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Influence of communal alpine pasturing in Styria, Austria, on the development of gastrointestinal strongylid infections over the grazing season in sheep – a pilot study

Einfluss der Alpung in der Steiermark, Österreich, auf die Verbreitung von gastrointestinalen Strongyliden-Infektionen während der Alpungssaison bei Schafen – eine Pilotstudie

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Summary

The aim of the present study was to determine the prevalence of ovine gastrointestinal strongylids (GiSt) and the influence of communal alpine pasturing during the grazing season on the development of GiSt infections in the region of Styria, Austria. The investigation included 243 adult sheep from 16 different farms pastured on a single communal alpine pasture. Individual fecal samples were taken at different time points. Before deworming and after pasturing fecal samples were examined qualitatively (flotation and larval culture) and quantitatively (fecal egg counting). In addition during pasturing fecal samples were analyzed two times (fecal egg counting). Before deworming and pasturing, the prevalence of GiSt infection in the sheep examined was 94.7%, after pasturing it was 100%; this increase was significant ($p < 0.001$). In addition, a statistically significant increase ($p < 0.001$) in egg shedding was observed during the observation period. Animals with low eggs per gram of feces (epg) values before pasturing also had lower values at the end of the grazing season and sheep with higher values had higher values at the end of the grazing season. *Trichostrongylus* was the most dominant GiSt genus before deworming and pasturing (45.6% of larvae in coproculture) and also after pasturing (38.8%). *Haemonchus (H.) contortus* was the second most frequent nematode both before and after grazing with 18.9% and 31.2%, respectively, and more commonly found than in previous studies from Austria. The proportions of *Trichostrongylus* ($p = 0.004$) and *Nematodirus* ($p = 0.032$) decreased and *H. contortus* ($p = 0.005$) increased significantly between the two different sampling dates. The results of this survey indicate that communal alpine pasturing management practices for sheep in the investigated area of Austria may promote proliferation of nematode infections during the pasturing season.

Keywords: Fecal egg count, *Haemonchus contortus*, *Trichostrongylus*, ovine strongylids

Zusammenfassung

Das Ziel dieser Studie war es, die Prävalenz von ovinen gastrointestinalen Strongyliden (GIS) zu bestimmen und den Einfluss der Alpung auf die Verbreitung der GIS-Infektion während der Weidesaison in Österreich zu ermitteln. Die Untersuchung umfasst 243 adulte Schafe von 16 verschiedenen Betrieben, die gemeinsam gealpt wurden. Einzelkotproben wurden zu verschiedenen Zeitpunkten gewonnen. Vor der Entwurmung und nach der Alpung wurden Kotproben qualitativ (Flotation und Kotkultur) und quantitativ (fäkale Eizahlbestimmung) untersucht. Zusätzlich wurden während der Alpung Kotproben zweimal quantitativ (fäkale Eizahlbestimmung) analysiert. Vor der Entwurmung und der Alpung lag die Prävalenz von GIS bei den untersuchten Schafen bei 94,7 %, nach der Alpung bei 100%, dieser Anstieg war signifikant ($p < 0,001$). Zusätzlich konnte ein signifikanter Anstieg ($p < 0,001$) bei der Eiausscheidung während der Alpung nachgewiesen werden. Tiere, die vor der Alpung einen niedrigen „eggs per gram of feces" (epg-Wert) aufwiesen, zeigten tendenziell auch niedrigere Werte am Ende der Weidesaison, und Tiere mit höheren epg-Werten hatten auch höhere Werte am Ende der Weidesaison. *Trichostrongylus* war sowohl vor (45,6 %) als auch nach der Alpung (38,8 %) der dominanteste GIS in der Kotkultur. *Haemonchus contortus* war der zweithäufigste Nematode vor und nach dem Weidegang mit 18,9 % und 31,2 % und wurde in dieser Arbeit deutlich häufiger nachgewiesen als in vorangegangenen Studien. Das Vorkommen von *Trichostrongylus* ($p = 0,004$) und *Nematodirus* ($p = 0,032$) sank und *H. contortus* ($p = 0,005$) stieg signifikant zwischen den beiden Beprobungszeitpunkten. Die Ergebnisse dieser Untersuchung geben erste Hinweise darauf, dass die Alpung von Schafen in der untersuchten Region Österreichs die Verbreitung von Nematoden möglicherweise fördert.

Schlüsselwörter: fäkale Eizahl, *Haemonchus contortus*, *Trichostrongylus*, ovine Strongyliden

Introduction

Communal alpine pasturing of ruminants is a traditional farming practice in Austria and other alpine regions. Generally, cattle, sheep and goats graze on communal alpine pastures from around May or June until September or October and return to their home farms in autumn. The duration of the grazing season on alpine pastures depends on climatic conditions and altitude. All grazing animals are at risk from infections with gastrointestinal strongylids (GiSt) with substantial morbidity as a possible consequence (Bishop and Stear, 2003). Common clinical signs of GiSt infections in sheep include anorexia, diarrhea and emaciation (Steel et al., 1982). A high worm burden may even lead to the death of the infected animals. In some cases, especially in infections with the blood-sucking nematode *Haemonchus (H.) contortus*, severe anemia develops (Crab et al., 2002). *H. contortus* is highly pathogenic and prevalence rates vary between countries. Generally, it is widely spread in tropical and subtropical climatic zones (O´Connor et al., 2006). In cooler temperate areas, *Teladorsagia circumcincta* is usually the dominant species (Stear et al., 2009). Recent changes in climatic conditions seem to have encouraged the dissemination of GiSt, particularly of *H. contortus* (van Dijk et al., 2008; Kenyon et al., 2009; Bolajoko et al., 2015). In Europe, high prevalences have been reported from Switzerland (77%) and Italy (73%) with higher infection rates in the south and frequent outbreaks in the central lowlands (Musella et al., 2011, 2014; Rinaldi et al., 2015) while in Ireland prevalences were low (4%). The distribution of *H. contortus* is very heterogeneous and depends on a variety of factors. Variation occurs from area to area and from farm to farm (Musella et al., 2011).

Under field conditions, most infections consist of several different species of GiSt (Shubber et al., 1981; Bishop and Stear, 2001). The effect of infections on the host animal depends on the one hand on the intensity of the infection and the nematode species involved and on the other hand on the immunological and physiological status of the host. Young and immunocompromised animals are most susceptible to infections with endoparasites in general (Shubber et al., 1981; Bishop and Stear, 2001). Infected lambs vary in the quantity of eggs they excrete and the distribution of egg counts is usually skewed in the population, with most of the lambs in a herd shedding relatively few eggs, while a small proportion excretes high numbers of nematode eggs. These so called "super-shedders" are of vital significance for the transmission of an infection in the herd (Stear et al., 2009). The production and excretion of eggs is determined by the number and fertility of adult female worms in each host. Genetically resistant or immune sheep show a decreased production and excretion of eggs, fewer adult nematodes and more inhibited larvae. Usually one or more of these features can be observed. In many cases, there is a negative correlation between the number of adult nematodes and their fertility so that increasing the amount of nematodes leads to a decline in fecundity (Stear et al., 2009). The reasons for this are unclear. Competition between the nematodes for food and other resources and an increased immune response in heavily infected animals are discussed as possible explanations (Stear et al., 2009).

To date, only few studies have been performed on the prevalence and distribution of ovine endoparasites in Austria. A study from 1977 showed that *Teladorsagia, Nematodirus* and *Trichostrongylus* were the most common genera of GiSt in sheep, while *Haemonchus* and *Cooperia* only played a minor role (El-Moukdad, 1977). Recent works have shown a regional prevalence of GiSt in sheep of up to 100% (Biermayer, 1996; Gergely and Wehowar, 2008; Feichtenschlager et al., 2014). In the German federal state of Bavaria, with an agricultural structure, climate and landscape similar to Austria, the most common GiSt genera infecting sheep were *Trichostrongylus, Haemonchus, Teladorsagia, Nematodirus* and *Cooperia* (Benesch, 1993; Rehbein et al., 1996).

The faecal egg count (FEC) for quantification of infections with gastro-intestinal nematodes is commonly used in sheep. Evaluation of worm burden is, however, limited with this technique, since multiple factors may influence the production of eggs, and the level of egg excretion differs between the different species of GiSt. *Nematodirus*, for example, excrete low numbers of eggs compared to *H. contortus* or *Teladorsagia* spp. While in some genera (e. g. *Nematodirus*) egg production is not strictly related to the level of worm burden, in others

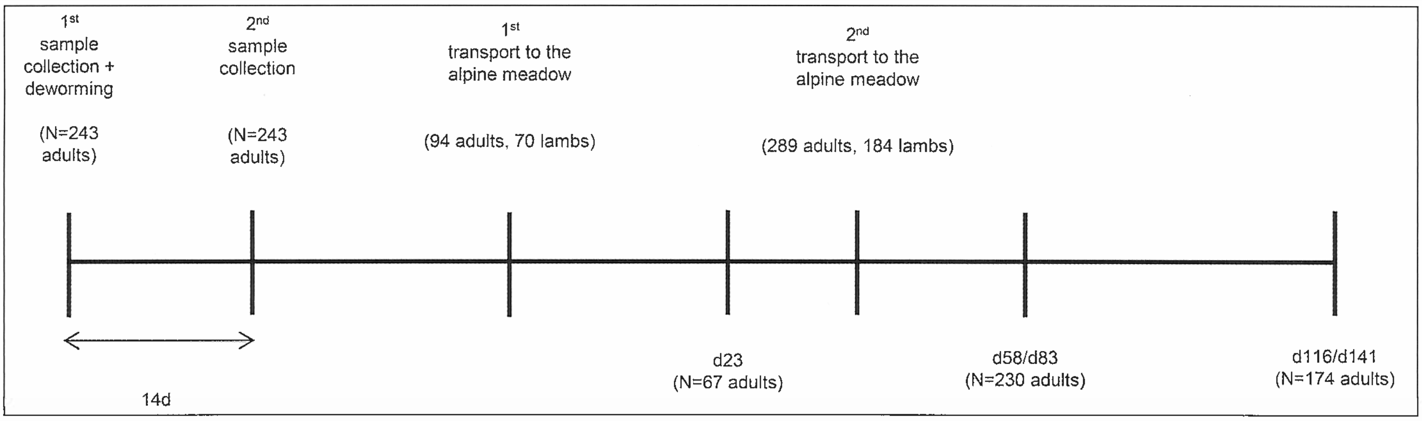

FIGURE 1: *Schedule for animal movement, time points of sample collection and treatment. Horizontal line indicates the period between first sample collection and last sample collection; vertical lines indicate times of sample collection and transport to the alpine meadow; 14d means a period of 14 days, d23 means the 23 day of alpine pasturing, d58/d83 means the 58/the 83 day of alpine pasturing, d116/141 means the 116/the 141 day of pasturing. N indicates the amount of animals which where sampled or transported.*

(e. g. *Teladorsagia*) the nematode's fertility and, as such, the subsequent egg production per female worm is higher when the worm burden in the gut is lower. Different genera and species also vary in their pathogenicity and fertility. For example, *H. contortus* is very pathogenic and also very fecund (Taylor, 2010). Older animals have been shown to develop immunity against the parasite, suppressing egg excretion (Bath, 2011). These considerations may limit the interpretation of the results of the FEC. Apart from the mentioned limitations, however, the FEC is, in addition to the clinical symptoms, still a good indicator as to whether an anthelmintic treatment is indicated (Taylor, 2010). More than 1000 eggs per gram of feces (epg) are generally considered indicative of a severe infection, between 500–1000 epg for a moderate infection (Taylor, 2010).

The aim of the present pilot study was to get first data on GiSt egg excretion and the presence of different strongylid genera in sheep managed by communal alpine pasturing in the investigated Styrian Alp (Schladming; alpine pasture "Hauser Kaibling") during the pasturing season.

Materials and Methods

This investigation was discussed and approved by the institutional ethics and animal welfare committee in accordance with Good Scientific Practice guidelines and national legislation.

Study area animals and management

The study area where sheep grazed during summer (May until September) was located in the Austrian federal state of Styria (Schladming; alpine pasture "Hauser Kaibling"), at an altitude of 1500 to 2150 meters above sea level. The mean temperature in summer (July) was 6.7°C (http://www.zamg.ac.at/cms/de/produkte/klima/buecher-karten-cds/klimakarten). The total size of the area was 271 ha including about 95 ha of pure pasture. Grazing management can be described as follow: at the beginning of pasturing all sheep graze on the lowest area of the meadow and during summer sheep travel further up to higher areas of the grazing area. At the end of pasturing all sheep travel down to the lowest point of the grazing area again. This traveling is controlled by a sheepherder.

During the grazing period, sheep were transported to the alpine pastures in two batches. In May 2015, 94 adult sheep and 70 lambs were transported and in June 2015 289 adult sheep and 184 lambs were taken to the pasture (Fig. 1). In total, 383 adult and 254 juvenile sheep grazed together on this area over summer.

The study was carried out between March and September 2015. The animals investigated were adult sheep from different farms in the federal states of Styria (n = 13) and Salzburg (n = 3). In total, 243 of the 383 adult sheep (75.5%) were included in this investigation. In addition, 254 lambs grazed in the same area together with their ewes. At the explicit request of the owners lambs were not included in the current study because of animal welfare reasons (rectal fecal sampling). All sheep were identified by individual earmarks.

Sample collection and parasitological examination

Individual fecal samples were collected directly from the rectum of the animals. Data recording and the first two sample collections (before deworming and 14 days after deworming) took place on the individual farms before turn-out to pasture. These samples were then examined quantitatively by a modified McMaster counting technique with a lower detection limit of 50 epg. For a better overview, values were classified into four categories: epg values between 0 and 49 (i. e. negative in the McMaster examination) were categorized as "negative infection", values between 50 and 450 as "mild infection", values between 500 and 1000 as "moderate infection" and FECs over 1000 epg of feces were generally considered indicative of "heavy infections" (Taylor, 2010). Third stage larvae of nematodes (L_3) were cultivated and identified according to van Wyk and Mayhew (2013) in pooled flock samples (one from each farm). In addition, 14 days after deworming with a macrocyclic lactone (0.2 mg of Moxidectin per kg of body weight; Cydectin®, Zoetis Switzerland, Zürich, Switzerland) based on the individual body weight of the animals (all animals were weighted using

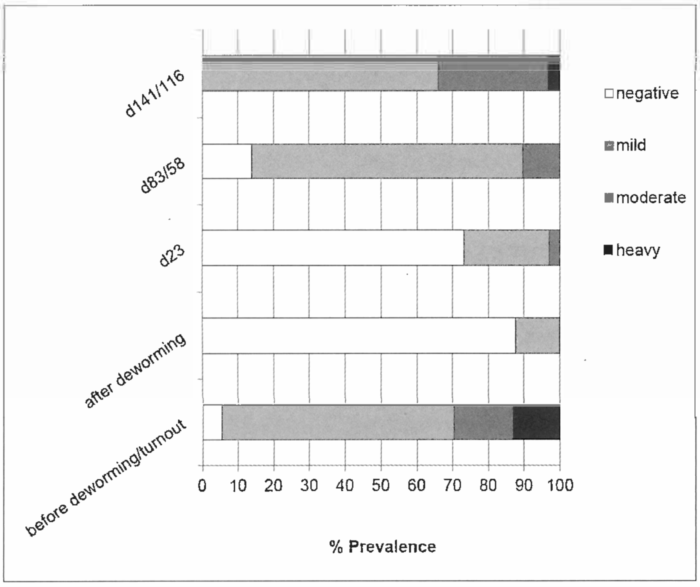

FIGURE 2: *Nematode fecal egg counts from samples of adult sheep in Styria before deworming (243 animals), after deworming (243 animals) and during pasturing (d23 = 67 animals; d83/58 = 230 animals; d141/116 = 174 animals). d23 means the 23 day of alpine pasturing, d83/58 means the 83/the 58 day of alpine pasturing, d141/116 means the 141/ the 116 day of pasturing.*

a mobile scale), individual fecal samples were collected to determine egg excretion before movement to the summer pastures. The sheep then grazed the communal alpine pasture from May until September.

During the summer grazing season, individual fecal samples were collected twice from the animals (d23 [at day 23] after movement and d83/d58 [at day 83/ at day 58]; Fig. 1) and also at the end of grazing period (d141/d116 [at day 141/at day 116]; Fig. 1). On d23, the epg for 67 out of the 243 sheep included in the study and on d83/d58 the epg for 230/243 sheep were determined. At the final examination (d141/d116), the feces of 174/243 sheep were investigated. At the end of the grazing period, strongylid larvae (L_3) were cultivated and identified in pooled flock samples (one from each farm) as before (Fig. 1).

The lower number of investigated animals at the end of the study is a result of different reasons like animal sale, death or slaughtering.

Statistical analysis

Descriptive and inferential statistics were calculated using Microsoft® Excel 2010 and IBM SPSS Statistics (Version 20.0). Correlations were performed using Spearman's correlation coefficient. For all analyses a p-value < 0.05 (5%) was seen as significant.

Results

Prevalence of gastrointestinal nematode infections before deworming and pasturing

The overall prevalence of GiSt infections in the examined sheep (n = 243) at the first examination was 94.7%. The positive epg values ranged between 50 and 5800 (mean = 507; median = 250; SD = 713; 25 percentile = 150.00; 75 percentile = 600.00). Thirteen animals (5.3%) had values < 50 epg and were categorized as "negative", 65.0% had a mild infection while 16.5% and 13.2% had moderate or heavy infections, respectively (Fig. 2).

In this time 28 of 31 (90.3%) sheep with epg values over 1000 had a reduced body condition.

Nematode fecal egg counts after deworming and during pasturing

After anthelmintic treatment, 87.6% of sheep had epg values < 50, 12.3% of sheep had a mild infection with values between 50 and 150 epg (Fig. 2). The mean epg was 9.4, the median 0.0 and the standard deviation 28.4.

On d23 of grazing, 73.1% of the examined sheep were determined to have values < 50 epg, 23.9% had values between 50 and 300 epg and 3.0% showed values between 600 and 700 epg (mean = 47.8; median = 0.0; SD = 125.1; 25 percentile = 0.00; 75 percentile = 50.00).

After 83/58 days of grazing, 13.9% of sheep returned values < 50, 75.7% had values between 50 and 450 and 10.4% had values between 500 and 850 (mean 198.9; median 150.0; SD = 193.0; 25 percentile = 50.00; 75 percentile = 250.00).

At the end of the grazing period (d141/d116), 66.1% of the animals had epg values between 50 and 450, 30.5% had values between 500 and 1000 and 3.4% had values between 1100 and 1600 (mean 431.6; median 350.0; SD = 283.3; 25 percentile = 237.50; 75 percentile = 562.50) (Fig. 2).

There was a statistically significant increase (p < 0.001) in epg-values between the different time points of sampling (Fig. 3). The correlation coefficient between epg values before deworming and at the end of the grazing season was 0.403.

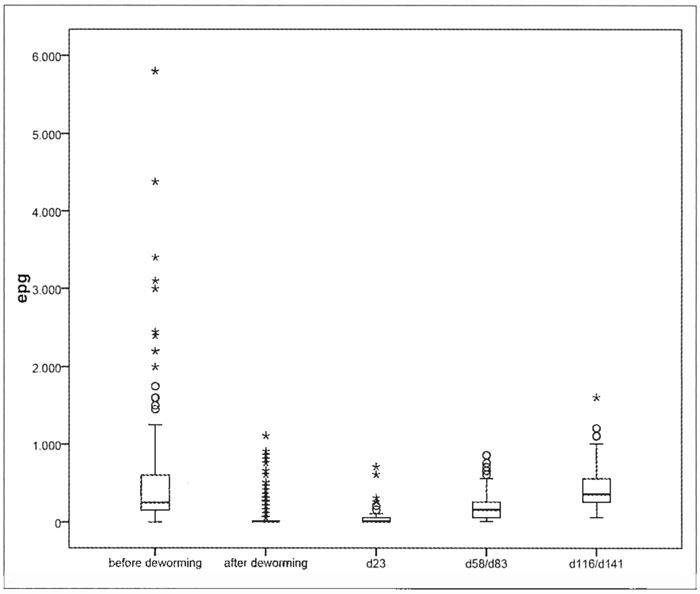

FIGURE 3: *Individual and mean epg values of examined sheep before deworming, after deworming and during pasturing. Boxes indicate quartiles, lines indicate the median, * and ° indicate outliers. d23 means the 23 day of alpine pasturing, d58/83 means the 58/the 83 day of alpine pasturing, d116/141 means the 116/ the 141 day of pasturing.*

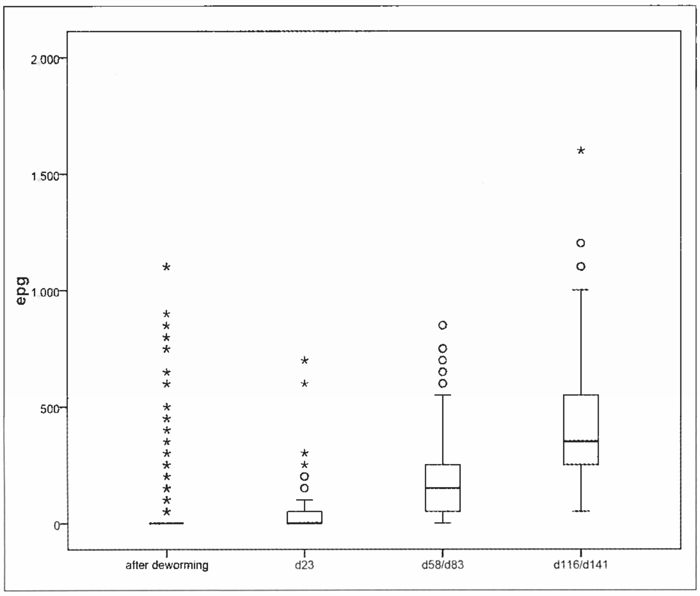

FIGURE 4: *Individual and mean epg values of examined sheep during pasturing. Boxes indicate quartiles, lines indicate the median, * and ° indicate outliers. d23 means the 23 day of alpine pasturing, d58/83 means the 58/the 83 day of alpine pasturing, d116/141 means the 116/ the 141 day of pasturing.*

Furthermore, a statistically significant difference was determined ($p < 0.001$) between epg values before deworming and at the end of the grazing season. With regard to individual development of egg excretion, animals with low values at baseline (before deworming) retained low values at the end of the investigation, and sheep with higher values continued to have high values after grazing ($p < 0.001$; correlation coefficient = 0.403) (Fig. 4).

Prevalence of nematode genera

The relative prevalences of third stage larvae (L_3) isolated from coprocultures before deworming and after pasturing are presented in Figure 5. *Trichostrongylus* spp. was the most dominant parasite both before deworming (found in 45.6% of samples) and after the grazing season (38.8%). *Haemonchus contortus* was the second most common nematode with 18.9% and 31.2% of all identified L_3, respectively. *Bunostomum* spp. (9.5% and 9.1%), *Teladorsagia* spp. (9.4% and 10.3%), *Cooperia* spp. (7.6% and 5.3%), *Nematodirus* spp. (3.2% and 2.6%), *Chabertia ovina* (3.0% and 1.3%) and *Oesophagostomum* spp. (2.9% and 1.4%) were found less frequently.

H. contortus significantly increased from the first to the last examination ($p = 0.005$), while *Trichostrongylus* spp. ($p = 0.004$) and *Nematodirus* spp. ($p = 0.032$) showed a significant decrease between the two samplings.

Discussion

In the present study we determined the prevalence of GiSt on a communal pasture used for pasturing of sheep in Styria, a federal state of Austria and observed an increase of infection intensity over time (determined by egg excretion) during pasturing.

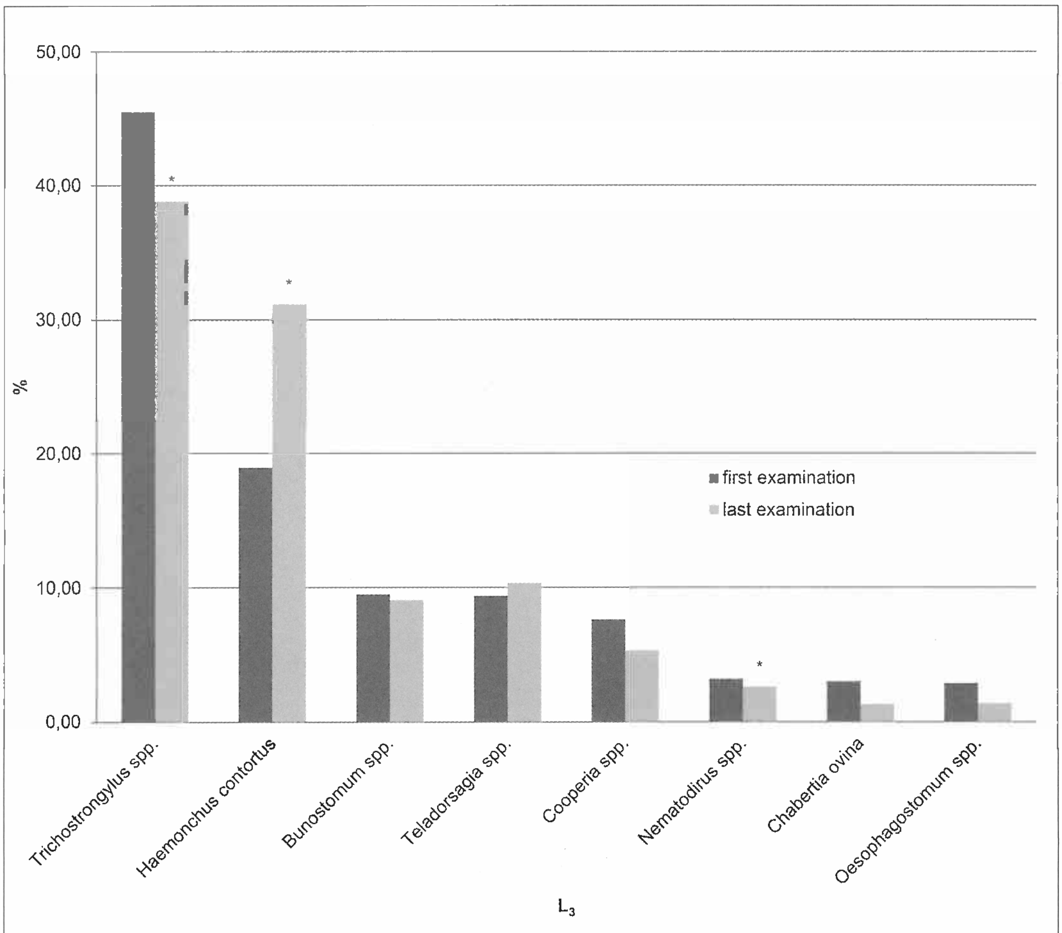

FIGURE 5: *Percentage of all identified third stage larvae of nematodes at the first and the last examination.*
** significant differences between the examination before deworming and after pasturing ($p < 0.005$)*

Communal alpine pasturing is a typical farming practice in Austria. Due to the joint use of grazing by sheep from different farms the risk of transmission of pathogens, including GiSt, is considerably higher than in closed systems. The duration spent on pasture depends on climatic conditions and covers the period of the maximum density of infective GiSt larvae on the grass (Amarante et al., 2004).

In Austria, the last comprehensive study of GiSt prevalence in Austrian sheep was published almost 40 years ago (El-Moukdad, 1977). The prevalence of 96.6% at that time is comparable with that determined in the current study, and recent investigations on smaller sheep populations also confirm these results (Biermayer, 1996; Gergely and Wehowar, 2008; Feichtenschlager et al., 2014). However, compared to the data from El-Moukdad (1977) with those from the present study, it appears that the species composition of GiSt has changed over the years. *H. contortus* was the second most prevalent nematode in the present study, while it was not of any significance in 1977. By contrast, *Nematodirus* spp. was dominant in the earlier investigation and was now only detected at a low rate (2.6–3.2%). In consequence of the way of cultivation the real infestation with *Nematodirus* spp. in this study may be considerably underestimated, because *Nematodirus* spp. (*N. battus, N. filicollis, N. spathiger*) ova need up to 14 days for cultivation and, in case of *N. battus*, a cold stimulus to hatch. Fungal overgrowth commonly makes the above culture method unsuitable for members of this genus (van Wyk and Mayhew, 2013). Of particular note is also the fact that a significant relative increase of *H. contortus* could be observed during grazing. This may be due to several reasons. *H. contortus* may have developed resistance to the applied anthelmintic drug used which resulted in an insufficient reduction of worms and maintenance of infection and egg excretion. Another reason for an increase of *H. contortus* might be changing climatic conditions which over the years may have led to more favorable conditions for this nematode species. In general, *H. contortus* is widely spread in tropical and subtropical regions (O´Connor et al., 2006) while *Teladorsagia circumcincta* predominates in cooler areas (Stear et al., 2009). The mean annual temperature in Austria increased from 10.1°C in 1977 to 14.0°C by 2014 (https://www.wien.gv.at/statistik/lebensraum/tabellen/temperatur-zr.html), and the increasing presence of *H. contortus* even at higher altitudes and cooler climate of the alpine pasture in this study indicates that it may be even more abundant in the temperate lowland regions of the Alps, as demonstrated already for Switzerland (Musella et al., 2011).

Generally, an increase in the FEC values (epg) during the alpine pasture period was observed. Considering the increase of *H. contortus* in larval cultures between the first to the last sampling, it must be assumed that this species contributes to this continuing increase of epg values, also because *H. contortus* has a high fecundity (Taylor, 2010). These findings indicate that the deworming regime applied in the investigated management system is insufficient to control GiSt infections in sheep during the grazing season. Communal grazing of animals from different farms may contribute to the infection pressure at the beginning of the grazing period if single "super-shedders" from farms with insufficient nematode control increase the initial contamination with eggs.

In addition, first-season grazing lambs are generally more susceptible to GiSt infections (Shubber et al., 1981; Bishop and Stear, 2001) and may contribute significantly to the egg contamination of the pasture and the transmission of GiSt within the flock (Stear et al., 2009). At the explicit request of the owners lambs were not included because of animal welfare reasons (rectal fecal sampling). Since lambs were not assessed during this study, their direct influence on the egg contamination of the pasture cannot be determined. Based on the large number of lambs present on the communal grazing land, it is expected that they also had an effect on the level of infection and its transmission within and between the flocks.

This investigation determined a statistically significant difference between epg-values after deworming and epg-values after the grazing period, indicating that although deworming decreased egg excretion initially, infection intensities increased again over time during summer with high epg values so that the treatment regime was not sufficient to control egg shedding until the end of the grazing season.

As observed in previous studies (Stear et al., 2009), animals with initially low epg were usually also low shedders by the end of the grazing season and high shedders remained so, and this was suggested to be due to genetic resistance (Stear et al., 2009). Apart from the genetic component, the genera of GiSt present in individual infected sheep could be causal for the different FEC values due to various fertility and productivity factors as discussed above.

Due to the high prevalence of *H. contortus* in the present study, the evaluation of mucous membrane color for the identification of anemic animals (e. g. by the FAMACHA scoring system; Kaplan, 2004; Burke et al., 2007) should be included in the decision making for sheep deworming management (Crab et al., 2002).

Conclusion

This study presents results on the current prevalence of GiSt in sheep in the investigated region in Styria ("Hauser Kaibling"). The applied deworming scheme did not prevent a substantial increase in fecal egg shedding during the time on pasture. Unexpectedly high prevalences of *H. contortus* especially by the end of the grazing season indicate that this parasite is probably becoming more important in the investigated area. When animals graze communal pastures, close endoparasite monitoring and effective anthelmintic treatment are necessary to ensure sufficient control of infections that might otherwise become a health threat to the sheep. This study was performed in a locally restricted area and so it is not possible to draw general conclusions from these experiments. Further studies in different geographic areas of Austria are needed to address the prevalence of GiSt in sheep in Austria and the influence of transhumant of the spread of GiSt in this country.

Acknowledgement

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Conflict of interest statement

The authors whose names are listed certify that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript. This investigation was done in cooperation with the Animal Health Service Styria and was a commissioned work. Karl Bauer is the managing director of the Animal Health Service Styria.

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