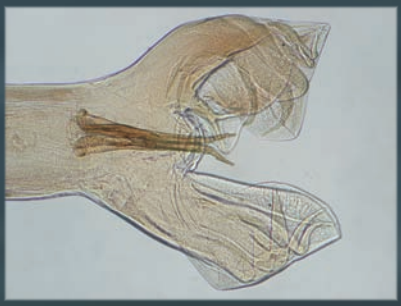
ANTHELMINTIC RESISTANCE OF *HAEMONCHUS CONTORTUS* AND THE FAMACHA©-METHOD AS A TOOL TO DELAY THE DEVELOPMENT OF ANTHELMINTIC RESISTANCE

MIRIAM SCHEUERLE

INAUGURAL-DISSERTATION
zur Erlangung
der veterinärbiologischen Doktorwürde
(Dr. rer. biol. vet.)
der Tierärztlichen Fakultät der
Ludwig-Maximilians-Universität München



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von

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In Erinnerung an meinen Opa

(1929-2008)

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ABBREVIATIONS

ABZ	Albendazole
BZ	Benzimidazoles
Cl	95% confidence limit of the mean
EHA	Egg Hatch Assay
epg	Eggs Per Gram faeces
EPR	Eprinomectin
ESSR	Extension and health service for small ruminants (Beratungs- und Gesundheitsdienst für Kleinwiederkäuer, BGK, Schweiz)
FAMACHA	Faffa-Malan-Chart
FAMs	FAMACHA score
FEC	Faecal Egg Count
FECR	Faecal Egg Count Reduction [%]
FECRT	Faecal Egg Count Reduction Test
FBZ	Fenbendazole
FNF	False negative fraction
FPF	False positive fraction
GIN	Gastrointestinal nematodes
L1	First stage larvae
L2	Second stage larvae
L3	Third stage larvae
L4	Fourth stage larvae
LEV	Levamisole
LMA	Larval Migration Assay
ML	Macrocyclic lactone
MOX	Moxidectin
NPV	Negative Predictive Value
PCV	Packed Cell Volume, i.e. Haematocrit
PPV	Positive Predictive Value
SD	Standard deviation
SE	Standard error
TNF	True Negative Fraction (sensitivity)
TPF	True Positive Fraction (specificity)
TST	Targeted Selective Treatment
WAAVP	World Association for the Advancement of Veterinary Parasitology

GLOSSARY

Anthelmintic	Pharmaceutical compound that is effective against helminth infections
FECRT	Faecal egg count reduction test: test to determine the reduction of eggs per gram faeces after a certain time post-treatment
In refugia	Parasites of a population, which are not reached by an anthelmintic treatment, i.e. eggs and larvae on the pasture
Resistance	Ability of a population to survive doses of a treatment that would be lethal to a susceptible population
Side resistance	The phenomenon that parasites resistant to one drug of a chemical class are also resistant to other drugs of the same class with a similar mode of action.
Targeted Selective Treatment	Part-flock anthelmintic treatment for only those animals in need of such a treatment (Cabaret, 2008).
Trichostrongylidosis	Infection caused by gastrointestinal nematodes

PRESENTATIONS

1. Scheuerle M.: „Monitoring und Management von Magen-Darm-Parasiten bei kleinen Wiederkäuern.“ Institut für veterinärmedizinische Untersuchungen, AGES; Innsbruck, Österreich, 16.04.07; oral presentation.

2. Scheuerle M., Pfister K.: „Resistenzen von *Haemonchus contortus* bei kleinen Wiederkäuern in Süddeutschland und der Schweiz.“ Tagung der Schafherden-gesundheitsdienste, Bad Sassendorf, 25.-26.09.07; oral presentation.

3. Scheuerle M., Pfister K.: „Three small ruminant flocks with anthelmintic resistance of *Haemonchus contortus* in Southern Germany and Switzerland.“ 6. Wissenschaftliche Türkisch-Deutsche Tagung, Tierärztliche Fakultät München 10./11.04.08; poster.

4. Scheuerle M., Pfister K.: „Resistenzen von *Haemonchus contortus* bei kleinen Wiederkäuern in Süddeutschland und der Schweiz.“ Tagung der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie, Innsbruck 20.-22.11.08; oral presentation.

5. Scheuerle M.,Mahling M., Pfister K.: „Die FAMACHA©-Anämieskala als Mittel zur Bestimmung des *Haemonchus contortus*-Befalls in Schweizer Ziegenherden“. DVG-Parasitologische Fachgespräche, Leipzig 17.-19.06.09; oral presentation.

INTRODUCTION AND OBJECTIVES

Infections with gastrointestinal nematodes (GIN) such as *Haemonchus contortus* are major causes of economic losses in small ruminant husbandry due to retarded growth, weight loss, disorder in fertility, loss in milk production and mortalities (Loyacano, 2002). These problems are enhanced by an increasing number of GIN species becoming resistant against anthelmintics. The frequent use of the three main anthelmintic classes, benzimidazoles (BZ), imidazothiazoles and macrocyclic lactones (ML), lead to an increasing development and spreading of resistance. In order to delay the appearance of resistance and to hopefully restore anthelmintic efficacy in the future, there is an urgent need to find alternatives and additions to pharmaceutical treatment. Targeted and selective treatments of individual animals are possibilities to reduce frequency of anthelmintic treatment and to maximise GIN populations in refugia. The FAMACHA©-method is an easy and practical on-farm method to identify animals in need of treatment in areas with a high proportion of *H. contortus*.

Hence, the present thesis was initiated with the following objectives:

(1) to get an overview of the gastrointestinal parasites of small ruminants in Southern Germany,
(2) to determine the status of anthelmintic efficacy in the selected flocks,
(3) to test the FAMACHA©-method as a possibility of minimising the application of anthelmintics with a view to manage GIN infections and
(4) to evaluate the prevalence of *H. contortus* and its role in the spreading of anthelmintic resistance.

LITERATURE REVIEW

1. Gastrointestinal parasites in small ruminants

Gastrointestinal parasite infections cause severe economic losses in wool, meat and milk production. Heavy infections can lead to acute clinical diseases and eventually to mortalities. Many species of nematodes, cestodes and coccidia cause parasitic gastritis and enteritis in sheep and goats. Trematode infections lead to damages of the liver. Expenses for treatment and prevention are a massive burden in animal husbandry.
The most important and frequent gastrointestinal parasites of small ruminants in Europe are listed on the following pages.

1.1. Gastrointestinal nematodes

The focus of the present thesis is on the nematodes residing in the gastrointestinal tract of small ruminants. These gastrointestinal nematodes (GIN) globally occur in sheep and goats. Depending on the local climatic conditions, different GIN species regionally predominate. They all have direct life cycles (fig. 1), which are similar in all species and enable the worms to be readily transmissible in livestock. For each of them the prepatent period is approximately 20 days. Adult worms live in the abomasum and the small intestine. The female worms lay eggs, which are excreted into the environment with the faeces. Under appropriate conditions, the eggs develop into first stage larvae (L1), second stage larvae (L2) and finally into infective third stage larvae (L3) that are ingested by the host during grazing. These larvae exsheath and migrate to their final location in the host's gastrointestinal system, where they develop into L4 and then into adult female or male worms. Under appropriate conditions of humidity and temperature the developmental stages, eggs and L1-L3, can survive several months on the pasture.
Trichostrongylids can not be differentiated by means of their eggs. Only adult worms and L3, respectively, allow the determination of the genera and species.

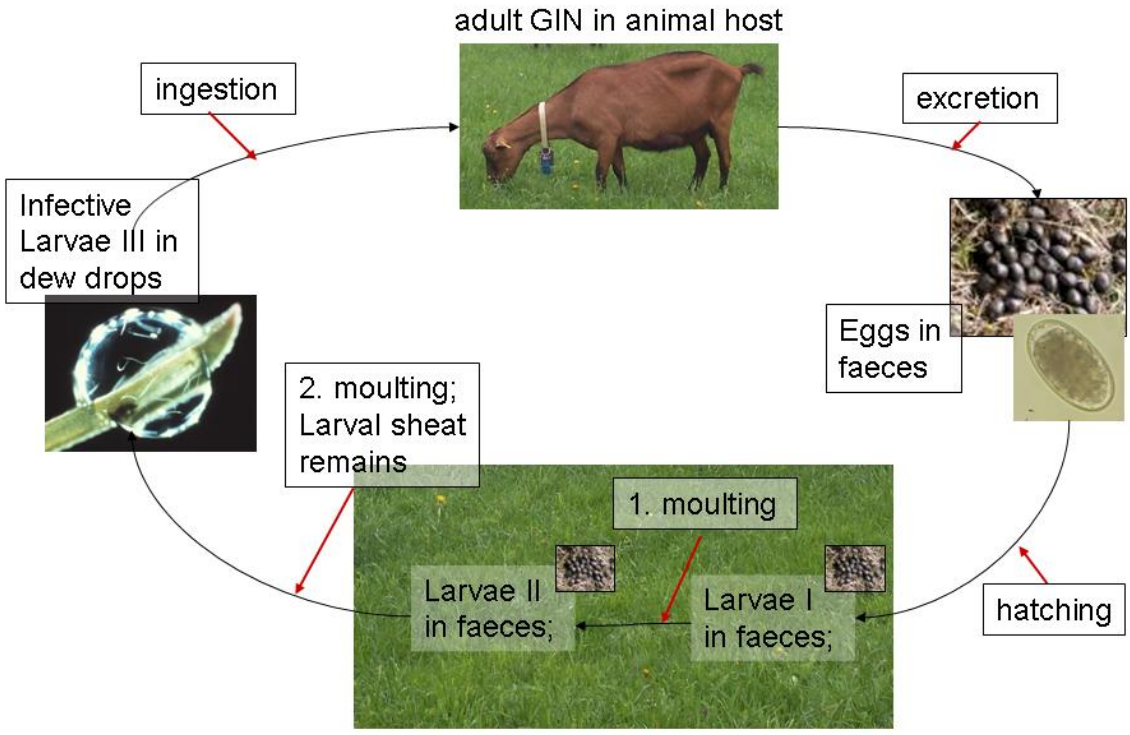

Figure 1: General life cycle of gastrointestinal nematodes of small ruminants.

1.2. Trichostrongylidosis

Infections with GINs can cause trichostrongylidosis in small ruminants, depending on the quantity and species of worms present, the general health, the nutritional and immunological status and the age of the animal. The infections occur mostly as mixed infections of different GIN species. Emaciation, persistent diarrhoea and weight loss are usually the main symptoms. Villous atrophy results in impaired digestion and malabsorption of nutrients. This leads to decreased live-weight gain, fibre and milk production and reproductive performance of small ruminants and therefore has a seriously impact on animal health and productivity. Hence, GIN parasitism represents the greatest economic constraint of small ruminant production (Perry and Randolph, 1999).

There are seven important causative agents of trichostrongylidosis in small ruminants:
Trichostrongylidae:

- *Haemonchus* spp.
- *Trichostrongylus* spp.
- *Teladorsagia* spp.
- *Cooperia* spp.

Molineidae:

- *Nematodirus* spp.

Chabertiidae:

- *Chabertia* sp
- *Oesophagostomum* spp.

1.2.1. *Haemonchus contortus* (Family: Trichostrongylidae)

Haemonchus contortus (known as the Barber's pole worm or the red stomach worm) is a very common parasite and occurs in nearly all subtropical and temperate areas of the world. In Middle Europe *H. contortus* is present in 50-75% of small ruminants (Eckert et al., 2008) Adult worms are attached to abomasal mucosa and feed on blood, which causes anaemia and eventually can lead to death, making *H. contortus* one of the most pathogenic nematodes of ruminants. Another reason that makes *H. contortus* dangerous is its ability to rapidly develop resistance against anthelmintics (Coles et al., 2005).
The female adult worms are 18-30 mm long and may lay over 5,000 eggs per day. The male adult worms are 10-16 mm long and thinner than the females. They are slender worms with a small buccal cavity, 3 lips and a slender tooth or lancet in the female. The vulva is in the posterior half of the body, covered by the vulval flap. The white ovaries wind spirally around the red intestine, giving the characteristic barber's pole appearance.
The main clinical signs of acute haemonchosis are anaemia, variable degrees of oedema, lethargy, dark coloured faeces and wool break. Chronic cases show weight loss and weakness. (Noble and Noble, 1982; Eckert et al., 2008).

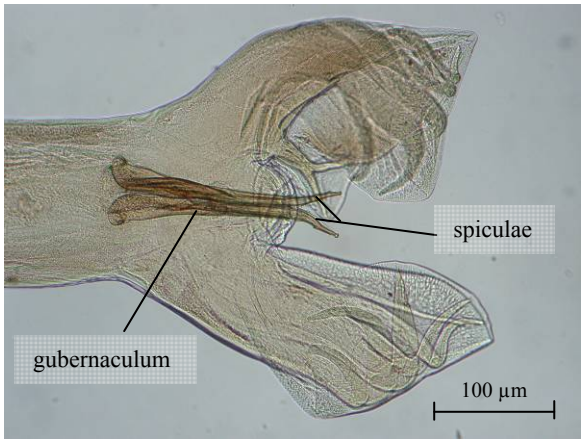

Figure 2: Copulatory bursa of male adult *H. contortus* found in abomasum of a goat died of acute haemonchosis.

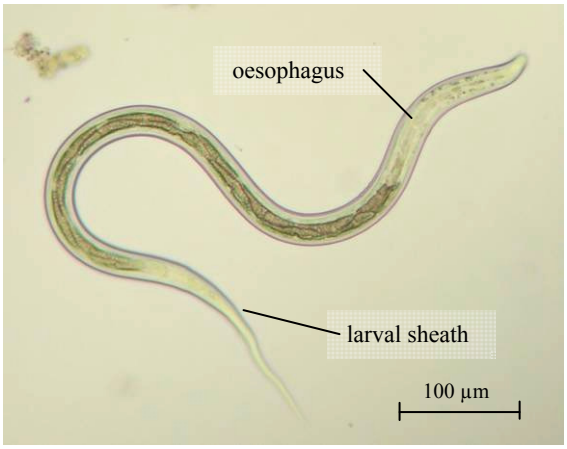

Figure 3: Third-stage larvae of *H. contortus* collected from coproculture.

1.2.2. *Trichostrongylus* spp., *Teladorsagia* spp. and *Cooperia* spp. (Family: Trichostrongylidae)

In Middle Europe several species of the *Trichostrongylus* genera occur and of these *T. colubriformis* and *T. vitrinus* are the most important ones in small ruminants with a prevalence of 50-75% (Eckert et al., 2008). The adults of both species live in the stomach of ruminants. *T. axei* is present in about 25-50% of the small ruminants and parasitises in the small intestine. (Rehbein et al., 1998).

Teladorsagia circumcincta and *T. trifurcata* are parasites of the stomach and are reported to be present in >75% of small ruminants in Germany (Rehbein et al. 1997a).

Cooperia curticei, a parasite of the small intestine, is present in >75% of European sheep and in between 25% and 50% of all goats (Eckert et al., 2008).

1.2.3. *Nematodirus* spp. (Family: Molineidae)

Nematodirus spp. is a parasite of the small intestine of ruminants. The species *N. battus*, *N. fillicollis* and *N. spathiger* are found in small ruminants. *Nematodirus* spp. is the only GIN, whose L3 larvae develop inside the egg. *N. battus* and *N. filicollis* are present in up to 75% of the sheep and in 25%-50% of the goats; *N. spathiger* is present in 25%-50% of the sheep in Europe (Eckert et al., 2008).

1.2.4. *Chabertia* spp. and *Oesophagostomum* spp. (Family: Chabertiidae)

Chabertia ovina is ubiquitous and a frequent parasite of the colon of small ruminants. Prevalence in South Germany is 98% in sheep and 84% in goats (Eckert et al. 2008).
The nodular worm of small ruminants in Middle Europe is *Oesphagostomum venulosum*. In South Germany, 97% of the slaughtered sheep and 76% of the slaughtered goats are infected with *O. venulosum* (Eckert et al. 2008, Rehbein et al., 1997b).

1.3. Other important gastrointestinal parasites of small ruminants

Further important gastrointestinal parasites of small ruminants are tapeworms (class: cestoda), liver flukes (class: trematoda) and coccida (class: coccidea).
The tapeworms found in small ruminants in Europe are *Monieza expansa* and *M. benedeni.* Heavy infections mostly occur in lambs and can lead to diarrhoea, emaciation, weight loss and retarded growth. In adults, infection generally shows no symptoms.
There are two parasites of the trematoda class that cause problems in small ruminant husbandry in Europe: the liver flukes *Fasciola hepatica* and *Dicrocoelium dendriticum*. Both induce damages of the liver, which have to be discarded at slaughter and therefore cause economic losses.
Infection with coccidia (genera: *Eimeria*) is one of the most prevalent infections of small ruminants. Numerous species of *Eimeria* are found in Europe, some of them are less or non-pathogenic, even when large numbers of oocysts are present in faeces. Pathogenic species are *Eimeria ovinoidalis* and others in sheep and *E. caprina* and *E. ninakohlyakimovae* in goats, respectively. Clinical signs include diarrhoea (sometimes containing blood or mucus), dehydration, fever, weight loss, anaemia and death.

Figure 4: Egg of a trichostrongylid and of *Moniezia expansa*.

2. Anthelmintic treatment schemes

The control of GIN-infections is largely based on preventive or therapeutic use of anthelmintic drugs (Williams, 1997). On most farms the flocks are traditionally treated two or more times per year, without coprological checking for the necessity of treatment or its efficacy afterwards. Mostly, these strategic treatments are performed in spring at the beginning of the grazing period to prevent the contamination of the pasture and at the end of summer, when infestation of the pasture with larvae and of the animals with adult worms usually is high. Additional treatments are administered whenever clinical signs become obvious. Pasture management specifically aiming at worm control includes clean-grazing systems (Rutter et al., 1984) and dose-and-move strategies (Michel, 1985, Coles et al., 1992, Barger 1997). Unfortunately, most farmers have only limited pastures available and can not perform rotational grazing with adequate resting intervals. In consequence, in heavy infected flocks some farmers have to treat their animals up to 6 times a year in order to prevent severe production losses and mortalities. However, these pasture management strategies rely on efficacious anthelmintics (Boa et al., 2001). When treatment is not fully efficacious only unsusceptible worms survive, produce eggs giving rise to resistant worms, which re-infect the flock and a further selection for anthelmintic resistance is the result.

3. Anthelmintic Resistance

The definition of resistance according to Prichard et al. (1980) is as follows: “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”.
This early definition still adequately describes the nature of resistance and hence was repeatedly reformulated and further extended to more molecular and genomic approaches (Gilleard, 2006).
Wood and Bishop (1981) classified four factors, which influence the rate at which resistance to a pesticide develops and spreads in a population:

(1) Genetic, including mutation rate and relative dominance of the trait.
(2) Reproductive, including generations per year and fluctuations in population size.
(3) Behavioural/ecological, including migration of the pest species and its ability to avoid the pesticide.
(4) Operational, including the proportion of the population exposed and the persistence of the chemical control agent.

Regarding the development of anthelmintic resistance in GINs, factors (1), (2) and (4) are very important and therefore have been studied thoroughly.
A repeated treatment of GIN populations with the same anthelmintics selects individuals that have innate or acquired resistance to the drugs. Resistance is inheritable by transmission of resistant alleles. Even point mutations can change the structure of proteins what may lead to a decrease of susceptibility to a pharmaceutical compound (Tiwari et al., 2006; Walsh et al., 2006; Prichard and Roulet, 2007). This can enable the resistant proportion of the parasite population to survive treatment. Treatment becomes ineffective when the proportion of resistant genes increases and thereby dilutes the susceptible genes (Sangster, 1999).
Anthelmintic resistance (AR) is likely to develop wherever the same substances are too frequently used. Treatments with less than the recommended dose rate may accelerate the development of AR (Chartier et al., 2000; Chartier et al., 2001). Furthermore, side resistance to other compounds in the same chemical group with a similar mode of action may occur.
Since the definition of AR by Prichard et al. in 1980, AR has become a major and still increasing problem in animal husbandry worldwide, especially in small ruminant livestock (Waller, 1994; Waller, 1997; Wolstenholme et al., 2004). All three current broad-spectrum anthelmintic families – the benzimidazoles, imidazothiazoles and macrocyclic lactones - are

concerned. The prevalence is high especially in Australia and South America, but also in Europe reports of AR in small ruminants are increasing (Bauer et al., 1987; Bauer, 1988; Bauer et al., 1988; Bauer, 2001; Hertzberg and Bauer, 2000; Schnyder et al., 2005; Artho et al., 2007; Cringoli et al., 2007). Particularly in large flocks with industrial production schemes and high stocking rates, which practise frequent treatment schemes, parasite populations tend to quickly lose the sensitivity to administered drugs (Borgsteede, 1997; Chartier and Hoste, 1994; Chartier et al., 2001).

3.1. Detection of anthelmintic resistance

Anthelmintic resistance in livestock is usually suspected, if the clinical condition of an animal does not improve after anthelmintic treatment. Often, this is due to factors like underdosing, faulty drenching equipment or application, and inaccurate assessment of the body weight. These factors have to be taken into account first, before considering an AR.

Various AR detection approaches are under discussion. Not every assay is suitable for detection of all anthelmintic classes. A variety of in vitro assays – like the egg hatch assay and larval motility tests – have been developed, in which the parasite stages are directly incubated in the chemical compound (Taylor et al., 2002). The egg hatch assay (Le Jambre, 1976), was used for BZ's and levamisole, but is unsuitable for the detection of resistance against avermectins and closantel. The incubation of the eggs in the drugs inhibits the hatching of the larvae and therefore further development. The larval motility tests are useful for detecting BZ and ML resistance. The larvae are incubated in the drugs and then motility of the larvae is measured by electronic detectors (Folz et al., 1987), by migration through a sieve (Sangster et al., 1988) or by observation (Gill et al., 1991). However, none of these methods takes into consideration the bioavailability and efficacy of the drugs in the treated host animal.

The faecal egg count reduction test (FECRT) is the most efficient in vivo assay, which can be used to evaluate the efficacy of drugs under field conditions (Taylor et al., 2002).

3.1.1. Faecal egg count reduction test (FECRT)

The most widely used test for resistance detection and monitoring of GIN-infections is the FECRT, which is suitable for all anthelmintic classes. The FECRT estimates the degree of resistance by comparing the faecal egg counts (FEC) before and after treatment (see Material

and Methods, 1.5). The World Association for the Advancement of Veterinary Parasitology (WAAVP) established guidelines that give precise details and recommendations for the use of this detection method (Coles et al. 1992; Coles et al., 2006). The FECRT provides a good estimation of AR with comparatively low costs and labour input (Taylor et al., 2002; Cabaret and Berrag, 2004). Furthermore, this test allows to identify problems with the application of the anthelmintic under field conditions.

One limitation of this method is that the number of GIN eggs excreted usually does not correlate with the actual worm burden. There is no correlation between FECs and worm counts for *Trichostrongylus colubriformis*. Although Sangster et al. (1979) reported a correlation between FECs and worm counts for *H. contortus*, no distinct proposition of the number of worms present in the host can be made by means of FECs.

In addition to the FECRT, it is advisable to identify the GIN species present after treatment by means of coproculture and L3 determination. The GIN population is usually composed of several species. If only one species survives treatment, the anthelmintic treatment seems to have been efficacious against susceptible worms and thus, AR is present in all likelihood.

4. Strategies for worm management

In order to control GIN infections and at the same time conserve the efficacy of anthelmintic treatment, new strategies for worm management have to be developed and introduced in livestock.

4.1. Targeted Selective Treatment

Targeted Selective Treatment (TST) focuses on minimising the percentage of treated animals in a flock by directing individual treatments towards those animals most susceptible to disease or towards those animals that have been identified as highly infected and therefore are responsible for major pasture contamination. It is assumed that animals with high worm burden show symptoms such as diarrhoea, emaciation, anaemia and reduced productivity, whereas animals with low worm burdens do not. Using TST, only clinically suspicious animals are treated. The identification of suspicious animals has to be reliable and can be accomplished by means of coprological examinations (FEC), body condition scoring, body weight, milk yield and anaemic pallor, respectively (Van Wyk et al. 2006).

Another approach to reduce the proportion of treated animals in a flock is the selective treatment of high producing individuals. High producing dairy animals and especially those in

their first lactation period are often less resistant to GIN infections (Hoste and Chartier, 1993; Chartier and Hoste, 1994). Therefore, the animals with the highest milk yield of the flock should be part of the treated proportion. If applied optimally, TST does not result in any significant production losses to farmers.

Since the amount of anthelmintics used is minimised, expenses for drugs can be reduced. As a further advantage of TST, a considerable percentage of the GIN population in the flock will remain susceptible to treatment, by keeping it in refugia, i.e. not exposed to the drugs. As a consequence, the selection pressure for the development of anthelmintic resistance can be reduced.

4.2. Refugia

Sustainable worm control strategies are largely based on the idea that a proportion of worms is not exposed to anthelmintics, i.e. survives "in refugia". Such worm populations are a source of reinfection and thus ensure that resistant worms do not become a dominant part of the total population (Boa et al., 2001; Pomroy, 2006; Waghorn et al., 2008). Usually, developmental stages of GIN in the environment, i.e. eggs and free-living larvae on infected pastures constitute the main part of the refugia. By means of TST this proportion can be reinforced with parasites in untreated animals. Refugia, defined by Van Wyk (2001) as the proportion of a parasite population that is not selected by drug treatment, is now considered to be probably the most important factor in delaying the development of anthelmintic resistance. Van Wyk (2001) and Coles (2002) hold the view that there should always be some animals left untreated. Thereby, also susceptible worm strains may survive in the flock and the population of resistant worms can be diluted. The higher the proportion of the GIN population is on the pasture, the slower the selection for resistance (Sangster, 2001). Above that, Sissay et al. (2006) and Leathwick et al. (2008) showed that through exploitation of refugia – i.e. by letting flocks graze on pastures infected with susceptible worms or by introducing bearers of susceptible strains in the flock anthelmintic efficacy can be restored.

Further investigations are necessary to identify the most appropriate indices for different situations and environmental conditions; so that the refugia effect is maximised for the least risk of disease and production loss and development of AR will be delayed or slowed down.

4.3. The FAMACHA©-method

The FAMACHA©-Method is a relatively simple and low-priced test that has been developed by scientists of the Onderstepoort Veterinary Institute in South Africa especially for small, resource-poor farms (Malan et al., 2001; Van Wyk and Bath, 2002). In Sub-Saharan Africa and in the Southern USA it has proved to be effective, provided that frequent inspection intervals are possible (Vatta et al., 2002a, b, c; Van Wyk and Bath, 2002; Kaplan et al., 2004; Burke et al., 2007). All animals of the flock have to be inspected for signs of haemonchosis every two weeks in order to be able to act on time when anaemic pallor becomes obvious.

The technique is being increasingly used as part of integrated parasite control programmes in the Southern hemisphere. By examining the colour of the inside of the lower eyelid of a sheep or goat, the degree of anaemia is estimated. By doing so, it is possible to approximately assess the anaemia, which is often caused by blood sucking gastrointestinal parasites, such as *H. contortus*. The treatment can then be administered selectively to animals with a considerable degree of anaemia. The FAMACHA©-method is therefore a practical tool for the accomplishment of Targeted Selective Treatments (TST), i.e. only animals showing clinical symptoms or reduced productivity should be anthelmintically treated in order to slow down the development of AR (Mahieu et al., 2007).

Unfortunately, so far only GIN-infections with a dominance of *H. contortus* can be monitored using the FAMACHA©-technique. Furthermore, it must be stated that other infections (liver flukes, blood parasites, conjunctivitis, any fever, etc.), environmental conditions (heat, drought and dust), stress and nutritional deficiencies can have influence on the colour of the eye's mucosa and thus may affect the FAMACHA©-scoring (Bath, 2000).

MATERIALS AND METHODS

1. Parasitological Techniques

1.1. Mode of Sampling

All faecal samples were taken directly from the rectum of the animals. For individual samples an average of 8 g of faeces was collected. Faeces for pooled samples of five to 10 animals were collected separately and thoroughly mixed in the laboratory. Faecal samples were kept cool during transport, in order to prevent trichostrongylid larvae from hatching. However, since *H. contortus* stages do not tolerate temperatures <4° C for more than 72 h (Smith-Buijs and Borgsteede, 1986), the samples were never stored at less than 8° C in order to maintain the ability to culture the larvae.

1.2. Standard coprological methods

The samples were screened for parasite stages by means of the flotation method according to Fülleborn, the sedimentation method according to Benedek and the larval emigration assay according to Baerman-Wetzel (Schnieder, 2006).

1.3. Collection of third-stage larvae

To determine the species of the gastrointestinal nematodes, coprocultures were performed according to Roberts and O'Sullivan (1950). Therefore, pooled faecal samples were mixed with vermiculite and kept in small plastic containers for a minimum of 12 days at room temperature. The samples were kept humid, mixed occasionally and were aerated every day for one hour. During this period the larvae hatched from the eggs and developed into L3. To collect third-stage larvae the cultures were filled with water and put upside down in a petri dish containing water. After a period of 24 h hours larvae had migrated towards the clear water and assembled in the reservoir of the petri dish. The L3 suspension was drained from the reservoir and filled in 300 ml beakers. The beakers were filled up with tap water and kept at 10° C for 12 h. After this sedimentation period, the supernatant was discarded and the cleaned larval suspension was filled into incubation flasks. These were kept at 10° C until the larvae were used for identification and further testing.

From each culture, at least 200 L3 were morphologically differentiated and identified according to keys of Bürger and Stoye (1968) and Van Wyk et al. (2004).

1.4. The McMaster Faecal egg count method

The samples were processed using a modified McMaster method (with a sensitivity of 30 eggs per gram faeces (epg) (Schnieder, 2006; QM-handbook, Institut für Vergleichende Tropenmedizin und Parasitologie, Mk07). Thereby, 4.5 g of faeces was suspended in 40.5 ml H_2O by using a plunger and filtered through a 300 µm sieve into a plastic container. While shaking the container (Vortex Genie 2, Scientific Industries) 10 ml of the suspension was pipetted into a centrifuge tube and centrifuged for 10 min at a RCF of 992.23g (Hettich Zentrifuge, Rotofix 32; 2500 rpm). After that, the supernatant was exchanged by saturated salt solution and the sediment was resuspended. By using a pipette a sample of the isolated fluid was taken, while vortexing the tube, and placed into a McMaster counting slide (Precision chambered counting slides, Advanced Equine Products, Chalex Corporation, USA). The number of eggs in one chamber (0.5 ml) was counted under a microscope. The epg was calculated by multiplying the number of eggs in the counted chamber by 30.

1.5. Faecal Egg Count Reduction Test

Faecal samples, taken rectally from the animals, were analysed by the Faecal Egg Count Reduction Test (FECRT) according to Coles et al. (2006). The FECRT provides an estimation of the anthelmintic efficacy by comparing faecal egg counts (FECs) before and after treatment (Boersema, 1983; Presidente, 1985).

The FECRT was calculated according to the following formula (BAUER, 1986):

$$FECR\ [\%] = (FEC_{\text{before treatment}} - FEC_{\text{post treatment}}) \times 100 / FEC_{\text{before treatment}}$$

An AR against an anthelmintic drug is considered present if the reduction after treatment is lower than 95% and the lower 95% confidence limit is below 90% (Coles et al., 1992).

1.6. Determination of packed cell volume (PCV)

An average of 5 ml sterile EDTA-blood per animal was collected from the jugular vein each month. The collected EDTA-samples were used to define the packed cell volume (PCV), of each goat by means of microcentrifugation (Kraft and Dürr, 1999).

2. Study flocks

2.1. Flocks selected for the coprological survey

The small ruminant flocks which initially took part in the coprological survey were mainly selected by the "sheep-flock health service" (Schafherden-Gesundheitsdienst) of Baden-Wuerttemberg. Most of those 19 flocks were transhumant herds which migrated through the "Schwäbische Alb" during the grazing season. In addition, 10 sheep and 10 goat herd-owners that made contact with our institute during the time of this thesis were included in the coprological survey.

2.2. Flocks selected for the anthelmintic resistance study

In a context of a regular coprological screening of 29 sheep and 10 goat flocks in Southern Germany and Switzerland, four cases of suspected anthelmintic resistance (AR) were found. In two goat and two sheep flocks FECR was not sufficient after the routine treatments performed by the farmers.

"Swiss-flock":

Goat flock nr. 1, consisting of 16 goats of various breeds (Chamoisee, Anglo-Nubian, Saanen and mixed breeds), lives in the Swiss Emmental and will be referred to as "Swiss-flock". All the goats are kept tied in winter and over night (Fig. 5) and are grazing on pastures in the summer.

"Blackforest-flock":

The "Blackforest-flock", consisting of 90 white German dairy goats, is located in a valley of the Black Forest, Germany. The goats are kept for organic milk production in a pen with a

small open air area. After an unsuccessful treatment with eprinomectin performed by the owner, 21 of these 90 goats were integrated in this study.

The two sheep flocks are situated in Southern Germany, one in Bavaria and one in Baden-Wuerttemberg.

"Allgaeu-flock":

The Bavarian flock consists of 80 Suffolk sheep and will be referred to as "Allgaeu-flock". It is a semi-professional livestock, composed of the Suffolk, the milk sheep and the mountain sheep breed. After a clinically observed inefficacious routine treatment with albendazole, 30 randomly selected sheep of the "Allgaeu-flock" were divided into three treatment groups of ten sheep each, which were sampled on the day of treatment and 12 days later.

"Alb-flock":

The sheep flock located in Baden-Wuerttemberg is called "Alb-flock" and consists of 45 Dorper yearlings. Similarly as in the "Allgaeu-flock", three randomly selected treatment groups of 10 sheep each were formed, after having noticed a decrease in the efficacy of moxidectin treatment and were sampled on the day of treatment and 13 days later.

Figure 5a: Individually tied goats in a stable in Switzerland.

Figure 5b: Goats kept in a pen in Southern Germany.

2.3. Goat flocks of the FAMACHA© study

Six goat flocks in Switzerland (Cantons of Bern, Luzern and Zug) were chosen for this study because of reports on insufficient or even lacking efficacy of treatment against gastrointestinal nematodes (GIN) by "Extension and health service for small ruminants" (ESSR) (fig. 6). 65 goats of various breeds (Chamoisee, Anglo-Nubian, Saanen and mixed breeds) were included in the study. Each flock consisted of at least one buck, one lamb group and 10 to 100 does with age range 1-13 years (tab. 1). All flocks spent the daytime in summer on pastures (fig. 7). The goats received additional food – hay, corn, and on the organic farms special herbage-mixtures – in the stable. The two big flocks were subdivided into several smaller groups. Of each goat flock, 8 to 13 goats were included in the study group and sampled at an interval of four weeks during a 6 month period.

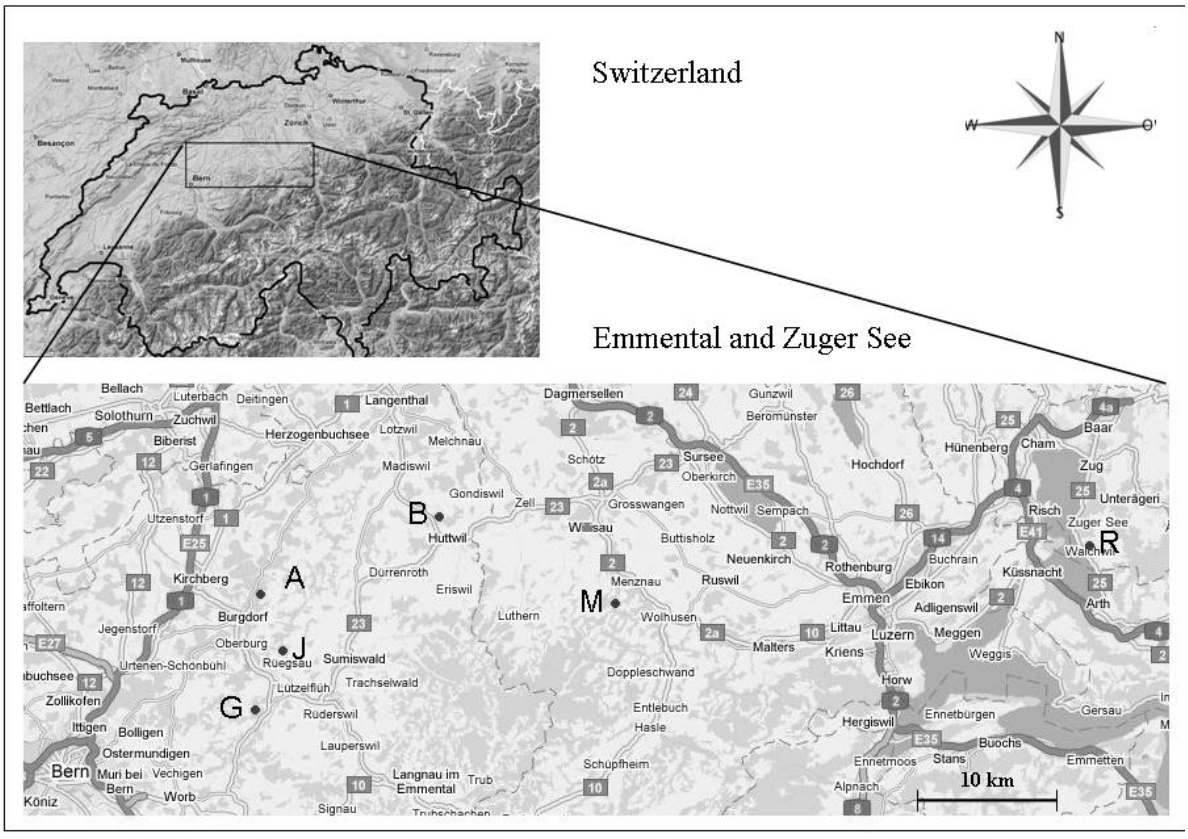

Figure 6: Location of the six study goat flocks of the FAMACHA©-study in Central Switzerland in the "Emmental" and at the "Zuger See" between Bern and Zug.

Figure 7: Characteristic pastures and a pen of the Swiss goat flocks that took part in the FAMACHA©-study.

Table 1: Characteristics of the six goat flocks of the FAMACHA©-study in Switzerland.

Flock	Canton	Size of flock /no. of goats tested	Housing system	Breeds	Use
A	Bern	25 head / 11	Pen, pasture	Mix, Cha, Tog,	Milk*
B	Bern	100 head / 11	Pen, pasture	Cha	Meat
G	Bern	13 head /13	Tied in stables, pasture	Cha, Tog, Mix, Nub, Saa,	Milk
J	Bern	15 head / 8	Pen, pasture	Boe	Breeding
M	Luzern	25 head / 11	Tied in stables, pasture	Mix, Tog, Cha, Saa	Milk*
R	Zug	100 head / 10	Pen, pasture	Saa, one Tog	Milk*

Mix = mixed breed; Cha = Chamoisee; Saa = Saanen; Tog = Toggenburg, Nub = Anglo-Nubian; Boe = Boer; * = organic farming;

3. The application of the FAMACHA©-Method

In the present study the colour of the ocular conjunctiva was evaluated following the recommendations of the FAMACHA©-method (Van Wyk and Bath, 2002). Thereto, the official FAMACHA©-anaemia guide was used (fig. 8). The guide shows 5 colour classes: 1 (red) and 2 (red–pink) being considered as non-anaemic; 3 (pink) mildly anaemic; 4 (pink–white) anaemic and 5 (white) severely anaemic.

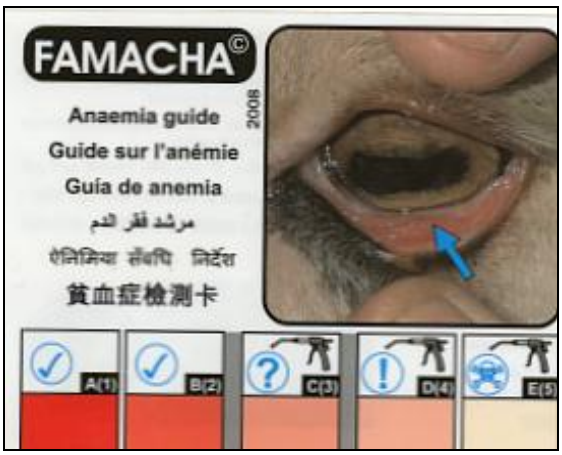

Figure 8: Official FAMACHA©-anaemia guide.

For FAMACHA© scoring, the lower eyelid of the animal is gently pulled down with the finger to expose the ventral conjunctiva. It can be helpful to push the upper eyelid down to cover the eyeball and the membrane nicitans. The colour of the lower conjunctiva is then evaluated by comparing it directly with the FAMACHA© chart (fig. 9).

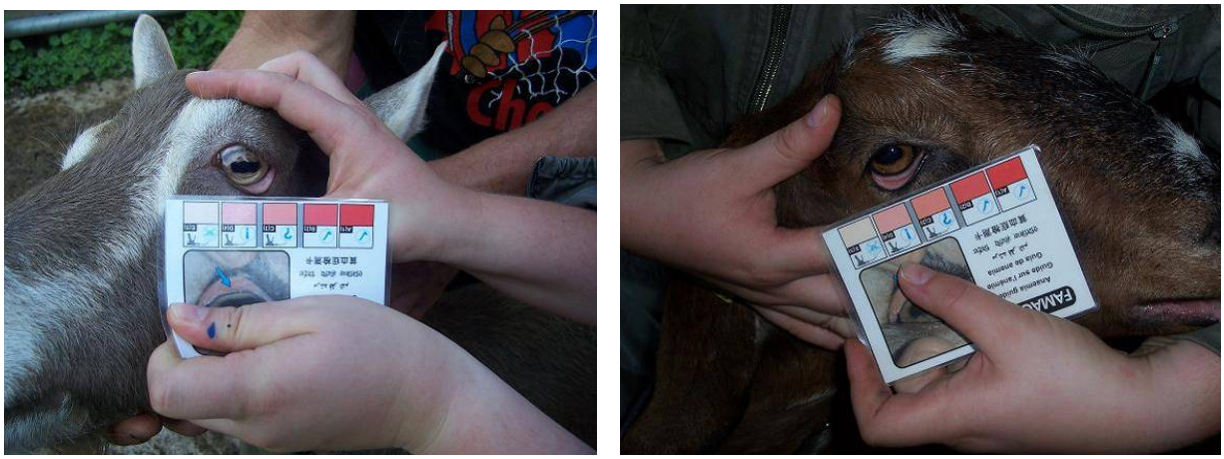

Figure 9: Application of the FAMACHA©-chart for the evaluation of the degree of anaemia on goats during the study.

4. Statistical analyses

4.1. Anthelmintic resistance

The efficacy of the treatment was calculated according to the methods described in the recommendations for the detection of anthelmintic resistance and efficacy of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al. 1992; Wood et al. 1995; Coles et al. 2006). Small flock sizes and health status did not allow leaving animals untreated as a control group. Resistance was considered as present if the faecal egg count reduction (FECR) was less than 95% and the lower 95% confidence limit (CI) for the reduction was less than 90%. If only one of the two criteria was met, anthelmintic resistance was suspected (Coles et al., 1992). Statistical analysis was run using SPSS 15.0 and 16.0, and Microsoft Excel 2003 software. Box-plots were performed to show the actual distribution of the FECR by means of its median, upper and lower quartile and minimal and maximal value. In some cases, faecal egg counts (FEC) were higher after treatment than before, due to natural, biological fluctuation of egg production by the GINs. The FECR post treatment (p.t.) of the individual animals was then considered as 0%, i.e. no reduction, to calculate the mean FECR of each treatment group.

4.2. The FAMACHA©-study

Spearman rank correlation coefficient and Pearson correlation were calculated (SPSS 15.0 and 16.0 software) to show the relationship between the four variables, FEC-overall, FEC-*H. contortus* fraction (FEC-Hc), FAMACHA©-score and PCV-value for each study month. Box plots were performed to display the relationships of these variables. Explorative and descriptive data analyses were performed for each variable in order to evaluate the mean, standard error of the mean, the standard deviation and the minimum and maximum of the values regarding each month and the entire study period.

Crosstabulations tables were drawn up with the different variables to evaluate the quality of the FAMACHA©-test. Sensitivity (TPF), specificity (TNF), the predictive value of a positive (PPV) and the predictive value of a negative (NPV) were calculated according to Vatta et al. (2001).

Test sensitivity was defined as:

TPV = (true positives/(true positives + false negatives))x 100;

specificity as:

TNV = (true negatives/(true negatives + false positives))x100;

the predictive value of a positive as:

PPV = (true positives/(true positives + false positives))x100;

and the predictive value of a negative as:

NPV = (true negatives/(true negatives + false negatives))x100.

The packed cell volume was used as the gold standard by which anaemia was measured and four cutoff values for anaemia (<15%, <22%, <24% <29% and <32% respectively) were assigned. In addition, FEC-overall and FEC-Hc were used to check the adequacy of the FAMACHA© scoring regarding egg excretion with the two cutoff values >300 epg and >600 epg, respectively. Stacked bar-charts were drawn to display the frequency of cases for the various categories.

RESULTS

1. Epidemiological survey of sheep flocks in Baden-Wuerttemberg

As a preparatory work of this thesis, a survey was started in transhumant and sedentary sheep flocks in order to get insight in the gastrointestinal parasite species present in Southern Germany. Initially, 19 herd-owners started to send pooled faecal samples of 10 animals each month. Unfortunately, only five of them sent the samples on a regular basis. Consequently only five flocks could be included in the study on the seasonal infection patterns.

1.1. Prevalence of parasite species in the flocks

In all investigated flocks, eggs of members of the *Trichostrongylidae* and oocysts of the *Eimeridae* family could be found (table 2). *Nematodirus battus*, N. *filicollis* and *Moniezia* species were primarily detected in lambs of most flocks. The lancet fluke was present in 79% of the study flocks. *Trichuris* spp. is present in most of these flocks grazing on the dry pastures of the "Schwäbische Alb".

Table 2: Gastrointestinal parasite prevalence in faecal samples of 19 sheep flocks in Baden-Wuerttemberg.

Parasites present	**In Flocks (percentage)**	**Range of FEC in ewes [epg /opg]**	**Highest FEC in lambs [epg/opg]**
Trichostrongylids	19 (100%)	0-3120	1920
***Nematodirus* spp.**	16 (84%)	0-120	300
***Trichuris* spp.**	14 (74%)	+ - ++	++
Monieza spp.	17 (90%)	+ - +++	+++
Fasciola hepatica	4 (21%)	+ - ++	
Dicrocoelium dendriticum	15 (79%)	+ - +++	++
Protostrongylids	7 (37%)	+ - ++	++
***Capillaria* spp.**	2 (11%)	+	
***Eimeria* spp.**	19 (100%)	0- 4380	35200

+ = sporadic; ++ = numerous; +++ = plentiful

General seasonal patterns were difficult to examine statistically because farmers followed no homogenous treatment scheme but treated whenever it was convenient for them, i.e. when the animals were assembled for reasons of counting, shearing, etc. In addition, the irregular sampling intervals made a statistical evaluation of the data difficult. However, GIN egg excretion peaks were found in both years between March and May and from September to November. During the present coprological survey, most farmers only treated when it was recommended according to a high egg excretion (>250 epg) which reduced the number of anthelmintic treatments to two treatments a year.

Five typical seasonal patterns of infection are displayed in figures 10a – e.

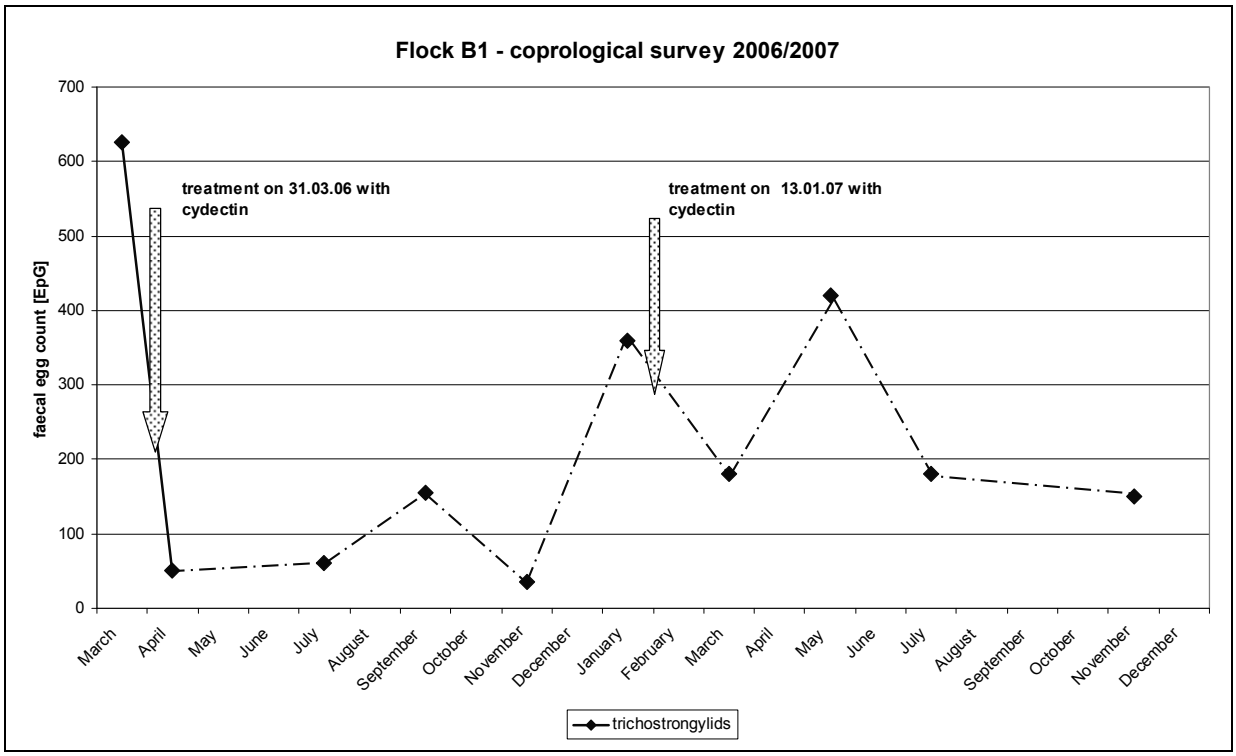

Figure 10a: Flock B1: Seasonal development of mean faecal egg counts of trichostrongylids during the study period from March 2006 to November 2007.

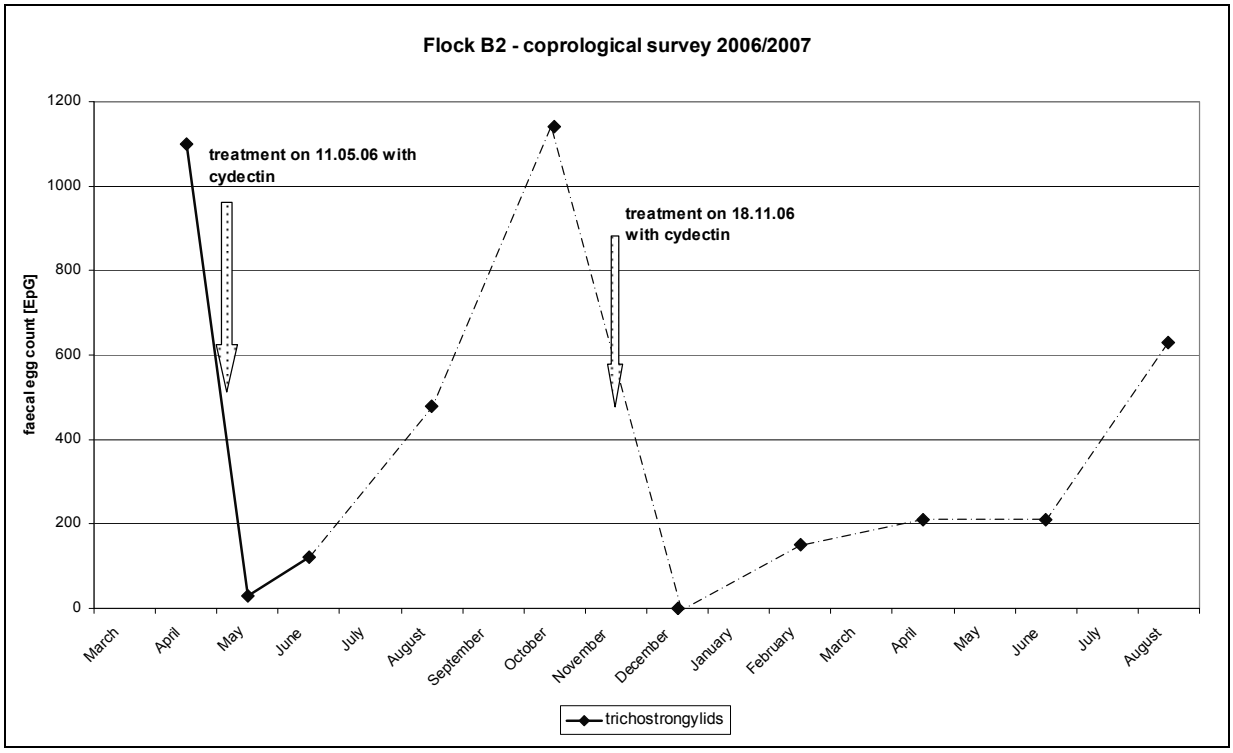

Figure 10b: Flock B2: Seasonal development of mean faecal egg counts of trichostrongylids during the study period from April 2006 to August 2007.

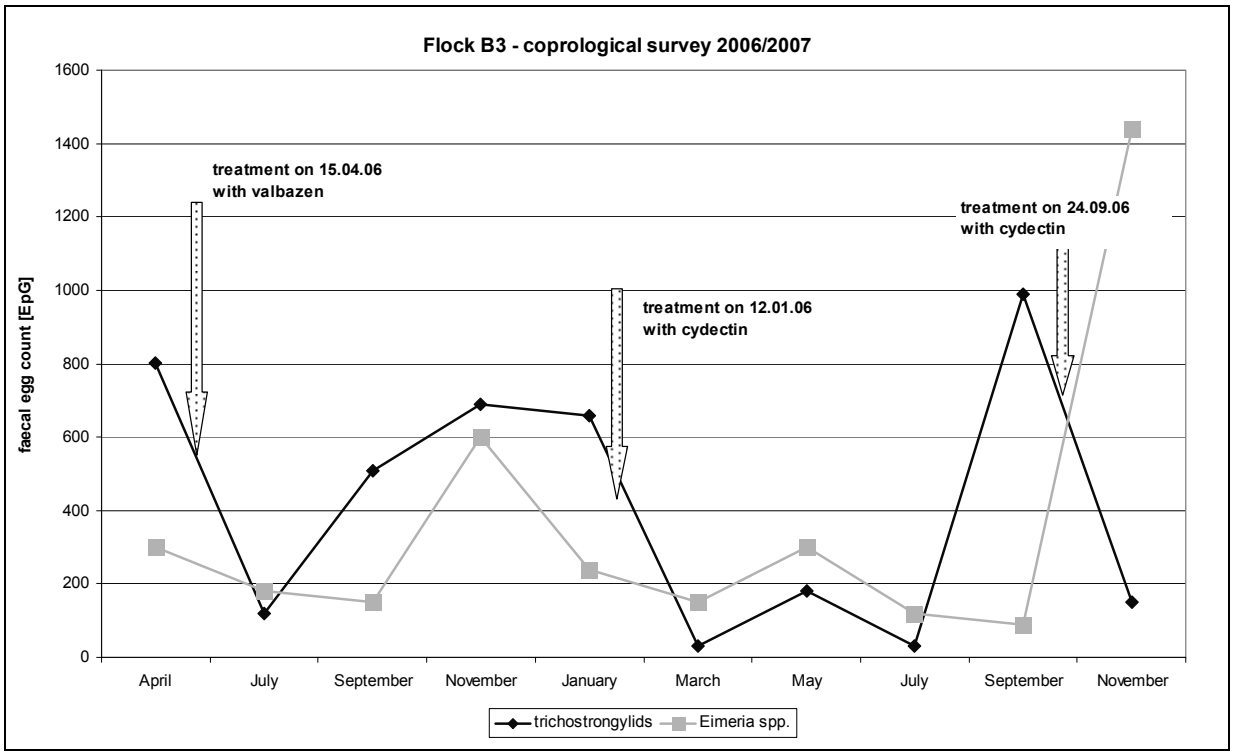

Figure 10c: Flock B3: Seasonal development of mean faecal egg counts of trichostrongylids and *Eimeria* spp. during the study period from April 2006 to November 2007.

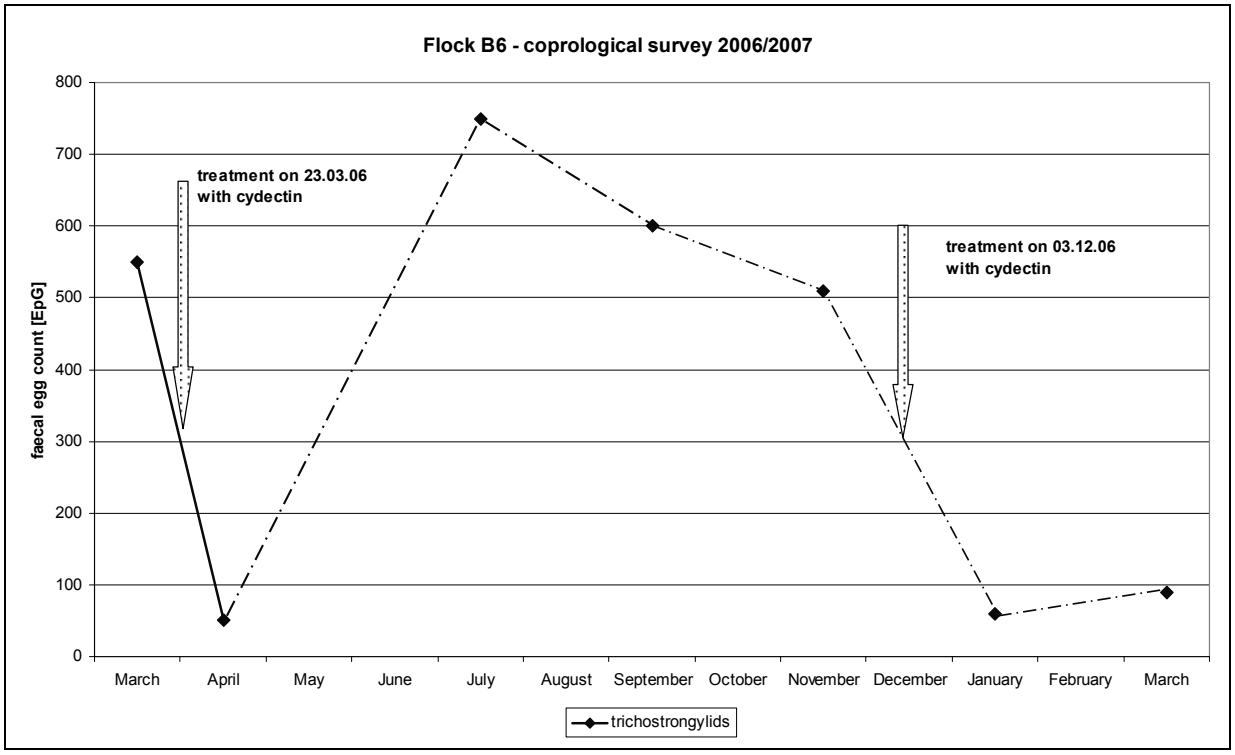

Figure 10d: Flock B6: Seasonal development of mean faecal egg counts of trichostrongylids during the study period from March 2006 to March 2007.

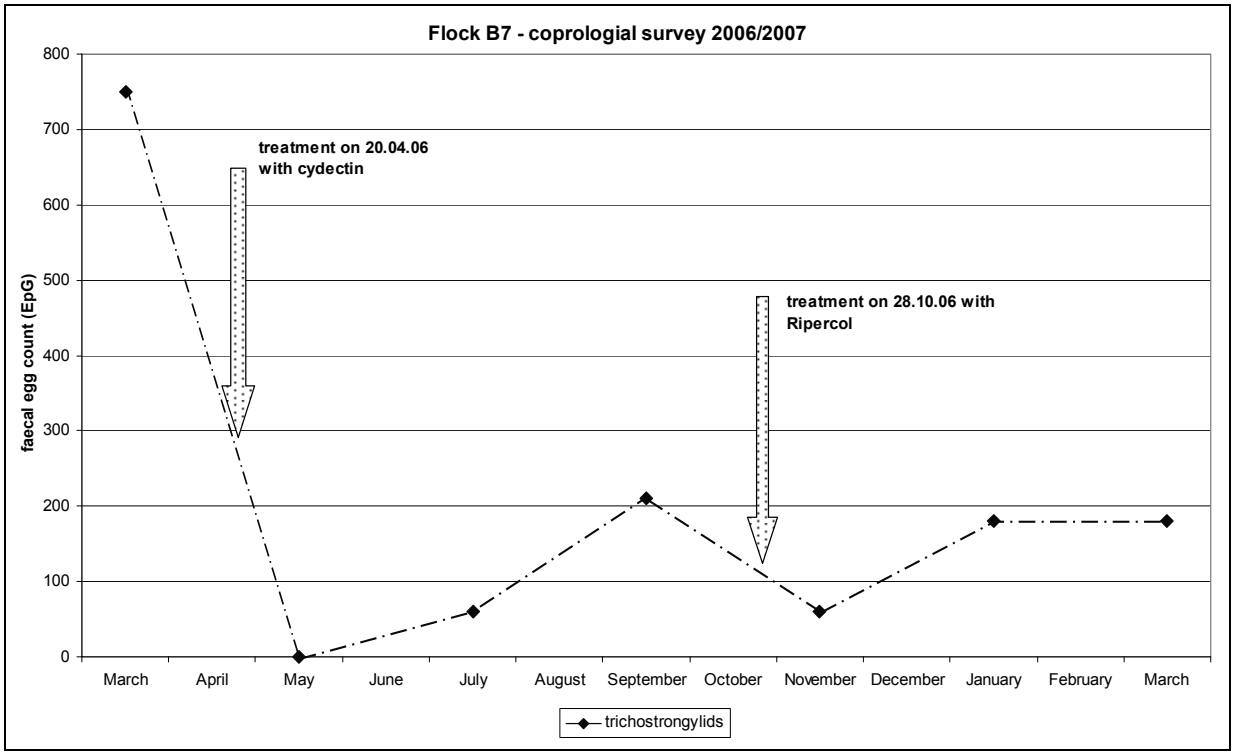

Figure 10e: Flock B7: Seasonal development of mean faecal egg counts of trichostrongylids during the study period from March 2006 to March 2007.

2. Anthelmintic resistance of GIN of small ruminants in Germany and Switzerland

Four small ruminant flocks infected with GINs resistant against various anthelmintic compounds have been identified during the AR study. The main results of this study are presented in the publication “Anthelmintic Resistance of *Haemonchus contortus* in small ruminants in Switzerland and Southern Germany” (see Results 5.1.). Additional data and figures can be found on the following pages.

2.1. Faecal egg count reduction in nine treatment groups

In order to test the anthelmintic efficacy, the FECRT according to WAAVP recommendations was performed (Coles et al., 1992). In figure 11 the mean FEC pre- and post-treatment of the six different treatment groups is presented. Only the moxidectin treated groups of the “Blackforest”- and the “Allgaeu-flock” had FECs of 0 epg after treatment. In the “Swiss-flock” mean FEC post-treatment were even higher than those pre-treatment, due to the high natural, biological variability of egg excretion (tab. 3).

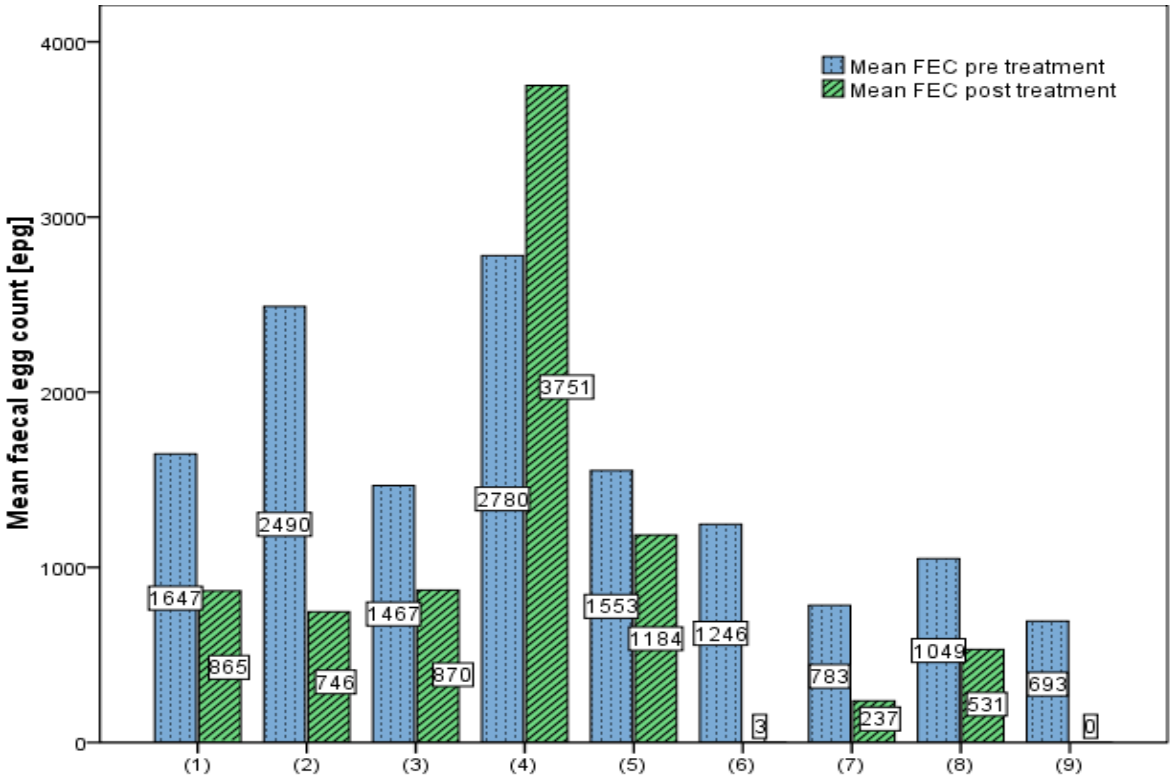

Figure 11: Mean faecal egg count pre- and post-treatment of the nine treatment groups: (1) "Alb-flock"-moxidectin, (2) "Alb-flock"-albendazole and (3) "Alb-flock"-Oxfendazole; (4) "Swiss-flock"-eprinomectin; (5) "Blackforest-flock"-eprinomectin and (6) "Blackforest-flock"-moxidectin; (7) "Allgaeu-flock"-albendazole, (8) "Allgaeu-flock"-fenbendazole and (9) "Allgaeu-flock"-moxidectin.

2.1.1. Negative faecal egg count reductions

A high variability of GIN egg excretion due to a natural biological fluctuation was observed. Thereby, several individual animals in all flocks showed even higher FECs after treatment than before. These negative reductions raised a problem in the statistical interpretation of the results. Hence, two different FECR schemes were defined and calculated, i.e. FECR-unmodified and FECR-classified.

For the calculation of the FECR-unmodified all reduction values were used as provided by faecal analysis to evaluate the efficacy of the anthelmintic treatment. For the calculation of the FECR-classified, on the other hand, all negative FECR-values were classified as having no efficacy, i.e. in those cases FECR is 0%. Table 3 presents the differences of FECR-unmodified

and FECR-classified. The median values of both calculation schemes are mostly identical. However, the means vary, when negative reductions are included.

Table 3: Median and mean faecal egg count reduction percentages (FECR) with standard error (S.E.) and standard deviation (S.D.) in two goat and two sheep flocks in Southern Germany and Switzerland treated with four different anthelmintics.

		FECR- classified*				**FECR- unmodified**			
	Treatment group	Median [%]	Mean [%]	(S.E.)	S.D.	Median [%]	Mean [%]	(S.E.)	S.D.
Goats	"Swiss-EPR"	0	**17.4**	(11.3)	43.7	-65.8	**-512.2**	(315)	1221
	"Blackforest"-EPR	25.9	**27.5**	(6.6)	30.4	25.9	**13.3**	(10)	48
	"Blackforest"-MOX	-	**100**	-	-	100	**99.1**	(0,5)	3
Sheep	"Allgaeu"-ABZ	83.9	**70.8**	(11.6)	36.8	83.9	**27.5**	(52)	164
	"Allgaeu"-FBZ	39.4	**52.4**	(12.1)	38.3	39.4	**44.9**	(17)	54
	"Allgaeu"-MOX	-	**100**	-	-	100	**100**	(0)	0
	"Alb"-MOX	43.1	**44.3**	(12.2)	38.6	43.1	**-11.6**	(42)	134
	"Alb"-ABZ	62.3	**55.3**	(10.4)	33.0	62.3	**52.9**	(12)	38
	"Alb"-OXF	45.9	**47.3**	(12.6)	39.8	45.9	**6.9**	(44)	138

* = Negative FECR values (<0) are classified as 0% reduction for the calculation of mean and median values.

Small flock sizes, the health status and therewith associated ethical reasons did not allow leaving animals untreated as a control group. Anthelmintic resistance was considered present if the FECR was less than 95% and the lower 95% confidence limit (CI) for the reduction was less than 90% (Coles et al., 1992). This was the case in seven of the nine treatment groups (tab. 4). Goats of the "Blackforest"-flock and sheep of the "Allgaeu"-flock are still susceptible to moxidectin. The anthelmintic efficacy was the same for both FECR calculation schemes in all treatment groups.

Table 4: Anthelmintic efficacy in the nine treatment groups. Faecal egg count reduction (FECR) and lower 95% confidence limit (Cl) as indicators of AR.

	Treatment group*	FECR-unmodified [%]	Lower 95% Cl	Status	FECR-classified [%]	Lower 95% Cl	Status
Goats	"Swiss-EPR"	-512.2	-1188.1	**resistant**	17.4	-6.8	**resistant**
	"Blackforest"-EPR	13.3	-8.4	**resistant**	27.5	13.7	**resistant**
	"Blackforest"-MOX	99.1	98.1	**susceptible**	100	-	**susceptible**
Sheep	"Allgaeu"-ABZ	27.5	-89.9	**resistant**	70.8	44.5	**resistant**
	"Allgaeu"-FBZ	44.9	6.3	**resistant**	52.4	25.0	**resistant**
	"Allgaeu"-MOX	100	-	**susceptible**	100	-	**susceptible**
	"Alb"-MOX	-11.6	-107.5	**resistant**	44.3	16.6	**resistant**
	"Alb"-ABZ	52.9	25.8	**resistant**	55.3	31.7	**resistant**
	"Alb"-OXF	6.9	-92.0	**resistant**	47.3	18.8	**resistant**

* Treatment groups: EPR = eprinomectin, MOX = moxidectin, ABZ = albendazole, FBZ = fenbendazole, OXF = oxfendazole

In the boxplot-diagram the faecal egg count reductions of all animals of a treatment group were used to show the distribution of all individual values of one treatment group, including its median, upper and lower quartile and minimal and maximal value (fig. 12). Apart from the groups "Blackforest"-flock-moxidectin and "Allgaeu"-flock-moxidectin, all other groups show FECRs of <70%.

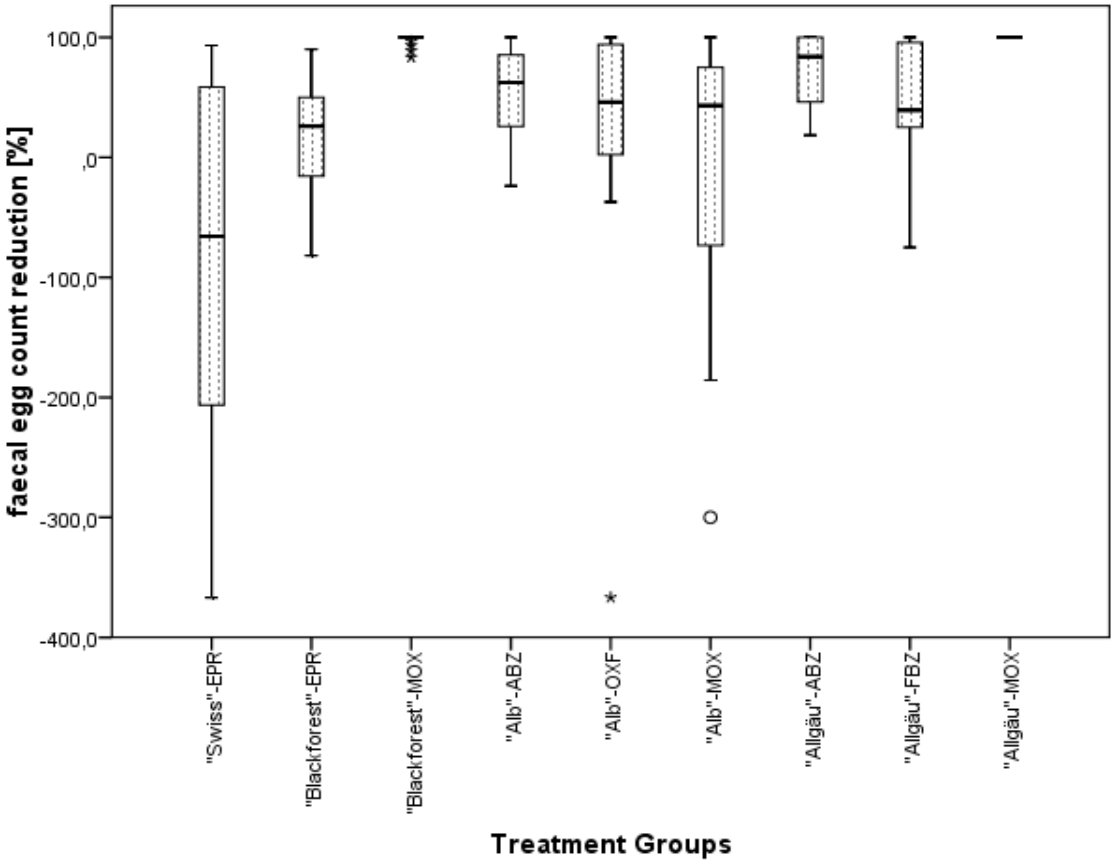

Figure 12: Box plots showing distribution of unmodified faecal egg count reductions in nine different treatment groups of the four study flocks (goat and sheep). Lower and upper borders of the box represent the 25th and 75th percentiles, respectively. Solid black lines and values in small boxes show median of FECR-unmodified. Whiskers above and below the boxes indicate the maximum and the minimum values, respectively. Symbols represent outliers; two outliers of group "Swiss"-EPR (-3400% and -3600%) are not shown.

EPR: eprinomectin; MOX: moxidectin; ABZ: albendazole; FBZ: fenbendazole; OXF: oxfendazole

2.1.2. Blackforest-flock

In the "Blackforest"-goat flock, haemonchosis was very severe and at an advanced stage, when AR against eprinomectin was detected. Milk yield had decreased considerably and some animals already died of anaemia and emaciation. After the inefficacious treatment with eprinomectin, coprocultures revealed exclusively, i.e. 100%, *H. contortus* larvae.

FECs were high and ranging from 270 epg to 4590 epg before treatment and from 300 epg to 4560 epg after the treatment with eprinomectin (fig. 13). The animals recovered after a subsequent, successful treatment with moxidectin (1mg/kg BW, pour-on formulation) that was administered one month later.

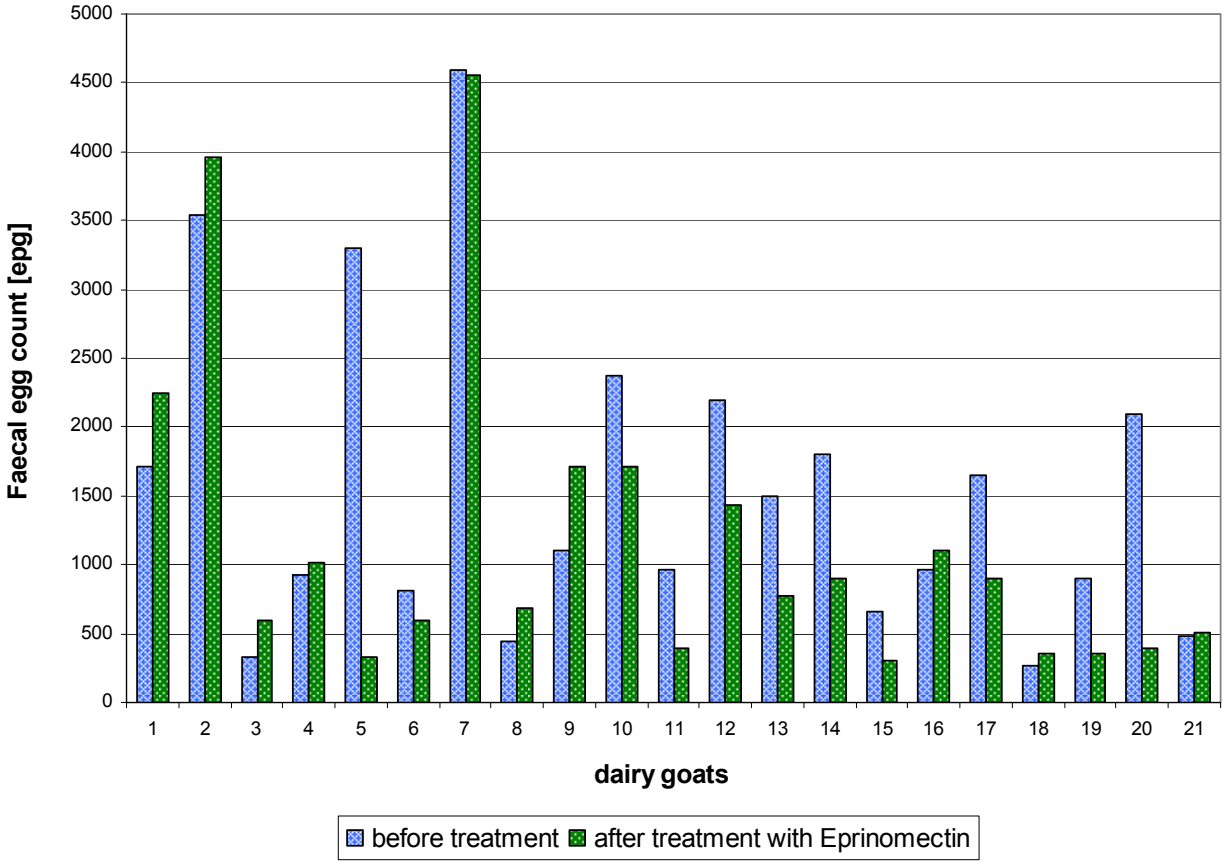

Figure 13: Comparison of faecal egg counts before and after treatment with eprinomectin (1mg/kg BW) of dairy goats of the "Blackforest"-flock.

3. Clinical signs of parasite infection

Various clinical signs of trichostrongylidosis like emaciation, scrubby fur and diarrhoea were observed in the flocks during the studies of this thesis (fig. 14, Fig. 15a). A young Boer-goat showed a significant submandibular oedema (fig. 15b). The oedema completely disappeared within two weeks following treatment.

Figure 14: Dairy goats suffering from severe haemonchosis.

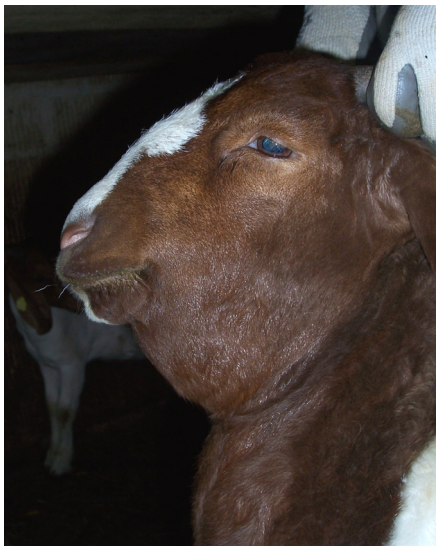

Figure 15 a und b: Boer-goat with a significant submandibular oedema („bottle jaw") and a goat with a scrubby fur and a reduced body weight.

4. Evaluation of the FAMACHA©-method in Switzerland

The FAMACHA©-method was evaluated in six goat flocks in Central Switzerland. The main results of this study are presented in the publication "The accuracy of the FAMACHA©-method in detecting anaemia and haemonchosis in goat flocks in Switzerland under field conditions" (see Results 5.2.). Additional data and figures can be found on the following pages. The raw data are presented in the appendix.

4.1. Packed cell volume and FAMACHA©-categories

In order to evaluate a potential correlation between the amount of red blood cells and the colour of the conjunctiva, the packed cell volume (PCV) of blood samples and FAMACHA©-scores were simultaneously and independently determined. The frequencies of detected PCV-categories, as defined in Materials and Methods for each FAMACHA©-score are presented in a stacked-bar chart (fig. 16).

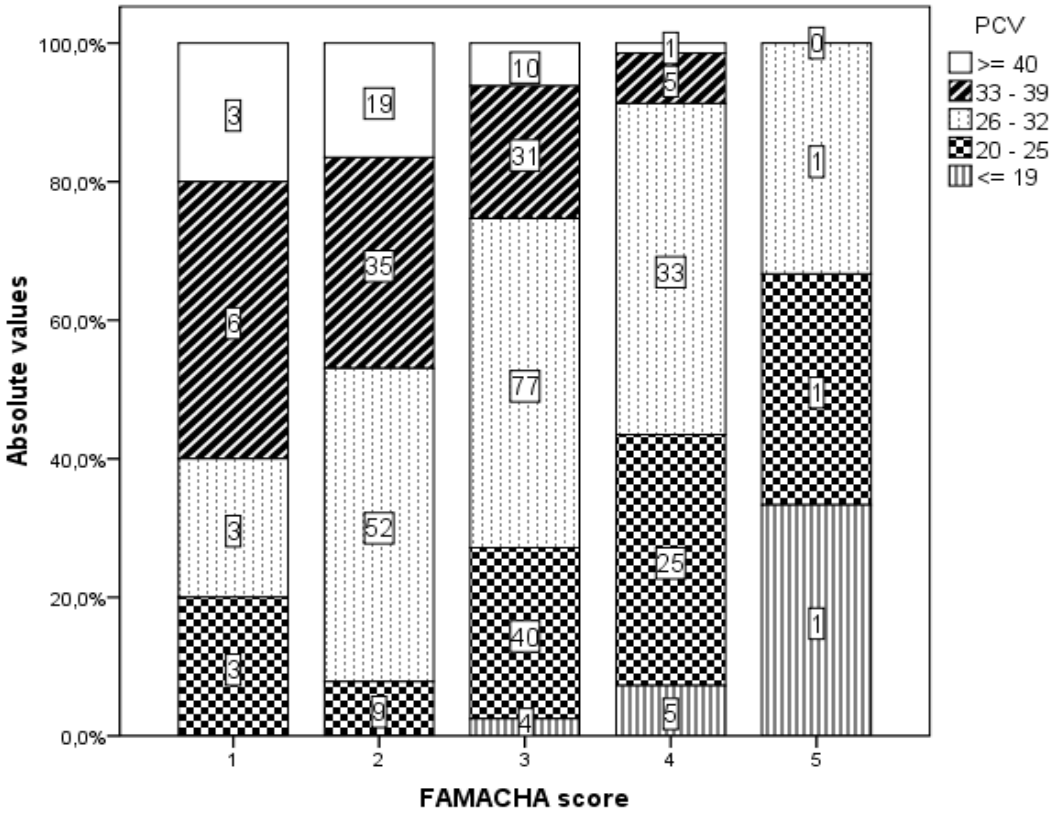

Figure 16: Frequency of packed cell volume categories for each FAMACHA©-score.

In all FAMACHA© categories there were animals with a PCV between 20% and 32%. Most animals showed a PCV >26%. PCV-values <19% could only be detected for FAMACHA©-categories 3 to 5. In category 5 no PCV higher than 32% was found.

4.2. Faecal egg count and FAMACHA©-categories

To additionally investigate a hypothesised correlation between FAMACHA©-scoring and severity of infection *H. contortus*, the number of excreted eggs and FAMACHA©-scores were independently determined. For each FAMACHA© category a range of FEC-values were assigned. For FAMACHA© category 1 a FEC ≤120 epg, for category 2 between 120 epg and 300 epg, for category 3 between 300 epg and 1020 epg, for category 4 between 1020 epg and 3330 epg and for category 5 a FEC >3330 epg, respectively, was defined. The frequencies of FEC categories for each FAMACHA©-score are presented in a stacked-bar chart (fig. 17). FEC ≤120 epg were found for each category. Most animals excreted between 300 epg and 3330 epg. This value exceeds the borderline value of 250 epg for which a treatment is recommended in ESSR-guidelines and by our institute.

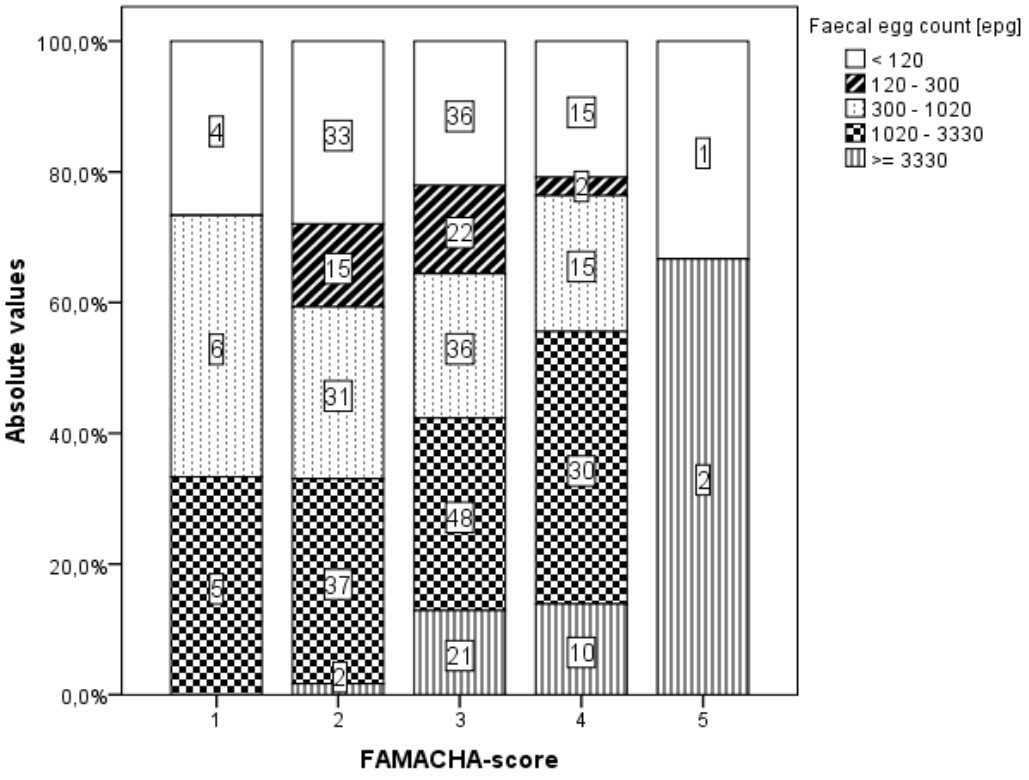

Figure 17: Frequency of animals of faecal egg count categories [epg] for each FAMACHA©-score.

4.3. Classification of packed cell volume ranges

To make PCV cutoffs applicable for European conditions, the ranges of PCV values for each FAMACHA©-category in this study were defined according to the standard blood values for goats in Central Europe (Kraft and Dürr, 1999) (tab. 5). Hence, the ranges deviated from the ones defined by Malan et al. (2001) in the original FAMACHA©-method. This adaptation was necessary because the goats in our study showed generally higher packed cell volume values than the animals involved in South-African studies. The mean packed cell volume of all goats over the entire study period was 30% ±6.9%.

Table 5: Range of PCV values for each FAMACHA©-category as defined by Malan et al. (2001) and in comparison to those defined in the present study with the respective FEC-categories.

FAMACHA©	PCV Malan et al.	PCV Study	FEC categories
1	>28%	>40%	<120 epg
2	23-27%	39 - 33%	120 - 300 epg
3	18-22%	32 - 26%	300 - 1020 epg
4	13-17%	25 - 20%	1020 – 3330 epg
5	≤ 12%	19 -13%	>3330 epg

4.3.1. Decisions for treatment according to different PCV-classifications

Unlike in studies conducted in Africa and in the USA (Malan et al., 2001, Van Wyk and Bath, 2002, Kaplan et al., 2004, Burke et al., 2007) with an anaemia cutoff-value set on PCV = 15, studies under European conditions require a higher cutoff-value. In our study, only two animals in FEC-category 5 would have received a treatment, if a cutoff-value of 15% had been considered (fig. 18). On the contrary, 237 goats with FEC >300 epg, that needed treatment, would have remained untreated.

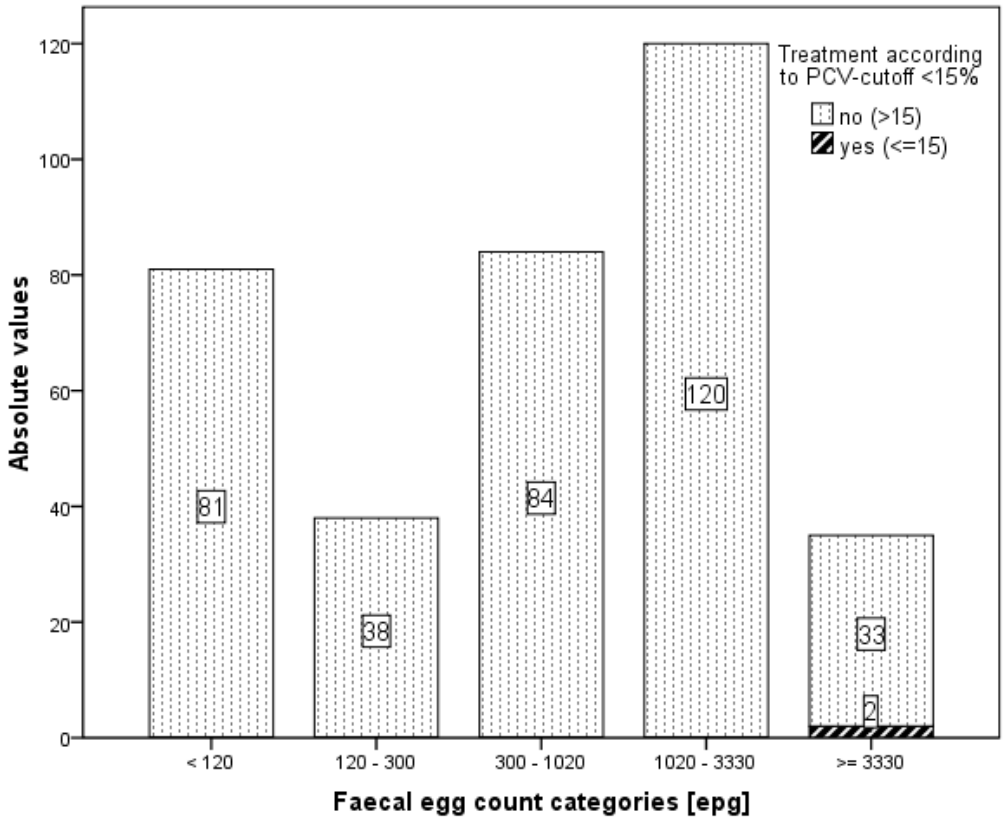

Figure 18: Distribution and frequency of decisions for treatment with a PCV cutoff = 15% as defined by Malan et al. (2001).

Relation of PCV-values to faecal egg count categories

Van Wyk et al. (2001) recommended that animals with FAMACHA©-scores >3 should be treated. According to Malan et al. (2001), a PCV of 22% is the highest PCV-value for FAMACHA©-score 3. Therefore, a PCV = 22% was tested as a further anaemia cutoff-value. However, if this cutoff was used, according to FEC-values 3, 4 and 5 only 7%, 15% and 37% of the study animals would have been treated (fig. 19, appendix tab. e). The mean percentage of treated goats according to this cutoff was only 12.0%. This implies that a higher cutoff-value is required in order to make a proper treatment decision.

Therefore the scheme of Tschuor et al. (2008), who defined a PCV of 24% as an indicator for anaemia in Swiss goats, was taken as higher anaemia cutoff-value. Using this cutoff, the mean percentage of treated animals went up to 19.8%. In detail, 7.6%, 17.6% and 59.1% of the goats with FEC-categories 3, 4 and 5, respectively, would have been recommended for treatment (fig. 20).

As the mean PCV of goats of our study was 30%, calculations using an anaemia cutoff of PCV = 29% were also performed. Thereby, 45%, 60% and 82% of the goats scored with FEC categories 3, 4 and 5 would have received treatment (fig. 21 appendix tab. e). The mean percentage of treated goats was 50.8%. This cutoff maximises the percentage of treated animals, but leaves enough goats untreated to ensure the maintenance of parasites in refugia. Thus, an anaemia-cutoff of PCV = 29% seems to be appropriate as a basic anaemia limit under European conditions.

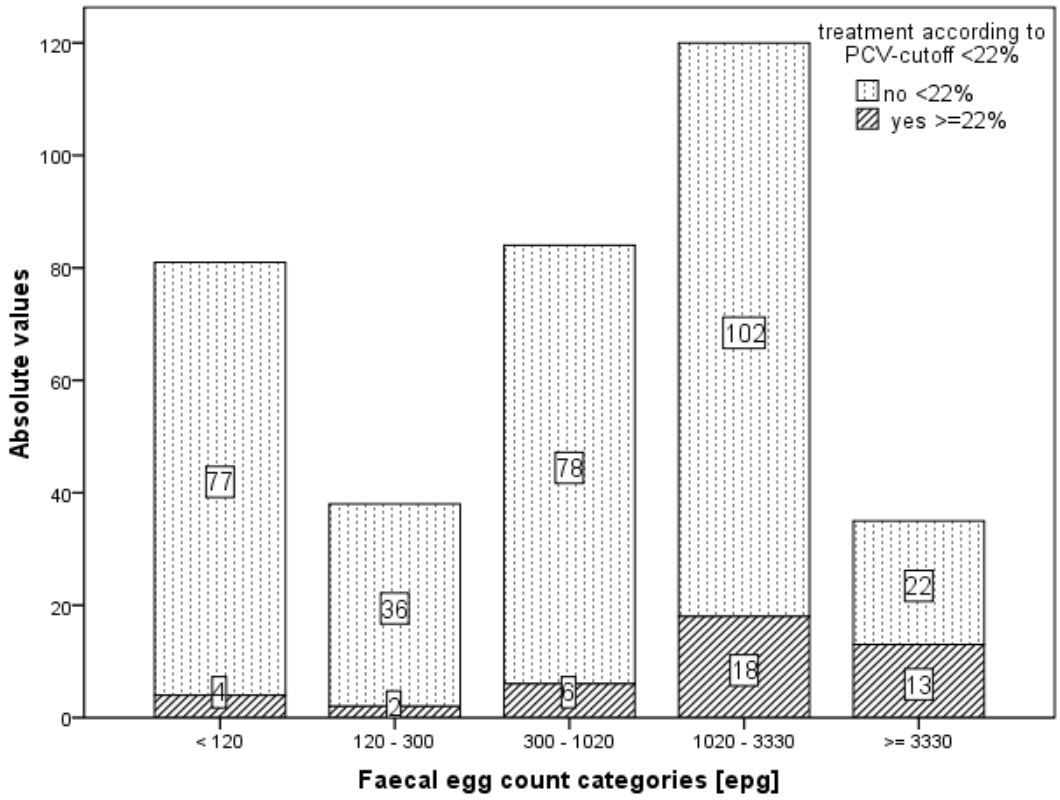

Figure 19: Distribution and frequency of decisions for treatment with a PCV cutoff = 22%.

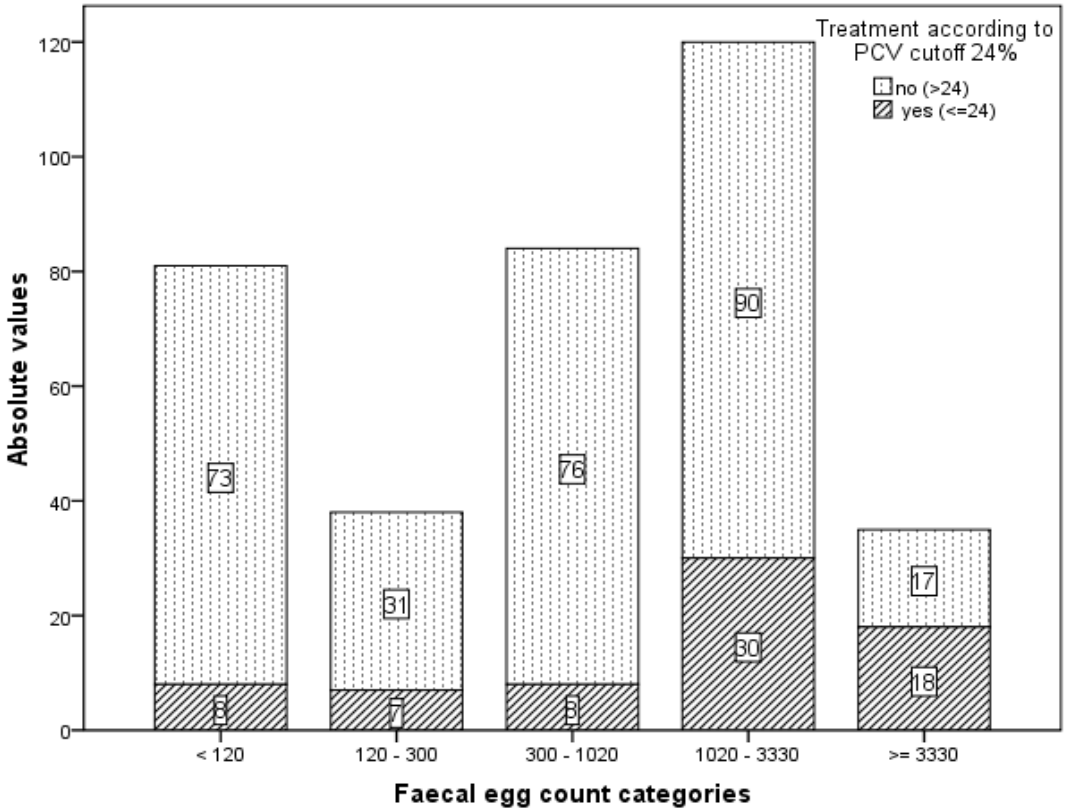

Figure 20: Distribution and frequency of decisions for treatment with a PCV cutoff = 24%.

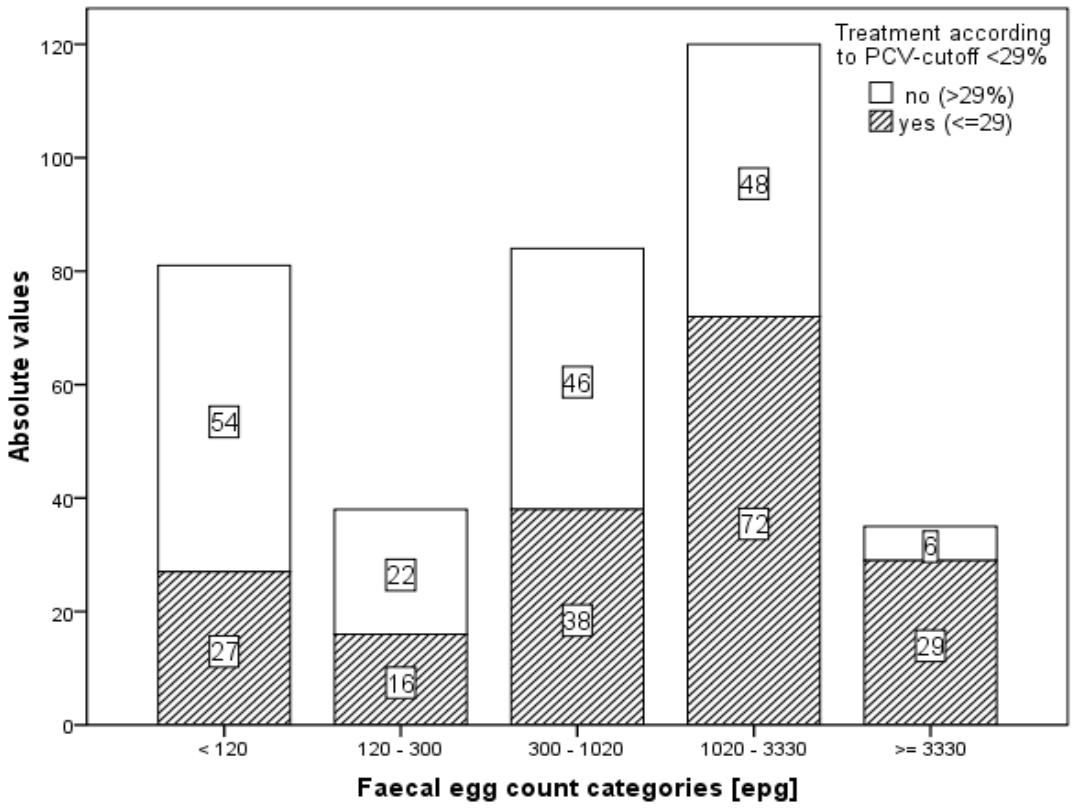

Figure 21: Distribution and frequency of decisions for treatment with a PCV cutoff = 29%.

Relation of PCV-values to FAMACHA©-scoring

If the different PCV cutoffs and their relation to the corresponding FAMACHA© categories are considered, a similar pattern shows. Likewise, a PCV-cutoff of 29% seems to be appropriate as a basic anaemia limit under European conditions and provided the best results. Thereby, 45%, 60% and 82% of the goats scored with FEC categories 3, 4 and 5 would have received treatment, respectively (fig. 22 appendix tab. f). The mean percentage of treated goats, using the cutoff of PCV = 29% was 51.7%.

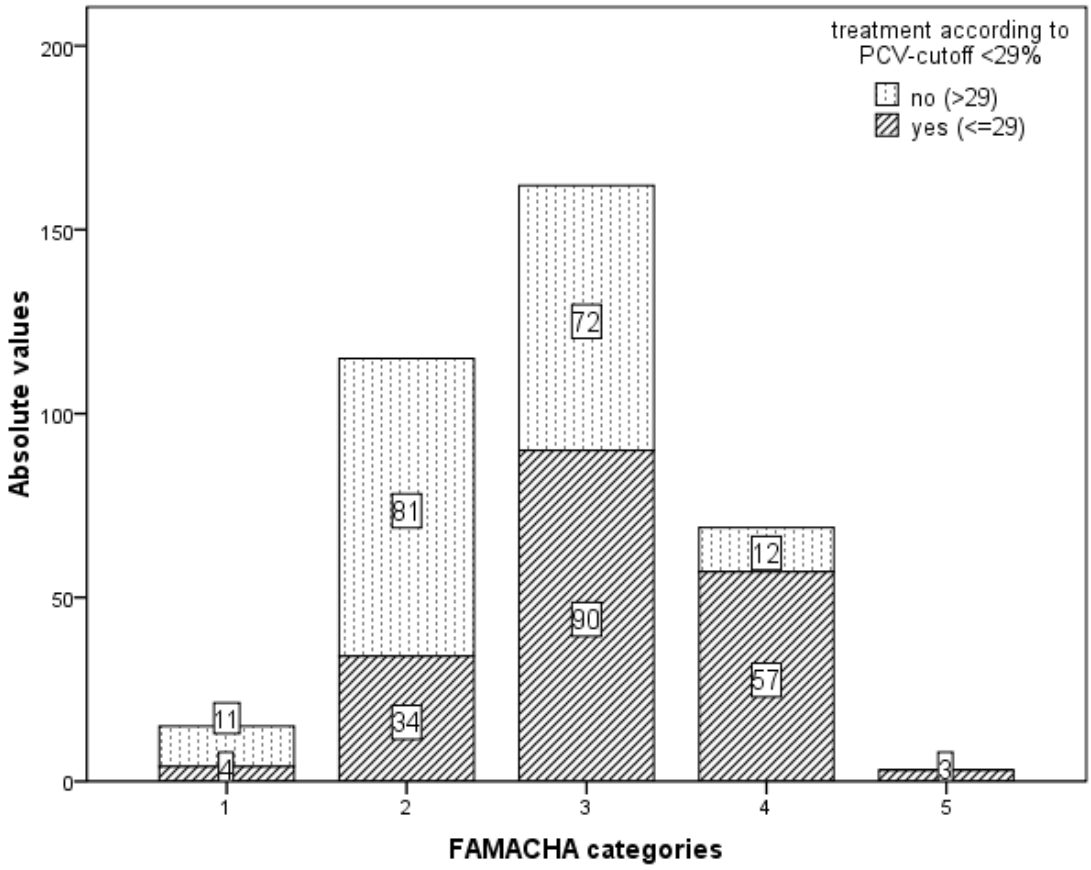

Figure 22: Distribution and frequency of decisions for treatment with PCV cutoff = 29%.

When using a cutoff of PCV = 15% only two of the 234 goats scored with treatment indicating FAMACHA-categories 3, 4 and 5 are treated (fig. 23).

When using the cutoff of PCV = 22% according to FAMACHA-category 3 as described by Malan et al. (2001), only 12.1% of all goats were treated. In detail 15.3% of the goats scored category 3, 21.7% of those scored category 4 and 33.4% of those scored category 5, are recommended for treatment (fig. 24, appendix tab. f).

The cutoff of a PCV = 24% value according to Tschuor et al. (2008) leads to a mean percentage of 20.1% of treated animals (fig. 25). Furthermore, 22.8%, 34.8% and 66.7% of goats in categories 3, 4 and 5, respectively, would have been treated (appendix tab. f).
Again, the calculations using an anaemia cutoff of PCV = 29%

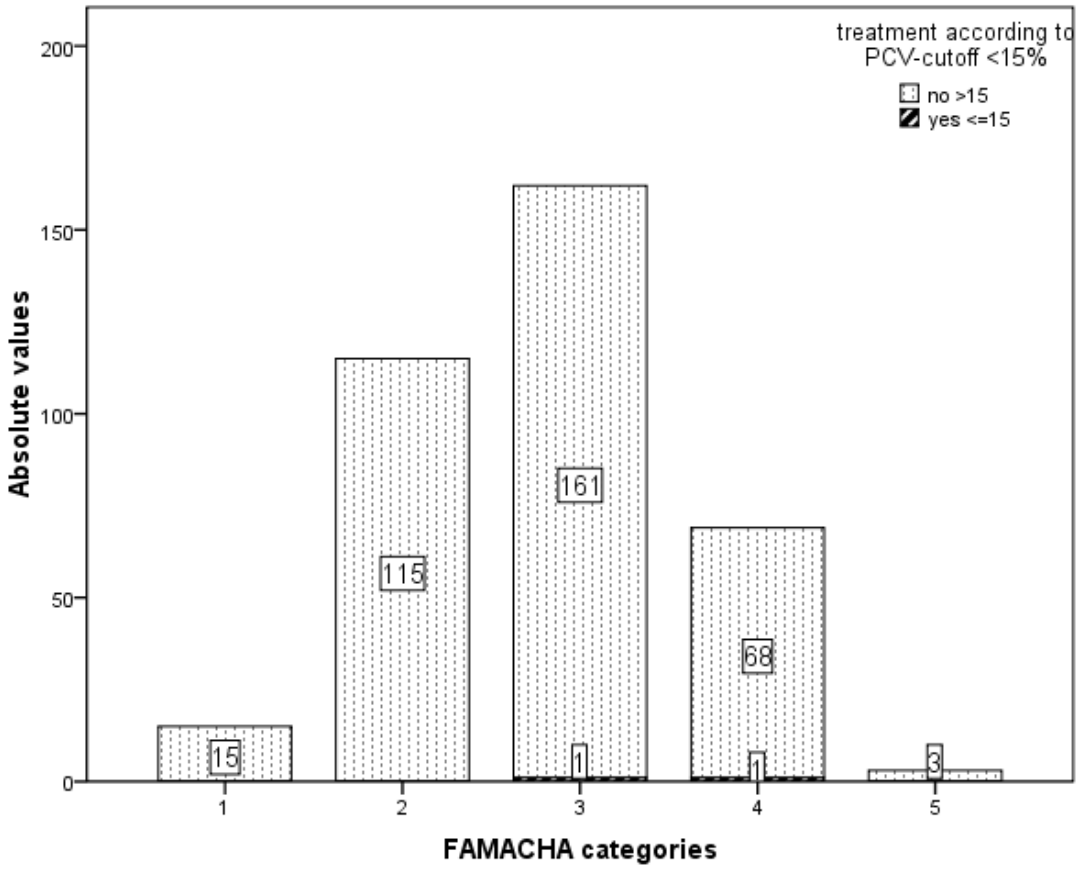

Figure 23: Distribution and frequency of decisions for treatment with PCV cutoff = 15% as assigned by Malan et al. (2001).

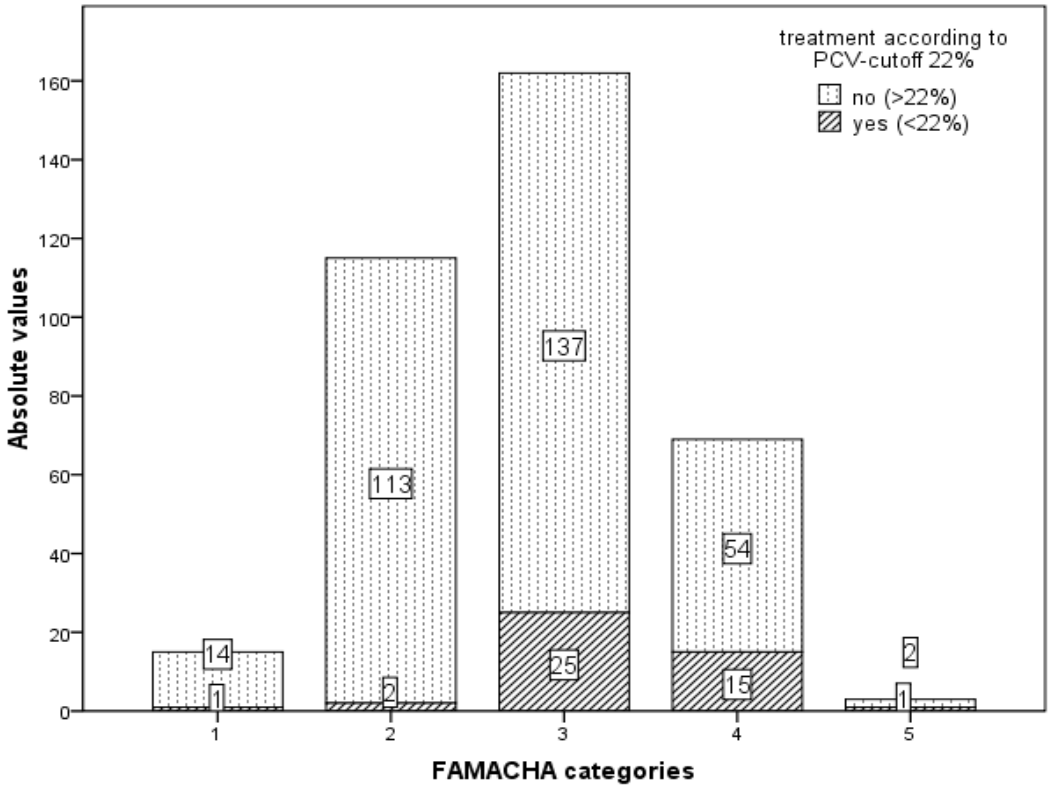

Figure 24: Distribution and frequency of decisions for treatment with PCV cutoff = 22%.

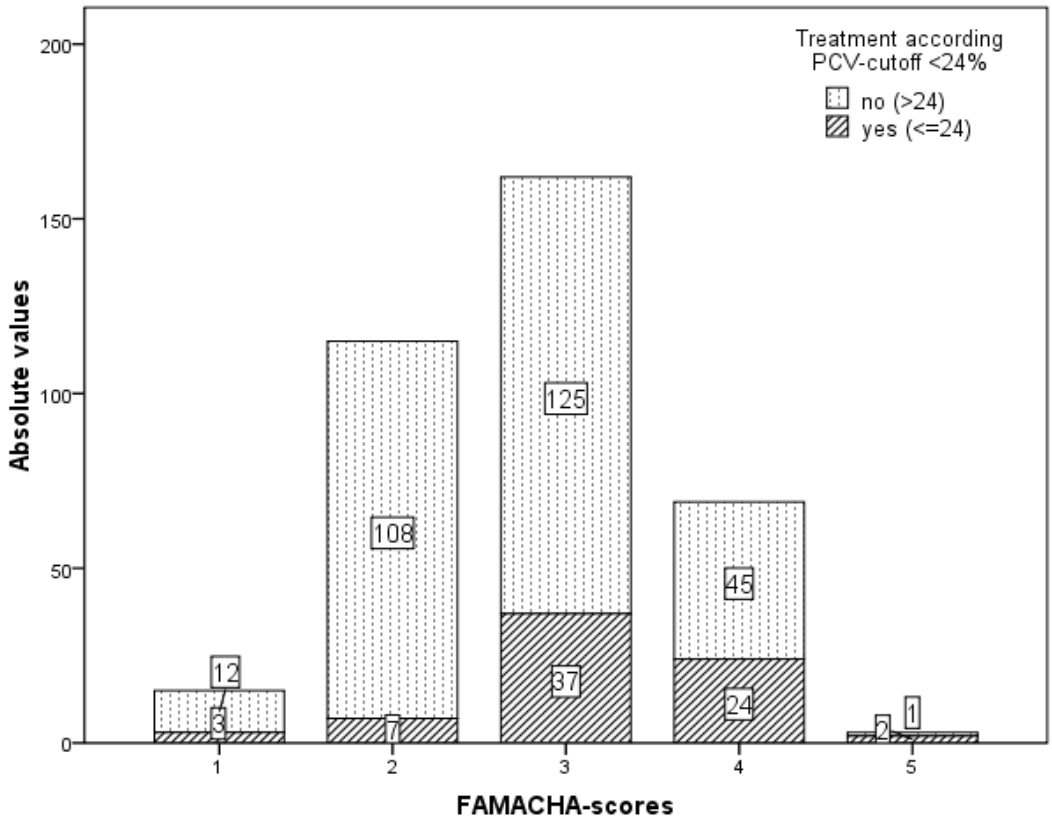

Figure 25: Distribution and frequency of decisions for treatment with PCV cutoff = 24%.

4.4. Prevalence of *Haemonchus contortus* in the study goats

In the flocks of the FAMACHA©-study *H. contortus* was the predominant species, based on the results of the coprocultures. The mean percentage of *H. contortus* was 58.9%. In four of the six flocks the percentage of H. contortus was >67% (compare tab. 4 of publication 2). The mean faecal egg counts of the *H. contortus*-proportion, calculated by using the percentage of *H. contortus* larvae of each flock in each month separately, ran in parallel to the FEC of all GINs during the study period (fig. 26).

The treatment of the goats by the farmers did not change this predominance. At the beginning of the study the mean FEC was 2681 epg. Based on these findings, animals with the highest FEC and with present clinical symptoms were treated by the farmers independently. Hence, the mean FEC in June had decreased. Over the summer months egg excretion gradually increased, until it decreased in September when some animals had been treated. Farmers of flocks G and R successfully treated all goats of their flocks at the end of September and therefore mean FEC was low in October (fig. 26).

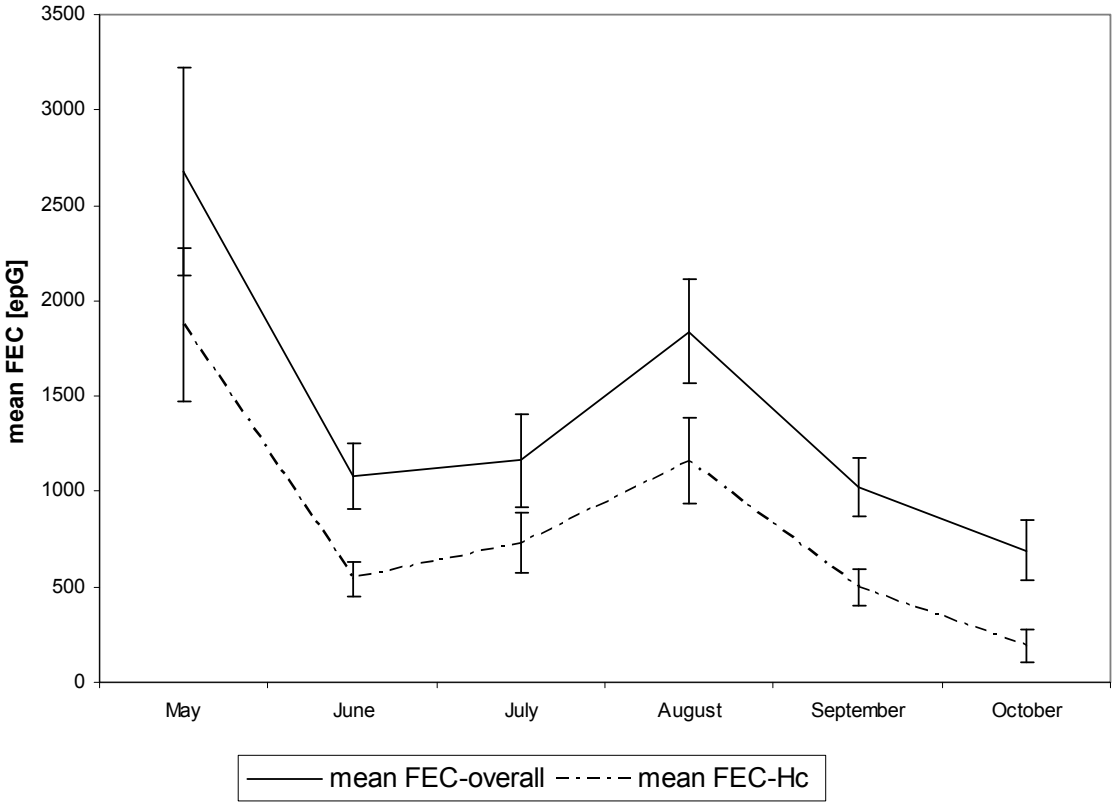

Figure 26: Pattern of mean GIN and of *Haemonchus contortus* epg excretion during the six study months. Whiskers show standard error of the mean.

5. Publications

5.1. Publication 1

Anthelmintic Resistance of *Haemonchus contortus* in small ruminants in Switzerland and Southern Germany

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

Wiener Klinische Wochenschrift

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Abstract

Two goat and two sheep flocks have been found to be suspicious of a clinically evident reduced anthelmintic efficacy, i.e. lacking improvement of gastrointestinal disorders, insufficient weight gain and continuing inappetence after anthelmintic treatments. In order to conduct an appropriate evaluation of the efficacy the following trials were performed: the faecal egg count reduction test on the studied goats of the two herds revealed a reduction of the egg-excretion after the eprinomectin-treatment (1 mg/kg BW, pour-on) of 17.4% and 27.5%, respectively, which clearly confirms the occurrence of anthelmintic resistance against eprinomectin in these two herds. The alternatively administered moxidectin-treatment (1 mg/kg BW, pour-on) of one flock resulted in a 99.1% faecal egg count reduction.In both sheep flocks, 30 randomly selected sheep were divided in three groups and each group was treated with a different anthelmintic, according to the instructions for use.

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

Zusammenfassung

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

In den beiden Schafherden wurden jeweils 30 zufällig ausgewählte Tiere in drei Gruppen eingeteilt, die jede mit einem anderen Anthelminthikum behandelt wurde. Der Eizahlreduktionstest erbrachte EpG - Reduktionen von 70,8% bzw. 55,3% (Albendazol-Gruppen), 52,4% (Fenbendazol-Gruppe) bzw. 47,3% (Oxfendazol-Gruppe). In den beiden

Moxidectin-Gruppen (0.2 mg/kg BW, oral) betrug die Reduktion 100% bzw. 44,3%. Vor und nach der Behandlung durchgeführte Koprokulturen zeigten, dass *Haemonchus contortus* die vorherrschende Helminthenspezies ist.

Keywords: Anthelmintic resistance, *Haemonchus contortus*, FECRT, goat, sheep,

Introduction

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

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

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

Materials and methods

Faecal egg counts

Faecal samples, taken from the rectum, were analysed by the faecal egg count reduction test (FECRT) according to Coles *et al*. [4]. The samples were processed using a modified McMaster-method with a sensitivity of 30 eggs per gram of faeces (epg). The efficacy of the treatment, i.e. the faecal egg count reduction (FECR) was calculated according to WAAVP-guidelines [4, 5, 6].

Larval culture

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

Animals

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

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

performed moxidectin treatment. Out of them three groups of 10 sheep each were formed and sampled on the day of treatment and 13 days later.

Treatment

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

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

Statistical analyses

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

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

Results

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

eprinomectin-treated goat flocks. Moreover, in the "Swiss-flock" the ranks are mostly positive, i.e. in most paired samples the FEC after treatment is higher than before.
The predominant species found in the post-treatment larval culture of all treatment-groups was *H. contortus* (table 2).

Discussion

The presented results on the reduced FECR clearly indicate the occurrence of anthelmintic resistance of *H. contortus* against eprinomectin in both goat flocks and against albendazole, oxfendazole and fenbendazole in the sheep flocks and in the "Alb-flock" also against moxidectin. In the 1990s, benzimidazole-resistant GINs in Switzerland, Germany and other European countries have been reported by Hertzberg *et al.* [10,11].
It is well known that the pharmacokinetics and the efficacy of anthelmintics vary significantly between sheep and goats [12, 13]. In goats, the metabolism of drugs is accelerated and thus leads to a reduced drug availability, which may contribute to the failure of treatment. As a consequence, the treatment-dose for goats has to be adapted to their particular metabolism by increasing the dose (double cattle dose) in order to reach higher plasma levels [14]. This was realized in the new Swiss regulations for eprinomectin [15]. In both goat flocks prior to this analysis the cattle-dose was used and this continuous under-dosing of eprinomectin could have contributed to the development of resistance.
Recent information of the GIN spectrum of sheep and especially goats in Germany and Switzerland is limited. Former studies mostly reported high prevalence levels of *Trichostrongylus* spp. and *Teladorsagia* spp., whereas *H. contortus*-infections have not been specified [16, 17]. However, in 1999 the first Swiss benzimidazole-resistant *H. contortus*-strain was isolated [11]. In the present flocks the prevalence of *H. contortus* with more than 74% was high. This could be due to the selection of the GIN-population as a consequence of the inefficacious anthelmintic treatments prior to this survey, i.e. only few GIN species survived, including *H. contortus*. Coles et al. [18] showed that *H. contortus* is able to rapidly develop resistance against anthelmintic drugs, if larvae which survived anthelmintic treatment can reinfect the animals. Consequently, this ability of *H. contortus* may lead to a gradual increase of its prevalence and therefore to a predominance of this species in affected areas of Europe. *H. contortus* is known as the predominant resistant GIN species in Southern USA, in Africa and in Australia, where a lot of imported small ruminant breeds come from [19]. In agreement with this it was speculated that *H. contortus*-carrying Boer goats, imported from Africa, introduced

benzimidazole- and ivermectin-resistant *H. contortus*-strains into Switzerland [3]. The "Alb-flock" with its Dorper sheep is a typical trading farm and similarly, sheep of the flock could easily have caught up resistant GINs from newly imported sheep. Comparatively, the "Allgaeu-flock" consists mostly of Suffolk and dairy sheep and generally grazed on its home pasture without any trading. It is not known whether this is the reason why moxidectin was still efficacious. It is most likely that the introduction of sheep carrying resistant worms into flocks with non-resistant worm populations might add to the spreading of resistance to previously unaffected farms. Imported animals and their parasite burden are being traded throughout Europe and thus potentially spread their worm infections, both susceptible and resistant ones. In all four flocks, newly introduced animals were anthelmintically treated against GIN-infections and held in quarantine, before integrating them into the flock. However, neither in these cases nor after routine anthelmintic treatment, a coproscopic analysis was performed to verify the efficacy of treatment. In addition, only one surface-limited pasture grazed without interruption, was available on all four farms. It is thus likely that the long-term use of the same anthelmintics and pastures without verifying anthelmintic efficacy, led to an increase of the proportion of resistant *H. contortus* larvae in refugia.

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

Acknowledgements

We are very grateful to the farmers for their help and interest in this study. Special thanks are given to our colleagues for the help with processing the samples.

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

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

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

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

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

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

Conflict of interest:

The authors declare that there is no conflict of interest.

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5.2. Publication 2

The accuracy of the FAMACHA©-method in detecting anaemia and haemonchosis in goat flocks in Switzerland under field conditions

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Veterinary Parasitology

Submitted

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Abstract

In this study, goats from six farms in Central Switzerland were examined for the evaluation of the FAMACHA©-method under middle European conditions. Individual faecal egg counts were determined at a four-week interval for a period of six months and the gastrointestinal nematode (GIN) genera were differentiated using larval culture. Simultaneously, the goats were bled for packed cell volume (PCV) determination and scored for anaemia of the conjunctiva according to the FAMACHA© -method. The three methods used for evaluating haemonchosis, namely FEC, PCV and FAMACHA© score, were compared to test the FAMACHA© -method for its accuracy and efficacy in detecting haemonchosis in Switzerland. PCV and FAMACHA© score correlated significantly during the entire period of six months, whereas PCV and FEC correlated significantly in four study months. The FAMACHA© score and FEC correlated significantly in June only. PCV served as the gold standard for evaluating the accuracy of FAMACHA©-method in detecting anaemic goats. The sensitivity of FAMACHA© in detecting anaemic goats was 93%, using the anaemia criteria cut-offs FAMACHA©-categories ≥3 and PCV <22%. The applicability of the method for detecting goats which needed treatment was tested with FEC >300 epg and >600 epg as cut-off values for treatment. The sensitivity of the method for detecting goats which needed a treatment was 76%, with regard to FEC of *Haemonchus contortus* (treatment cut-offs: FAMACHA© ≥3 and FEC >300 epg). The percentage of false negatives (FEC Hc-portion) was less than 11%. In addition, the use of FAMACHA© categories ≥3, as a treatment indicator, revealed that 64% of the animals were recommended for treatment. These results indicate the suitability of FAMACHA© as an additional part of an integrated anthelmintic control of goat flocks in Switzerland.

Keywords: FAMACHA©, anaemia, *Haemonchus contortus*, goat, Switzerland, Targeted Selective Treatment

Introduction:

In alpine and pre alpine areas, farmed goats mostly have access to pasture and hence are frequently infected with gastrointestinal nematodes (GIN). However, a drug treatment alone

cannot keep up with rapidly developing anthelmintic resistance (AR) in many areas. The escalation of AR in small ruminant husbandry calls for new methods for sustainable management of GIN infections. Among them, Targeted Selective Treatment (TST) using the FAMACHA©-system has been proposed as an important alternation (Van Wyk et al., 2006). The FAMACHA©-method is a diagnostic on-farm system, which facilitates farmers to identify individual animals that need an anthelmintic treatment, through comparison of the colour of the ocular mucous membranes against a dedicated colour chart. This method is based on the anaemia-resulting, blood-sucking activity of *Haemonchus contortus*. The FAMACHA©-categories range from 1 – red (non-anaemic) to 5 – practically white (severely anaemic) (Malan et al., 2001). Thereby, as suggested by Van Wyk and Bath (2002), only individual animals of the flock showing severe anaemia, i.e. goats with scored 3, 4 and 5 and sheep scored 4 and 5, respectively, have to be treated selectively. By using this system for the application of TST, some animals almost always remain untreated. These animals continue to void ova that have not been exposed to anthelmintic selection onto pasture and these are said to be in refugia. As a consequence, the GIN population in refugia could be exploited to regain anthelmintic efficacy. as discussed in theory by Van Wyk (2001). Although the FAMACHA©-method is used and considered as a valuable tool in sub-Saharan Africa, southern United States and South America ,where *H. contortus* is very common (Ejlertsen et al., 2006; Mahieu et al., 2007), it is still largely unknown in Europe. While only infections with *H. contortus* and possibly other haematophagous worm species (Van Wyk and Bath, 2002) can be monitored with this method, in Switzerland most flocks harbour only low burdens of *H. contortus*, and are co-infected with up to 5 different GIN genera, including *Trichostrongylus* spp., *Teladorsagia* spp., *Cooperia* spp., *Oesophagostomum* spp. and *Chabertia* spp. (Eckert et al., 2008). However, *H. contortus* is often the dominant species in flocks affected by AR. Therefore, the question arises whether the FAMACHA©-method is of use in Switzerland for the implementation of a more targeted anthelmintic dosing and for prevention of production losses in heavily infected flocks. Consequently, the objective of this study was to evaluate the applicability, the sensitivity and specificity of the FAMACHA©-method in 64 goats of six goat flocks, with a history of resistant GIN-infections, in Central Switzerland.

Materials and Methods

Goats and goat flocks

Sixty-four goats from six flocks in Switzerland (Cantons of Bern, Luzern and Zug) with a history of a reduced anthelmintic efficacy (personal observation) against GIN were chosen for

this study (Table 1). Each flock consisted of 10 to 100 heads, including bucks, one kid group and does with an age range from 2 months to 13 years. Of each goat flock, 8 to 13 individually tagged, randomly selected goats were included in the study group and sampled once a month in the grazing period, i.e. from May to October 2008. No kids were included in the study. Management and treatments of the flocks was always performed under the individual responsibility of the farmers and unrelated to FAMACHA©-results.

Table 1: Composition of the study goat flocks in Central Switzerland, 2008.

Flock	Size of flock /no. of goats tested	Age range of study group	Housing system	Breeds	Use
A	25 head / 11	1 - 8 years	Pen, pasture	Mix, Cha, Tog,	Milk*
B	100 head / 11	1 - 5 years	Pen, pasture	Cha	Meat
G	13 head /13	1 - 5 years	Tie-stalls, pasture	Cha, Tog, Mix, Nub, Saa,	Milk
J	15 head / 8	1 - 5 years	pen, pasture	Boe	Breeding
M	25 head / 11	1 - 7 years	Tie-stalls, pasture	Mix, Tog, Cha, Saa	Milk*
R	100 head / 10	1 - 9 years	Pen, pasture	Saa, one Tog	Milk*

Mix = mixed breed; Cha = Chamoisee; Saa = Saanen; Tog = Toggenburg, Nub = Anglo-Nubian; Boe = Boer; * = organic farming;

Sampling, data collection

At the beginning of each month, over the study period from May to October 2008, the same individually tagged goats were sampled.

The colour of the conjunctiva was clinically evaluated following the recommendations of the FAMACHA©-method (Malan et al., 2001; Van Wyk and Bath, 2002). Simultaneously, faecal samples were directly taken from the rectum and 5 ml sterile EDTA-blood were collected from the jugular vein of each goat and transferred to the laboratory.

Faecal analysis

A modified McMaster-method was used to determine the faecal egg counts (FEC) with a sensitivity of 30 eggs per gram faeces (epg) for each individual goat. Differential larval counts were done on larvae recovered according to Roberts and O'Sullivan (1950) from pooled cultures of 3 g of faeces per goat, incubated for a minimum of 12 days at 22° C. Every month 200 L3/flock were identified to the genus or species level for each month according to Bürger

and Stoye (1968) and VanWyk et al. (2004). By this means the percentage of infection with every GIN genus/species level was determined and the FEC-portion/flock of *H. contortus* (FEC-Hc) was estimated accordingly. Pooled faecal samples of each flock were also screened for *Fasciola hepatica* eggs by means of the sedimentation method after Benedek (Bauer, 2007), in order to exclude *F. hepatica* as an anaemia-causing factor (Van Wyk and Bath, 2002). In 12 individual cases throughout the study no faeces could be obtained.

Packed cell volume

The collected EDTA-samples were used to determine the packed cell volume (PCV) of each goat by means of microcentrifugation according to Kraft and Dürr (1999). The range of PCV values for each FAMACHA©-category was adapted in this study according to the standard blood values for goats (Kraft and Dürr, 1999) and to Middle European conditions (Table 2).

Table 2: Range of PCV values for each FAMACHA©-category as described by Malan et al. (2001) and adapted in this study (Kraft and Dürr, 1999).

FAMACHA© category	**PCV Malan**	**PCV Study**
1	>28%	>40%
2	23-27%	39 - 33%
3	18-22%	32 - 26%
4	13-17%	25 - 20%
5	≤ 12%	19 -13%

Statistical analysis

Spearman rank correlation coefficient and Pearson correlation were calculated (SPSS 15.0 and 16.0 software) to show the relationship between the four variables, FEC-overall, FEC-*H. contortus* portion (FEC-Hc), FAMACHA© score and PCV for each study month. Box plots were performed to display the relationships of the variables. Crosstabulations were drawn up with the variables to calculate sensitivity (TPF = true positive fraction), specificity (TNF = true negative fraction), the predictive value of a positive (PPV) and the predictive value of a negative (NPV) according to Vatta et al. (2001). Test sensitivity was defined as [(true positives/(true positives + false negatives))x 100]; specificity as [(true negatives/(true negatives + false positives))x100]; NPV as [(true negatives/(true negatives + false negatives))x100];

PPV as [(true positives/(true positives + false positives))x100]. PCV was used as the gold standard by which anaemia was measured and four cut-off values for anaemia (<15%, <22%, <29% and <32% respectively) were assigned. FEC-overall and FEC-Hc (i.e. the portion of *H. contortus*) were also used to check the adequacy of the FAMACHA© scoring with the two cut-off values more than 300 epg and more than 600 epg, respectively.

Results

Clinical conditions

The goats were generally in good condition and healthy throughout the study. No infections with *F. hepatica* could be determined. Only few goats were drenched under the responsibility of the farmers because of bad condition, diarrhoea and high FECs.

Faecal egg count and determination of PCV

The faecal egg output of all GIN genera was high for this region throughout the study (mean FEC = 1406 epg). Based on the results of the larval cultures, *H. contortus* was the predominant GIN species on all farms (mean FEC = 879 epg). FEC fluctuated over the study period with a maximum at the beginning of May, when several goats were treated, and another maximum in August (fig.1). Animals with high FAMACHA© scores generally had high FECs, and vice versa. However, FECs associated with categories 1 and 2 were nearly identical (Table 3). Most animals showed FEC of around 1500 epg and a FAMACHA© score of 3. In 12 cases no FEC was determined because faeces could not be obtained.

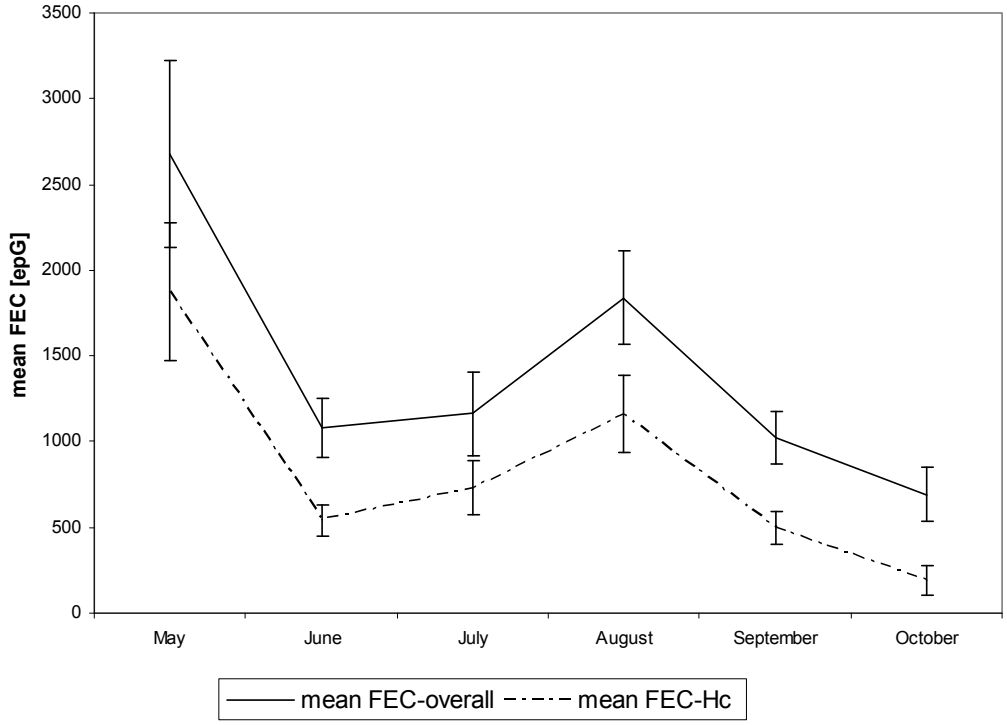

Figure 1: Pattern of mean faecal egg counts (epg) of mean FEC-overall and of the *H. contortus* portion (mean FEC-Hc) during the study period with pointwise 95% confidence intervals.

The mean PCV of all measured values was 30%, the lowest detected PCV-value was 12% and the highest was 59%, respectively. Because of rapid coagulation in 20 cases no PCV could be determined. Generally, animals with higher FAMACHA© scores revealed low PCV values (Table 3). There was a slight increase of mean FEC as PCV-categories decreased, as defined in this study (Table 2). In general, when PCV fell below 19%, FEC were higher than 1020 epg and the majority of FEC were above 3330 epg.

Table 3: Mean and standard error of the mean (S.E.) of faecal egg counts (FEC) and packed cell volume (PCV), respectively, for each FAMACHA©-score.

FAMACHA© score	FEC			PCV		
	n	Mean [epg]	(S.E.)	n	Mean [%]	(S.E.)
1	15	886	(241)	15	34	(2.2)
2	118	849	(90)	115	33	(0.6)
3	163	1493	(168)	162	29	(0.5)
4	73	2160	(446)	69	26	(0.7)
5	3	2850	(1426)	3	23	(2.0)
total	372	1406	(121)	364	30	(0.4)

Larval cultures

H. contortus, *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum*/*Chabertia* spp. and *Strongyloides papillosus* were detected in larval cultures of pooled faecal samples of the individual flocks. With the exception of flock A, *H. contortus* always was the predominant species (Table 4). The percentage of *H. contortus* larvae of each flock and each month was used to calculate the FEC portion of *H. contortus* (FEC-Hc).

Table 4: Mean percentage of GIN larvae genera/species detected by means of coproculture in pooled faecal samples of each flock during the study period.

	Mean percentage of GIN in each flock						
Flock	**A**	**B**	**G**	**J**	**M**	**R**	**Mean**
Haemonchus contortus	25,6	71,8	72,8	77,5	38,7	66,7	**58,9**
Trichostrongylus spp.	56,2	22,6	25,1	15,5	30,5	20,5	**28,4**
Oesophagostomum / Chabertia spp.	12,6	3,3	0,1	0,2	23,9	0,0	**6,7**
Teladorsagia spp.	5,2	1,0	0,0	0,7	1,2	0,0	**1,3**
Cooperia spp.	0,2	0,7	0,0	3,8	2,1	9,4	**2,7**
Strongyloides spp.	0,2	0,4	2,0	2,3	3,6	3,5	**2,0**

Relationship between the three clinical indicators of GIN infection

Correlations between the four variables are listed in table 5. PCV and FAMACHA©-score correlated significantly and negatively in all six study months. PCV and FEC and PCV and FEC-Hc, respectively, correlated significantly negative in the same four of six study months. Correlations between FEC and eye scores were positive in all study months but only in June the correlation was significant. FECs of the *H. contortus* portion and FAMACHA©-score correlated significantly in June, August and September.

Table 5: Correlation after Pearson of the four study variables: PCV, FEC, FEC-Hc and FAMACHA©.

	May	June	July	August	September	October
Correlation						
PCV-FAM	R = -0.387**	R = -0.462**	R = -0.441**	R = -0.318*	R = -0.432**	R = -0.368**
PCV-FEC	R = -0.344**	R = -0.171	R = -0.296*	R = -0.388**	R = -0.162	R = -0.314*
PCV-FEC-Hc	R = -0.308*	R = -0.119	R = -0.296*	R = -0.333*	R = -0.185	R = -0.312*
FEC-FAM	R = 0.233	R = 0.300*	R = 0.143	R = 0.126	R = 0.148	R = 0.023
FEC-FAM-Hc	R = 0.194	R = 0.261*	R = 0.164	R = 0.247*	R = 0.288*	R = 0.118

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

As shown in fig. 2 a, FEC values of the individual FAMACHA©-scores overlapped particularly in scores 1 to 4. Values overlapped less when only FECs of the *H. contortus*-portion were considered (fig 2 b). Especially for the *H. contortus* portion FEC ranges were narrow for FAMACHA©-scores 1 and 2, but were wide for the treatment category (FAMACHA© 3-5).

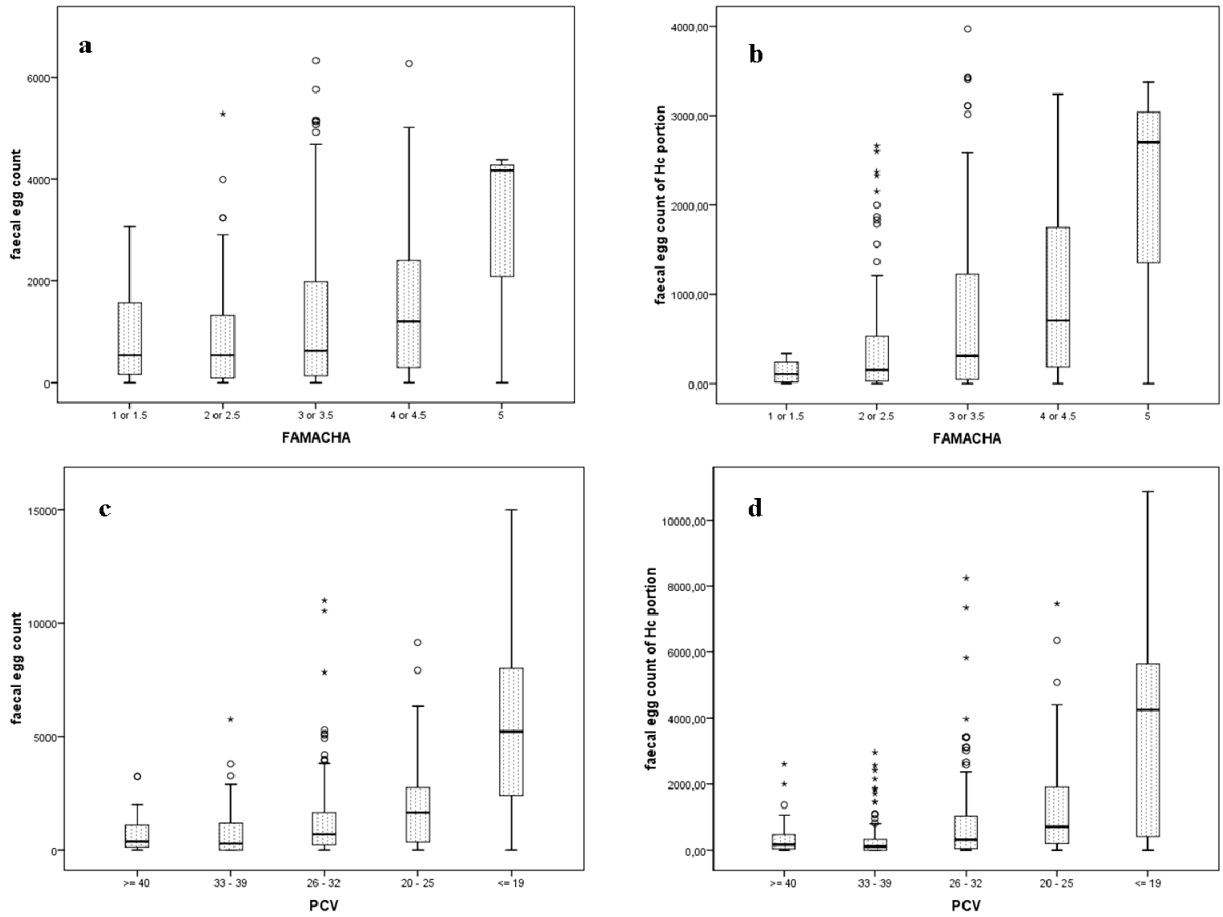

Figure 2: Box plots demonstrating the relationship between FEC, PCV and FAMACHA©-category in goats. Relationship between (a) Total faecal egg count (FEC-overall) and FAMACHA©, between (b) *Haemonchus* spp. faecal egg count (FEC-Hc) and eye score, between (c) Total faecal egg count (FEC-overall) and PCV category and between (d) *Haemonchus* spp. faecal egg count (FEC-Hc) and PCV. Lower and upper borders of the box represent the 25th and 75th percentiles, respectively. Median (solid line) values are presented within the box. Whiskers above and below the box indicate the maximum and the minimum non-outlier observation. Circles indicate outliers.

FEC associated with low PCV values were high for both FEC-overall and FECs of the *H. contortus* portion, respectively (Fig. 2c and d).

Small differences in PCV were noted for FAMACHA© score 4 (n = 69) and especially 5 (n = 3) (fig. 3). PCV values were high for FAMACHA© 1 and 2. Explorative analyses found that only 20% of the animals were scored FAMACHA© value 4 and 5, respectively.

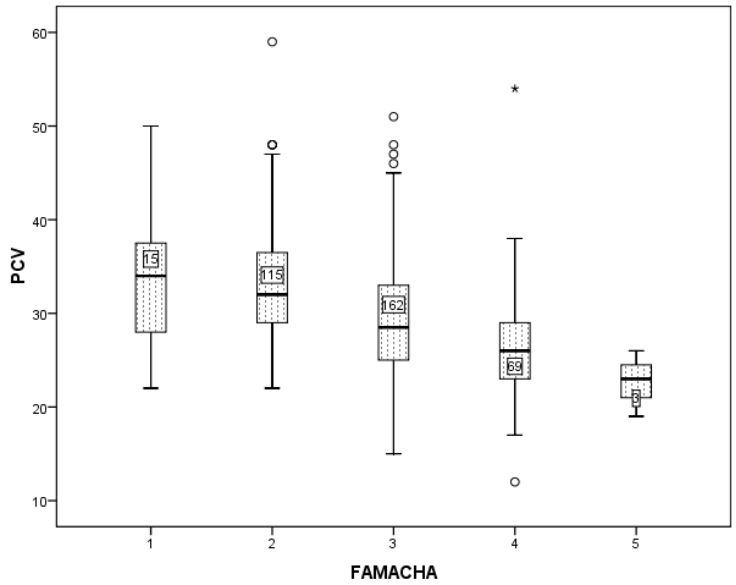

Figure 3: Box plot demonstrating the relationship between PCV and FAMACHA© eye score category in goats. Lower and upper borders of the box represent the 25th and 75th percentiles, respectively. Median (solid line) values are presented within the box. Whiskers above and below the box indicate the maximum and the minimum non-outlier observation. Circles indicate outliers.

Evaluation of the FAMACHA©-method

Number of true positives, false positives, true negatives and false negatives are shown in tables 6 and 7. The percentage of goats recommended for treatment decreased from 64.2% for FAMACHA© score 3, 4 and 5 to 20.4% for scores 4 and 5 [(TP + FP)/ total number x 100]. In 43.5% of all cases, the decision for a treatment according to FAMACHA© scoring was in agreement with recommendations for treatment of animals with FEC higher than 300 epg and in 36.6% with FEC higher than 600 epg .

Sensitivity, specificity, the predictive values of a positive and of a negative, respectively, of the FAMACHA©-test with reference to FEC and PCV are given in table 8. The number of false negatives decreased when only FEC-Hc was considered and thus, sensitivity increased. Sensitivity was lower when FAMACHA© scores 4 and 5 were considered positive test results. In this case, however, specificity was higher (Table 9).

Table 6: Crosstabulations of FEC-overall and FEC-Hc, respectively, by FAMACHA© with FEC of >300 epg and >600 epg and FAMACHA© scores 3, 4 and 5 were considered positive test results.

FAMACHA© score	**FEC-overall**					
Treatment*	No (<300)	Yes (> 300)	total	No (<600)	Yes (> 600)	total
no (1,2)	52	81	133	70	63	133
yes (3-5)	77	162	239	103	136	239
total	129	243	372	173	199	372
FAMACHA© score	**FEC-Hc**					
Treatment	No (<300)	Yes (> 300)	total	No (<600)	Yes (> 600)	total
no (1,2)	92	41	133	108	25	133
yes (3-5)	110	129	239	140	99	239
total	202	170	372	248	124	372

*: animals with FAMACHA-category 1 and 2 and with FECs >300 were recommended for anthelmintic treatment.

Table 7: Crosstabulations of FEC-overall and FEC-Hc, respectively, by FAMACHA© with FEC of >300 epg and >600 epg and FAMACHA© scores 4 and 5 were considered positive test results.

FAMACHA© score	**FEC-overall**					
Treatment*	No (<300)	Yes (> 300)	total	No (<600)	Yes (> 600)	total
no (1-3)	110	186	296	145	151	296
yes (**4, 5**)	19	57	76	28	48	76
total	129	243	372	173	199	372
FAMACHA© score	**FEC-Hc**					
Treatment	No (<300)	Yes (> 300)	total	No (<600)	Yes (> 600)	total
no (1-3	173	123	296	214	82	296
yes (**4, 5**)	29	47	76	34	42	76
total	202	170	372	248	124	372

*: see footnote Table 6.

Table 8: Quality of FAMACHA©-tests judged by FECs with two cut-offs and by PCV with four different cut-offs. FAMACHA©-scores 3, 4 and 5 were considered positive test results.

Treatment	FEC-overall cut-off		FEC-Hc cut-off		PCV cut-off			
FAMs 3, 4 and 5	>300	>600	>300	>600	15%	22%	29%	32%
Sensitivity [a] [%]	66.7	68.3	75.9	79.8	100	93.2	79.8	73.6
Specificity [b] [%]	40.3	40.5	45.5	43.6	35.9	39.7	52.3	57.3
(a + b)/2	53,5	54.4	60.7	61.7	67.95	66.45	66.05	65.45
positive predictive value (PPV)	67.8	56.9	54.0	41.4	0.9	17.5	64.1	79.9
negative predictive value (NPV)	39.1	52.6	69.2	81.2	100	97.7	70.8	48.5

FAMs = FAMACHA©-score; Sensitivity = (TP/(TP + FN))x 100; Specificity = (TN/(TN + FP))x 100; PPV = (TP/(TP+FP)x100); NPV = (TN/(TN+FN))x100; PPV = [(true positives/(true positives + false positives))x100]. NPV = [(true negatives/(true negatives + false negatives))x100];

Table 9: Quality of FAMACHA©-tests judged by FECs with two cut-offs and by PCV with four different cut-offs. FAMACHA© scores 4 and 5 were considered positive test results.

Treatment	FEC-overall cut-off		FEC-Hc cut-off		PCV cut-off			
FAMS 4 and 5	>300	>600	>300	>600	15%	22%	29%	32%
Sensitivity [a] [%]	23.5	24.1	27.7	33.9	50	36.4	31.9	25.9
Specificity [b] [%]	85.3	83.8	85.6	86.3	80.4	82.5	93.2	94.6
(a + b)/2	54.4	54.0	56.7	60.1	65.2	59.5	62.6	60.3
positive predictive value (PPV)	75.0	63.2	61.8	55.3	1.4	22.2	83.3	91.6
negative predictive value (NPV)	37.2	49.0	58.5	72.3	99.7	90.4	56.2	35.6

See footnote of Table 8.

Discussion:

The FAMACHA©-method is considered to be a useful tool for on-farm evaluation of anaemia and therefore competent for detecting small ruminants suffering from haemonchosis in Africa, in Southern USA and in the Caribbean (Van Wyk and Bath, 2002, Kaplan et al., 2004, Burke et al., 2007, Mahieu et al., 2007). Within this study the FAMACHA©-method was evaluated in parallel with the ongoing livestock management. The farmers treated their animals independently of the FAMACHA©-recommendations, but considering clinical signs of trichostrongylidosis, such as scrubby fur, reduced milk production and loss of appetite and weight. This was, however, of no concern since the objective of this study was to test the quality of the method and its applicability under Middle European conditions.

Earlier studies verified the correlation of PCV and the FAMACHA© values (Kaplan et al., 2004 Burke et al., 2007, Riley and Van Wyk, 2009). In this study it was additionally investigated, whether a reasonable coherence of FAMACHA© scoring and the number of excreted eggs exits. Such coherence might give an indication of the intensity of infection with *H. contortus*.

Except for two flocks, *H. contortus* was the predominant GIN species found in coprocultures (mean: 59% larvae, Table 4). This is more than the percentage of *H. contortus* of 37% in French dairy goats (Chartier and Reche, 1992). In three of the six goat flocks the percentage of *H. contortus* was over 75%. Given such a high percentage and since liver fluke infections were excluded as a further reason of anaemic pallor, it seems likely that haemonchosis is the major causative agent of anaemia in these studied flocks. Consequently, this should enable the FAMACHA©-system to detect animals that are unable to withstand current *Haemonchus* spp. challenge.

The sensitivity of the FAMACHA©-method varies from 73.6% to 100% for the different PCV ranges, depending on the PCV cut-off value used (Table 8). The highest average of sensitivity and specificity, calculated according to Vatta et al. (2001), was achieved when PCV values were lower than 29% and 22%, respectively, and FAMACHA© scores of 3, 4 and 5 were classified as representing anaemia. These PCV thresholds are higher than those in previous studies carried out in South Africa and Guadeloupe (Vatta et al., 2001; Mahieu et al., 2007) based on the fact that only in 10 cases during our study a PCV of 19% or lower was detected and the mean value of all PCV over the entire study was 30%. Koopmann et al. (2006) reported similar findings in sheep and goats in Northern Germany. In addition to differences in *H. contortus* infection levels, these differences could be due to the better health and alimentary

conditions in Germany and Switzerland compared to the resource-poorer farms in South Africa As a consequence, we assigned adapted PCV ranges for each FAMACHA©-score according to standard goat blood-values (Kraft et al., 1999). Negative correlation between FAMACHA©-scoring and PCV was significant in all study months and allowed to conclude that the FAMACHA©-system was used correctly. Furthermore this provided a proof of the general usefulness of the method for the detection of anaemia under European conditions.

The sensitivity of FAMACHA© with regard to FECs over 300 epg and 600 epg was 66.7% and 68.3%, respectively. As mentioned above, sensitivity was higher and the number of false negatives decreased when considering only FEC of the *H. contortus* portion. However, the correlation of the two variables was significant only in June in the first case and in June, August and September in the second case, respectively. The sensitivity was lower when FAMACHA© scores 4 and 5 were considered positive test results. Under these conditions, however, the specificity of the results was higher. A high sensitivity of a clinical test is more important than high specificity. An animal classified as false positive and hence treated will not be negatively affected by the drug, even if an unnecessary treatment does not support the approach of targeted treatment (Van Wyk et al., 2006). On the other hand, an animal classified falsely negative that is not treated will continue to contaminate the pasture, reinfect treated animals or could even die.

PCV as a measure of anaemia and FEC correlated significantly during a period of four months. The distribution showed the estimated tendency (fig.1); the higher the FECs the lower the detected PCV. Only few animals showed FAMACHA© scores 4-5 or PCV lower than 19%. Before such extreme levels were reached, goats showed other clinical signs of trichostrongylidosis, including scrubby fur and emaciation; all being more obvious to the farmers on cursory inspection than anaemia. Goats in this study seem to tolerate a quite high infection rate with *H. contortus*; presumably because of good care, high qualitiy nutritional level and the absence of other blood parasites as often present in tropical and subtropical areas. Indeed, several studies report the negative effects of malnutrition to the response to parasitism (Houdijk et al. 2001, Coop and Kyriazakis, 2001).

It seems that the FAMACHA©-system is working at its best under resource-poor conditions and where infections with *H. contortus* reach lethal levels more quickly. However, from the present study it appears that in flocks harbouring resistant *H. contortus*, the application of the FAMACHA©-method as a guidance for the administration of anthelmintic drugs can be an important tool in Europe as well. In addition, many farmers treat their animals against GIN infections without a coprological analysis of the treatment efficacy with the consequent danger

that worm resistance may not be identified. Under these circumstances, infections with GINs can quickly cause severe production losses. Here, a simple checking of the eye according to the FAMACHA©-method could prevent heavy infections and even from mortalities. Consequently, the present study demonstrates that FAMACHA©, under consideration of the obvious clinical signs of trichostrongylidosis and provided that *H. contortus* is present, can be used as tool of integrated treatment schemes.

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GENERAL DISCUSSION

Infections with gastrointestinal nematodes (GIN) represent a major constraint in small ruminant husbandry. The control of GIN infections is therefore an important part of livestock management. Unfortunately, a lot of GIN strains have become resistant against anthelmintic drugs. To date, anthelmintic resistance (AR) has been reported for all three broad-spectrum anthelmintic groups available for the control of gastrointestinal nematodes of small ruminants (i. benzimidazoles, ii. imidazothiazoles and hydropyrimidines, iii. macrocyclic lactones) worldwide and its impact on sheep and goat farming is dramatically increasing (Kaplan, 2004; Wolstenholme et al., 2004). Hence, the objectives of the present thesis were:

(i) to determine the prevalence of GIN infections in various small ruminant flocks,
(ii) to screen them for the occurence of anthelmintic resistance
(iii) to evaluate the FAMACHA©-method as a tool for minimising the application of anthelmintics in order to better manage GIN infections and
(iv) to evaluate the role of *H. contortus* in the spreading of anthelmintic resistance.

The family Trichostrongylidae constitutes an important part of gastrointestinal parasites of small ruminants. This epidemiological field-survey on GIN infections in small ruminants in Southern Germany and Switzerland has confirmed again that almost every goat and sheep grazing on pastures is infected with trichostrongylids. Among them, *H. contortus* is one of the most pathogenic members of this family, mainly due to its blood-sucking activity. Heavily infected animals may die due to severe anaemia, inappetence and rapid emaciation. Milder cases of haemonchosis cause economic losses due to reduced productivity.

During the present study, clinically suspected AR in two goat and two sheep flocks was found and gave rise to further analyses. The results clearly indicate the existence of AR of *H. contortus* against eprinomectin in goats of both studied flocks. The FECRT performed in the two sheep flocks revealed a substantial degree of resistance of *H. contortus* against albendazole, oxfendazole and fenbendazole. In addition, the GINs of the “Alb-flock” are also resistant against moxidectin. All these results are in line with previous findings of AR in sheep and goats in Western Europe (Artho et al., 2007; Bauer, 2001; Hertzberg and Bauer, 2000) and thus indicate a high prevalence of AR.

In this study, small flock sizes, the health status and ethical reasons did not allow leaving animals untreated as control groups. Therefore, resistance was considered present, if the FECR

was <95% (Coles et al., 1992, Artho et al., 2007). Accordingly, anthelmintic resistance was detected in seven out of the nine treatment groups, i.e. in all four flocks.

There are several reasons for the appearance of AR. One possibility is that a genetic mutation spontaneously develops within a parasite population that enables it to survive treatment. Together with too frequent and/or inappropriate dosing, this may lead to a selection of unsusceptible strains and to a continuous buildup of a resistant population. A further possibility is that animals carrying resistant GINs are introduced into a flock. The former is a very common reason because many farmers still do not consistently follow recommendations for the application of pharmaceutical products. Many farmers do not weigh the animals, and if the real weight is underestimated, under-dosing of anthelmintics is the consequence. A lot of goats are still treated according to the sheep dose, although it is well known that the pharmacokinetics and the efficacy of anthelmintics vary between sheep and goats (Sangster et al., 1991, Chartier et al., 2000). The metabolism of drugs is much quicker in goats, thus reducing drug availability (Alvinerie et al., 1999), a fact which may contribute to a reduced efficacy or even a failure of treatment. Because of this, it is recommended to adapt the treatment dose to the special metabolism of the goats by increasing it to two fold of the sheep dose (Hennessy et al., 1993, Alvinerie et al., 1999). Chartier and Pors (2004) showed that the eprinomectin treatment of goats with 1.0 mg/kg BW was highly effective. In the “Blackforest” goat flock the standard cattle dose (eprinomectin: 0.5 mg/kg BW) was used in the past and this permanent under-dosing seemed to have resulted in a rapid development of resistance. In the “Alb-flock” moxidectin has been systematically used for two years, and while the initial treatments were clinically effective, today the FECR has decreased to 44.3%. This indicates a rapid emergence of AR, as discussed by Coles et al. (2005), when substances are being used too frequently. The rotation between drugs of the different anthelmintic classes on an annual basis can help to slow down the development of resistance (Coles and Roush, 1992; Sangster, 2001).

The introduction of resistant GIN strains into a herd with non-resistant worm populations by integrating newly bought or imported animals is a widespread source of treatment failure in previously unaffected farms. Two of the resistant flocks consist of African breeds, i.e. Boer goats and Dorper sheep, which are traded on a regular basis. Artho et al. (2007) found avermectin-resistance of GIN (species not determined) in African Dorper sheep imported into Switzerland. Moreover, African Boer goats were reported to be infected with benzimidazole- and ivermectin-resistant *H. contortus* in Switzerland (Schnyder et al., 2005). Interestingly, *H. contortus* is the predominant resistant GIN species in Southern USA, in Africa and in Australia, where those imported breeds come from. In all four flocks, newly introduced

animals were treated against GIN infections and held in quarantine before integrating them into the flock. However, neither in these cases nor after a routine anthelmintic treatment, coprological analyses were performed to check the efficacy. Under such conditions, resistant GIN-strains can easily be introduced.

A lot of farmers follow the recommendations for "preventive or evasive worm management", by treating the whole flock and then moving it on a clean pasture (Michel, 1985, Coles et al., 1992, Barger, 1997). This was thought to keep the contamination level of the pastures low. Unfortunately, it turned out that "the dose and move system" may even enforce the development of AR, when inefficacious anthelmintics are used (Boa et al., 2001). As a consequence, the selection of resistant strains might be supported and the GIN population on the pasture will increasingly be composed of resistant strains. Particularly, in temperate areas, where GIN larvae are able to survive for several months, the system fails on the long run, especially if the time between grazing periods is too short (Bairden et al., 1995).

A possible reason of resistance development was that in all four flocks only a limited range of pastures, being grazed on permanently, was available. Due to the long-term application of the same anthelmintics and the grazing on the same pastures without monitoring the efficacy, the proportion of resistant *H. contortus* larvae in refugia was steadily increasing. Consequently, the decreasing proportion of susceptible larvae in refugia could not delay the development of AR, as it is expected according to Van Wyk, (2001). "In refugia*"* are those parasitic stages not reached by anthelmintic treatment, i.e. larval stages and eggs on the pastures. These parasites escaped the treatment, and thus the term "in refugia" has been introduced. This part of the parasite population can be increased by leaving several individuals of the flock untreated. If the percentage of parasites in refugia is kept high, enough GIN-strains susceptible to anthelmintics can survive treatment and as a consequence are not under selection pressure for anthelmintic resistance. Parasites in refugia provide a source of susceptible gene alleles to dilute resistant alleles in the population and can thereby decrease the development of anthelmintic resistance (Sangster, 1999; Van Wyk, 2001). Since the anthelmintic treatment will put a significant selection pressure for resistance on worm populations, treatment should be avoided whenever possible. This is of particular importance when refugia on pasture are small. Thus, it has become important to identify animals which really need a treatment and leave the others untreated. This leads to an increase of parasitic stages in refugia.

Targeted or selective application of anthelmintic treatment might be an important tool to keep susceptible GIN strains in livestock and to delay the development of AR. Several ways to accomplish this are discussed. Hoste et al. (2002) suggested to reduce the percentage of treated

animals of a flock by treating exclusively the goats in their first lactation and the multiparous ones with the highest potential of milk production. Goats of these two groups are supposed to be most susceptible to GIN infections. Accordingly, this selective treatment scheme resulted in no significant changes in the amount of excreted eggs or in milk production. However, the application of anthelmintics could be considerably reduced. By restricting anthelmintics to two-thirds of the flock, the method reduced substantially the cost of deworming. However, the main advantage of this mode of anthelmintic application is to delay the onset of resistance by preserving alleles of susceptibility within the GIN populations (Sangster, 1999; Jackson and Coop, 2000).

Another proven method of targeted treatment is to routinely sample all animals and treat only sheep with FECs higher than 300 epg and goats with FEC higher than 600 epg. Unfortunately, this diagnostic work is cost intensive and the sampling is very laborious for the farmers. Consequently, the routine individual sampling of all animals is only applicable in small herds, whereas in larger herds, easier, cheaper and faster methods are necessary to identify animals which have to be treated in a TST-system.

The FAMACHA©-system is such a method (Van Wyk, 2001), which can be used by the farmers themselves by checking their animals for signs of anaemia. The examination of the anaemia status of the conjunctiva with the FAMACHA©-colour-chart can be performed whenever the animals are assembled for means of counting, claw cutting, shearing or any other treatment.

Therefore, as a second part of this thesis the accuracy and feasibility of FAMACHA© was tested in six goat flocks in Switzerland. The FAMACHA©-categories, the packed cell volume (PCV) and the faecal egg counts of 64 goats were individually and separately determined at a four-week interval from May to October 2008. Earlier studies verified the correlation of PCV values and the FAMACHA©-scores (Burke et al. 2007, Kaplan et al., 2004). In the present study it was also investigated, if there is a reasonable correlation of the FAMACHA©-scoring and the number of excreted GIN eggs. Such an association might allow to draw some conclusions on the intensity of infection with *H. contortus* (Sangster et al., 1979).

In our study, the sensitivity of the FAMACHA©-method was either 86%, (considering an anaemia cutoff of a PCV = 24% and FAMACHA© score ≥3) or 80% (anaemia cutoff of a PCV = 29% and FAMACHA© score ≥3), respectively. Correlation between FAMACHA©-scoring and PCV was significant in all study months and thereby allows to conclude, that the FAMACHA©-system was applied correctly. Both PCV and FEC and PCV and FEC-Hc-portion, respectively, correlated significantly in May, July, August and October. This suggests

that there is some correlation of infection rate with blood-sucking *H. contortus* and the degree of anaemia also under European conditions.

By contrast, Koopmann et al. (2006) working in Northern Germany, reported that the FAMACHA©-system is not applicable in Germany. In their study, a prevalence of *H. contortus* larvae of only 25% in sheep and 12% in goats was found, whereas in the Swiss flocks *H. contortus* was, with one exception, the predominant GIN species (59% of larvae are *H. contortus*). In three of the six Swiss goat flocks the proportion of *H. contortus* larvae was even >75%. This coincides with results from a FAMACHA©-study conducted in Southern Italy that reported a *H. contortus* proportion of 55% in sheep (Di Loria et al., 2009). Due to this high percentage and since liver fluke infections or any other relevant pathogens were not the cause of anaemic pallor, haemonchosis seems to be the major causative agent of anaemia in the presently studied goats. Thus, it could clearly be shown that the FAMACHA©-system was able to detect highly infected animals in the present study.

To further adjust the FAMACHA©-system to European conditions, adapted PCV-ranges were defined for each FAMACHA©-category. The PCV-ranges and the anaemia cutoffs were higher than in previous studies conducted in Africa, in the USA and in Guadeloupe (Vatta et al., 2001; Burke et al., 2007, Mahieu et al., 2007). In these studies, they used PCV-values ≤15% or ≤19% as indicators for treatment, which are set much too low for European conditions. This disparity could be due to the better health and alimentary conditions in Germany and Switzerland compared to resource-poorer farms in South Africa and other subtropical or tropical regions. In the present study, only 10 animals had a PCV ≤19%. A clinical study of small ruminant blood parameters in Switzerland assigned PCV-values of <24% as an indicator for anaemia (Tschuor et al., 2008). The mean PCV in the Swiss study was 29%, which coincides with the mean PCV of 30% determined in the present study. To take this into consideration, the accuracy of FAMACHA© was tested for five different PCV-values as anaemia-cutoffs. For PCV-values <24%, the percentage of treated animals in treatment-indicating FAMACHA© categories 3 to 5 was low. When using an anaemia cutoff of a PCV of <29% the percentage of true positives increased. In addition, when using a PCV = 29% as anaemia-cutoff the percentage of correct treatment decisions and both, the positive predictive value (PPV) and the negative predictive value (NPV), respectively, could be maximised (PPV = 64%; NPV = 71%). Consequently, the FAMACHA©-method is working at its best in Europe, when anaemia is defined as PCV ≤29%. Although some animals, which require treatment, might remain undetected using FAMACHA©, this method offers an improvement compared to

conventional dosing practices, where all animals are treated (Vatta et al., 2001). The untreated proportion of the flock will maintain an additional reservoir of GINs in refugia.

Furthermore, the present study showed that it is also important to check the individual health status of the animals. Especially for animals scored with FAMACHA© category 3, it is essential to consider other clinical signs of trichostrongylidosis, such as emaciation, bottle jaw, diarrhoea and scrubby fur, for the decision for an anthelmintic treatment. This simple and self-evident fact can enhance the abilities of the FAMACHA©-method and provide a valuable tool for the realisation of an integrated anthelmintic livestock management.

The goal has to be to find a holistic management strategy for each livestock, which achieves an optimum in productivity and profitability in spite of worm infection (Bath, 2006). Targeted treatment, control of efficacy and preservation of susceptible worms in refugia are additional but crucial elements to the traditional treatment schemes, in order to manage both worm infections and the preservation of effective anthelmintics.

CONCLUSION

The results confirmed the presence of multiple anthelmintic resistances of *Haemonchus contortus* in small ruminants in the surveyed area. In the future, the molecular analysis of the respective resistant gene loci will be of great importance in order to characterise the resistant GIN strains. This information may be used to investigate the distribution and the provenance of resistant GIN strains. In addition, the detailed knowledge of the molecular mechanisms of anthelmintic resistance might allow to design new anthelmintics.

Moreover, the present thesis found that, provided a high prevalence of *H. contortus*, the FAMACHA©-method is capable of detecting the animals in need of anthelmintic treatment under European conditions. Further studies in Europe are urgently needed to evaluate whether the practical application of the FAMACHA©-method by the farmers can reduce the number of anthelmintic treatments without decreasing the productivity of the flock. Furthermore, it is of interest whether by utilising the FAMACHA©-system the increase of susceptible GINs in refugia indeed leads to a longer maintenance or a partly restoration of anthelmintic efficacy.

And above all, these new findings concerning GIN control strategies should be communicated to the farmers and veterinarians.

SUMMARY

In the context of an epidemiological study in Southern Germany and Switzerland, two sheep and two goat flocks with clinically reported AR were investigated. Therefore, faecal samples were analysed by Faecal Egg Count Reduction Tests (FECRT) following WAAVP recommendations. In the sheep flocks three randomly selected treatment groups of ten sheep each were chosen, which were sampled on treatment day and ten days later. Mean FECR was 70.8% and 55.3% in albendazole-groups (3.8 mg/kg BW), 52.4% in the fenbendazole-group (5 mg/kg BW), 47.3% in the oxfendazole-group (5 mg/kg BW), and 100% and 44.3% in moxidectin-groups (0.2 mg/kg BW), respectively. All goats were treated with eprinomectin (PourOn-formulation, 1 mg/kg BW). Mean FECR was 28.2% and 27.5% on day 13 after treatment. Coprocultures of all 9 individual treatment-groups revealed that the predominant species after treatment was *Haemonchus contortus*. The study confirmed the existence of *H. contortus*-strains resistant against eprinomectin in goats and against albendazole, oxfendazole, fenbendazole and moxidectin in sheep in Southern Germany and Switzerland. Therefore, it is important to establish new treatment schemes, like targeted treatment, to prevent the further spreading of resistance.

FAMACHA© is a system, which can be used for the accomplishment of Targeted Selective Treatment. The method was developed in sub-Saharan Africa for the clinical evaluation of anaemia in ruminants and is competent of detecting infections with blood-sucking *Haemonchus* spp.. The system determines the degree of anaemia by scoring the colour of the eye mucosa from category 1 (red = non-anaemic) to 5 (white = highly-anaemic), based on the FAMACHA©-colour-chart. Goats from six farms in Central Switzerland were scored for anaemia at four-week intervals, from May to October 2008. Simultaneously, PCV and FEC were individually ascertained. FEC, PCV and FAMACHA©-scores were statistically compared to evaluate the efficacy of FAMACHA© in detecting *H. contortus* infections. The FAMACHA©-scoring and PCV correlated significantly in all months of the study. The sensitivity of FAMACHA© in detecting anaemic goats was 86%, using the anaemia criteria cutoffs FAMACHA©-categories ≥3 and PCV <24%. The sensitivity of the method for detecting goats in need of treatment was >76%, with regard to FEC of *H. contortus* (treatment cutoffs: FAMACHA© ≥3 and FEC >300 epg or >600 epg, respectively). These results indicate the suitability of FAMACHA© as a tool for a Targeted Selective Treatment of goat flocks in Switzerland. Thus, this method might contribute to a slowdown of the development and the spreading of resistance.

ZUSAMMENFASSUNG

Im Rahmen einer epidemiologischen Studie über den Endoparasitenbefall von kleinen Wiederkäuern in Süddeutschland und der Schweiz wurden zwei Schaf- und zwei Ziegenherden mit einem klinischen Verdacht auf Anthelmintika-Resistenzen entdeckt und näher untersucht. In den beiden Schafherden (Suffolk und Dorper) und in den beiden Ziegenbeständen (16 Buren- und 21 Saanenziegen) wurde der Eizahlreduktionstest (FECRT) nach den Empfehlungen der WAAVP durchgeführt. Aus den Schafherden wurden drei Behandlungsgruppen á 10 Tiere zufällig ausgewählt, bei denen jeweils am Tag der Behandlung und 13 Tage später Kotproben rektal entnommen wurden. Die Mittlere Eizahlreduktion in den beiden Albendazol-Gruppen (3,8 mg/kg KGW) betrug 70,8% und 55,3%, in der Fenbendazol-Gruppe (5 mg/kg KGW) 52,4%, in der Oxfendazol-Gruppe (5 mg/kg KGW) 47,3% und in den beiden Moxidectin-Gruppen (0,2 mg/kg KGW) 100% bzw. 44,3%. Die Behandlung der beiden Ziegenherden mit Eprinomectin (1 mg/kg KGW) führte zu einer Mittleren Eizahlreduktion von 28.2% bzw. 27.5%. Bei der Auswertung der Larvenkulturen aller Behandlungsgruppen wurde *Haemonchus contortus* als vorherrschende Trichostrongyliden-Art nachgewiesen. Die Studie bestätigte somit die Resistenz von *H. contortus* gegen Albendazol, Fenbendazol, Oxfendazol und Moxidectin bei Schafen und gegen Eprinomectin bei Ziegen in Süddeutschland und der Schweiz. Neue Behandlungsstrategien wie z.B. gezielte Behandlung (TST) sind deshalb erforderlich.

FAMACHA© ist eine Methode, die man zur Durchführung von gezielter Behandlung einsetzen kann. Sie kann – entwickelt zur klinischen Bewertung des Anämie-Grades - dazu verwendet werden, den Befall von kleinen Wiederkäuern mit dem blutsaugenden Magenwurm *H. contortus* zu schätzen. Mit Hilfe von FAMACHA© wurde der Anämie-Grad von Ziegen aus 6 Beständen in der Schweiz einmal im Monat von Mai bis Oktober 2008 festgestellt. Gleichzeitig wurden bei jedem Tier Eiausscheidung (FEC) und Hämatokrit (PCV) bestimmt. Diese drei Werte wurden verglichen und ausgewertet, um die Anwendbarkeit und Genauigkeit von FAMACHA© bei der Ermittlung des *H. contortus*-Befalls unter Schweizer Bedingungen zu untersuchen. FAMACHA©-Wert und PCV korrelierten signifikant in allen sechs Monaten. Die Sensitivität von FAMACHA© bei der Erkennung von anämischen Ziegen lag bei 86%, wenn Anämie als FAMACHA©-Kategorie ≥3 und PCV <24% definiert wird. Die Sensitivität der Methode bei der Erkennung von Ziegen, die eine Behandlung benötigen, lag bei 76%, unter Berücksichtigung der Eizahlen des *H. contortus*-Anteils (Behandlung ab FAMACHA© ≥3 und

FEC >300 epg). Diese Ergebnisse deuten darauf hin, dass die FAMACHA©-Methode bei Ziegenbeständen im untersuchten Raum eine wertvolle Basis für die Durchführung von gezielten anthelmintischen Behandlungen darstellt.

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FIGURES

TABLES

APPENDIX

1. FAMACHA-study

Table a: Genus, amount and percentage of larvae found in pooled coprocultures of the six FAMACHA study flocks each month.

Flock A

	May		June		July		August		September		October		Mean	
Larvae	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Haemonchus contortus	**129**	**64,5**	**33**	**16,5**	**40**	**19,3**	**15**	**7,5**	**35**	**14,8**	**64**	**31,1**	**37,4**	**25,6**
Trichostongylus spp.	55	27,5	118	59	160	77,3	169	84,1	137	58,1	65	31,6	129,8	56,2
Oesopagostomum / Chabertia	14	7	17	8,5		0,0	12	6,0	61	25,8	58	28,2	37,0	12,6
*Ostertagia*spp.		0	32	16	7	3,4	5	2,5		0,0	19	9,2	15,8	5,2
Strongyloides spp.		0		0		0,0		0,0	3	1,3		0,0	3,0	0,2
Cooperia spp.	2	1		0		0,0		0,0		0,0		0,0	1,0	0,2
Total	**200**	**100**	**200**	**100**	**207**	**100**	**201**	**100**	**236**	**100**	**206**	**100**	**224,0**	**100**

Flock B

	May		June		July		August		September		October		Mean	
Larvae	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Haemonchus contortus	**44**	**37,6**	**169**	**84,5**	**144**	**66,7**	**183**	**80,3**	**176**	**87,6**	**175**	**74,5**	**148,5**	**71,3**
Trichostongylus spp.	73	62,4	31	15,5	65	30,1	21	9,2	14	7,0	27	11,5	38,5	24,8
Oesopagostomum / Chabertia		0,0		0		0,0	21	9,2	3	1,5	22	9,4	15,3	2,1
*Ostertagia*spp.		0,0		0	4	1,9	3	1,3		0,0	7	3,0	4,7	0,6
Strongyloides spp.		0,0		0		0,0		0,0	2	1,0	4	1,7	3	0,2
Cooperia spp.		0,0		0	3	1,4		0,0	6	3,0		0,0	4,5	0,9
Total	**117**	**100**	**200**	**100**	**216**	**100**	**228**	**100**	**201**	**100**	**235**	**100**	**214,5**	**100**

Flock G

	May		June		July		August		September		October		Mean	
Larvae	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Haemonchus contortus	**180**	**78,3**	**141**	**69,1**	**163**	**74,1**	**175**	**81,0**	**127**	**61,7**	**0**		**131**	**72,8**
Trichostongylus spp.	50	21,7	62	30,4	40	18,2	36	16,7	79	38,3	0		44,5	25,1
Oesopagostomum / Chabertia		0,0	1	0,5		0,0		0,0		0,0	0		0,5	0,1
*Ostertagia*spp.		0,0		0,0		0,0		0,0		0,0	0			0,0
Strongyloides spp.		0,0		0,0	17	7,7	5	2,3		0,0	0		7,3	2,0
Cooperia spp.		0,0		0,0		0,0		0,0		0,0	0			0,0
Total	**230**	**100**	**204**	**100**	**220**	**100**	**216**	**100**	**206**	**100**	**0**	**0**	**183,3**	**100**

Flock J

	May		June		July		August		September		October		Mean	
Larvae	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Haemonchus contortus	**170**	**85,0**	**144**	**66,4**	**125**	**61,3**	**180**	**85,3**	**66**	**73,3**	**197**	**93,8**	**147,0**	**74,3**
Trichostongylus spp.	24	12,0	34	15,7	51	25,0	28	13,3	19	21,1	13	6,2	28,2	17,4
Oesopagostomum / Chabertia		0,0		0,0		0,0		0,0	1	1,1		0,0	1,0	0,2
*Ostertagia*spp.	6	3,0		0,0		0,0	2	0,9		0,0		0,0	4,0	0,8
Strongyloides spp.		0,0		0,0	18	8,8	1	0,5	4	4,4		0,0	7,7	2,7
Cooperia spp.		0,0	39	18,0	10	4,9		0,0		0,0		0,0	**24,5**	**4,6**
Total	**200**	**100**	**217**	**100**	**204**	**100**	**211**	**100**	**90**	**100**	**210**	**100**	**212,333**	**100**

Flock M

	May		June		July		August		September		October		Mean	
Larvae	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Haemonchus contortus	**169**	**81,6**	**108**	**51,4**	**135**	**64,0**	**27**	**11,0**	**38**	**16,6**	**8**	**7,7**	**80,8**	**44,9**
Trichostongylus spp.	38	18,4	59	28,1	62	29,4	43	17,5	45	19,7	73	70,2	53,3	22,6
Oesopagostomum / Chabertia		0,0	17	8,1	14	6,6	161	65,4	145	63,3	0	0,0	67,4	28,7
*Ostertagia*spp.		0,0		0,0		0,0	8	3,3		0,0	4	3,8	6,0	0,7
Strongyloides spp.		0,0		0,0		0,0	7	2,8	1	0,4	19	18,3	9,0	0,7
Cooperia spp.		0,0	26	12,4		0,0		0,0		0,0	0	0,0	13,0	2,5
Total	**207**	**100**	**210**	**100**	**211**	**100**	**246**	**100**	**229**	**100,0**	**104**	**100**	**230**	**100**

Flock R

	May		June		July		August		September		October		Mean	
Larvae	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Haemonchus contortus	**33**	**33,0**	**87**	**42,0**	**192**	**92,8**	**187**	**89,0**	**163**	**76,5**	**0**		**110,3**	**66,7**
Trichostongylus spp.	67	67,0	23	11,1	15	7,2	20	9,5	16	7,5	0		23,5	20,5
Oesopagostomum / Chabertia		0,0		0,0		0,0		0,0		0,0	0			0,0
*Ostertagia*spp.		0,0		0,0		0,0		0,0		0,0	0			0,0
Strongyloides spp.		0,0		0,0		0,0	3	1,4	34	16,0	0		12,3	3,5
Cooperia spp.		0,0	97	46,9		0,0		0,0		0,0	0		48,5	9,4
Total	**100**	**100**	**207**	**100**	**207**	**100**	**210**	**100**	**213**	**100**	**0**	**0**	**194,7**	**100**

Table b: Data of FAMACHA-study: FAMACHA-score, packed cell volume and faecal egg count (FEC) of flock A and B for all six study months.

Flock A

	FAMACHA- Category						Haematocrit						Gastrointestinal nematodes					
	1-5 (red - white)						[%]						[epg]					
Study month	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
A1	4	3,5	2,5	3	3,5	2,5	22	27	28	29	37	34	342	270	510	2070	1170	1038
A2	3	3,0	3	3,5	3	3	22	22	24	20	22	23	330	90	150	1050	309	Kein kot
A3	3	2,5	2,5	2,5	2,5	2	30	38	31	38	43	40	480	990	1620	2010	1080	330
A4	4	3,5	3,5	3,5	3	2,5	24	22	26	27	48	30	1830	1170	0	60	210	30
A5	4	3,0	2,5	2,5	2,5	2	22	24	28	32	36	30	2790	150		720	720	630
A6	4	4,0	2,5	3	3	3,5	20	29	33	45	51	37	5010	1650	0	180	210	450
A7	3	3,0	3,5	2,5	2	2,5	21	19	21	27	27	27	0	1950		210	290	720
A8	2	2,0	2	2	2,5	2	32	31	30	31	47	36	1690	0	60	1021	240	420
A9	3	3,0	3	2,5	2,5	2,5	29	31	35	33	38	33	2010	300	222	1080	148	840
A10	4	4,0	3,5	3,5	3	3	25	26	27	26	39	28		0	150	450	1620	1080
A11	2	2,0	1,5	2,5	2	1,5	25	48	33	46	59	48	0	30	30	900	1080	540
Mean	**3,27**	**3,05**	**2,73**	**2,82**	**2,68**	**2,45**	**24,73**	**28,82**	**28,73**	**32,18**	**40,64**	**33,27**	**1448,20**	**600**	**304,67**	**886,45**	**643,36**	**607,80**

Flock B

	FAMACHA- Category						Haematocrit						Gastrointestinal nematodes					
	1-5 (red - white)						[%]						[epg]					
Study month	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
B1	4	4	1,5	3	2	2	12	17	36	43	39	35	15000	0	0	360	0	360
B2	3	2,5	2	2,5	3,5	2,5	35	38		33	38	42	0	0	0	300	0	660
B3	3	2,5	2	3	3,5	2	34	31		36	39	40	0	0	30	660	0	90
B4	4	3	3	3,5	3	4	26	25	28	16	23	26	0	0	90	13530	270	810
B5	3	3	3	3	3	3	34	37	31	20	35	40	120	270	90	7920	30	600
B6	2	2	1,5	2	2,5	2	44	48	42	44	37	38	1980	0	360	3240	270	2460
B7	3	2,5	4	3,5	3	3,5	35	36	18	25	32	35	30	30	8010	6330	3920	990
B8	4	3	2,5	4	3	3,5	22	32	27	26	30	27	510	60	360	2100	600	330
B9	3	2,5	3	4	2,5	3	32	32	31	26	33	26	1500	90	11010	2550	2460	7830
B10	4	3,5	3	4,5	3	3	27	30	26	25	40	41	0	60	30	1380	0	630
B11	4	2,5	2,5	2,5	3,5	2	23	35	28	30	35	34	1410	60	3990	570	120	1440
Mittelwert	**3,36**	**2,82**	**2,55**	**3,23**	**2,95**	**2,77**	**29,45**	**32,82**	**29,67**	**29,45**	**34,64**	**34,91**	**1868,18**	**51,82**	**2179,09**	**3540,00**	**697,27**	**1472,73**

Table c: Data of FAMACHA-study: FAMACHA-score, packed cell volume and faecal egg count (FEC) of flock G and J for all six study months.

Flock G

	FAMACHA- Category						Haematocrit						Gastrointestinal nematodes					
	1-5 (red - white)						[%]						[epg]					
Study month	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
G1	3	3	3	4	4,5	2	37	31	32	21	21	30	1860	900	690	2160	1860	0
G2	4	3,5	4	3,5	4	3	29	24	28	23	27	32	10530	3120	840	1260	2250	0
G3	3	2,5	2,5	3,5	4	1,5	28	32	31	25	25	34	5070	210	0	690	4380	0
G4	4	4	4	4	3,5	3	26	29	32	23	30	28	30	1740	2340	240	930	0
G5	4	3,5	3,5	3,5	4	4	37	26	22	20	29	35	3780		840	1680	360	0
G6	4	3	3	4	4	4	22	30	31	24	27	29	4140	180	150	240	360	0
G7	3	3	3	3	3,5	2	37	29	39	34	27	36	3270	210	120	0	600	0
G8	3	2,5	2,5	2,5	2	3	28	23	32	26	28	33	840	750	60	1170	920	0
G9	2	2	3	2,5	2	sold	42	35	22	32	43		1740	1170	1080	990	3240	
G10	4	3,5	3	4	4,5	2,5	23	30	32	23	29	37	26730	60	1140	870	1140	0
G11		3,5	4	4	5	3,5		29	25	23	23	31		1650	2070	2400	4380	0
G12		3	3	2,5	3	3		29	33	26	31	31		60	90	270	630	0
G13		3,5	4	5	4	2,5		28	26	19	25	31		2130	1170	4170	1620	0
Mittelwert	**3,38**	**3,12**	**3,27**	**3,54**	**3,69**	**2,83**	**30,90**	**28,85**	**29,62**	**24,54**	**28,08**	**32,25**	**5799,00**	**1015,00**	**814,62**	**1241,54**	**1743,85**	**0,00**

Flock J

	FAMACHA- Category						Haematocrit						Gastrointestinal nematodes					
	1-5 (red - white)						[%]						[epg]					
Study month	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
J1	4	4,5	4	3,5	4	3,5	27	26	29	24	24	23	3030	690	2850	5130	0	4680
J2	4	3	3	3	4	3	26	32	26	22	22	20	1710	5130	4920	5160	0	2760
J3	4	4	3	3	3,5	3,5	18	26	28	28	28	28	2400	840	3030	1500	0	630
J4	4	3,5	3	4	3,5	3	38	47	32	27	27	22	1710	120	840	60?	120	1626
J5	3	2,5	3	2	2,5	2	30	48	21	29	29	34	2310	60	2130	1830	60	0
J6	3	4	2	2	3	2	30	54	34	35	35	29	3660	1590	150	2190	0	420
J7	3	2,5	3,5	2	2,5	2	16	37	25	23	23	26	7920	750	600	2730	0	630
J8	3	3	3,5	2			25	46	33	34			2760	450	1290	0		
Mittelwert	**3,50**	**3,38**	**3,13**	**2,69**	**3,29**	**2,71**	**26,25**	**39,50**	**28,50**	**27,75**	**26,86**	**26,00**	**3187,50**	**1203,75**	**1976,25**	**2648,57**	**25,71**	**1535,14**

Table d: Data of FAMACHA-study: FAMACHA-score, packed cell volume and faecal egg count (FEC) of flock M and R for all six study months.

Flock M

	FAMACHA- Category						Haematocrit						Gastrointestinal nematodes					
	1-5 (red - white)						[%]						[epg]					
Study month	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
M1	3	3	2	1,5	2,5	1,5	23	32	29	23	30	38	9150	3090	2790	3060	2400	660
M2	4	3	3	3	3	3	19	21	22	15	21	26	6270	2540	3720	3720	270	600
M3	4	3,5	3	2,5	2,5	3	20	28	27	24	32	30	2280	3300	1860	30	420	240
M4	3	2	2,5	2,5	2,5	2	28	26	25	22	28	27	60	510	1890	120	120	780
M5	3	2,5	2	2,5	2,5	2,5	26	44	40	33	33	45	4200	1740	1110	2700	1440	60
M6	2	2	2,5	2,5	1,5	1,5	30	43	29	24	50	30	2910	270	1380	1650	1740	1380
M7	3	2	2	1,5	2	2	25	30	26	24	30	30	2100	870	810	2130	240	600
M8	2	2	2,5	1,5	2,5	2	29	31	32	26	27	27	1320	540	540	1770	5280	2100
M9	3	2,5	3	2	3	3	27	30	28	22	24	28	3810	1590	1410	1650	2010	1380
M10	3	2,5	2,5	3	3	2,5	28	33	30	24	27		570	1380	1860	2340	1080	340
M11	3	3	3	1,5	3,5	1,5	22	21	25	22	21	36	1050	4320	0	960	2460	360
Mittelwert	**3,00**	**2,55**	**2,55**	**2,18**	**2,59**	**2,23**	**25,18**	**30,82**	**28,45**	**23,55**	**29,36**	**31,70**	**3065,45**	**1831,82**	**1579,09**	**1830,00**	**1587,27**	**772,73**

Flock R

	FAMACHA- Category						Haematocrit						Gastrointestinal nematodes					
	1-5 (red - white)						[%]						[epg]					
Study month	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
R1	4	3,5	3,5	3,5	3,5	5	28	29		27	29	26	330	540	30	1110	570	0
R2	3	3	2,5	2,5	3	2	33	35		29	35	34	540	1740	570	1320	1440	0
R3	4	3,5	3	3	4	3	32	35		31	30	28	720	5760	180	2160	2580	0
R4	3	4,5	4	4,5	4,5	4	32	25		26	28	23	350	4380	480	1200	1050	0
R5	2	3	3	3	3	2	33	26		35	32	33	90	690	300	1920	1650	0
R6	3	4	4	3	3	3,5	37	27		23	28	31	60	450	90	2640	1170	0
R7	4	4	4	3,5	4	4	37	22		32	36	31	2880	1560	60	390	300	0
R8	4	3	4	2,5	2,5	3,5	32	27		38	41	42	3000	960	Ø	150	120	0
R9	4	3,5	4	3	3	sold	31	29		29	34		510	2640	30	1200	390	
R10	3	2,5	3	1,5	2,5	1,5	33	27		30	33	37	150	240	0	300	90	0
Mittelwert	**3,40**	**3,45**	**3,50**	**3,00**	**3,30**	**3,17**	**32,80**	**28,20**		**30,00**	**32,60**	**31,67**	**863,00**	**1896,00**	**193,33**	**1239,00**	**936,00**	**0,00**

Table e: Number and percentage of treatment decisions, according to the four PCV cutoff values 15%, 22%, 29% and 32% for each of the five FEC-categories.

		Treatment according to PCV cutoff 15%			
		Yes	**Yes-[%]**	**No**	**No-[%]**
FEC category [epg]	**total**				
1: <120	81	0	0%	81	100%
2: 120 - 300	38	0	0%	38	100%
3: 300 - 1020	84	0	0%	84	100%
4: 1020 - 3330	120	0	0%	120	100%
5: > 3330	35	2	5.7%	33	94.3%

		Treatment according to PCV cutoff 22%			
		yes	**Yes-[%]**	**No**	**No-[%]**
FEC category [epg]	**total**				
1: <120	81	4	4.9%	77	95.1%
2: 120 - 300	38	2	5.3%	36	94.7%
3: 300 - 1020	84	6	7.1%	78	92.9%
4: 1020 - 3330	120	18	15%	102	85%
5: > 3330	35	13	37.1%	22	62.9%

		Treatment according to PCV cutoff 24%			
		yes	**Yes-[%]**	**No**	**No-[%]**
FEC category [epg]	**total**				
1: <120	81	8	9.9%	73	90.1%
2: 120 - 300	38	7	18.4%	31	81.6%
3: 300 - 1020	84	8	9.5%	76	90.5%
4: 1020 - 3330	120	30	25%	90	75%
5: > 3330	35	18	51.4.9%	17	48.6%

		Treatment according to PCV cutoff 29%			
		yes	**Yes-[%]**	**No**	**No-[%]**
FEC category [epg]	**total**				
1: <120	81	27	33.3%	54	66.6%
2: 120 - 300	38	16	42.1%	22	57.9%
3: 300 - 1020	84	38	45.2%	46	54.8%
4: 1020 - 3330	120	72	60%	48	40%
5: > 3330	35	29	82.9%	6	17.1%

		Treatment according to PCV cutoff 32%			
		yes	**Yes [%]**	**No**	**No-[%]**
FEC category [epg]	**total**				
1: <120	81	45	55.6%	36	44.4%
2: 120 - 300	38	21	55.3%	17	44.7%
3: 300 - 1020	84	60	71.4%	24	28.6%
4: 1020 - 3330	120	89	74.2%	31	25.8%
5: > 3330	35	33	94.3%	2	5.7%

Table f: Number and percentage of treatment decisions, according to the four PCV cutoff values 15%, 22%, 24%, 29% and 32% for each of the five FAMACHA© categories.

		Treatment according to PCV cutoff 15%			
		yes	Yes-[%]	No	No-[%]
FAMACHA ©	**total**				
Cat. 1	15	0	0%	15	100%
Cat. 2	115	0	0%	115	100%
Cat. 3	162	1	1.6%	161	99.4%
Cat. 4	69	1	1.5%	68	99.5%
Cat. 5	3	0	5.7%	3	100%

		Treatment according to PCV cutoff 22%			
		yes	Yes-[%]	No	No-[%]
FAMACHA ©	**total**				
Cat. 1	15	1	6.7%	15	93.3%
Cat. 2	115	2	1.7%	113	98.3%
Cat. 3	162	25	15.4%	137	84.6%
Cat. 4	69	15	21.7%	54	78.3%
Cat. 5	3	1	33.3%	2	66.7%

		Treatment according to PCV cutoff 24%			
		yes	Yes-[%]	No	No-[%]
FAMACHA ©	**total**				
Cat. 1	15	3	20%	12	80%
Cat. 2	115	7	6.1%	108	93.9%
Cat. 3	162	37	22.8%	125	77.2%
Cat. 4	69	24	34.8%	45	65.2%
Cat. 5	3	2	66.7%	1	33.3%

		Treatment according PCV cutoff 29%			
		yes	Yes-[%]	No	No-[%]
FAMACHA ©	**total**				
Cat. 1	15	4	26.7%	11	73.3%
Cat. 2	115	34	29.6%	81	70.4%
Cat. 3	162	90	55.6%	72	44.4%
Cat. 4	69	57	82.6%	12	17.4%
Cat. 5	3	3	100%	0	0%

		Treatment according PCV cutoff 32%			
		yes	Yes [%]	No	No-[%]
FAMACHA©	**total**				
Cat. 1	15	6	40%	9	60%
Cat. 2	115	61	53%	54	47%
Cat. 3	162	121	74.7%	41	25.3%
Cat. 4	69	63	91.3%	6	8.7%
Cat. 5	3	3	100%	0	0%

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