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ORIGINAL PAPER

Gastrointestinal nematode infections in German sheep

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Abstract The objective of the present study was to determine the prevalence and variation of natural gastrointestinal nematode (GIN) infections in lambs according to birth type, gender and breed based on individual faecal egg counts (FEC) from various regions in Germany. A total of 3,924 lambs (3 to 15 months old) with different genetic backgrounds (Merinoland, German Blackhead Mutton, Rhoen, Texel and Merino long-wool) were individually sampled during the grazing period between 2006 and 2008. Furthermore, pooled faecal samples from each of the farms were cultured in order to differentiate the third-stage larvae of the nematode spp. Sixty-three percent of the lambs were infected with GIN. The infections were mostly low to moderate and involved several nematode species. The *Trichostrongylus* spp. was the predominant species based on the percentage of larvae in faecal cultures. Only 11.4% of the lambs were free of *Eimeria* oocysts. Tapeworm eggs were encountered in 13.2% of all samples. The prevalence of GIN infections varied significantly ($P<0.001$) among farms. A significantly higher FEC ($P<0.05$) was observed in multiple-born lambs when compared with singletons. Moreover, male lambs were more susceptible to infection than females ($P<0.001$). No significant differences ($P>0.05$) were observed between breeds regarding FEC. Inter-individual variations were higher than inter-breed differences, which may indicate the possibility of selection within these breeds for parasites resistance as described in earlier studies.

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Abbreviations

FEC	Faecal egg counts
GBM	German Blackhead Mutton
GIN	Gastrointestinal nematode
LS-means	Least square means
ML	Merinoland
MLW	Merino long-wool
OPG	Oocysts per gram of faeces
RH	Rhoen
TX	Texel

Introduction

Infections with gastrointestinal nematodes (GIN) can negatively affect the health and the overall productivity of infected animals (Levya et al. 1982; Holmes 1987; Suarez et al. 2009). Therefore, they can be a major cause of economic losses in small ruminant production (Coop et al. 1977). The common clinical signs of an infection with GIN are anorexia, diarrhoea, emaciation and anaemia (Steel et al. 1982; Behrens et al. 2001). The high level infections with nematodes may lead to the death of the infected animals. Under field conditions, most infections are usually mixed and include different species of nematodes. However, the impact of nematode infections on the animal is dependent on the intensity of infection as well as the physiological and immunological status of the host. Growing lambs and periparturient ewes are most susceptible to the infection by nematodes (Bishop and Stear 2001; Shubber et al. 1981). Today, the control of GIN in sheep has become less effective due to the development of anthelmintic resistance (Fleming et al. 2006). Moreover, there is a potent orientation towards organic farming, where the use of anthelmintics is limited Anonym (2010). Furthermore, the climate change will probably lead to changes in epidemiology and intensity of parasite infections (Hudson et al. 2006; van Dijk et al. 2010). Due to these facts, the economic impact of nematode infections will possibly increase in sheep farms in the future.

Most studies focussing on nematode infestations in Germany have reported a moderate to high prevalence of infection (51% and 100%) in sheep (Benesch 1993; Grzonka et al. 2000; Moritz 2005). The most common nematode genera infecting sheep in Germany are *Haemonchus, Trichostrongylus*, *Telodorsagia*, *Nematodirus* and *Cooperia* (Benesch 1993; Rehbein et al. 1996). It might be that some sheep breeds in Germany are more or less resistant to nematode infections than others. Gauly et al. (2002) reported that Merinoland had a lower FEC compared with Rhoen sheep following an experimental infection with *H. contortus.* However, differences between sheep breeds under conditions of natural infection in Germany have not yet been shown.

Therefore, the objective of the present study was to determine the prevalence of GIN in naturally infected lambs of five German sheep breeds based on individual faecal egg counts and to evaluate the predictable influence of birth type, gender, and breed on faecal nematode egg output.

Materials and methods

Animals and study areas

A total of 3,924 lambs of different breeds aged from 3 to 15 months were used in the study. Breeds used were Merinoland (ML), German Blackhead Mutton (GBM), Texel (TX), Rhoen (RH) and Merino long-wool (MLW) (Table 1). Data collection took place on various farms. Those were located in different federal states of Germany (Lower Saxony, Saxony-Anhalt, Thuringia, Baden-Wuerttemberg, Brandenburg and Hesse). The samples were collected once during the grazing seasons in 2006, 2007 and 2008. The farms were visited either once (ten farms), twice (nine farms) or three times (two farms) during the study period. Four farms kept more than one sheep breed. For statistical analysis, grazing season was divided into two periods (summer: June to August; autumn: September to December). The lambs were not dewormed at least 45 days before the sampling time.

Parasitological measurements

Fresh faecal samples were taken once directly from the rectum of the individual lambs. Eggs per gram of faeces (FEC) were determined using a modified McMaster method (Maff 1986) to quantify FEC with saturated NaCl as the flotation fluid (specific gravity of 1.2 kg/m^3). The eggs were counted with a sensitivity of 50 eggs per gram of faeces. Intensity of coccidia infection was semi-quantitatively scored via a four-score scaling system. The scaling evaluated samples as class 1 (coccidian-free), class 2 (<1,800 oocysts per gram (OPG)), class 3 (1,800 to 6,000 OPG) and class 4 (>6,000 OPG). For tapeworm infections, lambs were differentiated as non-infected and infected.

For the identification of nematode spp., 25 to 50 g of pooled faeces from each breed/farm were cultured for third-stage larvae (L3). For each pooled sample, 100 of these L3 were enumerated.

Data analyses

Individual FEC were $\log_e$ (n+10) transformed to produce approximately normally distributed data. All the statistical analyses were performed with SAS (9.1). The prevalence rates for the eggs of endoparasites in faecal samples and the confidence intervals were calculated with the FREQ procedure. *Pearson's correlation coefficients* between FEC and OPG as well as between FEC and age of lambs were determined using the *CORR procedure.*

Table 1 Total number of lambs and farms used in the study over 3 years and their breed

[a]		2006	2007	2008	Total no. of lambs
ML	No. of farms	2	6	6	1455
	No. of lambs	198	625	632	
GBM	No. of farms	3	4	6	851
	No. of lambs	63	193	595	
TX	No. of farms	1	4	2	377
	No. of lambs	16	208	153	
RH	No. of farms	1	3	4	557
	No. of lambs	71	157	329	
MLW	No. of farms	0	2	1	684
	No. of lambs	0	287	397	

ML Merinoland, *GBM* German Blackhead Mutton, *TX* Texel, *RH* Rhoen, *MLW* Merino long-wool

[a] The farms were visited either once (ten farms), twice (nine farms) or three times (two farms) during the study period. Four farms kept more than one sheep breed

Differences in FECs were analysed using the GLIMMIX procedure by using the following model:

$$Y_{\mathrm{ijklmnop}} = \mu + G_{\mathrm{i}} + F(G)_{\mathrm{j}} + S_{\mathrm{k}} + B_{\mathrm{l}} + \mathrm{SE(AN)}_{\mathrm{m}} + A_{\mathrm{n}} + \mathrm{FA}(B)_{\mathrm{o}} + e_{\mathrm{ijklmnop}}$$

Where Y_{ijklmnop}=observed value; μ=overall mean; G_{i}= fixed effect of breed (i=ML, GBM, TX, RH, MLW); $G(F)_{\mathrm{j}}$= fixed effect of breed nested within farms; S_{k}=fixed effect of sex (k=male, female); B_{l}=fixed effect of birth type (l= singleton, multiple); $\mathrm{SE(AN)}_{\mathrm{m}}$=fixed effect of season nested within years; A_{n}=age of lambs as covariate; $\mathrm{FA}(B)_{\mathrm{o}}$=sire nested within breeds as random; e_{ijklmnop}=random error.

ML lambs of one farm were not infected with GIN, and therefore, the data of this farm were not used for comparison of breeds, sex and birth type. Likewise, 600 lambs were excluded from the analyses due to missing values of fixed effects.

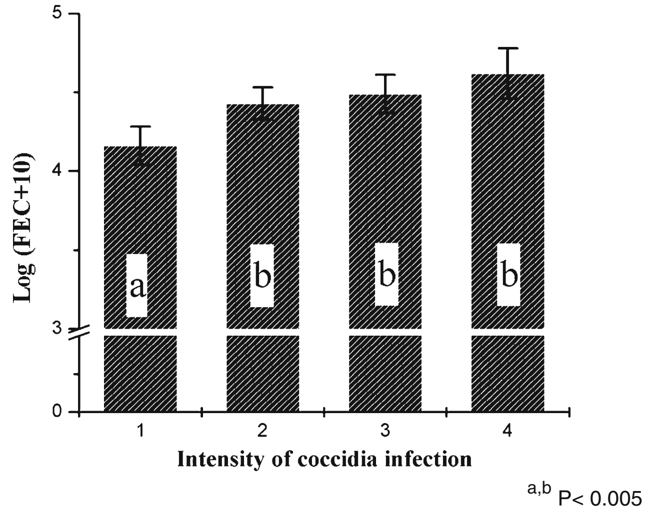

Fig. 1 Least-squares means and standard error of transformed FEC of lambs according to intensity of their coccidia infection

Results and discussion

Faecal egg counts

Table 2 summarises the results of faecal examinations. The prevalence of GIN infection is relatively high, where 62.8% of lambs were infected with at least one GIN species. However, the variations among farms were high and ranged from 0% to 100%. The infection level of GIN based on FEC was various and ranged from 50 to 17,000 with a mean value of 315.3 (±776.8) eggs per gram of faeces. Eleven samples had more than 5,000 eggs. Only 11.4% of the samples were free of *Eimeria* oocysts. Tapeworm eggs (*Moniezia* spp.) were encountered in 13.2% of the samples.

Earlier studies which were performed in Germany reported prevalences of GIN in sheep to be greater than 50% (Benesch 1993; Grzonka et al. 2000; Moritz 2005). Similar findings were obtained in the present study. Schwenk (1998) found *Eimeria* prevalence of 70% and for *Moniezia* spp., 6%. In other studies, all animals older than 10 weeks were infected with *Eimeria* spp. (Barutzki 1990); likewise, high infections with *Moniezia* spp. (57%) were reported by Graenzer et al. (1979). Between- and within-study variations in the infection rate of parasites under natural field conditions are not unexpected. These may be due to an inequality of the contamination of pasture with infective larvae, seasonal climatic fluctuations (Suttle 1994; Torina et al. 2004; Al-shaibani et al. 2008), feeding quality (Coop and Holmes 1996), husbandry systems (Wassmuth et al. 2001; Gauly et al. 2004) and genetic backgrounds of animals (Gray 1995; Gauly and Erhardt 2001; Reeg et al. 2005).

The samples of *Eimeria* oocyst-free lambs had less nematode eggs when compared with those of infected lambs (Fig. 1). No significant differences could be observed between the FEC values in the other classes of *Eimeria*-infection. However, FEC seemed to increase partially with increasing *Eimeria* infection. The phenotypic correlation between *Eimeria* oocysts and FEC values was 0.05 (P= 0.001). But the semi-quantitative estimation of coccidial infection limits the informational value. Kanyari (1988) reported a negative relation between the coccidial and helminth infection in goat. The author suggested that this negative relationship is a logical result of deworming against GIN and no treatment against coccidia. However, a positive correlation was estimated in sheep and goats in Kenya (Kanyari 1993).

Table 2 The prevalence of internal parasite eggs in faecal samples from lambs, as well as the 95% confidence interval of prevalence estimates (CI), the mean, standard deviation (SD) and the maximum value

	Prevalence (% positive)	CI	Mean	SD	Max. value
FEC	62.8	61.3–64.4	315.3	776.8	17,000
Nematodirus spp.	13.0	11.9–14	15.52	63.67	1,374
Trichuris spp.	3.4	2.9–4.1	2.99	19.78	432
Strongyloides papillosus	0.7	0.5–1	0.79	11.93	400
OPG	88.6	87.6–89.6	–	–	–
Tapeworm eggs	13.2	12.2–14.3	–	–	–

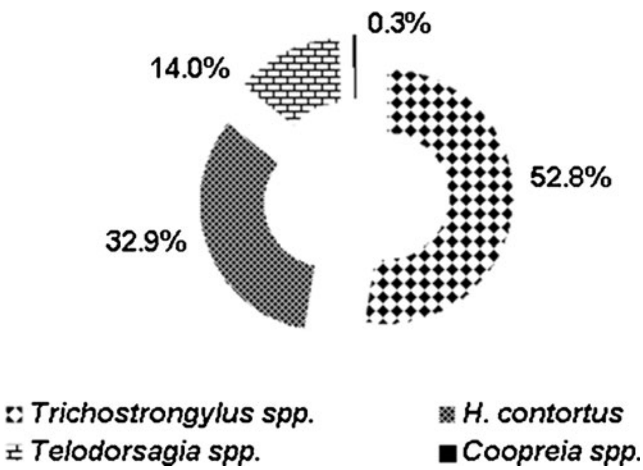

Fig. 2 Contribution of the nematode genera *Trichostrongylus* spp., *Teladorsagia* spp., *H. contortus* and *Cooperia* spp. according to the L3 differentiation

Larval cultures

Depending on the results of larval cultures, *Trichostrongylus* spp. were the predominant nematode species with 52.8% of all the larvae (Fig. 2), whereas 32.9% of third-stage larvae were *H. contortus*, 14% *Teladorsagia* spp. and only 0.3% *Cooperia* spp. The distribution of nematode species by farm is given in Fig. 3. Larvae of *Trichostrongylus* spp. were found in all farms, with at least with 10% of the larvae. *H. contortus* was predominant in six farms, and only one farm was free of *H. contortus*. In three farms, *Teladorsagia* spp. was not detected while they were predominant in one farm. *Cooperia* spp. larvae were found in five farms. The larval culture method is used to determine the genera of worms and their proportion. However, the results of larval cultures may not describe the proportion of worms exactly (Amarante 2000). This may be due to the different fecundities between nematode species (Coyne et al. 1991), the pooled faecal samples (Amarante 2000) and the differential survival rates of larvae in faecal culture for species (Dobson et al. 1992).

Effect of sex, type of birth and age of lambs

Female lambs were less susceptible to infections with GIN ($P<0.001$) compared with male lambs (Table 3). Many studies reported that male lambs are more susceptible to natural or experimental nematode infections around or after age of puberty when compared with females. Courtney et al. (1985) and Barger (1993) reported that host sex may have no consistent effect on susceptibility to infection in pre-pubertal lambs. Differences between females and males in susceptibility to parasite infection are probably caused by a difference in behaviour, morphology or physiological status of sex (Zuk and McKean 1996). Gauly et al. (2006) suggested that the different hormonal status of sexes may affect the immunological responses of lambs to *H. contortus*.

FEC values were affected by birth type of lambs (Table 3). Multiple-born lambs had higher FEC when compared with singleton-born lambs ($P<0.05$). Similar findings were reported by Romjali et al. (1997) and Haile et al. (2007). These authors explained the differences by better rearing and nutrition conditions in the singletons in comparison to the multiples. However, Gauly and Erhardt (2001) as well as McManus et al. (2009) did not find a significant effect of birth type on FEC following natural infections.

The faecal egg counts were negatively correlated with age of lambs (−0.23; $P<0.001$) as indicated in Fig. 4. Development of acquired immunity to nematode infection is positively related with age of animals (Kambara et al. 1993; Kambara and McFarlane 1996). Furthermore, weaning, live weight, fat reserves or nutritional factors that are

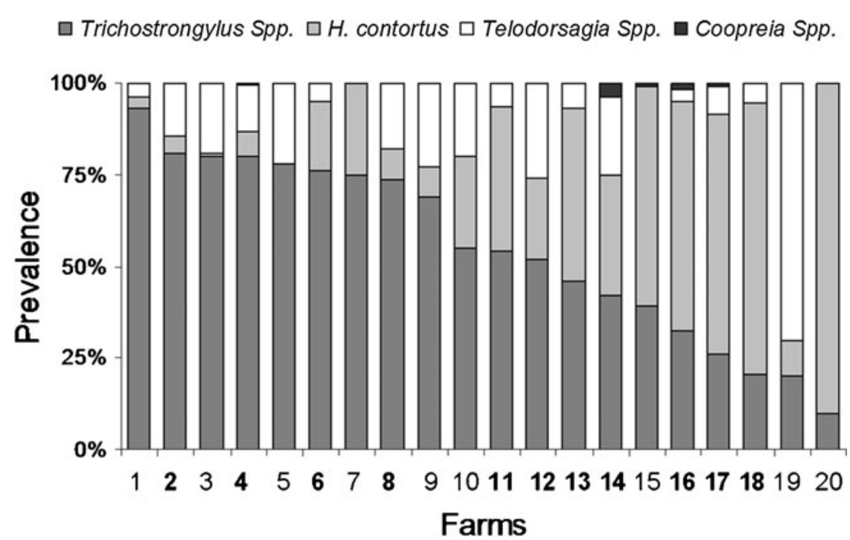

Fig. 3 Prevalence of various genera of gastrointestinal nematode in lambs depending on the results of larval cultures by farms. Bold numbers indicate the farms were visited more than once during the study period. Lambs of one farm were not infected, and therefore this farm was not presented in this figure

Table 3 Least squares means and standard error (SE) of transformed FEC of lambs considering sex and birth type

	Log (FEC+10)	SE
Sex		
Female	4.15*	0.10
Male	4.66*	0.12
Type of birth		
Single	4.35**	0.11
Multiple	4.46**	0.10

*$p<0.001$; **$p<0.05$

associated with age might also contribute to the positive relation between age and parasite resistance (McClure 2000).

Between- and within-breed variations

The MLW lambs were not compared with any other breeds because of the small number of farms included in the study and the time of sample collection month (December 2007 and 2008 only). No significant differences (P=0.42) were observed between the four breeds ML, GBM, TX and RH (Table 4), where the LS-means of log (FEC+10) ranged from 4.40 (±0.18) to 4.92 (±0.25). Very little information has been published about the variation of nematode resistance between sheep breeds in Germany. Gauly et al. (2002) reported that Merinoland had a lower FEC compared with Rhoen sheep following an experimental infection with *H. contortus*. However, worm burden, hematocrit and IgG antibody of the breeds did not differ significantly. In the present study, no differences between these two breeds in FEC appeared. A possible reason for that could be

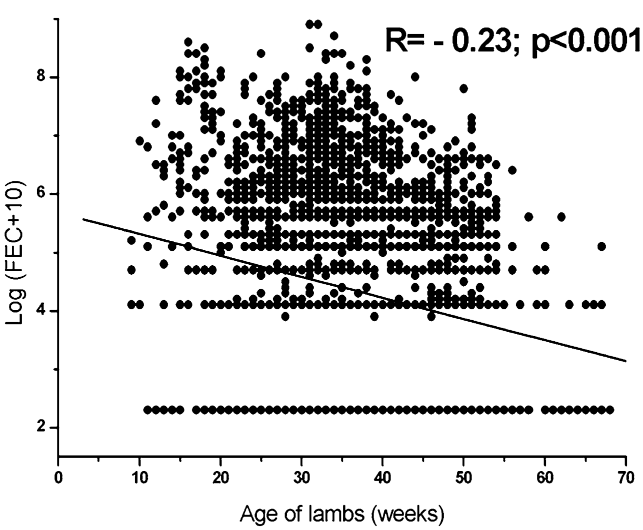

Fig. 4 The relationship of transformed fecal egg counts with age of lambs

Table 4 Least squares means, standard error (SE) and variation coefficient of FEC of lambs by breed

	Log (FEC+10)	SE	CV%
ML	4.50	0.19	45.22 (225.28)
GBM	4.40	0.18	39.97 (288.09)
TX	4.92	0.25	34.34 (167.56)
RH	4.48	0.32	33.00 (131.89)
MLW*	3.77	0.16	37.77 (164.32)

*The MLW lambs were not compared with any other breeds because of the small number of farms included in the study and the time of sample collection month; numbers within brackets represent the variation coefficient of untransformed FEC

ML Merinoland, *GBM* German Blackhead Mutton, *TX* Texel, *RH* Rhoen, *MLW* Merino long-wool

the differences in the infection (artificial, natural), such as the availability and sources of infectious larvae. Differences in the responses of lambs to mono- or mixed-infection may also be a determinant.

In Ireland, it has been reported that Texel sheep are less susceptible to natural nematode challenge when compared with Suffolk sheep (Good et al. 2006). In the present study, Texel lambs had in tendency greater FEC compared with the other breeds (P>0.05). Rhoen sheep are a local breed, whereas Merinoland and German Blackhead Mutton resulted from crossing native sheep breeds with Merinos and British meat breeds, respectively. Furthermore, the ML and GBM breeds are known in Germany since the eighteenth and nineteenth century, while the Texel sheep were imported to Germany in the 1960s (Roesicke et al. 2007). Accordingly, this may have led to a natural selection and adaptation of RH, ML and GBM breeds.

High variation coefficients in FECs within breed were found (132–288%). The transformation of FEC reduced the variations (33–45%) but nevertheless remained high. The high variation in FEC within breeds has been early reported in different breeds and regions (Bisset et al. 1996; Bouix et al. 1998; Gauly and Erhardt 2001). Animals under natural conditions are unevenly exposed to nematode infection due to the differences in pasture contamination with infectious larvae. The animals in this study were kept in various farms with different feeding quality and husbandry systems. These factors could promote this variation in infection within breeds (Coop and Holmes 1996, Wassmuth et al. 2001). Even so, the obtained variation of FEC in this study might indicate partly the differences in host animal resistance against gastrointestinal nematode infections, which can be explained by the variety in the genetic basis of animals. Therefore, selection for resistance to nematode infections in German sheep under natural conditions might be possible.

Conclusion

The results showed that the natural infections with gastrointestinal nematodes in lambs in Germany are common, vary between farms, and are influenced by age, birth type and gender of lambs. A high prevalence of *Eimeria* infection was detected, and coccidia-free lambs appear to be less susceptible to nematode infections. The infection level of GIN based on FEC was low to moderate and involved multi-species infections. *Trichostrongylus* spp., *H. contortus* and *Teladorsagia* spp. were the predominant species. Inter-individual variations in susceptibility to natural nematode infections were found to be more important than inter-breed differences.

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