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J Vet Res 68, 373-379, 2024
DOI:10.2478/jvetres-2024-0044

The herd-level prevalence of *Fasciola hepatica* infection in the goat population of Poland

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Received: February 27, 2024 *Accepted: July 31, 2024*

Abstract

Introduction: *Fasciola hepatica*, also known as the common liver fluke, is a globally distributed trematode parasite responsible for high economic losses in ruminants. Infection with *F. hepatica* occurs in Polish cattle and sheep; however, very little is known about its occurrence in goats. Therefore, a serological and coproscopic survey was carried out in Polish goats to determine the herd-level prevalence of *F. hepatica* infection in the goat population of Poland. **Material and Methods:** Between 2014 and 2022, 33 randomly selected goat herds were serologically screened in the regions of Poland for which risk of *F. hepatica* infection was estimated as increased based on the spatial distribution model developed within the frame of the GLOWORM project. Virtually all adult goats (>1 year-old) were tested using a commercial MM3-SERO ELISA. Risk factors for seropositive herd status were analysed in contingency tables. Also, faecal samples from 214 goat herds monitored for gastrointestinal nematode infections and anthelmintic resistance were examined using a sedimentation method. **Results:** At least one seropositive goat was detected in 11 of 33 herds, indicating herd-level seroprevalence of 33.3% (95% confidence interval (CI 95%): 19.7%–50.4%). At the animal level, only 17 of 1,464 tested goats were seropositive (1.2%, CI 95%: 0.7%–1.9%). The within-herd seroprevalence ranged from 0.8% to 11.1%. The serological status of the herd was not significantly associated with the characteristics of the herd or the extent of contact with sheep. In one herd, located in central Poland, a single positive faecal sample was found indicating a herd-level prevalence of *F. hepatica* infection of 0.5% (CI 95%: 0.1%–2.6%). The animal's post-mortem examination revealed liver lesions typical of chronic fasciolosis. **Conclusion:** *F. hepatica* infection occurs sporadically in Polish goat population and its prevalence is much lower than in cattle or sheep. Therefore, treatment or prevention of fasciolosis should only be considered if it has been reliably confirmed by an accurate diagnostic test. This applies also to goats inhabiting geographical areas where *F. hepatica* infection appears to be widespread in cattle and sheep, very likely due to the fact that goats avoid wet areas.

Keywords: coproscopy, liver fluke, MM3-SERO ELISA, sedimentation, seroprevalence.

Introduction

Fasciola hepatica, also known as the common liver fluke (in contrast to the giant liver flukes – *Fasciola gigantica* and *Fascioloides magna*), is a globally distributed trematode parasite. It poses a risk to human and animal health, as well as contributes to losses in livestock production in Europe and globally (8, 27). A wide range of mammals may serve as definitive hosts of *F. hepatica*, however the disease plays a major role in domestic and wild ruminants (27). *F. hepatica* is a digenetic trematode of the Trematoda class and Digenea subclass

with an indirect life cycle, in which the intermediate hosts are water snails of the *Lymnaeoidea* superfamily. The risk of disease depends on climatic factors such as optimal temperature (above 10°C) and high soil moisture which enable the intermediate hosts and free-living larvae of *F. hepatica* to thrive. Infection of the definitive host occurs *via* the oral route by ingestion of infective metacercariae – encysted larvae attached to plants growing near water pools. Fasciolosis may follow an acute, subacute (both mainly involving traumatic hepatitis), or chronic course (due to hyperplastic cholangitis) depending on the rate of parasite ingestion. Contrary to sheep and cattle, goats are unlikely to suffer from an acute form of the disease, as their browsing habits preclude massive ingestion of metacercariae in a short period of time. However, chronic infection by a low number of trematodes may lead to production losses because of reduced growth rates, fertility, milk yield, and carcass weight and inferior carcass composition (45, 51).

Traditionally, the diagnosis of fasciolosis was based on direct detection of fluke eggs in fresh faeces using the faecal sedimentation method (coproscopy). The method is considered to have very high diagnostic specificity (Sp) but low diagnostic sensitivity (Se) due to the 8–12-week prepatent period of fasciolosis, the paucity of eggs present frequently in a faecal sample and the intermittency of egg shedding (26). Several molecular diagnostic methods detecting *Fasciola* DNA have recently been developed; however, their reproducibility is still limited (hence their use is restricted to reference laboratories) and they have not overcome the main problem of the long prepatent period because *Fasciola* eggs are the source of DNA to be detected (6). Immunoenzymatic assays (sandwich ELISAs) detecting in feces metabolites released by late immature and adult flukes into the bile (coproantigen) currently ensure higher Se (4, 24, 28, 29, 49). Also, ELISAs detecting specific antibodies against the common liver fluke in the serum or milk overcome the problem of false negative results during the prepatent period, because antibodies against *F. hepatica* are detectable in serum as early as 3–4 weeks post infection and remain detectable for a very long time (1, 28, 31, 38, 44, 48). Therefore, serological ELISAs appear to be the most optimal diagnostic tools for large-scale surveys.

The prevalence of *F. hepatica* infection in Polish cattle has been thoroughly studied over the previous 20 years (17, 18, 21, 22, 23, 32, 47), and it is known to vary between geographical regions and seasons within a range from 7% to 80%. Data regarding fasciolosis in small ruminants are limited, not only in Poland but generally in Europe (5), although they suffice to suggest that the prevalence differs considerably between geographical regions (42). Very few local studies conducted on *F. hepatica* infection in sheep and goats in Poland have shown that prevalence is below 10% (15, 40); however, data from large-scale epidemiological surveys are lacking. Therefore, the aim of the study was to determine the herd-level prevalence of *F. hepatica* infection in the goat population of Poland by serological and coproscopic examinations.

Material and Methods

Serological survey. A serological survey was conducted in the years 2014–2022. The herds were randomly selected for the study (according to simple random method) in the regions of Poland for which the risk of *F. hepatica* infection was estimated as increased based on the spatial distribution model developed within the frame of the GLOWORM project (12). The minimum number of herds to be tested was set at 25, so that it was representative for the goat population assuming the expected herd-level seroprevalence of 50%, expected precision of ±20%, and the 95% level of confidence (16). Goat herds were categorised as small (≤30 adult goats), medium (31–100 adult goats) or large (>100 adult goats).

Blood samples were collected from all adult goats (>1 year-old) kept in the herd at the moment of the visit of the scientific team. Blood collection was a part of caprine arthritis-encephalitis control programme described elsewhere. Blood sampling was approved by the Local Ethics Committee in Warsaw (approval nos 31/2013 and WAW2/048/2021). Blood was collected from the jugular vein into 10 mL clot activator tubes (BD Vacutainer; Becton Dickinson, Plymouth, UK), left overnight at +4°C for clotting, and centrifuged at 3,000 rpm (1,390 × *g*) for 10 min. The serum was harvested to 2 mL Eppendorf tubes and stored frozen at −20°C until testing. Serum samples were tested using a commercial MM3-SERO ELISA (Monoscreen Ab-ELISA *Fasciola hepatica* indirect double wells kit; BioX Diagnostics, Rochefort, Belgium) performed according to the manufacturer's instruction. The MM3-SERO ELISA is coated with highly specific proteins from a 7–40 kDa fraction (mainly cathepsins L1 and L2) from the excretory/secretory products of *F. hepatica* captured by the monoclonal antibody MM3 (31, 37). This ELISA yields results as the sample-to-positive control ratios (S/P%) and its interpretation is as follows: samples with S/P% <10% are considered negative, 10%–15% as inconclusive (doubtful) and ≥15% as positive, between 15% and 45% indicating weak infestation, 45%–75% indicating medium infestation, and >75% severe infestation. The MM3-SERO ELISA is considered to have a very high Se of 99.2% (CI 95%: 97.0%–99.9%) and virtually perfect Sp of 100% (CI 95%: 98.6%–100%) in cattle and sheep (9, 11, 28, 30, 31). Therefore, the herd was considered seropositive if at least one goat in this herd tested positive in serology (*i.e.* had S/P% ≥15% in the MM3-SERO ELISA). The herd-level seroprevalence was calculated as the proportion of seropositive herds. The within-herd seroprevalence was calculated as the proportion of seropositive goats in the herd.

Coproscopic survey. Coproscopic survey (based on faecal egg count) was carried out in the years 2015–2023. Faecal samples were obtained within the frame of the voluntary monitoring of gastrointestinal nematode infections and anthelmintic resistance run by the Division of Veterinary Epidemiology and Economics at the Institute of Veterinary Medicine of the Warsaw University of Life Sciences (35). Therefore, the number of herds to be tested was based on the non-random convenience method (46) and the minimum number of herds was not predetermined.

Faecal samples were collected directly from the rectum or immediately after defecation by farmers or by the scientific team during veterinary consultations on goat farms. If collected by the owners of the goats, faecal samples were placed individually in zip-lock plastic bags. Samples were delivered to the parasitological laboratory within 24 h at room temperature and processed within the next 24 h so that the total time which had elapsed between sample collection and examination did not exceed 48 h. The sample pooling procedure was the same whether the owner or a researcher had collected the samples. Faecal samples in each herd were combined into pooled samples consisting of samples of five goats each. The number of pooled samples for each herd varied depending on the overall number of examined animals. In the parasitological laboratory, five 3-g portions of faeces, one from each of five goats, were mixed and subsequently 3 g of this pooled sample was combined with 10 mL of water. The faecal samples were examined by centrifugal sedimentation test (50). The faecal solution was placed in a 15 mL test tube with 3 mL of (*R*)-(+)-Limonene 97% (Sigma-Aldrich/Merck, Darmstadt, Germany), and the tube was capped. The filled test tube was shaken several times and centrifuged for 5 min at approximately 500 × *g*. After centrifugation, the sediment was resuspended in a few drops of water, and two drops of the sediment were placed on a slide, covered with a coverslip, and examined at 100× magnification under a light microscope (Eclipse E200; Nikon, Tokyo, Japan) (50). A sample was classified as positive when at least one egg was found in the sample. In the case of a positive result for the pooled sample, all individual samples constituting the pool were tested separately using the aforementioned method to identify an infected animal. The herd was considered positive if at least one goat from this herd tested positive in coproscopy. The herd-level prevalence was calculated as the proportion of herds positive in coproscopy.

Statistical analysis. Numerical variables (the numbers of goats in herds and prevalences) were not normally distributed (according to normal probability Q-Q plots and the Shapiro–Wilk W test); therefore, they were summarised using the median, interquartile range (IQR), and range. Categorical variables (serological and coproscopic status of herds and goats, geographic locations of herds, herd size categories, grazing on the pasture, access to water pools on the pasture and contact with sheep and cattle on the farm) were presented as counts and proportions. Associations between categorical variables were analysed using the maximum-likelihood G test or Fisher exact test (if the minimum expected value of the contingency table was <5), and the size of effect was presented as the odds ratio (OR). The Haldane–Anscombe correction for zero cells in the contingency table was used, if necessary. The 95% confidence intervals (CI 95%) for proportions were calculated using the Wilson score method (3). A significance level (α) was set at 0.05. The analysis was carried out in TIBCO Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

Results

The serological survey enrolled 33 randomly selected herds counting from 9 to 183 adult goats with the median (IQR) of 35 (23–61) goats. Twelve herds (36.4%) were small, 17 (51.5%) were medium, and 4 (12.1%) large. In 32 of 33 herds, between 1 and 34 male goats (median of 3) were kept. The herds were located in 9 of the 16 provinces of Poland (Table 1). Between 9 and 168 adult goats with the median (IQR) of 33 (19–49) were tested per herd, which amounted to 1,464 adult goats (134 males and 1,330 females) in total.

Seropositive goats were detected in 11 of 33 herds, which corresponded to a herd-level seroprevalence of 33.3% (CI 95%: 19.7%–50.4%). At the animal level, a positive result was found in only 17 of 1,464 tested goats (1 male and 16 females) (1.2%, CI 95%: 0.7%–1.9%). Two positive MM3-SERO ELISA results were indicative of a medium infestation and 15 positive results corresponded to a weak infestation. In 8 herds only a single goat was seropositive, while two, three and four goats were seropositive in the 3 remaining herds. The within-herd seroprevalence ranged from 0.8% to 11.1% with the median (IQR) of 2.3% (1.5%–4.6%) (Table 2). There were 14 inconclusive serological results, 12 of them in herds in which positive results were also found.

The serological status of the herd was not significantly associated with its geographical location (with the province in which the farm was located) (P = 0.291) or with any other investigated risk factor (Table 3). The proportion of seropositive males (0.7%; 1/134) was not significantly different from the proportion of seropositive females (1.2%; 16/1330; P = 0.617).

Faecal samples of 2,770 goats from 214 herds were tested, including 17 of 33 serologically tested herds (51.5%; 8 of 11 seropositive and 9 of 22 seronegative herds). Between 1 and 83 faecal samples (median (IQR) of 10 (5–15)) per herd were tested. The herds were located in all provinces of Poland (Table 1). The herd-level prevalence of *F. hepatica* infection according to coproscopy turned out to be 0.5% (CI 95%: 0.1%–2.6%), which was a significantly lower figure compared to the seroprevalence (P < 0.001) (Fig. 1).

In only one herd, located in central Poland, a single positive faecal sample was found. This goat was also

seropositive and its serological result indicated medium infestation. As the goat was emaciated, it was euthanised on the owner's request and subjected to a necropsy. The post-mortem examination revealed liver lesions typical of chronic fasciolosis. The organ was fibrotic, with an irregular nodular surface and an opaque capsule. Upon cutting the thickened biliary ducts filled with numerous mature flukes were found.

Table 1. Geographical distribution of goat herds tested for *Fasciola hepatica* infection

Province	Number of herds tested serologically (% of 33 herds)	Number of seropositive herds / Number of tested herds	Number of herds tested coproscopically (% of 214 herds)
Małopolskie	2 (6.1)	2 / 2	35 (16.4)
Mazowieckie	1 (3.0)	0 / 1	33 (15.4)
Wielkopolskie	-	-	19 (8.9)
Warmińsko-Mazurskie	14 (42.4)	3 / 14	16 (7.5)
Podkarpackie	-	-	15 (7.0)
Śląskie	1 (3.0)	0 / 1	12 (5.6)
Pomorskie	1 (3.0)	0 / 1	11 (5.1)
Lubelskie	4 (12.1)	1 / 4	11 (5.1)
Łódzkie	-	-	11 (5.1)
Lubuskie	-	-	9 (4.2)
Kujawsko-Pomorskie	-	-	9 (4.2)
Dolnośląskie	-	-	8 (3.7)
Podlaskie	5 (15.2)	2 / 5	8 (3.7)
Świętokrzyskie	3 (9.1)	2 / 3	6 (2.8)*
Zachodniopomorskie	2 (6.1)	1 / 2	6 (2.8)
Opolskie	-		5 (2.3)

* – the herd in which a goat positive in the MM3-SERO ELISA and coproscopy was found

Table 2. Herds in which at least one goat positive for *Fasciola hepatica* in the MM3-SERO ELISA was found

Herd	Number of adult goats in the herd	Sample size (sample fraction (%))	Number of seropositive goats (weak/medium/strong infestation)	Within-herd seroprevalence (95% confidence interval) (%)
1	158	153 (96.8)	2 (2/0/0)	1.3 (0.4–4.6)
2	66	66 (100)	1 (1/0/0)	1.5 (0.3–8.1)
3	62	62 (100)	4 (4/0/0)	6.5 (2.5–15.5)
4	27	27 (100)	3 (2/1/0)	11.1 (3.9–28.1)
5	36	35 (97.2)	1 (1/0/0)	2.9 (0.5–14.5)
6	61	51 (83.6)	1 (1/0/0)	2.0 (0.4–10.3)
7	22	22 (100)	1 (1/0/0)	4.6 (0.8–21.8)
8	33	33 (100)	1 (1/0/0)	3.0 (0.5–15.3)
9	48	48 (100)	1 (1/0/0)	2.1 (0.4–10.9)
10	127	127 (100)	1 (1/0/0)	0.8 (0.1–4.3)
11	58	44 (75.9)	1 (0/1/0)	2.3 (0.4–11.8)

Table 3. The analysis of risk factors for herd-level seropositivity for *Fasciola hepatica* infection in the goat population of Poland

Risk factor	Category	Number of seropositive herds/Number of herds in the category (seroprevalence (%))	P-value	Odds ratio (95% confidence interval)
Herd size	Small Medium Large	2 / 12 (16.7) 7 / 17 (41.2) 2 / 4 (50.0)	0.291	-
Grazing on the pasture	Yes No	9 / 31 (29.0) 2 / 2 (100)	0.104	0.08 (0.01–1.9)
Access to water pools on the pasture	Yes No	3 / 8 (37.5) 8 / 25 (32.0)	0.999	1.28 (0.24–6.7)
Contact with sheep on the farm	Yes No	4 / 10 (40.0) 7 / 23 (30.4)	0.696	1.52 (0.33–7.2)
Contact with cattle on the farm	Yes No	1 / 6 (16.7) 10 / 27 (37.0)	0.637	0.34 (0.04–3.3)

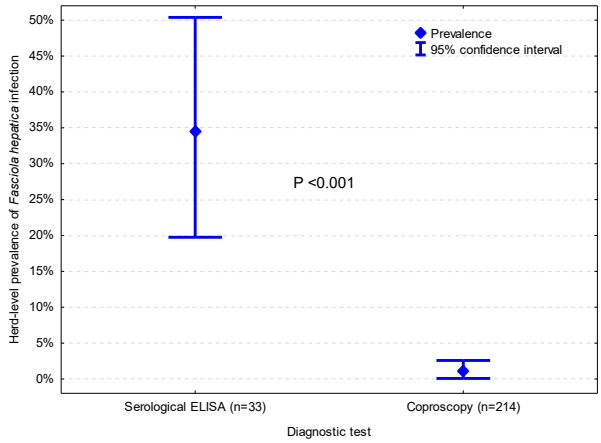

Fig. 1. The herd-level prevalence of *Fasciola hepatica* infection in the goat population of Poland according to serological testing with an MM3-SERO ELISA and a coproscopic test

Discussion

Our study shows that *F. hepatica* infection in goats in Poland occurs sporadically without any geographical pattern. Although seropositive goats were found in one third of tested herds, antibodies were detected only in single animals in each seropositive herd. Such a low number of seropositive results raises doubts as to the true status of these animals, given that coproscopy confirmed patent *F. hepatica* infection in only one seropositive animal. Only a test of a perfect Sp could ensure sufficiently high positive predictive value to draw reliable conclusions (45).

The MM3-SERO ELISA used in our study appears to be a very specific test according to studies so far carried out in sheep (31) and cattle (30). Such a high Sp is claimed to result from the use of a novel antigen captured by MM3 monoclonal antibodies and from controlling for potential unspecific cross-reactions by the use of additional wells on a plate which are coated solely by MM3 monoclonal antibodies. The very optimistic results presented in these studies should, however, be treated with great caution. They were all obtained by the same scientific team and have not been verified later by any other studies. Moreover, the MM3-SERO ELISA has never been validated on goats, although a large-scale cross-sectional study based on this ELISA was published for the goat population of Galicia, Spain (39). The MM3-SERO ELISA is based on anti-ruminant IgG1 conjugate; therefore the chance is low that it would work worse in goats than in sheep, but still an assumption that it is perfectly specific in goats should be made very cautiously.

Undoubtedly, our study shows that *F. hepatica* infection occurs in the Polish goat population, since the infection of one serologically positive goat was confirmed by the coproscopy and then by the post-mortem examination. A very low prevalence in coproscopy compared to seroprevalence has been observed in previous studies from other countries (13) and may be explained in two ways. First, it may be the consequence of the low Se of a single coproscopic examination. Coproscopy is known to detect *F. hepatic* infection from 8–9 weeks post infection at the earliest (2, 25), and only if a sufficient number of eggs is excreted in faeces. As a consequence, the Se of coproscopy is rarely estimated at more than 50% although few studies on the diagnostic accuracy of coproscopy in small ruminants have been published (25). The use of a coproantigen ELISA instead of coproscopy would have undoubtedly increased the Se, as it has been shown to detect even more positive goats than serological ELISAs (49). Secondly, it may result from past infections which have already been cleared by animals' immune systems. As antibodies to *F. hepatica* are known to persist long beyond the period of active infection (31), the seroprevalence from our study may better correspond to the actual level of goat exposure to *F. hepatica* infection in Poland than do the estimates based on the coproscopy. The exposure to *F. hepatica* in goats is certainly very low compared to the situation observed in Polish cattle (19) and probably also in Polish sheep, although data on fasciolosis in sheep are rather scarce (15). The risk of fasciolosis is mostly determined by the presence and extensity of infected snails, in Europe mainly freshwater snails of the species *Galba truncatula* (10, 41, 43). According to previous studies, the prevalence of *F. hepatica* infection in Polish snails is quite high (20). Nevertheless, these gastropods can only survive in wet conditions, while goats tend to avoid wetlands and damp pastures. Moreover, goats prefer browsing to grazing, which is associated with frequent changes of place of feeding and precludes ingestion of large quantities of metacercariae even on highly contaminated pastures (45). According to our observations resulting from many years of veterinary service on Polish goat farms, goats rarely share pastures with reservoir hosts of *F. hepatica* such as sheep and cattle.

Moreover, the common use of benzimidazoles on goat farms in Poland may lead to unintentional and somehow accidental reduction of active *F. hepatica* infection in goats. Most antiparasitic treatments are implemented based on common opinions about the high risk which tapeworms or liver flukes pose for ruminants without any attempt to seek medical confirmation of a true infection prior to treatment (36). Our study indicates that such a practice is totally pointless in light of the extremely rare occurrence of *F. hepatica* infection in Poland. Furthermore, the unjustified use of anthelmintics stimulates the development of resistance of gastrointestinal nematodes to benzimidazoles, which is already a substantial problem of goat herds worldwide and also in Poland (33, 34, 35). This observation makes proper parasitological diagnostics in a goat herd crucial to maintain the effectiveness of anthelmintic drugs as well as animal health and welfare.

Conclusion

F. hepatica infection occurs sporadically in the Polish goat population and its prevalence is much lower than in cattle or sheep. Therefore, treatment or prevention

against fasciolosis should only be considered if it has been reliably confirmed by a positive result of a highly accurate diagnostic test. This also applies to goats inhabiting geographical areas where *F. hepatica* infection appears to be widespread in cattle and sheep.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The study was partially financed by a grant from the National Science Centre (decision no. DEC-2020/37/B/NZ6/00457) and a grant from the Ministry of Science and Higher Education of the Republic of Poland (decision no. 9506/E-385/R/2018). The latter grant provided financial support for the modernisation of the parasitological and serological laboratories in which the diagnostic procedures were performed. The research internship of one of the co-authors (Adrian-Valentin Potărniche) was supported by the Polish National Agency for Academic Exchange (NAWA) (decision no. BPN/ULM/2022/1/00087/U/00001). The funding bodies had no direct role in the design of the study, the collection, analysis or interpretation of the data or the writing of the manuscript.

Animal Rights Statement: Blood sampling was approved by the Local Ethics Committee in Warsaw (approval nos 31/2013 and WAW2/048/2021).

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