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Short Communication

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Trematode and lung worm prevalence in slaughtered lambs in Styria

Prävalenzermittlung für Trematoden und Lungenwürmer bei Schlachtlämmern in der Steiermark

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Summary

In this study we set out to estimate the prevalence for trematodes and lung worms in slaughtered lambs in Styria.
In total 218 lambs were investigated from two abattoirs in Styria. All animals were examined clinically. Faecal samples, condemned lungs and livers and the bile of all selected animals were collected during the slaughter process. *Fasciola hepatica* and *Dicrocoelium dendriticum* were the target species. Additionally, samples were checked for the presence of lungworms.
Fasciola hepatica and *Dictyocaulus filaria* were not detected in any of the samples taken. The total number of *Dicrocoelium dendriticum* was 14.5%. *Protostrongylus rufescens* was detected in five out of 218 animals (2.3%).

Keywords: small ruminant, *Fasciola hepatica, Dicrocoelium dendriticum, Dictyocaulus filaria, Protostrongylus* spp.

Zusammenfassung

In dieser Studie wurde eine Prävalenzermittlung für Trematoden und Lungenwürmer bei Schlachtlämmern in der Steiermark durchgeführt.
Insgesamt wurden 218 Lämmer von zwei Schlachthöfen untersucht. Alle Tiere wurden bei der Anlieferung an den beiden Schlachthöfen einer klinischen Untersuchung unterzogen. Kotproben, als genussuntauglich befundete Lungen und Lebern sowie die Galle aller ausgewählten Tiere wurden während des Schlachtprozesses gesammelt. Es wurde auf *Fasciola hepatica* und *Dicrocoelium dendriticum* sowie auf das Vorhandensein von Lungenwürmern untersucht.
Fasciola hepatica und *Dictyocaulus filaria* wurden in keiner der entnommenen Proben nachgewiesen. Die Gesamtanzahl von *Dicrocoelium dendriticum* betrug 14,5 %. *Protostrongylus rufescens* wurde bei fünf aus 218 Tieren (2,3 %) nachgewiesen.

Schlüsselwörter: Kleiner Wiederkäuer, *Fasciola hepatica, Dicrocoelium dendriticum, Dictyocaulus filaria, Protostrongylus* spp.

Introduction

Aim of this study was to investigate the current prevalence of *Fasciola (F.) hepatica, Dicrocoelium (D.) dendriticum, Dictyocaulus (D.) filaria* and *Protostrongylus* spp. in lambs in two abattoirs in Styria, Austria. The federal state Styria now farms the third-largest livestock numbers in Austria and infestations with pastoral parasites present a great risk to animal health (Gahleitner 2016, Huber et al. 2014).

Liver fluke

Fasciolosis is of enormous economic importance since infected animals often show poor performance (Beesley et al. 2018). The incidence of fasciolosis has increased in recent years. It is assumed that the main reasons are climate change and increased animal trafficking (Rojo-Vázquez et al. 2012). Apart from some resistant sheep breeds, most sheep are not able to develop sufficient immunity to eliminate *F. hepatica*. As a result, high-grade infections often lead to serious illnesses and even death (Deplazes 2012). Lack of immunity in sheep to *F. hepatica* also leads to enormous contamination of pastures with eggs (Schnieder 2006).

Although the economic damage caused by dicrocoeliosis is much lower, infestation with *D. dendriticum* is of great importance worldwide (Khanjari et al. 2014). Besides loss of performance, discarded livers at the abattoir are economically damaging (Rojo-Vázquez et al. 2012, Schweizer et al. 2003). In a previous study in the eastern part of Austria, only two out of 33 sheep tested positive by faecal sedimentation for *D. dendriticum* (Schoiswohl et al. 2017). Nevertheless, it is difficult to estimate the degree of infection of an animal with *D. dendriticum* or *F. hepatica* from merely the egg numbers in the faeces (Campo et al. 2000, Rehbein et al. 2002, Rojo-Vázquez et al. 2012). This is because the eggs are intermittently excreted with the biliary fluid. Further, post- mortem examinations of liver and biliary fluid are considered to be sensitive methods for the detection of liver flukes (Braun et al. 1995, Rapsch et al. 2006, Rehbein et al. 2002, Rojo-Vázquez et al. 1981).

Lungworm

D. filaria can result in enormous economic losses (Hiepe 2009). The mortality of young animals can be as high as 25% (Baker et al. 2001). While the large lungworm is often found in (sub-)tropical areas, such as Ethiopia (Abebe et al. 2016), Hertzberg and Sager (2006) found sporadic cases in small ruminants in central Europe. However, neither Feichtenschlager et al. (2014) nor Schoiswohl et al. (2017) were able to detect large lungworms in Austrian small ruminant herds. They nonetheless detected infection rates for the small lungworm to be 18.8% (Feichtenschlager et al. 2014) and 9.4%, respectively. While infestation with small lungworms generally does not result in clinical disease in sheep, it may result in impaired pulmonary gas exchange (Berrag and Cabaret 1996). The reliabilities of detection techniques also differ. According to a study conducted by Alemu et al. (2006), where 104 animals underwent detailed pathological examinations, there was a lower prevalence of 9.8% for *Protostrongylus (P.) rufescens* and a higher prevalence of 49.7% for *Muellerius (M.) capillaries* compared with coproscopic examinations.

Materials and methods

This study was approved by the institutional ethics committee and the national authority according to § 26ff of Law for Animal Experiments, Tierversuchsgesetz 2012 – TVG 2012.

Using WinEpiscope 2.0 (Thrusfield et al. 2001), we calculated a minimum convenience sample size of 205 animals based on an assumed total population of approximately 1,200 lambs. This is the projected number that is usually slaughtered in both study locations over the projected six-months sampling period, taking into account an estimation within +/– 5% at the 90% confidence level for an expected prevalence of 20%.

The study was performed between June and November 2017. Samples from 218 lambs, 42.5% males and 57.5% females, from 52 different farms, 12 organic and 40 conventional, were obtained for examination at two abattoirs in Styria. The majority of the animals were crossbreeds (44.3%), followed by Krainer mountain sheep (20.8%). All animals eligible for the study had to meet the principle inclusion criteria, which were no treatment with antiparasitic drugs within the previous three months and less than six month of age. The faecal samples and the bile of all animals participating in this study were collected during the slaughter process. All livers and lungs were examined for macroscopic pathological changes (unfit for human consumption). All gallbladders were removed and closely examined at the university clinic, as were all organs declared unfit for consumption. Organs fit for consumption remained at the abattoir after on-site examination and entered the food chain. The sample materials were stored in plastic bags at 8 °C until processing. Faecal samples were analysed in the laboratory using the sedimentation technique after Benedek for trematodes (Bauer 2006) and with the Baermann/Wetzel technique using 100 x light microscopy magnification (Optiphot-2, Nikon) for lungworms (Eckert 2000, Prosl and Joachim 2006).

All study animals were clinically examined (body condition score (BCS), lung auscultation, presence or absence of nasal discharge, signs of anaemia or icteric mucous membranes and coughing) and condemned livers and lungs were examined macroscopically for signs of endoparasitosis (worm nodes). For this, the bronchi as well as the lung parenchyma were palpated and cut at multiple sites. Pathological findings were clarified microscopically. Biliary fluid from every study animal was analysed for the presence of Fasciola eggs and adult trematodes using 40 - 100 x light microscopy magnification (Optiphot-2, Nikon). Additionally, the bile ducts and the main bronchi were opened and secretions examined for the presence of adult endoparasites.

Data were analysed using the statistical program SPSS (version 20, IBM SPSS Statistics). Differences between groups in frequency distribution were analysed using Chi-Square-tests. For all analyses a p-value below 5% ($p < 0.05$) was considered significant.

Results

In total, 31 lungs (14.3%), eight from organic and 23 from conventional farms, and 27 livers (12.4%), nine from organic and 18 from conventional farm had pathological changes. They were unfit for consumption and underwent further investigation at the university clinic. *F. hepatica* and *D. filaria* were not detected in any of the samples. In sediments, the total prevalence of *D. dendriticum* was 14.68% (32 lambs). *P. rufescens* was detected in five out of the 218 animals, corresponding to a prevalence of 2.3%.

Five out of 16 animals with adult flukes in the gall bladder had eggs in their faeces and three animals had eggs in their bile. In the 27 unsuitable livers investigated, *D. dendriticum* could be detected four times in bile ducts. Two of these animals additionally possessed adult flukes in their biliary fluid. Eggs were found in the biliary fluid of eleven animals with satisfactory livers and adult small liver flukes could be extracted from the gall bladders of 13 livers classified as fit for human consumption. In addition, clinical symptoms of liver fluke infection, such as emaciation or anaemic mucous membranes, could not be associated with the actual infection.

Pneumonia-induced changes (e.g. atelectatic regions) and hemorrhages were found in 31 lungs, but there was no evidence of lungworm disease. Only one animal with lungworm disease showed a low body condition score. Three animals positive for *P. rufescens* showed moderately increased lung sounds.

Discussion

Liver fluke

In contrast to a previous prevalence study in the same region of Austria (Schoiswohl et al. 2017), we found a high prevalence for *D. dendriticum* (14.68%) in sediments. Besides, we have shown that infestations of *D. dendriticum* cannot be detected purely macroscopically during organ examination at the slaughterhouse. Adult stages or eggs of *D. dendriticum* could be found in animals with livers that were considered fit for human consumption. Since four livers positive for *D. dendriticum* showed only slight pathological changes, such as punctual haemorrhages or infarction, low grade infestations can easily be overlooked. Massive, substantial damage to the liver, as described by Otranto and Traversa (2003), was not observed and the symptoms of dicrocoeliosis can often be masked by other parasitoses. For this reason, we could not conclude from the clinical examination alone the degree of infestation with *D. dendriticum*.

Lungworm

In contrast to a previous study in Styria (Feichtenschlager et al. 2014) with a calculated prevalence of 18.8% in the present study the prevalence for *P. rufescens* was low by 2.3%. Only two lungs out of the five positive tested sheep in the coproscopy were deemed unfit for human consumption. All lungs were negative for adult lungworms and only pneumonia-induced pathological changes were visible. Due to the lack of typical pulmonary symptoms in lungworm-positive animals in this study, we assume that infestation is often manifest without clinical symptoms and can therefore remain unnoticed (Hiepe 2009). Additionally, coproscopy seems to be more predictive of infestation than direct lung examination. Alemu et al. (2006) published similar results. In contrast, Regassa et al. (2010) were able to detect higher prevalence rates *post mortem* (62.3%) than from coproscopy (36.9%).

In keeping with findings of Pelletier et al. (2005), we were unable to confirm a correlation between body condition score and a lungworm infestation.

In addition it is also noteworthy that *F. hepatica* and *D. filaria* have not been found in any of the samples. As these parasites are of high pathogenicity, this is an important information particularly for veterinarians and farmers in this region.

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Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical approval

This study was approved by the institutional ethics committee and the national authority according to § 26ff of Law for Animal Experiments, Tierversuchsgesetz 2012 – TVG 2012.

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Authors' contribution

Bianca Lambacher and Reinhild Krametter-Frötscher conceived of the presented idea.
Bianca Lambacher, Christina Ambros, Lisa-Maria Karner, Barbara Hinney, Julia Schoiswohl, Alexander Tichy, Josef Elmer, Josef Frei, Reinhild Krametter-Frötscher developed the theory and performed the computations.
Bianca Lambacher and Alexander Tichy verified the analytical methods.
All authors discussed the results and contributed to the final manuscript.
Bianca Lambacher wrote the manuscript.
All authors approved the final version of the manuscript.

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