# Genetic differences in resistance to gastrointestinal nematodes in different sheep breeds under conditions of natural infection

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Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

Aus dem Department für Nutztierwissenschaften
der Georg-August-Universität Göttingen

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Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Agrarwissenschaften
der Georg-August-Universität Göttingen

vorgelegt von

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**Göttingen, Juli 2011**

**Bibliografische Information der Deutschen Nationalbibliothek**
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.d-nb.de abrufbar.
1. Aufl. - Göttingen : Cuvillier, 2011
Zugl.: Göttingen, Univ., Diss., 2011

978-3-86955-854-7

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Tag der mündlichen Prüfung: 21. Juli 2011


Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de


1. Auflage, 2011
Gedruckt auf säurefreiem Papier

978-3-86955-854-7

**Acknowledgments**

I would like to express my sincere and deepest gratitude to my supervisor Prof. Dr. Dr. Matthias Gauly, for his continuous support and guidance in every aspect of my PhD study. His ever willingness to solve encountered personal and technical problems will not be forgotten. It has been a great privilege to be one of his PhD students. I also would like to sincerely thank Prof. Dr. Sven König for his kind help during the data analysis and for taking over the co-referee. I owe a heavy debt of gratitude to Prof. Dr. Wolfgang Holtz for his constructive criticism, valuable guidance and for agreeing to act as third examiner. My greatest personal debt is due to my supervisor in Syria Prof. Dr. Bassam Issa for his unlimited help and prudent advice.

My heartfelt thanks goes to Dr. Eva Moors for her support, encouragement, and great assistance with farm connections and data collection. I feel very grateful that I have had the opportunity to work with her in this project. Many thanks go to Ms. Birgit Sohnrey, Mr. Erwin Tönges, Mr. Knut Salzmann and all technical staff of the Department of Animal Sciences for their enormous assistance and help during my work.

Very personal and heartfelt thanks go to Dr. Gürbüz Daş, Dr. Falko Kaufmann, Dr. Christina Münch, Dr. Alexander Riek and Dr. Azzam Al Yacoub for their friendship and the enjoyable times together, which will definitely be a phase of my life I will look back to with great pleasure.

I am very grateful to my friends, Dr. Christian Lambertz and Abdussamad Muhammad Abdussamad for proof-reading my thesis, and to Pilasrak Panprasert, Dr. Chakrapong Chaikong, Kalyakorn Wongrak, Mazhar Shahin, Dr. Mohammed Saleh, Simon Riedel, Phonwon Singhaphan, Dr. Amphon Warittitham, Dr. Morten Friedrich, Dr. Uta König von Borstel, Dr. Jan Maxa, Christine Friedrich, Susanne Theis, Katrin Hille, Sabrina Weber, Kerstin Wegner, Katharina Tiersch, Christine Sanker, Patricia Graf, Daniela Marthold, Dr. Sebastian Hoppe, Björn Tönepöhl, Sören Düvel for their contributions and for the friendly and warm working atmosphere.

I would also like to thank the University of Damascus for the financial support I received during my study and my stay in Germany. Special thanks goes to all the sheep farmers who helped us to make this study possible.

No words can express my gratitude to my loving mother and father, for their endless love, care, sacrifices and all they have given me throughout my life. I would like also to express my deepest gratitude to my brother Youssef and my sisters Fatima, Mariem and Mona for their moral support und motivation. This work would have been unachievable without the understanding, support, patience and love of my wife. My thanks, love and respect to you, Mariem. A special acknowledgment goes to my children, Roua & Amgd, for bringing so much happiness and strength into my life.

If I have left out anyone, it was inadvertently, so please accept my sincere gratitude.

*Ahmad*

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# CHAPTER I

## General Introduction

## 1.1. Gastrointestinal nematode infection in sheep

The major species of nematodes infecting the gastrointestinal tract of ruminants belong to the family *Trichostrongylidae.* This family includes the genera *Teladorsagia*, *Cooperia, Haemonchus, Trichostrongylus* and *Nematodirus* (Schnieder, 2006). These nematodes have a simple direct life cycle consisting of an egg stage, five larval stages and an adult stage (Figure 1.1). They do not need an intermediate host. The life cycle of a nematode can be divided into two phases, the free-living phase in the external environment and the parasitic phase in the host (Waller, 1998). After the adult worms mate, the females lays eggs in the abomasum or the small intestine of the infected animal, which are excreted with the faeces and contaminate the pastures. The eggs hatch into the first stage larvae (L1), which develop and moult for the first time to the unsheathed second larval stage (L2). Without casting the sheath they moult to the sheathed third larval stage (L3) (Schnieder, 2006). The first and second larval stages have the ability to feed on soil microbes and bacteria but they are not able to move. Whereas, the third stage larvae are active and can crawl onto the grass leaves to be taken up by grazing animals (Waller, 1998; Schnieder, 2006). Under ideal conditions of temperature and humidity, the development from the hatching of the eggs to the third larval stage takes about 4 to 6 days. Exceptionally for *Nematodirus* spp., this development takes more than two months.

The parasitic phase starts after the ingestion of infective larvae (L3) by the host. These larvae lose their sheath in the host to become parasitic L3 and migrate to their host organ (abomasum or small intestinal). The larvae enter the gastric glands where they moult for the third time to the fourth larval stage (L4) and emerge back into the lumen of the gastrointestinal tract. After another moulting, the L4 develop into immature adults (L5). Afterwards, they become mature adults **(**Schnieder, 2006; Sutherland and Scott, 2009**)**. The adult stages of nematodes survive in the lumen of the gastrointestinal tract of ruminants. The prepatent stage, which is the period from the infection of the host until the first eggs are excreted with the faeces, is about 3 weeks. The fecundity of adult female worms ranges form 40 for *Nematodirus* spp. (Coyne *et al.,* 1991) to over 5000 eggs per day for *H. contortus* (Le Jambre, 1995).

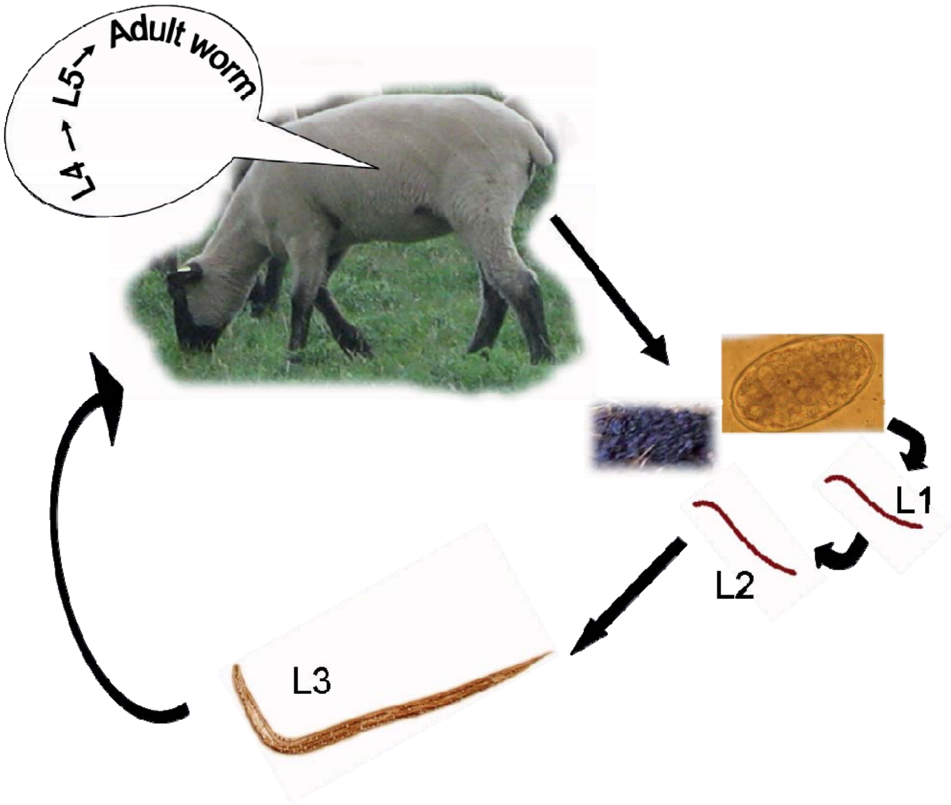


**Fig. 1.1** The life cycle of gastrointestinal nematodes

Infections with gastrointestinal nematodes cause severe economic losses in the sheep industry (Davies *et al.*, 2005). Beside the direct costs of these infections including anthelmintic drugs and veterinary services, indirect costs are caused. These are due to reduced performances of clinically and sub-clinically infected animals. Infections with gastrointestinal nematodes in the field are usually mixed infections and involve several different species of nematodes. Its impact on the animals is mainly influenced by the intensity of the infection and the physiological status of the host. Growing lambs and periparturient ewes are most susceptible to the infection by nematodes (Shubber *et al.*, 1981; Bishop and Stear, 2001). The common clinical symptoms of the infection with gastrointestinal nematodes are anorexia, diarrhea, emaciation, anaemia. Heavy infections may cause the death of infected animal (Steel *et al.*, 1982; Behrens, 2001).

Resistance and resilience explain the responses of the host animal to parasitic infections. Woolaston and Baker (1996) defined resistance as “the ability of a host to initiate and maintain responses to suppress the establishment of parasites and/or eliminate the parasite load", and resilience as "the ability of the host to maintain a relatively undepressed production level under parasite challenge". Faecal egg counts (FEC) is used worldwide to measure host animal resistance against gastrointestinal nematode infections and to estimate the breeding value of host resistance (Rahmann and Seip, 2006; Stear *et al.*, 2007). Resilience can be assessed by comparison between the performance of nematode-infected and non-infected animals (Albers *et al.*, 1987).

**Table 1.1** Prevalence of gastrointestinal nematode infections in sheep reported by different authors from various regions in Germany

| **Region** | **Prevalence of GIN %** | **Author** |
|---|---|---|
| Taunus | 80 | SCHNEIDER (1942) |
| Württemberg | 95 | FORSTNER (1960) |
| Bayern | 99 | GRÄNZER *et al.* (1979) |
| Hessen | 51 | BENESCH (1993) |
| Oberbayern | 100 | REHBEIN *et al.* (1996) |
| Bonn | 54 | SCHWENK (1998) |
| Schwäbische Alb | 100 | REHBEIN *et al.* (1998) |
| Sachsen-Anhalt | 100 | GRZONKA *et al.* (2000) |
| Niedersachsen | 71 | MORITZ (2005) |

Most studies focussing on nematode infestations in Germany have reported a moderate to high prevalence of infection in sheep (Table 1.1). The predominant nematode species were *Teladorsagia* spp., *Haemonchus contortus, Trichostrongylus* spp. *Nematodirus* spp. and *Cooperia* spp. (Benesch, 1993; Rehbein *et al.,* 1996).

## 1.2. Strategic control of gastrointestinal nematodes

There are several ways of controlling nematodes in either the host animal or in the environment to which the host animal or the parasite is exposed. In the following, the most widely used control methods will be described.

### 1.2.1. Anthelmintic treatments

The traditional method to control the gastrointestinal nematodes is the use of chemical dewormers. In most farms in Germany, the animals are usually treated two or more times per year. The major two treatments take place at the beginning of the grazing season and at the end of the summer. Some farmers treat their animals additionally at the end of the grazing period before the animals return to the barns. The treated animals are generally moved to clean pasture, if it is available, to minimise the re-infections. There are three major classes of anthelmintics that are widely used for the control of nematodes in small ruminants: Benzimidazoles, Avermectins / Milbemycins and Imidazothiazoles / Tetrahydropyrimidines (Kaplan, 2004; Hale, 2006). However, the frequent use of these compounds has caused resistance to the anthelmintics in numerous nematode species. Recently, a new chemical class of synthetic anthelmintics (the Amino-Acetonitrile Derivatives) was discovered (Kaminsky *et al.*, 2008). Nematode resistance to this anthelmintic has not yet been observed.

#### 1.2.1.1. Anthelmintic resistance (AR)

The anthelmintic resistance was defined by Prichard *et al.* (1980) as: “Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable”. The use of anthelmintics may inevitably lead to AR (Alvarez-Sanchez *et al.*, 2006). The parasites can become resistant to an anthelmintic in a very short period of time. The period between the approval of an anthelmintic and the first report of its resistance varied from 3 to 9 years (Kaplan 2004). The phenomenon of AR in gastrointestinal nematodes of small ruminants is world wide observed (Fleming *et al.*, 2006). Resistance being developed by nematodes of sheep was first reported in Germany by Bauer *et al.* (1987) and Düwel *et al.* (1987).

### 1.2.1.2. Targeted selective treatment

In practice, if an animal population is infected by nematodes all the animals will be treated. In the selective treatment approach only those animals are treated that require the treatment most. Thus, highly susceptible or infected animal, which are responsible for the heavy environmental contamination with nematode eggs, will be drenched. Through this approach the frequency of anthelmintic treatments and the excessive exposure of the worms to the anthelmintics can be sustainably reduced. This in turn will slow down the spread of AR (Hoste *et al.*, 2002; Gallidis *et al.*, 2009). Worms that have not been exposed to the drugs are supposed to be in refugia and are, therefore, still susceptible to the anthelmintics (Jackson *et al.*, 2009; Kenyon *et al.*, 2009). As the proportion of larvae in refugia increases, the development of AR decreases extensively.

The identification of animals, which are suspected to be highly infected with nematodes, relies mainly on the faecal eggs count (FEC). However, this method is time consuming and also cost-intensive. That led to looking for simpler, cheaper and safe indicators of nematode infections, such as scoring mucosal colour (FAMACHA©), scoring body condition as well as dag score (van Wyk and Bath, 2002; Broughan and Wall, 2007; Riley and van Wyk, 2009; Bath and van Wyk, 2009).

### 1.2.2. Grazing management

The management of pastures basically aims to reduce the extent of infections and re-infections by reducing the intake of infective larvae of the grazing animals from the pasture. To obtain a safe pasture, grazing be must inhibited for 6 months during cold weather or for 3 months during hot, dry weather (Fleming et al; 2006). Michel (1985) classified strategies of the grazing management as following: preventive grazing is based on raising worm-free animals on a clean pastures; evasive grazing relies on the movement of animals to a clean pasture just before the infectious larvae are evolved on the original pasture; diluting grazing exploits the concurrent grazing of susceptible animals with a greater population of relative resistant animals of the same or different species in order to reduce the contamination of the pasture with parasite eggs. However, these strategies can be only applied if a large pasture size is available.

Barger (1997) suggested that the control of nematodes based on the grazing management may be unsustainable when considered in isolation. Nevertheless, the use of this strategy combined with anthelmintic treatments probably will be more effective in parasite control. However, it may not reduce AR.

### 1.2.3. Dietary supplementation

The importance of nutrition for the control of nematodes is firstly expressed as the influence of a parasite on host metabolism and secondly as the effect on resistance or tolerance of the host against the parasites (Coop and Kyriazakis, 1999). The infection with gastrointestinal nematodes reduces the feed intake and at the same time the efficiency of feed utilization, whereas the main impact of the infection is the loss of endogenous protein in the form of whole blood, plasma, sloughed epithelial cells and mucus (Coop and Holmes, 1996; Knox *et al.*, 2006). In young animals, infections may also disrupt the absorption of some minerals, e.g. phosphorus, calcium, copper and magnesium (Sykes and Greer, 2003).

Many studies reported that sheep show a higher resilience when fed a high level of metabolizable protein. The negative impact of the infection on weight gain and wool growth as well as on blood parameters such as hematocrit, total serum protein and albumin concentrations was smaller in animals supplemented with high protein levels when compared to animals supplemented with low protein levels (Abbott *et al.*, 1988; van Houtert *et al.*, 1995; Coop and Holmes, 1996; van Houtert and Sykes, 1996). In the case of protein scarcity, a high level of protein can also improve the resistance of the parasitised host animals (Coop and Kyriazakis, 2001). Through higher protein supplementations the immune response of infected sheep can be increased resulting in reduce the infection parameters (van Houtert *et al.,* 1995b; Datta *et al.,* 1998; Steel, 2003).

However, the effect of the protein supplementation on the resistance of sheep against GIN is less pronounced than the effect on the resilience, especially in breeds which are relatively resistant to nematode infections (Coop and Kyriazakis, 1999). Since the ruminants are known to use plants with low protein contents and less degradable fibres effectively, protein supplementation as an alternative or supportive strategy to control GIN in sheep seems to be relatively uneconomical.

### 1.2.4. Vaccination

Helminths molt several times throughout their developmental cycle and the adult worms of most species live free in the gut lumen. Additionally, nematodes may often change their antigenic structures at different stages of their life cycle and they are much larger in body size than other pathogens (de Veer *et al.*, 2007). Due to these facts, the mechanisms of naturally acquired immunity against GIN infections remain not fully understood. Accordingly, the development of vaccines against nematodes is more complex than those against other pathogens. However, 3 different strategies were used to develop nematode vaccines: attenuated vaccines, vaccines based on natural immunity and the hidden antigen approach (Stear *et al.*, 2007). Irradiated larval vaccines against nematodes are the only commercially available vaccines (Bain, 1999). Vaccines against *H. contortus* can only stimulate the natural mechanisms but are not able to create immunity in animals which are naturally susceptible to GIN or in lambs (Bain, 1999; Stear *et al.*, 1999; Stear *et al.*, 2007).

The antigen vaccines against *H. contortus* had no effect on FEC in five month old lambs. Additionally, the vaccination negatively affected the productivity of animals that were not undrenched (Eady *et al.*, 2003). The vaccination had some experimental success (Jacobs *et al.*, 1999; Smith *et al.*, 2003; Redmond and Knox, 2004) but still unidentifiable (Jackson *et al.*, 2009).

### 1.2.5. Biological control

The increasing AR together with the growing number of organic farms which rely on non-chemical alternatives to anthelmintics necessitates an effective biological strategy to control nematodes. The aim of a biological control strategy is not to eliminate the parasites, but to reduce their populations to harmless levels for the host animals. The nematode-destroying microfungus *Duddingtonia flagrans* is one of the most common biological control against nematodes in livestock (Waller and Thamsborg, 2004). This fungus feeds on the larval stages of nematodes and reduces the pasture contamination with infective third stage larvae. This in turn results in a decreased intensity and severity of nematode infections (Larsen *et al.*, 1997; Rahmann and Seip, 2006). Another promising biological approach to control nematodes is the use of the medicinal plants with anthelmintic activity such as *Azadirachta indica, Caesalpinia crista, Veronia anthelminthica, Embelia ribes, Fumaria parviflora* and

*Ananas comosus* (Hördegen *et al.*, 2003; Githiori *et al.*, 2006; Chandrawathani *et al.*, 2006; Athanasiadou *et al.*, 2007). In principle, either the whole plant is fed or plant extracts are administered to parasitised animal (Athanasiadou *et al.*, 2007). Some of these medicinal plants have been reported to be very effective against nematode infections. For example, *F. parviflora* reduced the faecal egg counts of *H. contortus* by up to 100% and the worm burden by 72% (Hördegen *et al.*, 2003). However, the biological control of nematodes cannot be fully recommended due to the significant variability among different studies (Rahmann and Seip, 2006; Stear *et al.*, 2007).

### 1.2.6. Breeding for resistance

Since resistance can be considered as a qualitative trait of the host animal, the variation between and within host populations can be used to breed more resistant hosts. The advantage of breeding for resistance results in a reduction of the worm burden, which in turn can reduce the impact of parasites on the productivity of the host, as well as reduces the use of anthelmintics and the contamination of pastures with infective larvae (Gray, 1997). Breeding for resistance against nematodes in livestock is classified into three strategies: the selection among breeds, cross-breeding and the selection within breeds (Stear *et al.*, 2007).

#### 1.2.6.1. Selection among breeds

Breed differences in resistance against gastrointestinal nematodes have been reported since seventies (Bradley *et al.*, 1973; Altaif and Dargie, 1978). Many sheep breeds have been reported as relative resistant breed to nematode infections in different countries following experimental or natural infections. For example, Lohi sheep in Pakistan (Saddiqi *et al.,* 2010a,b), Red Maasai in Kenya (Mugambi *et al.*, 1997), Texel in Ireland (Good *et al.*, 2006) and Santa Ines in Brazil (Amarante *et al.*, 2004). In Germany, differences in FEC among German sheep breeds indicating different levels of resistance were observed. Merinoland had a lower FEC compared to Rhoen sheep following an experimental infection with *H. contortus.* However, worm burden, hematocrit and IgG antibody of these breeds did not differ significantly (Gauly *et al.,* 2002). For this breeding strategy, two key issues are still under discussion. Firstly it has to be examined, whether the performance of relative resistant breeds will always be comparable or superior to susceptible breeds. Secondly, it has to be taken into consideration whether farmers will accept to replace their original breeds with the more resistant breeds (Stear *et al.,* 2007).

**1.2.6.2. Cross-breeding**

The aim of crossbreeding is to combine the superior characteristics of two breeds and to obtain a new genotype with an optimal level of desirable traits (Cartwright, 1970). The crossing of relatively resistant and susceptible breeds may be an alternative strategy to the replacement of the susceptible breeds. Nevertheless, only a few studies focused on the responses of crossbreeds to the resistance against nematodes. Hielscher *et al.* (2006) reported that crossbreed lambs of Rhoen × Merinoland are more resistant against *H. contortus* infection when compared to the purebreds and the crossbreeds Merinoland × Rhoen. Crossbreeding between the relatively resistant breed Florida Native and the susceptible breed Rambouillet resulted in ewes that were more resistant against nematode infections than the purebreeds of the susceptible breed (Amarante *et al.*, 1999). The FEC and packed cell volume of Suffolk x Gulf Coast Native crossbreeds have been shown to be intermediate between the purebreds (Li *et al.*, 2001). However, more studies are needed to prove the effect of crossbreeding on the productivity and resistance in successive generations.

**1.2.6.3. Selection within breeds**

In order to breed for general disease resistance in a given breed, either some members of this breed must be resistant to this disease (Cole, 1933) or the population of this breed must have enough genetic variation for resistance. Gray (1997) reported that it is possible to use the genetic variation in resistance to nematode parasites of sheep by selection. A few studies on within-breed variation in response to helminth infections have been conducted in Germany. Gauly and Erhardt (2001) estimated the heritability for FEC between 0.11 and 0.44 in Rhoen sheep following natural infection with GIN. Following an experimental infection in lambs at 12 weeks of age with 5000 L3 *H. contortus,* heritabilities for FEC at 4 and 8 weeks post-infection were estimated between 0 to 0.35 and 0.07 to 0.17 in Rhoen and in Merinoland sheep, respectively (Gauly *et al.* 2002). These results are widely in agreement with other values of heritability estimated in studies from several countries of the world (Morris *et al.*, 1995; Eady *et al.*, 1996; Bishop *et al.*, 2004).

Karlsson and Greeff (2006) reported that the average genetic gain for the estimated breeding value of FEC is 2.7% per year for the resistant line of Rylington Merino. This line was selected for low FEC following natural challenge. Following artificial infection, this resistant selected line had 93% fewer adult *T. colubriformis* and 44% fewer adult *T. circumcincta* in comparison to the unselected control animals (Kemper et al., 2010).

Another example of the successful selection for resistance to GIN in sheep is the line of Merino sheep selected for increased resistance in Australia. The selection was based on FEC following an artificial *H. contortus* infection at 4 to 6 months of age. Woolaston *et al.* (1990) reported that after approximately four generations of selection FECs were lower and PCV were higher in the resistance line when compared to the unselected control line and the susceptible line. Whereas, the increased resistance line had 79% lower FEC and the susceptible line had 37% higher FEC than the control line. In the same experiment and following an artificial infection with *T. colubriformis* the line selected for increased resistance to *H. contortus* had 42% lower FECs than the control. This indicates that the animals which are resistant to the infection with one parasite species may also be resistant to some extent to other species. Moreover, the ewes of the line selected for increased resistance had lower FECs in the periparturient period compared to the unselected control line (Woolaston, 1992).

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# CHAPTER II

Gastrointestinal nematode infections in German sheep

**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 3924 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 farm were cultured for third-stage-larval differentiation of the nematodes spp..

Sixty-three percent of lambs were infected with gastrointestinal nematodes and the infections were mostly low-moderate and involved several different species. The *Trichostrongylus* spp. were the predominant species based on the percentage of larvae in faecal cultures. The samples of only 11.4% of lambs were *Eimeria* oocyst free. 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 in regard to 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.

## 2.1. Introduction

Infections with gastrointestinal nematodes 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 gastrointestinal nematodes 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 ((EG) Nr. 889/2008). Further, 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 e*t al*. 1996). It might be that some sheep breeds in Germany are more or less resistance 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 gastrointestinal nematodes 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.

## 2.2. Materials and methods

### *2.2.1. Animals and study areas*

The study was carried out using 3,924 lambs of different breeds aged from 3 to 15 months. Breeds used were Merinoland (ML), German Blackhead Mutton (GBM), Texel (TX), Rhoen (RH), and Merino long-wool (MLW) (Table 2.1). Data collection took place on various farms. Those were located in different 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 (9 farms), twice (10 farms) or three times in these years (2 farms). 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.

### *2.2.2.. Parasitological measurements*

Fresh faecal samples were taken once directly from the rectum of the individual lamb. Eggs per gram of faeces were determined using a modified McMaster method (Maff, 1986) to quantify faecal egg counts (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 (coccidia free), class 2 (<1800 oocysts per gram, OPG), class 3 (1800 to 6000 OPG) and class 4 (>6000 OPG). For tapeworm infections it was only differentiated between non-infected and infected lambs.

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

**Table 2.1** Total number of lambs and farms used in the study over three years and their breeds. Namely Merinoland (ML), German Blackhead Mutton (GBM), Texel (TX), Rhoen (RH) and Merino long-wool (MLW)

| | | **2006** | **2007** | **2008** | **Total no. of Lambs** |
|---|---|---|---|---|---|
| **ML** | No. of farms | 2 | 6 | 6 | 1,455 |
| | 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 | |

### *2.2.3. Data analysis*

Individual faecal egg counts (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 CORR procedure.

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

$$Y_{ijklmnop} = \mu + G_i + G(F)_j + S_k + B_l + SE(AN)_m + A_n + FA(B)_o + e_{ijklmnop}$$

$Y_{ijklmnop}$ = observed value;

$\mu$ = overall mean;

$G_i$ = fixed effect of breeds (i = ML, GBM, TX, RH, MLW);

$G(F)_j$ = fixed effect of breeds nested within farms;

$S_k$ = fixed effect of sex (k= male, female);

$B_l$ = fixed effect of birth type (l = singleton, multiple);

$SE(AN)_m$ = fixed effect of season nested within years;

$A_n$ = age of lambs as covariate;

$FA(B)_o$ = sire nested within breeds as random;

$e_{ijklmnop}$ = random error.

ML lambs of one farm were not infected, 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 the missing values of fixed effects.

## 2.3. Results and Discussion

### *2.3.1. Faecal egg counts*

Table 2.2 summarises the results of faecal examinations. The prevalence of gastrointestinal nematode infection is relatively high, where 62.8% of lambs were infected with at least one species of GIN. However, the variations among farms were high and ranged from 0 to 100%. Intensity of infection was various and ranged from 50 to 17000 eggs per gram of faeces with a mean value of 315.3 (±776.8). 11 samples had more than 5000 eggs. Only the samples of 11.4% of lambs were *Eimeria* oocyst free. Tapeworm eggs (*Moniezia* spp.) were encountered in 13.2% of all samples.

**Table 2.2** 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 (S.D.) and the maximum value

| | Prevalence (% positive) | CI | Mean | S.D. | Max. value |
|---|---|---|---|---|---|
| **FEC** | 62.8 | 61.3 – 64.4 | 315.3 | 776.8 | 17000 |
| ***Nematodirus* spp.** | 13.0 | 11.9 – 14 | 15.52 | 63.67 | 1374 |
| ***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 | - | - | - |

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 an *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 variation in the infection rate of parasites under natural field conditions are not unexpected. These may due to an inequality on the contamination of pasture with infective larvae, 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).

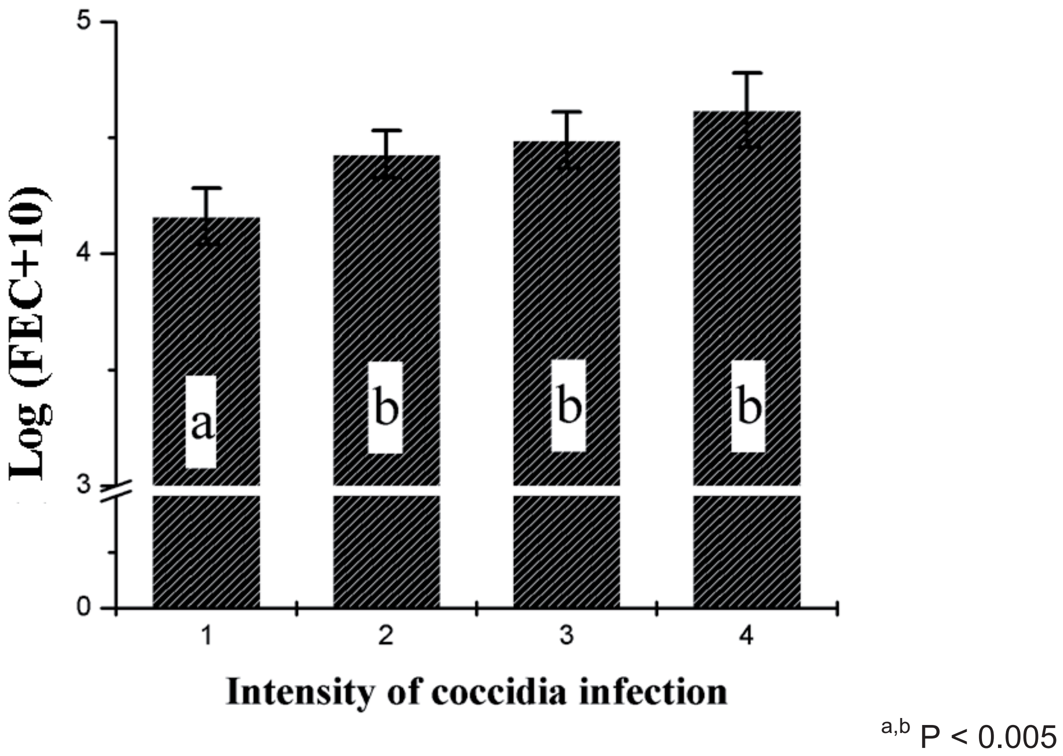


**Fig. 2.1** Least squares means and standard error of transformed FEC of lambs according to intensity of their coccidia infections; class 1 (coccidia free), class 2 (< 1800 oocysts per gram, OPG), class 3 (1800 to 6000 OPG) and class 4 (> 6000 OPG)

The samples of *Eimeria* oocyst free lambs had less nematode eggs when compared to those from *Eimeria* infected lambs (Figure 2.1). No significant differences could be observed between the FEC values in the other *Eimeria* intensity classes. However, FEC seemed to increase partially with increasing of *Eimeria* infection. The phenotypic correlation between *Eimeria* oocysts and log transformed FEC values was 0.05 ($P = 0.001$). But the semi quantitative estimation of coccidial infection may make this value less important. 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 treating against coccidia. However, a positive correlation was estimated in sheep and goats in Kenya (Kanyari 1993).

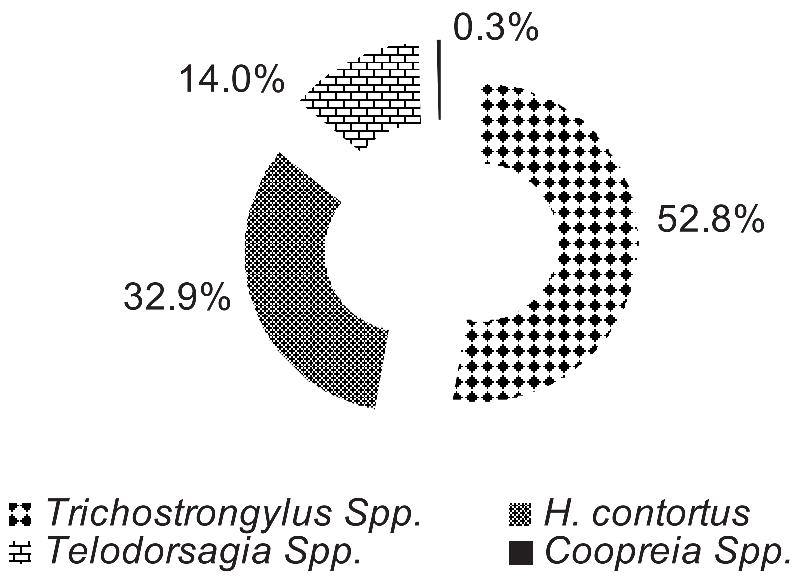


**Fig. 2.2** Contribution of the nematode genera *Trichostrongylus* spp., *Teladorsagia* spp., *H. contortus*, and *Cooperia* spp. according to the L3 differentiation

### *2.3.2. Larval cultures*

Depending on the results of larval cultures, *Trichostrongylus* spp. were the predominant nematode species with 52.8% of all the larvae (Figure 2.2). 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 Figure 3. Larvae of *Trichostrongylus* spp. were found in all farms, at least with 10% of all larvae. *H. contortus* was predominant in 6 farms and only one farm was free of *H. contortus*. In 3 farms *Teladorsagia* spp. were not detected while they were predominant in only one farm. *Cooperia* spp. larvae were found in 5 farms. The larval culture method is used to determine the genera of worms and their proportion. However, the results of larval cultures may do not exactly describe the proportion of worms (Amarante, 2000). Due to the fecundity differences 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).

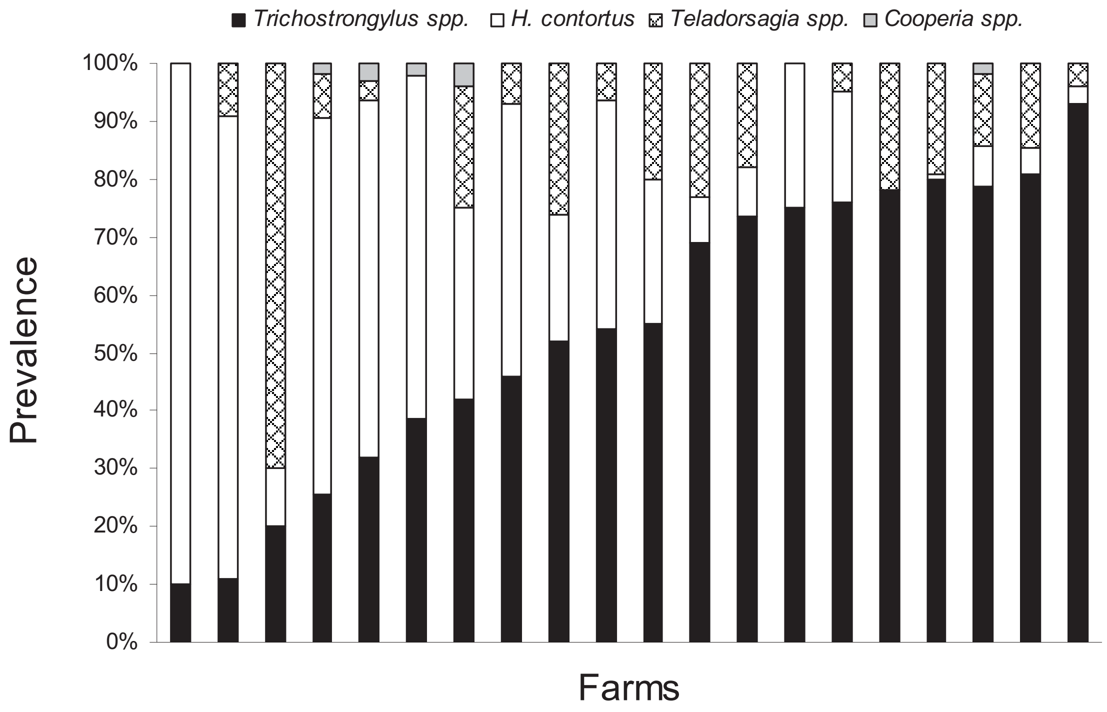


**Fig. 2.3** Prevalence of various genera of gastrointestinal nematodes in lambs depending on the results of larval culture from the different farms. Lambs of one farm were not infected, therefore, this farm was not presented in this figure

### *2.3.3. Effect of sex, type of birth and age of lambs*

Female lambs were less susceptible to infections with gastrointestinal nematodes (P < 0.001) compared to male lambs (Table 2.3). 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 parasites 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*.

**Table 2.3** Least squares means and standard error (S.E.) of transformed FEC of lambs considering sex, birth type and season

| | Log (FEC+10) | S.E. |
|---|---|---|
| **Sex** | | |
| Female | 4.15 $^{A}$ | 0.10 |
| Male | 4.66 $^{B}$ | 0.12 |
| **Type of birth** | | |
| Single | 4.35 $^{a}$ | 0.11 |
| Multiple | 4.46 $^{b}$ | 0.10 |
| **Season** | | |
| Autumn 2006 | 6.59 $^{A}$ | 0.15 |
| Summer 2007 | 3.41 $^{B}$ | 0.15 |
| Autumn 2007 | 4.88 $^{C}$ | 0.11 |
| Summer 2008 | 4.12 $^{D}$ | 0.13 |
| Autumn 2008 | 3.39 $^{B}$ | 0.11 |

$^{A, B}$ $P < 0.001$; $^{a,b}$ $P < 0.05$

FEC values were affected by birth type of lambs (Table 2.3). Multiple born lambs had higher FEC when compared to singleton lambs ($P < 0.05$). Similar findings were reported by Romjali *et al.* (1997) and Haile *et al.* (2007). These authors explained these differences to be due to better rearing and nutrition conditions in the singletons compared 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 figure 2.4. Development of acquired immunity to nematode infection is positively related with age of animals (Kambara *et al.*, 1993; Kambara and McFarlane, 1996). However, weaning, live weight, fat reserves or nutritional factors that are associated with age might also contribute to the positive relation between age and parasites resistance (McClure, 2000).

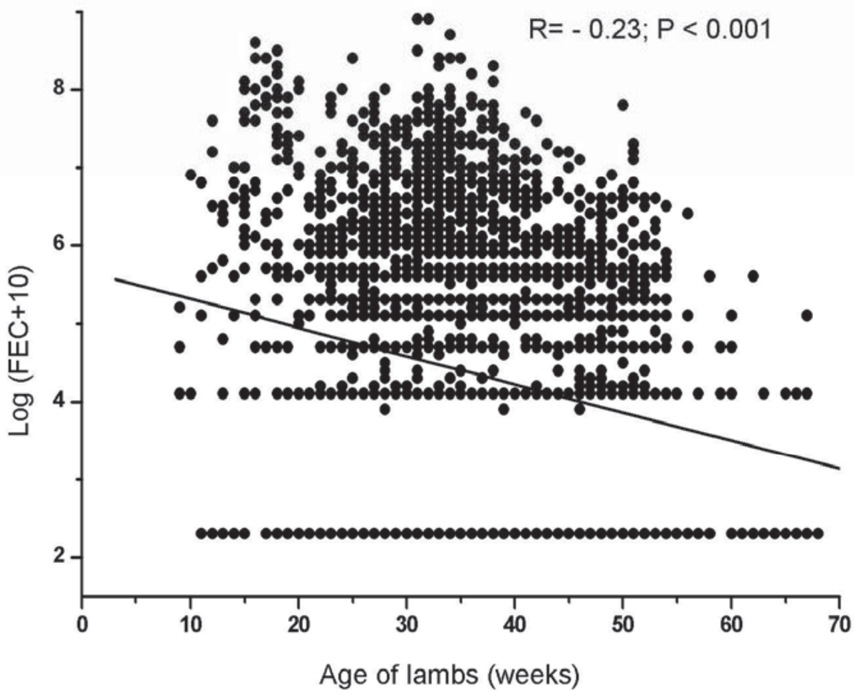


**Fig. 2.4** The relationship between transformed faecal egg counts and age of lambs

### *2.3.4. 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). No significant differences (P = 0.42) were observed between the four breeds ML, GBM, TX and RH (Table 2.4). 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 to 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 the differences in the infection (artificial; natural), such as the availability and sources of infectious larvae. It might also be that the responses of lambs to mono- or mixed-infection are different.

**Table 2.4** Least squares means, standard error (S.E.) and variation coefficient of FEC of lambs by breeds; Merinoland (ML), German Blackhead Mutton (GBM), Texel (TX), Rhoen (RH) and Merino Long-wool (MLW)

| | **Log (FEC+10)** | **S.E.** | **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

In Ireland, it has been reported that Texel sheep are less susceptible to natural nematode challenge when compared to Suffolk sheep (Good *et al.*, 2006). In the present study, Texel lambs had in tendency greater FEC compared to the other breeds (P > 0.05). Rhoen sheep are a local breed, Merinoland and German Blackhead Mutton resulted from crossing native sheep breeds with Merinos and British meat breeds, respectively. Furthermore, the Texel sheep were imported to Germany in the 60's, while the other two breeds are known in Germany since the eighteenth and nineteenth century (Roesicke *et al.*, 2007). That could be an advantage for RH, ML and GBM breeds under natural conditions according to natural selection and adaptation.

High variation coefficients in faecal egg counts within breed were found (132 – 288%). The transformation of FEC reduced the variations (33- 45%) nevertheless, remained high. ML lambs showed a greater variation in transformed FEC compared to other breeds. The 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). The animals under natural conditions are unevenly exposed to nematode infection due to the differences in pasture contamination with infectious larvae. The animal in this study were kept in various farm 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 though, the obtained variation of FEC in this study might indicate partly differences in host animal resistance against gastrointestinal nematode infections**,** which can be due to the variety in the genetic basis of animals. That can be evidence that selection for resistance to nematode in German sheep under natural condition is possible.

## 2.4. Conclusion

The results showed that the natural infections with gastrointestinal nematodes in lambs in Germany are common, vary between farms, and 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 intensity of GIN infections was low to moderate and involved multi-species infections. The *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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# CHAPTER III

## Indirect indicators of gastrointestinal nematode infections in lambs

**Abstract**

Targeted selective anthelmintic treatment is a successful strategy to slow down the spread of anthelmintic resistance by reducing the frequency of treatments. Eggs per gram of faeces (EPG) has been widely used to determine lambs requiring an anthelmintic treatment. Nevertheless, the cost for EPG may limit the potential benefit of the selective treatment. Therefore, the aim of this study was to evaluate the use of some potential infection indicator traits, either separately or combined, to detect individually lambs in need for drenching when exposed to natural nematode infections.

A total of 3,924 lambs from different breeds were sampled for faecal egg counts expressed as eggs per gram of faeces (EPG). At the time of faecal collection, the FAMACHA© score, the body condition score (BCS), the dag score (DS) and the faecal consistency (FCS) were also determined and served as potential infection indicator traits. Lambs with EPGs of 400 or higher were classified as animals requiring drenching. The combination of these four indicators was used to investigate if the decision making whether an animal requires drenching can be improved by using this combination. Accordingly, an animal is treated if at least one indicator trait supports drenching. A second indicators combination only relied on FAMACHA©, BCS and DS values to make the decision.

The overall average EPG ranged from 118 to 512 among breeds. Only 22% of the lambs required drenching. The decision of drenching based on each of the potential indicator traits yielded low sensitivities (13 to 39%) and high ratios of false negative results (13 to 19%). The sensitivities of the indicator combinations were higher (67 and 69%) than those for the individual indicators and were positively associated with the intensity of infection. However, the indicator combinations were not specific enough and had high ratio of false positives (37 and 39%) results. Therefore, it can be concluded that decision of drenching for lambs under moderate levels of mixed natural nematode infections based on these alternative infection indicator traits cannot be recommended.

### 3.1. Introduction

The traditional method to control the gastrointestinal nematodes of small ruminants is the use of chemical treatments, but the intensive application of these drugs results in a growing number of resistant nematode populations (Alvarez-Sanchez *et al.*, 2006). According to the definition of Prichard *et al.* (1980) 'resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable'. The phenomenon of anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) of small ruminants is observed world wide (Fleming *et al.*, 2006), Germany included (Bauer *et al.* 1987; Hertzberg and Bauer 2000).

In the routinely used parasite control the whole herd is treated in case an animal population is found to be infected with GIN. The aim of a selective treatment is to drench only the animals that require treatment and which are highly susceptible to GIN infections. Thus, these animals are responsible for the heavy environmental contamination with infective nematode larvae (Kenyon *et al.*, 2009). The 'smart drenching' approach using selective chemical treatments to control GIN infections in sheep can reduce the frequency of anthelmintic treatments and the excessive exposure of the worms to the anthelmintics. This in turn will slow down the spread of AR (Hoste *et al.*, 2002). Worms that have not been exposed to the drugs are supposed to be in refugia and, therefore, are still susceptible to the anthelmintics. As the proportion of larvae in refugia increases, the development of AR decreases extensively (Martin *et al.*, 1981).

The decision to treat an animal is usually based on eggs per gram of faeces (EPG). EPG is the most widely used indicator for detecting GIN infections as well as to measure host animal resistance. However, EPG is time- and cost-intensive. Therefore, looking for other indicators, which could be cheaper, simpler and giving direct reliable decisions, is required. Many traits have been suggested to play this role, such as mucosal colour scoring (FAMACHA©), body condition score, Dag score as well as using differences in performance traits e.g. body weight and milk yield as indicators (van Wyk *et al.*, 2006; Bath and van Wyk, 2009; Riley and Van Wyk, 2009). In Germany, these possible indicators have not been evaluated extensively under conditions of a natural infection.

The aim of this study was therefore to investigate the possibility to use the FAMACHA© score, the body condition, the faecal consistency and the dag score as indicators to detect the intensity of infection with gastrointestinal nematodes in lambs under the conditions of natural infection.

## 3.2. Materials and Methods

### *3.2.1. Animals*

The study was carried out on 3,924 lambs aged from 3 to 15 months. Data were collected at different farms and from lambs of the five breeds Merinoland (ML), German Blackhead Mutton (GBM), Texel (TX), Rhoen (RH) and Merino Long-wool (MLW). Samples were collected only once from the rectum of the animals during the grazing seasons in 2006, 2007 and 2008. The lambs were not dewormed at least 45 days prior to the sampling.

### *3.2.2. Parasitological measurements*

Eggs per gram of faeces (EPG) were determined using a modified McMaster method (Maff, 1986) as described in Chapter II.

### *3.2.3. Tested potential indicator traits (TPIT)*

At the time of faeces collection, the dag score (DS), the body condition (BCS) and the FAMACHA© were also determined. Additionally, the faecal consistency (FCS) was determined in the laboratory on the same day with a scale ranging from 1 to 5; where 1 indicates dry faecal pellets and 5 watery and fluid faeces. With the DS scale the degree or extent of faecal contamination of the fleece was described. Dags were scored visually from 0 for no faecal contamination to 5 for extensive dags (Larsen *et al.*, 1994). BCS was scored from 1 for emaciated lambs to 5 for obese lambs as described by Thompson and Meyer (1994). To determine the FAMACHA© value the colour of conjunctiva mucosa was individually compared with a FAMACHA© chart. This chart has scale of 1 to 5 with 1 and 2 indicating an optimal mucosal colour with no evidence of anemia, and 5 indicating a white conjunctiva with severe anemia (Van Wyk and Bath, 2002). All the potential indicator traits were assessed by the same person.

### *3.2.3. Data analysis*

Individual EPGs were $\log_e$ (n+10) transformed in order to normalize the data. Transformed EPG values were used to perform box and whisker plot analysis using the program MicroCal Origin© 6.0 as well as to determine the phenotypic correlations of EPGs with the TPIT using the CORR procedure of the program SAS 9.1.

The accuracy of the potential indicator traits in detecting the nematode infection was evaluated with EPG as the standard method. 400 eggs per gram were suggested as a trigger level for drenching (Younie *et al.*, 2004). The cut-off values of required drenching for the examined traits are shown in Table 3.1. The combination of the four indicators was used to investigate if the decision making for drenching can be improved by using FBDC. Accordingly, an animal is treated if at least one of the indicator traits supports the drenching. Since FCS is time-intensive, the combination FBD was tested as well. The combination FBD relied on FAMACHA©, BCS and DS values to make the decision. The combinations FBDC and FBD were also separately evaluated for the farms with moderate (mean EPG $\geq$ 400) and low (mean EPG < 400) intensities of infection.

A true positive case (TP) was defined if drenching is required according to EPG and confirmed by TPIT values. Whereas, a true negative case was defined when drenching is not required according to EPG and TPIT values. A false positive case was defined when drenching is not required according to EPG but according to TPIT values. A false negative case (FN) was defined when drenching is required according to EPG but not to TPIT values. The sensitivity ratio of TPIT was calculated as following:

$$\text{Sensitivity} = \frac{TP}{(TP + FN)} \times 100$$

**Table 3.1** Cut-off values* of required drenching for eggs per gram of faeces (EPG), FAMACHA© score, body condition (BCS), dag score (DS) and faecal consistency (FCS)

| | Drenching | |
|---|---|---|
| | **YES** | **NO** |
| **EPG** | **≥ 400** | **< 400** |
| **FAMACHA©** | **≥ 3** | **≤ 2** |
| **BCS** | **≤ 2** | **≥ 3** |
| **DS** | **≥ 3** | **≤ 2** |
| **FCS** | **≥ 4** | **≤ 3** |

* The cut-off values for EPG was suggested by Younie et al. (2004) and for FAMACHA and DS by Bath and van Wyk (2009). The lambs with BCS score lower than mean BCS minus one standard deviation (2.9-0.65) and the lambs with FCS score higher than mean FCS plus one standard deviation (2.39+0.98) were suggested to be drenched

### 3.3. Results

The intensity of infection was low to moderate (Table 3.2). The mean EPG ranged from 118 to 512 with high standard deviations (195 to 1,289) among breeds. The prevalence of tapeworm was 11% in ML and GBM lambs, 14% in TX and MLW lambs and 22% in RH lambs. The prevalence of *Eimeria* was high in all breeds**.** *Nematodirus* eggs were encountered with a low intensity in 11, 7, 18, 20 and 3% of the samples in ML, GBM, TX, RH and MLW, respectively.

Depending on the results of the larval cultures, *Haemonchus contortus***,** *Trichostrongylus* spp. and *Teladorsagia* spp. were the predominant genera of nematodes (Figure 3.1). *H. contortus* constituted the largest proportion of larvae in ML (55%) and in RH (54%). The largest proportions of larvae in GBM, TX and MLW were *Trichostrongylus* spp. with 56%, 63% and 66%, respectively. According to the used trigger level for drenching, only 859 lambs (22%) required drenching.

**Table 3.2** Eggs per gram of faeces (EPG) (Mean ± standard deviation) and prevalence of tapeworms and coccidian infection by breeds

| | EPG | Tapeworm (%) | Coccidia (%) |
|---|---|---|---|
| **ML** | 252 ± 583 | 11 | 81 |
| **GBM** | 447 ± 1289 | 11 | 96 |
| **TX** | 326 ± 547 | 14 | 95 |
| **RH** | 512 ± 675 | 22 | 92 |
| **MLW** | 118 ± 195 | 14 | 90 |

ML: Merinoland, GBM: German Blackhead Mutton, TX: Texel, RH: Rhoen and MLW: Merino Long-wool

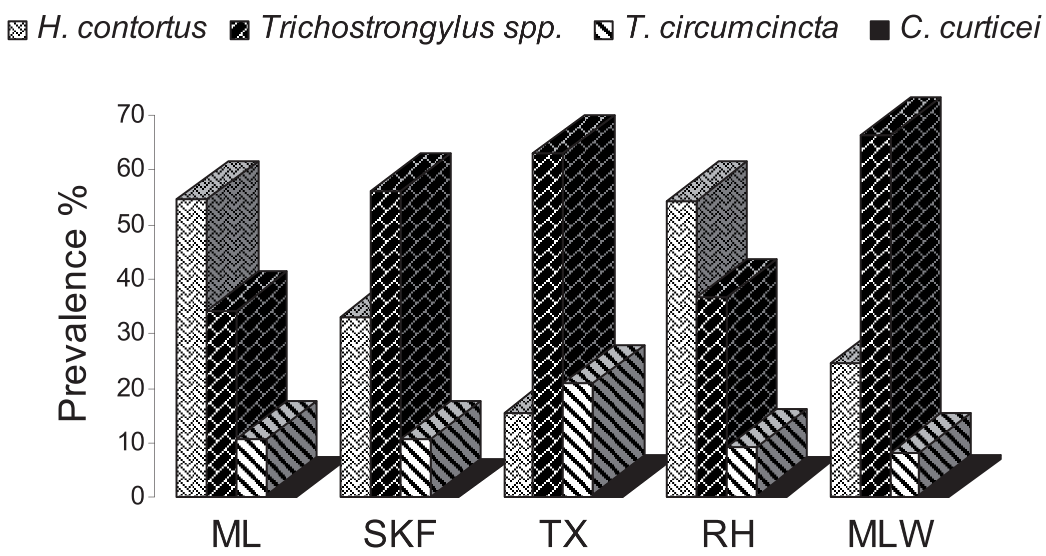


**Fig. 3.1** Prevalence (%) of nematode genera by breeds; ML: Merinoland, GBM: German Blackhead Mutton, TX: Texel, RH: Rhoen and MLW: Merino Long-wool

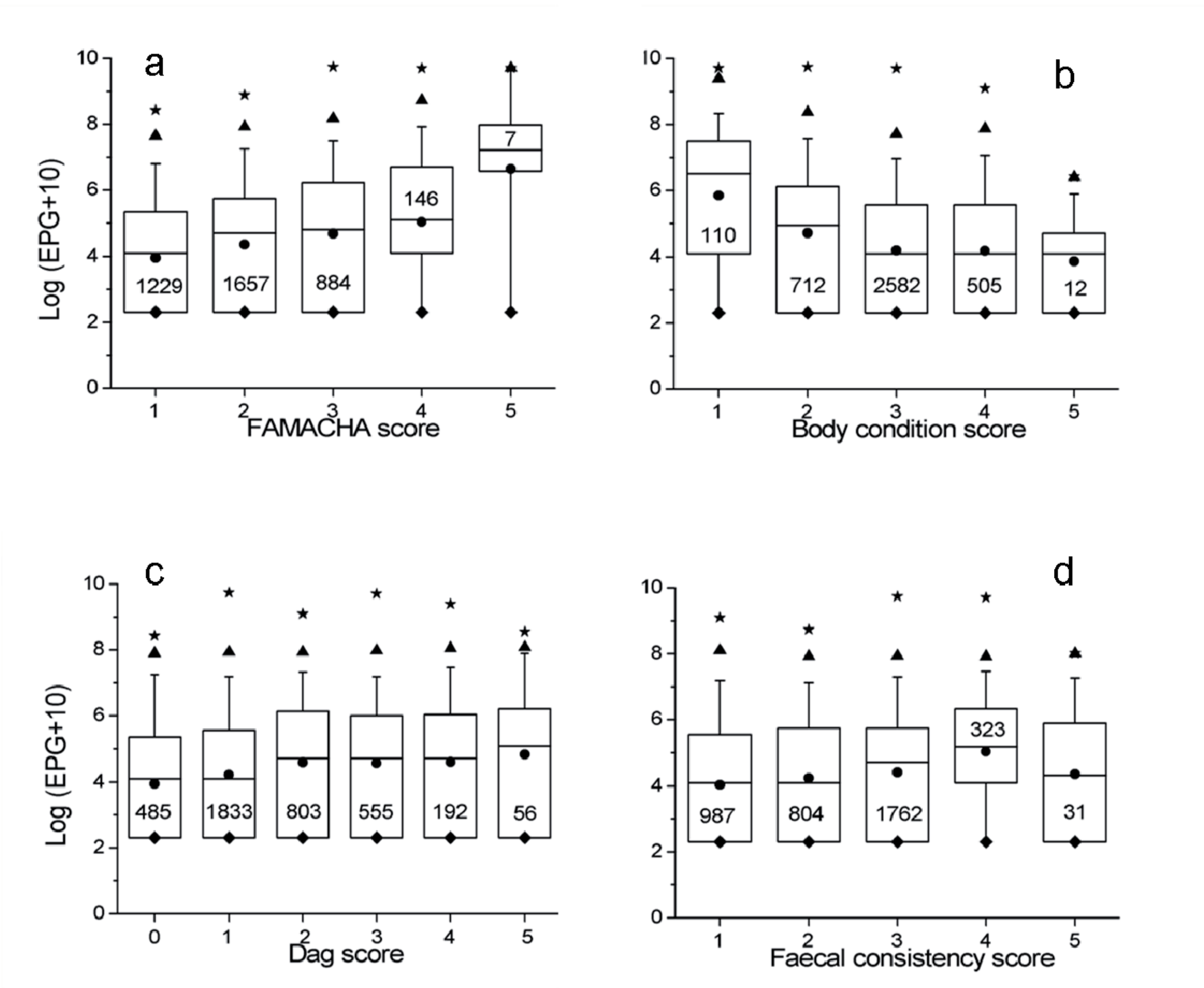


**Fig. 3.2** Box and whisker plots demonstrating the distribution of eggs per gram of faeces (log (FEC+10)) within the different scores of the FAMACHA© (a), the body condition score (b), the dag score (c), and the faecal consistency score (d). The lower and upper borders of the box represent the 25th and 75th percentiles, respectively. Mean (●) and median (▬ line) values are presented within the box. Minimum, outliers (99%), and maximum are indicated by (♦), (▲) and (*). The numbers of animals within each category are presented within the boxes

Figure 3.2 shows the distribution of transformed EPG by FAMACHA, BCS, DS and FCS. There was an increase of mean EPG as the FAMACHA© score increased but with high variations within each scale. Only seven animals had the FAMACHA© score 5 and most animals (2886) were scored 1 and 2. Few lambs had extreme values for BCS (1 and 5) and 2,582 lambs had score 3. The lambs with BCS 3, 4, and 5 had lower mean EPG than the lambs with BCS 1 and 2. Twelve percent of the lambs had a DS of 0 and 56 lambs had score 5. The lambs with DS 0 and 1 had lower mean EPG in comparison to the lambs with other scores. Less than 10% of the lambs had fluid faeces with a consistency score of 4 and 5. These lambs had higher mean EPG than the lambs with FCS 1 and 2.

Using the indicator combinations leads to the lowest ratio (7%) of false negative but to the highest ratio (37 – 39%) of false positive decisions (Table 3.3). The ratios of correct treatments of FAMACHA©, BCS, DS and FCS as indicators for drenching requirement vs. EPG were 69, 71, 68, and 75%, respectively. However, the sensitivities of the individual TPIT were in general low and ranged from 0% for FCS in MLW lambs to 63% for FAMACHA© in GBM lambs (Table 3.4). The indicators combinations FBDC and FBD had evidently higher sensitivity ratios in comparison to each individual trait. The highest estimated values were found in GBM (74 and 77%) and RH (78 and 80%) for FBD and FBDC, respectively.

**Table 3.3** Ratio of false negatives, false positives and correct treatments of FAMACHA© system, body condition score (BCS), dag score (DS), faecal consistency (FCS), and the combinations FBDC and FBD as indicators for drenching requirement vs. eggs per gram of faeces

| | False negative (%) | False positive (%) | Correct (%) |
|---|---|---|---|
| **FAMACHA©** | 13 | 18 | 69 |
| **BCS** | 15 | 14 | 71 |
| **DS** | 17 | 15 | 68 |
| **FCS** | 19 | 6 | 75 |
| **FBDC*** | 7 | 39 | 54 |
| **FBD†** | 7 | 37 | 56 |

**Table 3.4** Sensitivity of FAMACHA© system, body condition score (BCS), dag score (DS), faecal consistency (FCS), and the combinations FBDC and FBD as indicators for drenching requirement vs. eggs per gram of faeces by breeds

| | ML | GBM | TX | RH | MLW | Total |
|---|---|---|---|---|---|---|
| **FAMACHA** | 14 | 63 | 36 | 49 | 15 | 39 |
| **BCS** | 38 | 16 | 25 | 39 | 37 | 31 |
| **DS** | 17 | 20 | 18 | 39 | 11 | 23 |
| **FCS** | 8 | 14 | 10 | 23 | 0 | 13 |
| **FBDC*** | 59 | 77 | 62 | 80 | 50 | 69 |
| **FBD†** | 57 | 74 | 60 | 78 | 50 | 67 |

* FBDC: combination of FAMACHA, BCS, DS and FCS. Treatment is required if at least one of the indicator traits supports the drenching.
† FBD: combination of FAMACHA, BCS and DS. Treatment is required if at least one of these indicator traits supports the drenching.
ML: Merinoland, GBM: German Blackhead Mutton, TX: Texel, RH: Rhoen and MLW: Merino Long-wool.

**Table 3.5** Ratio of false negatives, false positives, correct treatments and the sensitivity of the combinations FBDC and FBD as indicators to identify the lambs requiring drenching vs. eggs per gram of faeces in farms with low (mean EPG < 400) and moderate (mean EPG ≥ 400) intensities of infection

| | False negative (%) | False positive (%) | Correct (%) | Sensitivity (%) |
|---|---|---|---|---|
| **Low infection** | | | | |
| FBDC* | 5 | 41 | 54 | 54 |
| FBD† | 5 | 39 | 56 | 53 |
| **Moderate infection** | | | | |
| FBDC | 10 | 35 | 55 | 77 |
| FBD | 11 | 33 | 56 | 74 |

* FBDC: combination of FAMACHA, BCS, DS and FCS. Treatment is required if at least one of the indicator traits supports the drenching. † FBD: combination of FAMACHA, BCS and DS. Treatment is required if at least one of these indicator traits supports the drenching.

The evaluation of drenching decision based on the indicator combinations in farms with low and moderate intensities of infection is presented in table 3.5. The sensitivities (77 and 74%) and the false negatives (10 and 11%) of FBDC and FBD were higher in the farms with moderate intensity than those in the farms with low intensity. The ratios of the false positives and the correct treatments were similar to some extent in the farms with low and moderate intensities of infection.

The EPG significantly ($P \leq 0.001$) correlated with all TPIT when estimated for all lambs (Table 3.6). The correlations between EPG and indicator traits were weak in the farms with a low intensity of infection. These correlations in the farms with moderate infections were only significant for FAMACHA© (0.19; $p \leq 0.001$) and BCS (-0.21; $p \leq 0.001$).

**Table 3.6** Phenotypic correlations of eggs per gram of faeces with FAMACHA© system, body condition (BCS), dag score (DS) and faecal consistency (FCS) in farms with low (mean EPG < 400) and moderate (mean EPG ≥ 400) intensities of infection

| | FAMACHA | BCS | DS | FCS |
|---|---|---|---|---|
| **Low infection (N=2611)** | 0.05 * | -0.03 | 0.09 ** | 0.04 * |
| **Moderate infection (N=1313)** | 0.19 ** | -0.21 ** | 0.001 | 0.02 |
| **Total (N=3924)** | 0.17 ** | -0.15 ** | 0.11 ** | 0.13 ** |

* $P \leq 0.05$; ** $P \leq 0.001$

### 3.4. Discussion

In this study only 22% of the lambs required treatment according to the suggested trigger level (EPG ≥ 400). This percentage is expected to be lower in older animals, which are less susceptible to parasite infection than lambs (McClure, 2000). The major distribution of nematode worms in an infected host population is over-dispersed in a small percentage of animals (Barger, 1985; Hoste *et al.,* 2001). Anderson and May (1982) suggested that the treatment of a heavily infected hosts (8% of the host population) can theoretically reduce mean worm burdens by up to 50% when the worms are highly clumped. Therefore, the use of the selective treatment could be considered as an economic approach. In addition it effectively slows down the spread of the anthelmintic resistance in sheep farms.

The mean EPG values increased as the FAMACHA© score increased. However, a low correlation coefficient between FAMACHA© and EPG was estimated. That may due to the high variations of EPG values within the FAMACHA© scores. This correlation is similar to that estimated under natural infection conditions in Germany (Koopmann *et al.*, 2006; Moors and Gauly, 2009). The sensitivity ratios of FAMACHA© in GBM and RH lambs were moderate. The intensities of infection in GBM and RH lambs were in tendency higher than other breeds. However, the FAMACHA© eye color chart seems to be unreliable for accurately diagnosing natural infection of nematode in Germany when alone used. This is probably due to the low intensity of infection and the comparatively low prevalence of *H. contortus*. This finding supports the suggestion that the practical use of the FAMACHA© system in detecting gastrointestinal nematode infections with low parasite pressure is limited (Gauly *et al.*, 2004).

The negative impact of nematode infections on body condition score was not fully clear. There was a significantly negative but low correlation between BCS and EPG. The impact of nematode infections on animal performance may be associated with the intensity and pathogenicity of the infection (Stear *et al.*, 2003), as well as with the nutritional and physiological status of the host animal (Bishop and Stear, 2003). The correlations between infection parameters and performance traits vary dramatically from one study to another. Vagenas *et al.* (2002) found no correlations between EPG and body weight in goats. Vanimisetti *et al.* (2004) reported a negative correlation between mean body weight and mean EPG in hair sheep. Following natural infections, daily weight gains of Rhoen lambs were negatively correlated with EPG (Gauly and Erhardt, 2001). Vatta *et al.* (2002) found no relationship between BCS and FEC, whereas a significant correlation was reported by Burke *et al.* (2007). Using BCS as a single trait to identify the lambs in need for drenching is excluded due to the high ratio of false negative and its low sensitivity.

The relations of EPG with DS and FCS seem to be complicated. Diarrhoea is one of the known signs indicating a high infection intensity with gastrointestinal nematodes. An infection with *Trichostrongylus* spp., *Teladorsagia* spp. or *Nematodirus* spp. can cause diarrhoea especially in young lambs (Sutherland and Scott, 2009) which then result in higher DS. Therefore, it will be expected that the EPG should have linear positive correlations with DS and FCS. On the other hand, Williams *et al.* (2010) reported that the immune response to nematode infection reduce the faecal dry matter due to the negative relation of faecal consistency with eosinophils and globule leukocytes. Thus, resistant animals are supposed to have high DS and FCS. A poor relationship between DS and FCS with EPG were reported earlier under Australian natural conditions of infections (Greeff and Karlsson, 1997; Pollott *et al.*, 2004). Under the same condition of the present study, the DS and FCS seem to be unsuitable to detect the nematode infection in lambs due to their low sensitivity and weak correlations with EPG. However, the faecal consistency may be influenced by other factors which can avert its relation to nematode infection, e.g. *Eimeria* infection can have an impact on FCS (Peregrine *et al.*, 2009). This could not be detected in our study due to the semi-quantitative determination of the coccidian oocysts.

The decision makings of drenching based on the indicator combinations gave more credibility with lower false negatives results and higher sensitivities than those for each individual indicator. However, the indicator combinations were not specific enough and had higher ratios of false positives. The false negatives and the sensitivities are more important than false positives, because they can confirm the reliability of these indicators to identify highly infected animals. The sensitivities of these indicator combinations were associated more or less with the infection intensity. The high values of the sensitivity were achieved in the breeds with high intensities of infection (GBM and RH). Whereas, a low value was observed in MLW lambs, which had the lowest intensity of infection. Additionally, there were clear differences between the sensitivities of the indicator combinations in the farms with low and moderate intensities of infection.

Changing the trigger level for drenching to lower or higher values will change this outcome. E.g. if the trigger level is increased to 500 EPG, then the sensitivity of the combination FBDC will increase to 72% and the false positives will increase to 41% (results not presented). In contrast, the sensitivity and false positives will decrease to 68% and 36%, respectively, if the trigger level is reduced to 300 EPG. Nevertheless, the obtained values did not reach the acceptable limit to recommend these indicator combinations for drenching decisions of lambs under moderate level of mixed, natural nematode infections in Germany.

A major problem of using these indicator traits is the resilient animals. These animals do not show any signs of nematode infections (Coffey *et al.*, 2007), and therefore cannot be detected by the tested indicator traits. The treatment of the resilient animals may not be required because their performance cannot be negatively affected during the infection (Woolaston and Baker, 1996). However, they may play an important role in the contamination of the pasture. Another problem is due to the fact that the EPG probably did not reflect the worm burden in the case of mixed nematode infections, especially if the larval cultures have not been done for each lamb individually (Amarante, 2000). Additional research to test these indicators according to worm burdens is therefore required.

### 3.5. Conclusion

The FAMACHA© system, body condition score, dag score and faecal consistency score have unacceptably low sensitivities when they are separately used to determine the intensity of nematode infections in lambs under field conditions. The sensitivities of the indicator combinations were higher than those of each individual indicator and positively associated with the intensity of infection. However, their ratios of sensitivity, false negatives and false positives seem to be unreliable. Therefore, the drenching decisions for lambs based on these alternative infection indicator traits under moderate levels of mixed natural nematode infections cannot be recommended.

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# CHAPTER IV

Genetic resistance to natural gastrointestinal nematode infections in German sheep

**Abstract**

The objective of this study was to evaluate the possibility of breeding for resistance against gastrointestinal nematode infections in German sheep, either based on faecal egg counts (FEC) or by using dag score (DS), faecal consistency score (FCS), body condition score (BCS) and FAMACHA© as indicator traits. Genetic analysis was carried out based on FEC of 3,459 lambs (from 180 sires) of four sheep breeds under the conditions of natural infections. Therefore, lambs from various farms were sampled once during the grazing period. At the time of sampling DS, FCS, BCS and FAMACHA© score were also determined and served as potential infection indicator traits. Heritabilities and genetic correlations were estimated applying restricted maximum likelihood (REML) methodology.

Estimated heritabilities of FEC ranged from 0.22 to 0.59 among breeds. Heritabilities of the indicator traits DS, FCS, BCS and FAMACHA© ranged from 0.15 to 0.50, 0.16 to 0.24, 0.02 to 0.55 and 0.01 to 0.25, respectively. The phenotypic correlations of FEC with the potential infection traits were low and ranging from -0.20 to 0.19. A significant ($P \leq 0.01$) negative correlation was found between BCS and FEC in three breeds. FEC did not show a consistent genetic correlation with other traits and in most cases estimated values had high standard errors. Genetic correlations between the indicator traits DS and FCS were high, i.e. ranging between 0.50 and 0.95.

It is concluded that breeding for resistance against gastrointestinal nematodes (low faecal egg counts) following a natural mixed infection in Merinoland, Merino Long-wool, German Blackhead Mutton and Texel breeds by selection is feasible. In opposite the indirect selection for nematode resistance using DS, FCS, BCS or FAMACHA© eye colour chart seem to be unsuitable.

## 4.1. Introduction

Since resistance to gastrointestinal nematode (GIN) can be considered as a qualitative trait of host animal, variations between and within host populations can be used to breed more resistant hosts. Breeding for resistance against nematodes in livestock can be based on the variation between or within breeds as well as crossbreeding (Nicholas, 1987). Breed differences in resistance against gastrointestinal nematodes were described since the seventies (Bradley *et al.*, 1973; Altaif and Dargie, 1978; Amarante *et al.*, 2004). Furthermore, within breed variation can be used in order to select resistant sheep (Gray, 1997; Woolaston and Baker, 1996).

Faecal egg counts (FEC) reflect the resistance of the host animal against GIN infections and are used worldwide to estimate breeding value of host resistance (Gruner and Lantier, 1995; Rahmann and Seip, 2007; Stear *et al.*, 2007). In addition to FEC, there are various indirect indicators that may be used to evaluate parasite infection as well as the resistance of the host animal. The FAMACHA© eye colour chart is one of the potential indicators which can reliably be used when blood sucking nematodes, such as Haemonchus species, are the predominant species (Malan and van Wyk, 1992; Riley and van Wyk, 2009). Since infections with GIN cause some degree of diarrhoea in sheep (Sutherland and Scott, 2009), dag and faecal consistency scores could be used as indicators of high intensity of nematode infections. Due to the negative effects of infection with internal parasites on lamb growth performance, Bath and van Wyk (2009) suggested the Back (Body condition) score as one of Five Point Check© that could be useful to detect the nematode infection. These afore-mentioned infection and/or host resistance indicators have not been thoroughly evaluated under conditions of natural GIN infections in Germany.

The variation among individuals in FEC regarding resistance to nematode infection may be partly due to the difference in their feeding behaviour (Hoste *et al.*, 2001). This cannot be adapted to studies based on artificial infection of animals. Furthermore in the case of artificial infections, the genotype x environment interactions may not be evident as infected animals are mostly kept under strict experimental/controlled conditions. Therefore, the reliability of estimates of genetic parameters based on natural infections may be superior to those based on artificial infection. However, it is important to keep in mind that the animals under natural conditions are unevenly exposed to nematode infection due to the differences in pasture contamination with infectious larvae. In Germany, Gauly *et al.* (2002) estimated heritabilities for FEC in Rhoen (0 and 0.35) and Merinoland (0.07 and 0.17) sheep at four and eight weeks post-infection, respectively, following an experimental infection with H. contortus. Under natural conditions of infection with GIN, Gauly and Erhardt (2001) estimated the heritability for FEC to be between 0.11 and 0.44 in Rhoen sheep. To our knowledge, there are presently no more published studies that have estimated genetic parameters of nematode resistance in most important German sheep.

Therefore, the aim of present study was to investigate the possibility of breeding of four German sheep breeds for resistance against nematodes. Such an aim implies to estimate the heritability of faecal egg counts, and to investigate possible genetic relationships of FEC with dag score, faecal consistency scores, body condition score and FAMACHA© score in naturally infected lambs.

## 4.2. Materials and methods

### *4.2.1. Animals*

The study was carried out using 3,459 lambs aged 3 to 15 months from 18 farms. Lambs from the following four breeds were used: Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX). An overview of the genetic structure within each breed is given in Table 1. Overall, average number of offspring per ram was 18. Faecal samples were collected only once during the grazing seasons of 2006, 2007 and 2008. Also, 376 faecal samples of MLW lambs were taken and examined in 2010. For statistical analysis the grazing season was divided into two periods (1: June to August; 2: September to December). The faecal samples were taken from lambs that had not been dewormed at least 45 days before sampling date.

**Table 4.1** Number of lambs, sires and offspring per sire within breeds; Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX)

| | Number of Lambs | Number of sires | Offspring per sire Mean (± SD) | Range |
|---|---|---|---|---|
| **ML** | 1,378 | 65 | 20.6 ± 18.7 | 1 - 91 |
| **MLW** | 1,054 | 59 | 17.5 ± 16.1 | 1 – 101 |
| **GBM** | 654 | 39 | 16.7 ± 13.1 | 1 – 81 |
| **TX** | 373 | 17 | 20.8 ± 17.2 | 1 – 94 |

### *4.2.2. Faeces analysis*

Eggs per gram of faeces (FEC) were determined using a modified McMaster method (Maff, 1986) as described in Chapter II.

### *4.2.3. Tested potential indicator traits (TPIT)*

At the time of faeces collection, dag score (DS), faecal consistency score (FCS), body condition score (FCS) and FAMACHA© were also determined as described in Chapter III. DS, FCS, BCS and FAMACHA© will be described in the present article as ’tested potential indicator traits (TPIT)’.

### *4.2.3. Data analysis*

Individual faecal egg counts (FEC) were $\log_e$ (n + 10) transformed to achieve a normal distribution of the data. The phenotypic Pearson correlation coefficients among traits were calculated using SAS, version 9.1. Heritabilities and genetic correlations were estimated by applying a multi-trait animal model and using REML-methodology as implemented in the program VCE, version 4.2.5 (Neumaier and Groeneveld, 1998). The following model was used:

$$y_{ijklmn} = \mu + L_i + F_j + S_k + B_l + SE_m + AGE + e_{ijklmn}$$

Where

$y_{ijklmn}$ = observation of FEC and of indicator traits

$\mu$ = overall mean effect

Li = random additive genetic effect of animal i

$F_j$ = fixed effect of farm j

$S_k$ = fixed effect of sex k

$B_l$ = fixed effect of birth type l

$SE_m$ = fixed effect of season m nested within years

AGE = age of lambs modelled as a covariate (linear regression)

$e_{ijklmn}$ = random residual effect.

## 4.3. Results

### *4.3.1. Faecal examination*

65 % of the examined lambs were infected with at least one species of GIN. The prevalence of nematodes ranged between 53 and 77% (Figure 4.1). The mean of FEC was 261 (±565). *Trichostrongylus* spp., *H. contortus* and *Teladorsagia* spp. were the predominant nematodes. 89% of the samples were *Eimeria* positive. Tapeworm eggs were encountered in 11 % of all faeces samples.

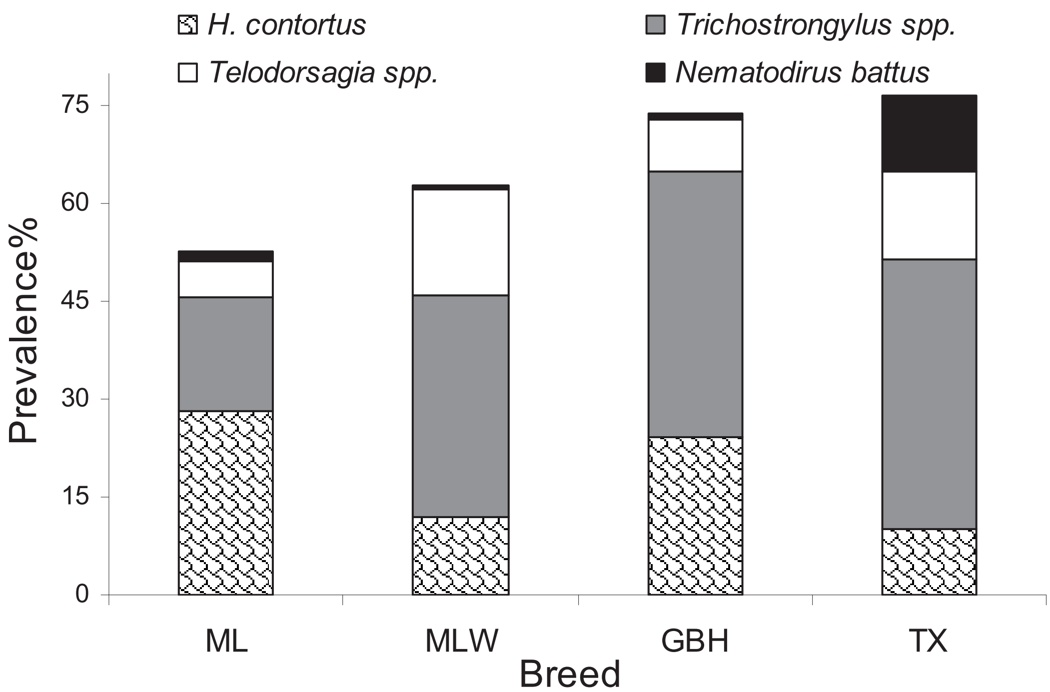


**Fig. 4.1** Prevalence and composition of nematode species by breeds; Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX). Prevalence of *Nematodirus battus* was calculated as ratio of *Nematodirus* mean to mean of total faecal egg counts (mean of *Nematodirus*/mean FEC). Prevalence of *Cooperia* spp. was less than 1% in all breeds and is therefore not presented in this Figure.

### *4.3.2. Heritabilities*

Heritability estimates of FEC, DS, FCS, BCS and FAMACHA© are given in Table 2. Heritability of the FEC ranged from low to moderate-high estimates among the breeds. GBM had the lowest (0.22 ± 0.09) and the ML had the highest (0.59 ± 0.06) heritability for FEC. Merino Long-wool and Texel sheep had moderate heritabilities of the FEC (0.37 ± 0.05 and 0.39 ± 0.12, respectively). Heritabilities estimated for DS varied among breeds from 0.15 ± 0.06 (GBM) to 0.50 ± 0.06 (MLW). Nevertheless, the estimates for FCS were lower and had less variation among breeds (0.16 to 0.24). The heritability of BCS ranged between 0.02 ± 0.03 and 0.55 ± 0.12. FAMACHA© score had a similar heritability in MLW and TX (0.16 ± 0.03 and 0.14 ± 0.09, respectively). The heritability of the FAMACHA© score was 0.25 (± 0.03) in ML and 0.01 (± 0.02) in GBM.

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**Table 4.2** Heritability estimates (± SE) of faecal egg counts (FEC), dag score (DS), faecal consistency score (FCS), body condition score (BCS) and FAMACHA© score (FAM) by breeds; Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX)

| | **ML** | **MLW** | **GBM** | **TX** |
|---|---|---|---|---|
| **FEC** | 0.59 ± 0.06 | 0.37 ± 0.05 | 0.22 ± 0.09 | 0.39 ± 0.12 |
| **DS** | 0.34 ± 0.04 | 0.50 ± 0.06 | 0.15 ± 0.08 | 0.24 ± 0.10 |
| **FCS** | 0.18 ± 0.03 | 0.24 ± 0.05 | 0.23 ± 0.13 | 0.16 ± 0.08 |
| **BCS** | 0.28 ± 0.05 | 0.28 ± 0.05 | 0.02 ± 0.03 | 0.55 ± 0.12 |
| **FAM** | 0.25 ± 0.03 | 0.16 ± 0.03 | 0.01 ± 0.02 | 0.14 ± 0.09 |

### *4.3.3. Relationship between FEC and potential indicator traits*

Most of the phenotypic correlations between FEC and the potential indicator infection traits were close to zero (Table 3). DS correlated significantly with FEC only in Texel breed (-0.14; $P \leq 0.01$). In ML, MLW and TX lambs BCS was negatively related to FEC (-0.09 to -0.20; $P \leq 0.01$), whereas no relation was detected in GBM lambs. FAMACHA© score did not show a significant correlation with FEC in ML and TX, whereas a positively significant ($P \leq 0.001$) correlation was estimated in MLW and GBM lambs.

The genetic correlation coefficients between FEC and other traits are shown in Table 4. A positive genetic correlation was estimated between FEC and DS in ML (0.16 ± 0.07). This relation was negative in GBM (-0.61 ± 0.22). FEC was not genetically related to FCS in MLW, GBM and Texel. However, FEC was negatively related with FCS in ML (-0.48 ± 0.08). A high genetic correlation found between FEC and BCS (-0.87 ± 0.15) in GBM. This correlation did not differ from zero in other breeds. The genetic correlation between FAMACHA© and FEC varied highly among breeds and ranged from -0.15 (± 0.14) in MLW to 0.60 (± 0.31) in GBM

**Table 4.3** Phenotypic correlations of faecal egg counts with dag score (DS), faecal consistency score (FCS), body condition score (BCS) and FAMACHA© score (FAM) by breeds; Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX)

| | ML | MLW | GBM | TX |
|---|---|---|---|---|
| **DS** | 0.01 | 0.01 | 0.04 | -0.14** |
| **FCS** | 0.08** | 0.06 | 0.09* | -0.09 |
| **BCS** | -0.20*** | -0.09** | -0.01 | -0.20*** |
| **FAM** | 0.03 | 0.16*** | 0.19*** | 0.07 |

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

**Table 4.4** Estimates of genetic correlations (± SE) of faecal egg counts with dag score (DS), faecal consistency score (FCS), body condition score (BCS) and FAMACHA© score (FAM) by breeds; Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX)

| | **ML** | **MLW** | **GBM** | **TX** |
|---|---|---|---|---|
| **DS** | 0.16 ± 0.07 | -0.05 ± 0.07 | -0.61 ± 0.22 | 0.03 ± 0.27 |
| **FCS** | -0.48 ± 0.08 | -0.16 ± 0.09 | 0.24 ± 0.30 | 0.04 ± 0.25 |
| **BCS** | -0.06 ± 0.08 | 0.01 ± 0.08 | -0.87 ± 0.15 | 0.03 ± 0.17 |
| **FAM** | 0.09 ± 0.07 | -0.15 ± 0.14 | 0.60 ± 0.31 | 0.13 ± 0.27 |

### *4.3.3. Relationships among potential indicator traits*

Tables 5 and 6 present the phenotypic and genetic correlations among DS, FCS, BCS and FAMACHA©. Positively and highly significant correlations between DS and FCS were recorded for all breeds (0.20 to 0.48; $P \leq 0.001$). Their genetic correlations were also high and positive (0.50 ± 0.11 to 0.95 ± 0.08). Phenotypic correlations between BCS and DS and FCS, and between FAMACHA© and DS and FCS, were low and mostly not significant. In ML lambs a negative genetic correlation was estimated between DS and BCS (-0.47 ± 0.13), whereas the genetic correlation between BCS and FCS was positive (0.18 ± 0.13). The relationship of BCS with DS and FCS revealed positive (MLW and GBM) and negative (TX) genetic correlations. Mostly, FAMACHA© showed an unreliable genetic correlation with DS and FCS. Negative and significant phenotypic correlations were calculated between FAMACHA© and BCS (-0.08 to -0.21; $P \leq 0.01$). The genetic correlation between FAMACHA© and BCS was strongly negative in MLW (-0.53 ± 0.13) and GBM (-0.96 ± 0.20).

**Table 4.5** Phenotypic correlations among dag score (DS), faecal consistency score (FCS), body condition score (BCS) and FAMACHA© score (FAM) in lambs by breeds; Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX)

| | **ML** | **MLW** | **GBM** | **TX** |
|---|---|---|---|---|
| **DS-FCS** | $0.20^{***}$ | $0.21^{***}$ | $0.29^{***}$ | $0.48^{***}$ |
| **DS-BCS** | $-0.05^{*}$ | $0.06^{*}$ | -0.06 | 0.04 |
| **FCS-BCS** | 0.01 | $0.11^{***}$ | $0.07^{*}$ | $0.15^{**}$ |
| **DS-FAM** | 0.01 | $-0.07^{*}$ | $0.10^{*}$ | 0.09 |
| **FCS-FAM** | -0.01 | $0.06^{*}$ | 0.03 | -0.03 |
| **BCS-FAM** | $-0.08^{**}$ | $-0.12^{***}$ | $-0.21^{***}$ | $-0.16^{**}$ |

$^{*}$ $P \leq 0.05$; $^{**}$ $P \leq 0.01$; $^{***}$ $P \leq 0.001$

**Table 4.6** Estimates of genetic correlations (± SE) among dag score (DS), faecal consistency score (FCS), body condition score (BCS) and FAMACHA© score (FAM) in lambs by breeds; Merinoland (ML), Merino Long-wool (MLW), German Blackhead Mutton (GBM) and Texel (TX)

| | **ML** | **MLW** | **GBM** | **TX** |
|---|---|---|---|---|
| **DS-FCS** | 0.50 ± 0.11 | 0.93 ± 0.04 | 0.77 ± 0.47 | 0.95 ± 0.08 |
| **DS-BCS** | -0.47 ± 0.13 | 0.62 ± 0.11 | 0.82 ± 0.47 | -0.34 ± 0.22 |
| **FCS-BCS** | 0.18 ± 0.14 | 0.72 ± 0.09 | 0.23 ± 0.26 | -0.55 ± 0.20 |
| **DS-FAM** | 0.04 ± 0.06 | -0.20 ± 0.12 | -0.83 ± 0.28 | -0.26 ± 0.31 |
| **FCS-FAM** | -0.06 ± 0.10 | -0.50 ± 0.14 | -0.45 ± 0.45 | -0.10 ± 0.37 |
| **BCS-FAM** | 0.07 ± 0.10 | -0.53 ± 0.13 | -0.92 ± 0.20 | 0.25 ± 0.30 |

## 4.4. Discussion

All animals were sampled during the grazing season, which provides favorable environmental conditions for the development of nematode parasites. Despite these conditions, prevalence and intensity of nematode infections in the current study remained on a low – moderate level. This is in agreement with earlier reports under comparable conditions in Germany (Benesch, 1993; Grzonka *et al.*, 2000; Moritz, 2005).

### *4.4.1. Heritabilities*

There is no report on the use of FEC and/or potential indicator traits for selection in any of the examined sheep in this study. There were only two published studies reporting heritability estimates for FEC in German sheep breeds. Gauly and Erhardt (2001) estimated heritabilities between 0.11 and 0.44 in Rhoen sheep based on natural infection. After artificial infection with Haemonchus contortus, heritabilities for FEC in Rhoen and Merinoland ranged between 0 and 0.35 (Gauly *et al.*, 2002). Regardless of genotype, heritability estimates for FEC can be influenced by many factors such as intensity and species composition of infections and age of host animal. Greater values of FEC heritability may be estimated under low or high intensity of nematode infections than under moderate infection (Pollott and Greeff, 2004). The composition and pathogenicity of parasite populations could also change the estimate. Pollott *et al.* (2004) estimated higher heritabilities of FEC as the age of the host animal increases. Thus, heritabilities increase from 0.2 at weaning to 0.65 at an age of 400 days. Despite differences in outcomes of FEC heritability estimates among different studies and genotypes, heritabilities of FEC estimated in the current study are mostly in the range of those reported in earlier studies (Gauly and Erhardt, 2001; Pollott *et al.*, 2004; Lobo *et al.*, 2009). The estimated heritabilities for FEC in the current work are indicative of a relatively high within-breed-variation. This sufficient variation in FEC provides the possibility to select animals for resistance against GIN infections.

Most of earlier studies reported that faecal consistency is less influenced by genetic factors. Wolf *et al.* (2008) estimated heritability of FCS between 0.06 and 0.11 for different breeds. In Australia, Greeff and Karlsson (1997, 1998 and 1999) also reported moderate to low values with 0.38, 0.04 and 0.13, respectively. Accordingly, low heritability estimates for FCS in the present study showed that environmental factors accounted for the most variation in FCS. The high percentage of fluid in the faeces (diarrhea) is the main reason for dag (Williams *et al.*, 2009) and the ability of breech to accumulate faecal soiling probably play an important role in variation of DS (French and Morgan, 1996). DS is negatively associated with breech bareness of lambs (Scobie *et al.*, 2007). The breech wrinkle could also increase the accumulation of dag (Morris, 2000). Based on these anatomical grounds, variability between individuals in DS seems to be more related to genetic factors than FCS. Researchers in Australia and New Zealand have estimated low to moderate heritability for DS **(**Larsen *et al.* 1995; Morris *et al.* 1995; Pollott *et al.*, 2004). The current study showed comparable results.

Body condition score is known as one of the best and simple procedure for describing nutritional status and potential performance of sheep. However, BCS is less considered in breeding programs when compared to direct performance traits. It is therefore not surprising that the heritability estimate of BCS has not been the focus of earlier studies. Since BCS is a measure of body fat content, it is closely associated to live weight of animal (Teixeira *et al.*, 1989; Frutos *et al.*, 1997). Maria *et al.* (1993) estimated heritability for weaning weight of lambs with 0.35 for males and 0.22 for females. In Hungary, Komlosi (2008) reported values between 0.07 and 0.62 in different breeds. Heritability of BCS obtained by Riley and Van Wyk (2009) ranged between 0.17 and 0.33. The heritability estimates of BCS in our study were moderate except in GBM. The low value in GBM is probably due to intensive selection pressure for meat production resulting in reduced genetic variation in the population.

The heritability of FAMACHA© varied from 0.01 to 0.26. Similar heritabilities have been reported by Riley and Van Wyk (2009) and Snyman (2007). The intensity of the GIN infections and the prevalence of *Haemonchus contortus* were low in the present study. Therefore, it could be suggested that the impact of infection on the haematocrit value of the lambs was low and therefore may not be quantified by the FAMACHA© system.

### *4.4.2. Relationship between FEC and potential indicator traits*

Phenotypically, faecal egg counts were weakly correlated to all potential indicator traits. That may be expected due to relatively low intensity of nematode infection. However, these relationships have been discussed in detail in Chapter III.

The genetic correlations of FEC with DS and FCS seem to be unreliable due to high standard errors. These were in some cases larger than the values of correlation coefficients. The high standard errors may be due to the limited number of animals available. FEC has been found to be unfavourably, genetically correlated with DS or FCS (Greeff and Karlsson 1997; Pollott *et al.*, 2004**;** Karlsson and Greeff, 2006), suggesting that sheep should be selected for both low FEC and low faecal scoring (Karlsson *et al.*, 2004). In the presented study, the correlation between FEC and FCS in Merinoland supports that selection against nematode infection in ML will be associated with an increased level of faecal fluid.

The impact of nematode infection on productivity and growth depends on environmental conditions, such as nutritional and physiological status of the host animal (Bishop and Stear, 2003). These authors assumed that the performance traits were not genetically related to resistance against parasite infection. Vagenas *et al.* (2002) found no genetic correlations between FEC and body weight in goats. The estimates of genetic correlation between FEC and BCS in the present study were very low in ML, MLW and TX.

No relationship between FAMACHA© and FEC score, neither genetically nor phenotypically, were found in the current study. Under field condition in Germany, the nematode infections are usually mixed infections with moderate intensity. The prevalence of *Haemonchus contortus* in current study was low (about 33% in this study) which may disqualify the FAMACHA© as a tool to detect gastrointestinal nematode infections in Germany (Koopmann *et al.*, 2006; Moors and Gauly, 2009). However, the high genetic correlation between FEC and FAMACHA© has been reported where *H. contortus* is the predominant nematode (van Wyk and Bath, 2002; Riley and van Wyk, 2009).

In GBM, the genetic variation between individuals in BCS and FAMACHA© were very small as reflected by estimated heritabilities. This variation was 0.001 for BCS and 0.003 for FAMACHA©. Also the genetic covariance between FEC and the both traits (BCS: -0.07, FAMACHA: 0.05) were low. Due to these facts, the high genetic correlations in GBM could not be relied upon.

#### *4.4.3. Relationship among the potential indicator traits*

DS and FCS revealed a strong genetic correlation. This finding support earlier suggestion that the same genes probably express these traits (Pollott *et al.*, 2004) and thus selection for decreased DS may decrease FCS in all breeds. Since FAMACHA© can indicate the level of anemia (diseases or nutrition problems) and BCS describes the potential performance of sheep, a negative relation between these two traits may be expected. Negative and significant correlations were found between FAMACHA© and body condition score in all breeds. However, the coefficients of correlation were relatively low. This is probably due to low variation in BCS and FAMACHA© among the lambs. A significant and negative genetic relation was only found in MLW and GBM lambs. This is in agreement with previous report by Riley and van Wyk (2009).

### 4.5. Conclusion

The genetic variations in faecal egg counts of the examined sheep breeds suggest that it is possible to select Merinoland, Merino Long-wool, German Blackhead Mutton and Texel sheep for improved nematode resistance (low FEC) under field conditions in Germany. The use of dag score, faecal consistency, body condition or FAMACHA© as indicator traits for nematode infection in breeding programmes for selecting resistant animals seem to be generally unsuitable. The genetic relationship among indirect indicator traits showed different patterns in each breed except those between dag score and faecal consistency, which was positive and high in all breeds.

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# CHAPTER V

Is the establishment rate and fecundity of *Haemonchus contortus* related to body or abomasal measurements in sheep?

**Abstract**

The relation among parasitological parameters, abomasal size and body size measurements were investigated in lambs following an experimental infection with *Haemonchus contortus*. In total, one hundred lambs from five different genotypes (Merinoland (ML), Texel × ML, Suffolk x ML, German Blackhead Mutton x ML and Ile de France × ML) were experimentally infected with 5000 infective 3rd stage larvae of *H. contortus* at time of weaning with twelve weeks of age**.** Four and six weeks after infection, individual faecal samples were collected for estimation of faecal egg counts (FEC). Furthermore wither height, shoulder width, heart girth, loin girth and body length were taken at eighteen weeks of life. Lambs were slaughtered and necropsied seven weeks post-infection, and worm counts, abomasal volume and surface area were determined.

Positive correlations were found between different body size parameters, body weights and abomasal sizes. FEC and worm counts were not significantly correlated either with body size parameters or with abomasal size. The mean worm burden in ML lambs was higher than does in crossbred lambs. There was no significant difference in abomasal size between ML and crossbred lambs. The results suggest that the variations between animals in worm burden following an experimental infection with *H. contortus* (worm resistance) are not influenced by body size parameters or abomasal sizes. Therefore other factors, including genetic based differences in resistance must cause these findings between and within breeds.

## 5.1. Introduction

Infections with gastrointestinal nematodes are a major source of economic losses in the sheep industry (Davies *et al*., 2005). The hematophagous *Haemonchus contortus* is considered to be one of the most pathogenic nematodes of sheep due to the blood-sucking habits of the adult worms (Williamson *et al.,* 2003). The average blood loss was estimated between 0.003 to 0.004 ml per worm per day (Dargie and Allonby, 1975), causing severe anaemia. It can also cause weight loss, reduced growth rate and even death in small ruminants (Schallig, 2000).

Abomasal nematodes can directly influence the abomasal environment to enable their survival and reproduction (Hertzberg *et al.,* 2000). Salman and Duncan (1984) described the main histological changes in abomasum of *H. contortus* infected lambs. Where, the primary infection caused loss of the superficial mucous layer, hyperplasia of mucus cells, congestion of mucosal blood vessels, haemorrhages, and an increase in the cellularity of the lamina propria. Erosions of the surface epithelial cells and oedema of the mucosa and sub mucosa were observed after re-infection. An increase in pH value and a decrease in oxygen content ($pO_2$) of the abomasal fluid after infection with a high number of *H. contortus* larvae have been reported by Nicholls *et al*. (1987). Moreover, Simpson (2000) reported that nematodes can decrease acid secretion in the abomasum and increase serum gastrin and pepsinogen concentrations in the abomasum.

Earlier studies were describing correlations between the sizes of parasites and the body size of hosts (Harrison, 1915). This suggestion or what is known now as Harrison's rule was also proved for different ectoparasites (Morand *et al*., 2000; Johnson *et al*., 2005). Johnson *et al*. (2005) for example estimated a positive relationship between wing feather size and wing louse body size. Studies in fish reported that the size of host fish has a strong impact on abundance and richness of common ectoparasites (Poulin and Rohde, 1997; Lo *et al*., 1998; Cable and van Oosterhout, 2007). These results led to controversial questions such as, whether endoparasites generally relate to the size of their environments or their hosts and whether differences in body and/or organ size of the host partly explains variation between animals with regard to worm burden or faecal egg counts in small ruminants.

However, there is no report of such correlations in lambs in the literatures we have so far consulted. Therefore, the objective of this study was to estimate the relation between parasitological parameters with body measurements and abomasal size in lambs experimentally infected with *H. contortus*.

## 5.2. Materials and methods

### *5.2.1. Experimental flock and design*

The experimental flock was located at the Research station 'Oberer Hardthof' of the University of Giessen. Lambing of all studied animals occurred indoor during three weeks in February, 2008. The animals were separated into three groups according to their birth week.

In total, 100 lambs (51 females and 49 males) from Merinoland (ML) and four crossbreds (20 lambs /genotype) were used in the study. Merinoland ewes were mated with 3 to 4 rams of Texel (TX), Suffolk (SU), German Blackhead Mutton (GBM) and Ile de France (IDF), respectively, to produce crossbred lambs.

The lambs were weaned at twelve weeks of age, separated from other sheep and housed indoor throughout the trial period. They were given water and hay *ad libitum* and about 500 grams/lamb/day concentrate diet (ME 10.8 MJ/kg, 18.0% crude protein). The infective larvae were supplied by the Institute of Parasitology, University of Giessen, Germany. The larvae were recovered from cultures of faeces derived from previously infected sheep. The larvae were microscopically counted in 100 µl (4x 25 µl) and that was used in calculating the volume that would contain 5000 *H. contortus* L3. The larvae were younger than four weeks. The lambs were each given orally a single-dose infection with 5000 L3 using a syringe at time of weaning (twelve weeks of age). All animals were slaughtered at 19 weeks of age. No anthelmintic treatments were applied during the study.

### *5.2.2. Body measurements*

One week before slaughter, five parameters (Figure 5.1) were measured to describe the body size of the animals (Fourie *et al.*, 2002; Janssens and Vandepitte, 2004). Wither height (WH) was measured as the vertical distance from the thoracic vertebrae to the ground;

shoulder width (SW), as the horizontal distance between the processes on the left and on the right shoulder blade; heart girth (HG) was obtained as the smallest circumference round the animal just behind the foreleg; loin girth (LG) was determined as the circumference round the animal just before the hind leg, and body length (BL) was measured from the humeri to the aitchbone (*Tuber ischiadicum*). All lambs were weighed at birth and at 12, 16 and 18 weeks of age.

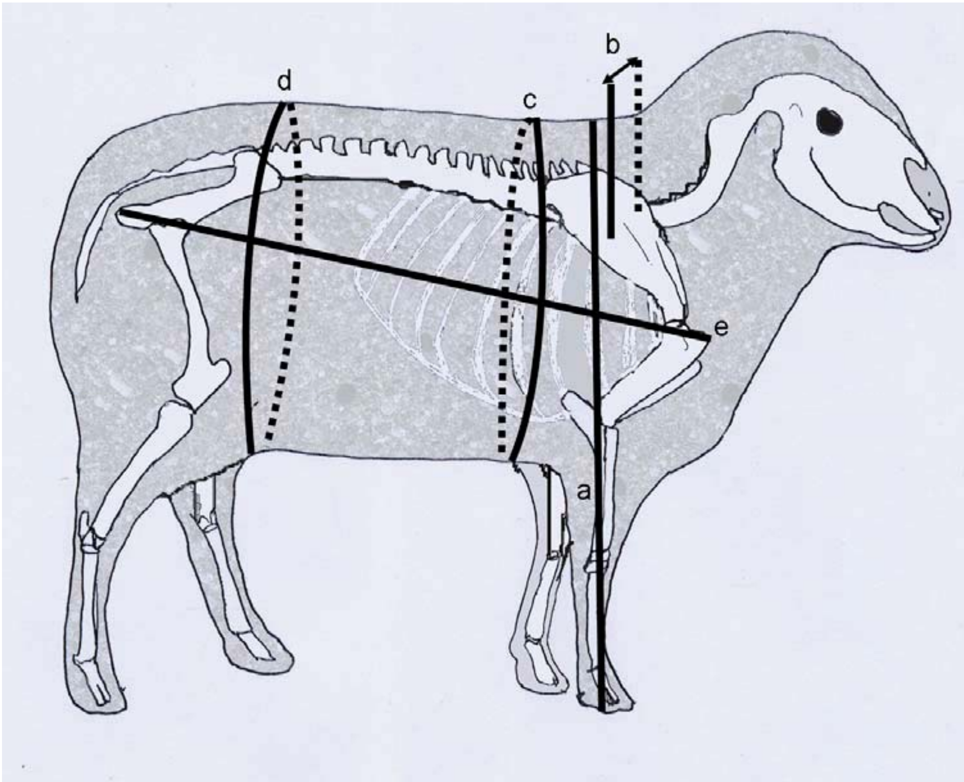


**Fig. 5.1** The body dimension measurements taken from lambs during the study- a: wither height, b: shoulder width, c: heart girth, d: loin girth and e: body length **(**modified from Koenig *et al.,* 1968**)**

### *5.2.3. Faecal egg counts*

Individual faecal samples were taken directly from the rectum on the infection day, as well as 4 and 6 weeks after infection (FEC-4, FEC-6) respectively. The lambs were sampled twice in morning of the first two days of 4$^{th}$ and 6$^{th}$ week post infection. The presented FEC were calculated as the average of FECs of the two samples. Faecal samples were analysed using a modified McMaster method (Bairden, 1991) to quantify faecal egg counts with saturated NaCl as the floatation fluid. The eggs were counted with a sensitivity of 50 eggs per gram of faeces. All samples were analysed on the day of sample collection or the next day.

### *5.2.4. Necropsy procedure and abomasal measurements*

Abomasums of slaughtered lambs were dissociated from the omasum and the duodenum. It was washed with water and then tied off at the junction with the duodenum. The abomasum was then completely filled up with water, which thereafter was emptied in a 5 liter beaker to determine the abomasal volume. Adult worms were collected from the abomasum following the guidelines of the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) for evaluating the efficacy of anthelmintics in ruminants (Wood *et al*., 1995). The abomasum was opened along the greater curvature and washed under moderate water pressure. The washing was filled up to 2.5 liter and two aliquots (10 % of the total content each) were withdrawn from the bucket and mixed with 10 % formalin for examination later. All worms in these aliquots were counted and identified according to their sex and then the individual total worm counts were estimated. After collecting the worms, a digital photo of the opened abomasum was taken to determine the abomasal surface area by using the software Adobe Photoshop® CS2 (Jarou, 2009).

### *5.2.5. Statistical analysis*

Individual faecal egg counts (FEC) and worm burdens were square root transformed to normalise distributed data. Differences between morphological and parasitological parameters were analysed with the GLIMMIX procedure of SAS by using the following model: $\boldsymbol{Y_{ijkl(m)n} = \mu + G_i + S_j + (G * S)_k + B_l + LW_{(m)} + e_{ijk(l)mn}}$ ($Y_{ijkl(m)\ n}$ = observed value; μ = overall mean; $G_I$ = fixed effect of sires (i = TX, SU, GBM, IDF, ML); $S_j$ = fixed effect of sex (j = Female, Male); $(G * S)_k$ = interaction of genotype and sex; $B_l$ = fixed effect of birth type (l = singleton, Multiple); $LW_{(m)}$ = body weight at $12^{th}$ weeks was only used to analyse parasitological parameters (covariate); $E_{ijkl(m)n}$ = random error).

The Pearson correlation coefficient was used to determine phenotypic correlations among parasitological and morphological parameters. All the statistical analyses were performed with SAS (9.1).

## 5.3. Results and Discussion

### *5.3.1. Body and abomasal measurements*

Table 5.1 shows the least-squares means of body and abomasal measurements for the different genotypes and gender. No significant difference ($P > 0.05$) was observed in body weight of the crossbreds. There were no significant ($P > 0.05$) effects of genotype on loin girth, body length or abomasal surface area. ML lambs had higher ($P < 0.05$) wither height than SU x ML crossbred. Significant differences ($P < 0.05$) for shoulder width and heart girth were found only between TX × ML and ML, whereas, ML lambs had narrower shoulder and smaller heart girth than TX × ML. TX × ML had significantly ($P < 0.05$) larger abomasal volume than SU x ML, GBM x ML and IDF × ML. The abomasal volume of ML was not significantly different from those of the crossbreds ($P > 0.05$). Female lambs had lower body weights and smaller body sizes when compared with males. Significant differences ($P < 0.01$) could be observed between male and female lambs for body weight, wither height, heart girth, body length, abomasal volume and abomasal surface area. Male lambs showed higher values in all measured body parameters which is in agreement with previous reports (Afolayan *et al*., 2006; Sowande and Sobola, 2008).

All body and abomasal measurements were highly ($P < 0.001$) correlated with liveweight at $18^{th}$ weeks of age (Table 5.2). Body traits exhibited positive correlations with each other (0.39 - 0.80, $P < 0.001$). Similar findings were reported earlier (Fourie *et al*., 2002; Janssens and Vandepitte, 2004). Liveweight was highly correlated with heart girth (0.80, $P \leq 0.001$). In previous studies, heart girth has already been suggested as the best indicator of body weight (Fourie *et al*., 2002; Janssens and Vandepitte, 2004; Sowande and Sobola, 2008).

**Table 5.1** Least squares means (± S.E.) for body and abomasal measurements of lambs by gender and sires - TX, SU, GBM, IDF and ML (BW at wk18 of age, WH, SW, HG, LG, BL, AV and AS)

| | **BW (Kg)** | **WH (cm)** | **SW (cm)** | **HG (cm)** | **LG (cm)** | **BL (cm)** | **AV (liter)** | **AS ($cm^2$)** |
|---|---|---|---|---|---|---|---|---|
| **Sires** | | | | | | | | |
| **TX** | 44.6 ± 1.21 | 65.0 ± 0.73 $^{ab}$ | 16.7 ± 0.39 $^{a}$ | 81.8 ± 0.99 $^{a}$ | 78.0 ± 1.45 | 74.6 ± 1.11 | 3.01 ± 0.13 $^{a}$ | 761 ± 22.4 |
| **SU** | 43.2 ± 1.25 | 63.8 ± 0.76 $^{a}$ | 16.0 ± 0.40 $^{ab}$ | 79.8 ± 1.03 $^{ab}$ | 75.1 ± 1.41 | 75.1 ± 1.14 | 2.54 ± 0.14 $^{b}$ | 716 ± 23.2 |
| **GBM** | 43.0 ± 1.41 | 65.8 ± 0.85 $^{ab}$ | 15.9 ± 0.45 $^{ab}$ | 80.0 ± 1.15 $^{ab}$ | 78.8 ± 1.47 | 75.9 ± 1.27 | 2.64 ± 0.15 $^{b}$ | 704 ± 26.0 |
| **IDF** | 42.7 ± 1.34 | 64.0 ± 0.81 $^{ab}$ | 15.8 ± 0.43 $^{ab}$ | 80.7 ± 1.09 $^{ab}$ | 77.6 ± 1.44 | 73.9 ± 1.22 | 2.57 ± 0.14 $^{b}$ | 711 ± 24.7 |
| **ML** | 42.2 ± 1.24 | 66.0 ± 0.75 $^{b}$ | 15.6 ± 0.40 $^{b}$ | 79.0 ± 1.02 $^{b}$ | 75.0 ± 1.49 | 76.1 ± 1.13 | 2.77 ± 0.14 $^{ab}$ | 754 ± 23.0 |
| **Sex** | | | | | | | | |
| **Male** | 46.2 ± 0.86 $^{A}$ | 66.5 ± 0.52 $^{A}$ | 16.3 ± 0.28 | 81.6 ± 0.66 $^{A}$ | 78.0 ± 0.91 | 76.7 ± 0.78 $^{A}$ | 2.93 ± 0.09 $^{A}$ | 765 ± 15.8 $^{A}$ |
| **Female** | 40.1 ± 0.88 $^{B}$ | 63.4 ± 0.53 $^{B}$ | 15.7 ± 0.28 | 78.9 ± 0.67 $^{B}$ | 75.8 ± 0.93 | 73.6 ± 0.80 $^{B}$ | 2.48 ± 0.09 $^{B}$ | 693 ± 16.3 $^{B}$ |

TX: Texel; SU: Suffolk; GBM: German Blackhead Mutton; IDF: Ile de France; ML; Merinoland; WH: wither height; SW: shoulder width; HG: heart girth; LG: loin girth; BL: body length; AV: abomasal volume; AS: abomasal surface.

$^{a,b}$: Different letters in the same column indicate significant differences between the genotypes ($P \leq 0.05$)

$^{A,B}$: Different letters in the same column indicate significant differences between male and female lambs ($P \leq 0.01$)

The correlation coefficients of abomasal volume and surface area with most body parameters were statistically significant ($P \leq 0.05$) (Table 5.2). The values of these correlations were in most cases lower than the correlation coefficients among body measurement traits. That, to some extent, can be expected particularly during growth. In general, the growth of tissues and organs of the body is allometric or proportional to each other (Marple, 2003). The body and abomasal size may grow not in the same rapidity or same ratio. According to Lyford (1988), the proportion of abomasal to body weight decreased from 6.3 g/kg in newborn lambs to 3.7 g/kg in adult animals. The correlations of loin girth with abomasal size were low and not significant in the case of abomasal surface area.

The measure of loin girth is not dependent on the skeleton dimensions. This may be a weakness of this measurement when compared with other body measurements. But the strong correlations of loin girth with other body measurements support that it as a reliable indicator for body size. However, why the loin girth did not significantly relate to the abomasal surface area cannot be explained. Abomasal size was highly correlated with body weight. This supports the findings of earlier studies in cattle (Huber, 1969; Clausen, 1999). The correlation between abomasal volume and abomasal surface area was 0.79 ($P < 0.001$). May be the variations in fold thickness which affect the volume of abomasum have an impact on the value of this parameter.

**Table 5.2** Phenotypic correlations among different parameters of body and abomasal measurements in lambs

| | **AV** | **AS** | **WH** | **SW** | **HG** | **LG** | **BL** |
|---|---|---|---|---|---|---|---|
| **AS** | 0.79 *** | | | | | | |
| **WH** | 0.33 *** | 0.31 *** | | | | | |
| **SW** | 0.36 *** | 0.36 *** | 0.49 *** | | | | |
| **HG** | 0.41 *** | 0.33 *** | 0.57 *** | 0.73 *** | | | |
| **LG** | 0.24 * | 0.12 | 0.50 *** | 0.53 *** | 0.65 *** | | |
| **BL** | 0.27 ** | 0.22 * | 0.49 *** | 0.55 *** | 0.62 *** | 0.39 *** | |
| **BW** | 0.55 *** | 0.43 *** | 0.62 *** | 0.70 *** | 0.80 *** | 0.69 *** | 0.65 *** |

WH: wither height; SW: shoulder width; HG: heart girth; LG: loin girth; BL: body length; AV: abomasal volume; AS: abomasal surface; BW: body weight at wk 18 of age
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

### *5.3.2. Parasitological parameters*

All lambs were free from helminthes before infection as confirmed by the nematode egg free samples on the infection day. The total number of worms varied from 280 to 3505, with a mean of 2138 (± 754). The worms sex ratio (female:male) was 0.91:1.0. High variation in worm burden among lambs was observed in the presented study, but such variations are generally not unexpected despite the lambs being experimentally infected. Many earlier studies reported high variation among individuals following an experimental infection with *H. contortus* in lambs; e.g. establishment rates ranged from 4% to 28% in 3-month old lambs infected with 10000 third larvae of *H. contortus* (Lacroux *et al.,* 2006). Mean FEC-6 (5596 ± 3077) was lower ($P < 0.05$) when compared with mean FEC-4 (8134 ± 3698) (Table 5.3). This observation agrees with those reported earlier in Merinoland lambs (Gauly *et al*., 2002). The peak of eggs excretion of *H. contortus* was observed on the 4$^{th}$ and 5$^{th}$ weeks post infection in two different breeds (Shakya, 2007). Estimated correlations ($P < 0.001$) were positive among all parasitological parameters (Table 5.3). The correlation coefficient between FEC-4 and FEC-6 was 0.55. Correlations between total number of worms and square root FEC were 0.69 and 0.51, respectively.

**Table 5.3** Mean (±SD) and phenotypic correlations* among parasitological parameters after an experimental infection with 5000 *H. contortus* L3 in lambs

| | FEC-4 | FEC-6 | Total worms |
|---|---|---|---|
| **FEC-4** | 8134 ± 3698 [a] | | |
| **FEC-6** | 0.55 | 5596 ± 3077 [b] | |
| **Total worms** | 0.69 | 0.51 | 2138 ± 754[†] |

[†] mean of establishment rate: 42.7 (± 15.1)
* All correlation coefficients are significant at $p \leq 0.001$
[a,b] Denote significant difference ($P \leq 0.05$) between faecal eggs counts at 4 and 6 weeks post infection

Effects of genotypes of lambs on FEC were generally insignificant, but the Merinoland lambs seems to be more susceptible to infection with *H. contortus* when compared ($P < 0.05$) with other crossbreds (Table 5.4) according to worm burden comparison. Hielscher *et al.* (2006) reported that the crossbreed lambs of Rhoen × Merinoland are more resistant against *H. contortus* infection compared to purebreds and Merinoland × Rhoen. Therefore, we can suggest that the crossbreeding of ML ewes with sires of other breed produce lambs that are probably more resistant against nematode infections than ML. However, studies have shown that crossbreds could be sometimes relatively resistant than the purebreds especially than the susceptible breed (Amarante *et al.*, 1999; Li *et al.*, 2001). Type of birth and liveweight at infection time as well as sex of lambs had no effect on any of the parasitological parameters (Table 5.4). Many studies reported that male sheep are more susceptible than females to natural or experimental nematode infections around or after age of puberty (Courtney *et al.,* 1985; Barger, 1993).

**Table 5.4** Effects of sires, sex, birth type and live weight at wk12 of age on transformed faecal egg counts (FEC-4; FEC-6) and worms burden after an experimental infection with 5000 *H. contortus* L3 in lambs

| | FEC-4 | FEC-6 | Total worms |
|---|---|---|---|
| ***Sires*** | | | |
| **TX** | 82.6 ± 5.35 | 72.7 ± 5.45 | 43.8 ± 2.15 [b] |
| **SU** | 86.6 ± 5.24 | 72.6 ± 5.53 | 43.9 ± 2.22 [b] |
| **GBM** | 88.6 ± 6.01 | 65.7 ± 6.24 | 43.1 ± 2.47 [b] |
| **IDF** | 83.2 ± 5.56 | 71.1 ± 5.87 | 42.6 ± 2.32 [b] |
| **ML** | 93.4 ± 5.22 | 69.9 ± 5.52 | 50.7 ± 2.24 [a] |
| ***P-values*** | | | |
| **Sires** | 0.581 | 0.892 | 0.074 |
| **Sex** | 0.492 | 0.741 | 0.745 |
| **Sires × sex** | 0.623 | 0.951 | 0.048 |
| **Birth type** | 0.631 | 0.583 | 0.506 |
| **Live weight*** | 0.501 | 0.600 | 0.760 |

[a,b]: Values with no common superscripts within a factor indicate significant differences ($P < 0.05$)
* live weight at wk12 of age

### *5.3.3. Relation between morphometric and parasitological parameters*

Pearson's correlation coefficients of faecal egg counts and worm counts with body and abomasal size measurements are presented in Table 5.5. The correlations between body measurements and parasitological parameters have been further weakened and not reliably different from zero, except the correlations between FEC-4 and loin girth, heart girth and body weight at 18 weeks of age ($P < 0.05$). These negative relations might indicate anorexia, which is a common response to parasite infection (Kyriazakis *et al.,* 1998). The second faecal samples (FEC-6) were collected at the same time when body dimensions were determined, therefore, FEC-6 and worm burden may give a more reliable statement about the infection status to estimate the correlations with body measurement than FEC-4. But the FEC-6 and worm burden do not show any significant relation with body parameters. Fecal egg count at 4 weeks post-infection was higher than the corresponding value at 6 weeks post-infection probably due to high worm recovery 4 weeks post-infection. This could also explain the differences in the relations of FEC-4 and FEC-6 with body measurements especially body weight. However, these results were not in agreement with the results obtained in a study performed by Coltman *et al*. (2001). The authors reported a negative correlation between hindleg length and FEC following natural infection in Soay sheep, which is a relatively ancient sheep breed and may differ from other more intensively selected breeds.

Since the abomasum is the host organ of *H. contortus*, thus the relation between the size of abomasum and parasitological parameters is more important than other relations. To the best of our knowledge, this relation was previously not detected in sheep. In the present study, the size of abomasum did not influence the establishment rate of *H. contortus*. Where, the abomasal volume and surface area did not significantly correlate with worm counts (0.06-0.16; $P>0.05$), respectively. As well as, the abomasal size did not significantly correlate with faecal egg counts at 4 and 6 week post infection (-0.17 to 0.13; $P>0.05$). The worm fecundity, as previously proved, is associated with the size of adult worm (Rowe *et al*., 2008). Therefore, we can suggest that the size of worms was not relevant to the abomasal volume depending on the weak correlations of FEC with volume and surface area of abomasum. This indicates that the Harrison's rule is not suitable to interpret the interaction between *H. contortus* and abomasal size.

**Table 5.5** Phenotypic correlation coefficients of parasitological parameters with body and abomasal measurements traits in lambs

| | FEC-4 | FEC-6 | Total Worms |
|---|---|---|---|
| **AV** | -0.17 | 0.02 | 0.06 |
| **AS** | -0.10 | 0.13 | 0.16 |
| **WH** | -0.14 | 0.02 | 0.05 |
| **SW** | -0.10 | 0.07 | 0.01 |
| **HG** | -0.24 * | -0.17 | -0.12 |
| **LG** | -0.20 * | -0.15 | -0.07 |
| **BL** | -0.16 | -0.05 | -0.09 |
| **BW** | -0.26 * | -0.09 | -0.07 |

WH: wither height; SW: shoulder width; HG: heart girth; LG: loin girth; BL: body length; AV: abomasal volume; AS: abomasal surface; BW: body weight at wk 18 of age
* $p \leq 0.05$

In theoretical predictions, the size of habitat can probably affect the establishment and productivity of parasites depending on food and space availability. Likewise, Morand *et al.* (2000) suggested that the size of parasites may be determined by some physical dimensions of habitat. Thus, a positive relation between abomasal size and parasitological parameters of *H. contortus* should be expected, but the results of the present study did not support this suggestion. The Merinoland lambs had high number of total worms even though they did not differ significantly from crossbreds in abomasal volume and surface area. In addition, the Texel lambs had a bigger abomasal volume in comparison to that in other crossbreds, where no significant differences in parasitological parameters could be observed among crossbreds. However, it may be possible that the different intensity of parasitic infections (e.g. increased worm burden) may change this outcome. Following an experimental infection with *H. contortus*, Yadav *et al.* (1993) reported a significant difference in abomasal volume between the Hisardale lambs, which were more susceptible to infection, and Munjal lambs. The abomasal volume was higher in Hisardale lambs when compared to that in Munjal lambs.

However, there was no clear evidence that the difference in abomasal volume affected the infection parameters. Whereas, the abomasal volume of control lambs was not compared between breeds and the relation between infection parameters with the abomasal volume was also not estimated. The organ size is limited by number and size of their cells (Conlon and Raff, 1999). Hence, the larger abomasum is constructed from larger or more cells in comparison to small ones. That may affect the local response of host against infections especially the cellular response. Furthermore, the parasites can change the environment of their organ host probably to create ideal conditions for their survival and reproduction; e.g. the fecundity of *H. contortus* related to abomasal acidity and their optimum pH range from 4 to 4.5 (Honde and Bueno, 1982). Abomasal nematodes can decrease acid secretion and increase serum gastrin and pepsinogen concentrations in the abomasum (Simpson, 2000). However, it is not clear if the nematode at different levels of infection can similarly alter the environment of the different size abomasum. Therefore, the interaction between abomasal size and *Haemonchus* probably is more complicated to be understood only by estimating a simple relation. Additional research with histological and immunological investigation is therefore required.

Lo *et al*. (1998) reported a positive relationship between host size and common endoparasite abundance in the digestive system of fish. The authors suggested that the larger fish consume more food and are more liable to infection. This suggestion is excluded for this study due to the experimental infection. Therefore, the result of this study should be applied cautiously under natural grazing conditions, where the body size can probably affect grazing behaviour or host dietary habits which, in turn, can influence the intake of infective larvae.

## 5.4. Conclusion

The results indicate that there is no direct relation between body size measurements and parasite infection in lambs of different genotypes, which were experimentally infected with *H. contortus*. Furthermore, the abomasal volume and surface area apparently do not indicate significant differences in establishment or fecundity of *H. contortus*, which suggest that the size of abomasum is not suitable to explain the variation in the susceptibility of lambs to *Haemonchus* infection

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# CHAPTER VI

## General discussion and conclusion

## 6.1. Prevalence of gastrointestinal nematodes

The qualitative and quantitative prevalence of gastrointestinal nematode (GIN) infections in lambs of different sheep breeds based on faecal egg counts (FEC) was investigated in the current study (**Chapter II**). The nematode infections were moderately prevalent, where 63% of the examined lambs being infected with at least one species of GIN. The intensity of infection varied from low to moderate.

The factors that influenced the faecal egg output are related to the host animal itself or to the environment which the host animal or parasite is exposed. Older lambs were less susceptible to nematode infection than younger lambs. The development of acquired immunity to nematode infection, as indicated with lower FECs, is positively related with the age of the animals (Kambara *et al.,* 1993; Kambara and McFarlane, 1996). However, weaning, live weight, fat reserves or nutritional factors that are associated with age might also contribute to this positive relation between age and parasite resistance (McClure, 2000). Male lambs were more susceptible to infections than females. That may be due to differences in behaviour, morphology or physiological and hormonal status of both sexes (Zuk and McKean 1996; Gauly et al. 2006). Singleton lambs were less susceptible to nematode infections in comparison to multiple born lambs. This may be explained by better rearing and nutrition conditions for singleton lambs (Romjali *et al.,* 1997; Haile *et al.* 2007).

The faecal egg counts varied significantly ($P < 0.001$) among the farms. This effect may be due to the differences in farm management including pasture management, nutrition, flock size, etc. The pasture management can reduce the contamination of pastures with infective larval stages (Bukhari and Sanyal, 2011) by using a rotation system (Burke *et al.,* 2009), alteration between species (Marley *et al.,* 2006), lowering the stocking density (Stear *et al.,* 2007), avoiding different age groups grazing the same pasture or moving dewormed animals to clean pastures (Barger, 1997). However, pasture management may have a limited impact as herd size increases. The differences in the quality of nutritional factors among farms may also contribute to the variation observed in nematode infections. It has been reported that supplementary feeding with protein improves the development of immunity of lambs as well as their resilience to parasites infections (Strain and Stear, 2001; Haile *et al.,* 2002; Louvandini *et al.,* 2006).

### 6.2. Selective treatment

Changing the strategy of animal drenching became inevitable due to the high spread of parasite resistance to anthelmintic drugs, which has been observed worldwide (Wolstenholme *et al.,* 2004). The major distribution of nematode worms in an infected host population is over-dispersed in a small percentage of animals (Barger, 1985; Hoste *et al.,* 2001a). This is in agreement with the results of the present study (**Chapter III**). Only 22% of the examined lambs required drenches according to the suggested trigger level for drenching (400 EPG). Anderson and May (1982) suggested that the treatment of heavily infected hosts (8% of the population) can theoretically reduce the mean worm burdens by up to 50% when the worms are highly clumped. Therefore, using selective treatment could be considered as an economic approach. In addition it effectively slows down the spread of the anthelmintic resistance (AR) in sheep farms.

FEC has been widely used to determine lambs requiring an anthelmintic treatment. However, the time- and money-intensiveness of FEC may limit the potential benefit of the selective treatment. Therefore, drenching all animals may be more favourable for the farmers than selective treatment based on individual faecal analysis. The FAMACHA© score, body condition score (BCS), dag score (DS) and faecal consistency (FCS), which are simpler and cheaper than FEC, were determined in the present study and served as potential infection indicator traits. The use of these traits were evaluated, either separately or combined, to detect animals in need of drenching when exposed to natural nematode infections. The estimated correlations between these traits and FEC, and their sensitivities were low. The sensitivities of the combinations of these traits were higher than that of each indicator alone and positively associated with the intensity of infection. In any case, the obtained values did not reach the acceptable limit to recommend using of these traits as a safe indicator for drenching decisions of lambs under moderate levels of mixed natural nematode infections. The determination of infection intensity in the present work was based on FEC, which probably does not perfectly correlate with the worm burden of animals infected with various nematode species (Amarante, 2000). Further research to examine these indicator traits according to the worm burden is therefore required.

Despite the relative high costs of individual faecal analysis, the application of selective treatment based on FEC can be recommended. In the long-term this strategy can reduce the worm population and keep the current anthelmintics effectively. Further developing new anthelmintic drugs will probably be more expensive than using of this approach. However, supporting the farmers by their governments to apply the selective treatment may be required.

### 6.3. Genetic variations between and within breeds

Due to the increasing development of AR in nematodes of small ruminants and the increasing the number of organic farms, non-chemical alternative strategies to anthelmintics to control nematodes have been became an urgent necessity. The use of the genetic variation in resistance against GIN to selection either between or within breeds as well as crossbreeding for an improved host resistance is one of the known alternative strategies (Nicholas, 1987; Gray, 1997; Amarante *et al.,* 2004). The advantage of breeding for resistance results in the reduction of worm burdens, which in turn can reduce the impact of parasites on the productivity of hosts as well as the utilizing of anthelmintics and the contamination of the pastures by infective larvae (Gray, 1997).

No significant differences could be observed between the Merionland (ML), German Blackhead (GBM), Texel and Rhoen sheep breeds in susceptibility to nematode infection under natural conditions in Germany (**Chapter II**). Under conditions of an experimental infection with *H. contortus*, Gauly et al. (2002) reported that Merinoland had a lower FEC compared to Rhoen sheep, even though the worm burden did not differ significantly. The variation among animals in the resistance to nematode infections may partly be due to the difference in their feeding behaviors (Hoste *et al.,* 2001b). This cannot be observed under conditions of artificial infections performed in non-pasture systems. In addition, the genotype x environment interactions may not appear evidently in the case of the artificial infection, where the infected animals are mostly kept under strict experimental conditions. Beyond these, the responses of lambs to mono- or mixed-infections may differ.

Previous studies that focused on the responses of crossbreeds to the resistance against nematodes in Germany have been performed under conditions of experimental infections. These studies report that the crossbreeds of some meat sheep breeds and Rhoen sheep with

ML have been found to be relatively resistant than ML purebred lambs (Hielscher *et al.* 2006; Herrmann, 2010). The results of present study (**Chapter V**) support these findings. However, more studies are needed to investigate the effect of crossbreeding on the productivity as well as the resistance to nematode infection under natural condition and in successive host generations.

The results presented in **Chapter II** indicate high inter-individual variations in FEC of all examined breeds. These variations indicate differences in host animal resistance against GIN infections, which are partly due to the variety in the genetic background of the animals (**Chapter IV**). However, the differences in body size parameters or abomasal sizes cannot explain the variations between animals in susceptibility to nematode infections (**Chapter V**).

The estimated heritabilities of FEC in the current study were relative high in ML (0.59), Merino Lang-wool (MLW) (0.37) and Texel (0.39) breeds. These values are in the same range of many earlier reports (Gauly and Erhardt, 2001; Pollot *et al.*, 2004; Lobo *et al.*, 2009). The estimated heritabilities indicate that breeding for an improved nematode resistance (low FEC) by selection under field conditions in Germany in these breeds is possible. The genetic relationships of FEC with production traits were not investigated in the present work. Bishop and Stear (2003) assumed that the performance traits are not genetically related to the resistance against parasite infections. In agreement with Bishop and Stear (2003) others reported that genetic relations between performance traits and resistance is low or unfavorable (Eady et al. 1998; Mandonnet *et al.,* 2001; Vagenas *et al.* 2002; Vanimisetti *et al.* 2004). Nevertheless, the relationships between FEC and production traits vary between different geographical regions (Stear *et al.,* 2007). Further research to estimate this relationship in German sheep is therefore required.

The heritability estimates for the potential infection indicator traits were high in some cases. For example, the heritability of DS in MLW was 0.50 and BCS in Texel was 0.55. However, FAMACHA, BCS, DS and FCS did not show consistence for the genetic correlations with FEC among breeds. These correlations were generally unfavourable and the estimated values had high standard errors. This may be due to low intensity of nematode infections. Therefore, it can be suggested that the use of these traits in breeding programmes as indicators for nematode resistance under German conditions seem to be unsuitable.

### 6.4. Conclusion

From the results of this study it can be concluded that the natural infections with GIN of lambs in Germany are common, vary between farms, and are influenced by age, birth type and sex of the lambs. The intensity of GIN infections was low to moderate and involved multi-species infections. A high prevalence of *Eimeria* infections was detected and coccidia-free lambs appear to be less susceptible to nematode infections.

The traits DS, FCS, BCS and FAMACHA© cannot be recommended as indicator traits for making decisions whether lambs require drenching when the animals are moderately infected with different nematode species.

Furthermore, the body size measurements, abomasal volume and abomasal surface area apparently do not indicate significant differences in the establishment or fecundity of *H. contortus*. This suggests that the size of body or abomasum is not a suitable parameter to explain the variations in the susceptibility of lambs to *Haemonchus* infections.

According to the estimated heritability for FEC, it is possible to select ML, MLW, GBM and TX sheep for an improved nematode resistance (low FEC) under field conditions of mixed infection in Germany. The use of DS, FCS, BCS or FAMACHA© as indicator traits for nematode infection in breeding programmes for selecting resistant animals seems to be unsuitable.

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**Summary**

The objectives of the present study were to determine the prevalence of gastrointestinal nematodes (GIN) in naturally infected lambs of five sheep breeds based on individual faecal egg counts (FEC) in Germany, and to evaluate the predictable influence of birth type, age, sex and breed of lambs on the excretion of nematode eggs in the faeces. Furthermore, the use of potential infection indicator traits such as the dag score (DS), the faecal consistency (FCS), the body condition (BCS) and the FAMACHA©, either separately or combined, to detect individually lambs in need for drenching when exposed to natural nematode infections was investigated. Additionally, the possibility of breeding for resistance against GIN in German sheep breeds directly based on faecal egg counts (FEC) or indirectly by using DS, FCS, BCS and FAMACHA© was also evaluated. Moreover, the relationships between the nematode infection with *Haemonchus contortus* and body and organ measurements of lambs were estimated.

The field trials under natural conditions of nematode infections (**Chapters II, III** and **IV**) were carried out on 3,924 lambs of different breeds aged from 3 to 15 months. The breeds used were Merinoland (ML), German Blackhead Mutton (GBM), Texel (TX), Rhoen (RH), and Merino long-wool (MLW). Data collection took place on various farms. The samples were collected once during the grazing seasons in 2006, 2007 and 2008. At the time of faecal sample collection, the FAMACHA© score, the BCS, the DS and FCS were additionally determined and served as potential infection indicator traits. The lambs were not dewormed at least 45 days before the sampling time.

Sixty-three percent of the lambs were infected with GIN. The infections were mostly low to moderate and involved different species. *Trichostrongylus* spp. were the predominant species based on the percentage of larvae in the faecal cultures. Only 11.4% of faecal samples were *Eimeria* oocysts free. Tapeworm eggs were encountered in 13.2% of all samples. The farms affected the prevalence of GIN infections significantly ($P < 0.001$). Significantly higher FECs ($P < 0.05$) were observed in multiple lambs when compared with singletons. Moreover, male lambs were more susceptible to infection than females ($P < 0.001$). The FECs were negatively correlated with the age of the lambs ($P < 0.001$). No significant differences ($P > 0.05$) were observed between breeds in regard to FEC.

The decision of drenching based on each of the potential indicator traits yielded low sensitivities (13 to 39%) and high ratios of false negative results (13 to 19%). The sensitivities of the indicator combinations were higher (67 and 69%) than those for the individual indicator traits and were positively associated with the intensity of infection. However, the indicator combinations were not specific enough and had high ratios of false positives (37 and 39%).

The estimated heritabilities for FEC ranged from 0.22 to 0.59 among breeds. Heritabilities for the indicator traits DS, FCS, BCS and FAMACHA© ranged between 0.15 to 0.50, 0.16 to 0.24, 0.02 to 0.55 and 0.01 to 0.25, respectively. The phenotypic correlations of FEC with the potential infection traits were on a low level. A negative correlation was found between BCS and FEC in three breeds ($P \leq 0.01$). FEC did not show a consistent genetic correlation with the other traits and in most cases estimated values had high standard errors. Genetic correlations between DS an FCS were high ranging between 0.50 and 0.95.

For determining the correlations among parasitological and morphological parameters (**Chapter V**) 100 lambs from five different genotypes (ML, TX × ML, Suffolk x ML, GBM x ML and Ile de France × ML) were experimentally infected with 5,000 infective 3rd stage larvae of *H. contortus* at the time of weaning (12 weeks of age). Four and six weeks after infection, individual faecal samples were collected for estimation of FECs. Furthermore wither height, shoulder width, heart girth, loin girth and body length were recorded at eighteen weeks of age. Lambs were slaughtered and necropsied seven weeks post-infection, and worm counts, abomasal volume and surface area were determined.

Positive correlations were found between different body size parameters, body weights and abomasal sizes. FEC and worm counts were neither significantly correlated with body size parameters nor with abomasal size. The mean worm burden in ML lambs was higher than does in crossbred lambs, whereas there was no significant difference in abomasal size between ML and crossbred lambs.

**Zusammenfassung**

Die Ziele der vorliegenden Untersuchungen waren: 1. Bestimmung der Befallshäufigkeit von Magen-Darm-Strongyliden (MDS) bei Schafen in Deutschland auf Basis der individuellen Eizahl pro Gramm Kot (EpG). Besondere Berücksichtigung fand dabei der Einfluss von Geburtstyp, Alter und Geschlecht der Schafe; 2. Überprüfung der Eignung der Merkmale Dag Score (DS), Kotkonsistenz (FCS), Körperkondition (BCS) und FAMACHA©-Score als Indikatoren einer Magen-Darm-Strongyliden-Infektion; 3. Nachweis von Rasseunterschieden bei den Indikatormerkmalen einer Parasitenresistenz sowie die Schätzung der Erblichkeiten für diese Merkmale; 4. Darstellung des Zusammenhangs zwischen Etablierungsrate, verschiedenen Körpermaßen und Labmagengröße nach künstlicher Infektion mit *Haemonchus contortus*.

Die Untersuchungen wurden unter natürlichen Infektionsbedingungen (**Kapitel II, III** und **IV**) auf 21 Schafbetrieben an insgesamt 3924 Tieren im Alter von 3-15 Monaten der Rassen Merinolandschaf (ML), Schwarzköpfiges Fleischschaf (SKF), Texelschaf (TX), Rhönschaf (RH) und Merinolangwollschaf (MLW) durchgeführt. Während der Weidesaison wurde pro Tier jeweils eine Kotprobe rektal entnommen. Zeitgleich zur Kotprobennahme wurde bei allen Tieren DS, FCS, BCS und der FAMACHA©-Score beurteilt. Die Tiere wurden mindestens seit 45 Tage vor dem Zeitpunkt der Probenahme nicht mehr entwurmt.

In 63% der untersuchten Schaf-Kotproben konnten Eier von MDS nachgewiesen werden. Die Ausscheidungs-Intensität variierte von 50 bis 17.000 Eiern pro Gramm Kot (Mittelwert 315 ± 777). Mit einem Anteil von 52,8 % war *Trichostrongylus* spp. die vorherrschende Nematoden-Gattung. Eimerien-Oozysten wurden in 88,6 % der Proben nachgewiesen, Bandwurmeier in 13,2 % der Proben.

Es bestand eine negative Korrelation ($P < 0,001$) zwischen dem Alter der Tiere und den EpG-Werten. Der Zeitpunkt der Probennahme (Jahr / Saison) und die Betriebe hatten einen signifikanten Einfluss ($P < 0,001$) auf die Prävalenz von MDS. Ein signifikanter Einfluss des Geburtstyps ($P < 0.05$) und des Geschlechts ($P < 0.001$) auf die Höhe der Eiausscheidung konnte ebenfalls nachgewiesen werden. Beim Merkmal Eiausscheidung pro Gramm Kot (EpG) unterschieden sich die Rassen nicht signifikant ($P = 0.412$).

Die Entscheidung zur Entwurmung der infizierten Tiere auf Basis der untersuchten Indikatormerkmale ergab geringe Sensitivitäten (13 bis 39 %) und hohe Falsch-Negativ-Raten (13 bis 19 %). Bei Kombination der Indikatormerkmale erhöhte sich die Sensitivität auf 67 und 69 %. Allerdings waren die kombinierten Indikatormerkmale nicht ausreichend spezifisch und zeigten eine erhöhte Falsch-Positiv-Rate von 37 bis 39 %.

Für das Merkmal EpG wurden für die verschiedenen Schafrassen Heritabilitäten im Bereich von 0,22 bis 0,59 geschätzt. Die geschätzten Heritabilitäten für die anderen Indikatormerkmale lagen bei 0,15 bis 0,50 für DS, bei 0,15 bis 0,24 für FCS, bei 0,02 bis 0,55 für BCS sowie 0,01 bis 0,25 für FAMACHA©. Bei Ml, MLW und TX waren BCS und die EpG-Werte negativ korreliert ($P \leq 0,001$). Die phänotypischen Korrelationen zwischen EpG und den anderen Indikatormerkmalen waren niedriger. Ebenso zeigte EpG keine konsistenten genetischen Korrelationen mit den untersuchten Indikatormerkmalen. In den meisten Fällen hatten die Schätzwerte einen hohen Standardfehler.

Um den Zusammenhang zwischen den morphologischen und parasitologischen Parametern zu ermitteln (**Kapitel V**), standen insgesamt 100 Schaflämmer auf der Lehr- und Forschungsstation (Oberer Hardthof) des Institutes für Tierzucht und Haustiergenetik der Justus-Liebig-Universität Gießen zur Verfügung. Die Tiere hatten folgende Genotypen: Texel × Merinoland (ML), Suffolk x ML, Schwarzköpfiges Fleischschaf x ML, Ile de France × ML und ML. Im Alter von 12 Wochen wurden die Lämmer abgesetzt und mit *H. contortus* Drittlarven (L3) oral infiziert. Vier und sechs Wochen nach der Infektion wurden von jedem Tier Kotproben entnommen und die EpG-Werte bestimmt. Im Alter von 18 Wochen wurden Körpergewicht, Schulterhöhe, Schulterbreite, Brustumfang, Bauchumfang und Körperlänge der Lämmer erfasst. Eine Woche später erfolgte die Schlachtung der Tiere. Es wurden die Würmer im Labmagen gezählt sowie Labmagen-Volumen und -Fläche bestimmt.

Signifikant positive Korrelationen wurden zwischen dem Körpergewicht, den Körpermaßen und der Labmagengröße berechnet. Die Wurmzahl und die EpG-Werte waren mit den Körper- und Labmagengrößen nicht signifikant korreliert. Die ML-Reinzuchttiere zeigten die signifikant höchste mittlere Wurmzahl und unterschieden sich signifikant von den Kreuzungstieren. In der Labmagengröße konnten keine signifikanten Unterschiede zwischen ML und den Kreuzungstieren gefunden werden.

Aus den vorgelegten Ergebnissen kann gefolgert werden, dass eine natürliche Infektion mit Magen-Darm-Strongyliden bei Jungschafen in Deutschland häufig, und zwar als Mischinfektion vorkommt. Die Intensitäten der MDS-Infektionen liegen im mittleren Bereich und sind von der Jahreszeit, dem Geburtstyp, dem Alter und vom Geschlecht der Tiere abhängig. Die Merkmale Dag Score, Kotkonsistenz, Körperkondition und FAMACHA©-Score sind als Indikatoren für den Grad eines Magen-Darm-Strongylidenbefalls unter natürlichen Infektionsbedingungen eher ungeeignet. Darüber hinaus konnte kein relevanter Einfluss der Körper- und Labmagengröße auf die Etablierungs- und Fruchtbarkeitsrate von *H. contortus* nachgewiesen werden. Die Körpermaße scheinen demnach nicht für die Unterschiede zwischen verschiedenen Genotypen hinsichtlich Wurmanzahl und/oder Eiausscheidung verantwortlich zu sein. Die geschätzten Heritabilitäten der Eizahl pro Gramm Kot bei Merionland-, Merinolangwoll-, Texel und Schwarzköpfiges Fleischschafen zeigen die grundsätzliche Möglichkeit der Zucht auf Parasiten-Resistenz basierend auf EpG-Werten unter natürlichen Infektionen.

## List of PhD-related publications

### *Journal papers*

Idris, A., Moors, E., Sohnrey, B., Gauly, M. 2011. Gastrointestinal nematode infections in German sheep. Parasitology Research, *under review*.

Idris, A., Moors, E., Budnick, C., Herrmann, A., Erhardt, G., Gauly, M. 2011. Is the establishment rate and fecundity of *Haemonchus contortus* related to body or abomasal measurements in sheep? Animal 5, 1276-1282.

### *Conference proceedings*

Idris, A., Moors, E., Erhardt, G., Gauly, M. 2011. Genetic resistance to natural gastrointestinal nematode infections in German sheep breeds. 62$^{nd}$ Annual Meeting of the European Association for Animal Production. Book of Abstracts, Page (1)149. Stavanger, Norway.

Idris, A., Moors, E., Erhardt, G., Gauly, M. 2011. Is the establishment rate and fecundity of *Haemonchus contortus* related to body or abomasal measurements in sheep? 62$^{nd}$ Annual Meeting of the European Association for Animal Production. Book of Abstracts, Page (2)149. Stavanger, Norway.

Idris, A., Moors, E., Gauly, M. 2011. Einsatz indirekter Hilfsparameter zur Erkennung von Schaflämmern für eine selektive anthelminthische Behandlung. DVG-Tagung, Fachgruppe Parasitologie und parasitäre Krankheiten. Berlin, Germany

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**Curriculum Vitae**

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## *WORK ACTIVITIES & EXPERIENCES*

| | |
|---|---|
| 2001 – 2006 | Teaching assistant (Sheep Breeding) in Faculty of Agriculture - Univ. of Damascus |
| 2000 – 2001 | Agricultural engineer in Ministry of Agriculture- Damascus. |

## *EDUCATION*

| | |
|---|---|
| 2006 - 2011 | Doctoral student, Department of Animal Sciences, Livestock Production Group, Georg-August-University, Göttingen, Germany |
| 1993 – 1999 | Special License in Agricultural Engineering "Animal Production" (Faculty of Agriculture - Univ. of Damascus) |
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