (Meyer, 2001) levamisole is presently the only remaining options.

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Table 2: Anthelmintic groups and frequency of anthelmintic treatments per year in the 3 types of farms

		Boer goat farms n =24	Dorper sheep farms n = 12	Domestic sheep farms n = 11
Employed anthelmintic groups (%)	only ML[1]	38	25	9
	ML, BZ[2]	54	50	64
	others	8	25	27
Annual treatment frequency (%)	1	0	0	0
	2	21	8	36
	3	21	50	18
	4	38	17	18
	> 4	12	17	0
	not known	8	8	27

[1]ML=macrocyclic lactones

[2]BZ=benzimidazoles

Table 1: FECR% of animals treated with doramectin on Boer goat farms (B 1-24), Dorper sheep farms (D1-12) and domestic sheep farms (S1-11).

B (n)	FECR% (95% CI)	B (n)	FECR% (95 % CI)	D (n)	FECR% (95% CI)	S (n)	FECR% (95% CI)
B1 (2)	96 [1,2]	B13 (6)	100 (100)	D1 (1)	94 [1,4]	S1 (1)	100 [1,2]
B2 (3)	94 [1,2]	B14 (6)	96 (0-99.7)	D2 (2)	19 [1,4]	S2 (3)	100 (100)
B3 (3)	100 (100)	B15 (7)	90 [1,4]	D3 (3)	53 [1,4]	S3 (3)	100 (100)
B4 (4)	95 [1,3]	B16 (7)	31 [1,4]	D4 (3)	96 [1,3]	S4 (4)	100 (100)
B5 (4)	83 [1,4]	B17 (7)	62 [1,4]	D5 (4)	84 [1,4]	S5 (5)	100 (100)
B6 (4)	100 (100)	B18 (8)	100 (100)	D6 (4)	47 [1,4]	S6 (9)	99 (97.0-99.9)
B7 (4)	36 [1,4]	B19 (9)	69 [1,4]	D7 (5)	52 [1,4]	S7 (9)	100 (100)
B8 (4)	97 (81.0-99.0)	B20 (12)	44 [1,4]	D8 (5)	100 (100)	S8 (10)	75 [1,4]
B9 (4)	48 [1,4]	B21 (12)	99 (93-99.9)	D9 (6)	100 (100)	S9 (11)	89 [1,4]
B10 (4)	100 (100)	B22 (13)	54 [1,4]	D10 (12)	93 (0-99.7)	S10 (13)	98 (0-99.9)
B11 (5)	89 [1,4]	B23 (19)	77 [1,4]	D11 (8)	67 [1,4]	S11 (14)	100 (100)
B12 (5)	100 (100)	B24 (22)	99 (96.0-99.9)	D12 (8)	100 (100)		

n = number of animals sampled

CI = confidence interval

[1] = CI not calculated

[2] = n too small

[3] = only group sampling

[4] = AVM-resistant

Table 3: Differentiation of third stage GIN larvae (%) from coprocultures after treatment with doramectin of Boer goats (B), Dorper sheep (D) and domestic sheep (S).

Farms (ID)	*H. contortus*	*Trichostrongylus* spp.	*Cooperia* spp.
B5	100	-	-
B7	99	1	-
B11	100	-	-
B16	89	11	-
B19	78	22	-
B20	91	9	-
B22	87	12	1
B23	4	96	-
D2	89	11	-
D3	100	-	-
D11	99	1	-
S8	100	-	-
S9	71	29	-