A Cross-Sectional Study of Fasciolosis in Autochthonous Cattle From NW Spain by Using a 2.9-kDa Recombinant Protein

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ABSTRACT

A cross-sectional study of fasciolosis was conducted on serum samples from 296 autochthonous Rubia Gallega cattle of 36 farms by using an enzyme-linked immunosorbent assay (ELISA) test with a 2.9-kDa recombinant protein. Results were examined on the basis of the age of the cattle (0-2, 3-8 and >8 years), farm characteristics (number of cattle, slope, altitude, rainfall) and *Fasciola*-control measures. The cattle-level prevalence was 71% and the highest values were observed in the oldest animals. Ninety percent (86%, 100%) of the farms had cattle positive to the ELISA test, and by estimating the odds ratio values, we observed the highest risk for fasciolosis in the biggest farms (more than 25 cattle), where cattle received treatment against this trematode, located above 1000 m altitude and where rainfall did not achieve more than 1000 mm.

INTRODUCTION

One of the main livestock systems consists of maintaining cattle under field conditions, so herds are maintained outside on pastures and are only brought into the paddocks during the night. The autochthonous cattle breeds of Spain are commonly maintained under this regime because of their great adaptation to the climatic conditions of their area of origin. They are focused to produce calves with outdoor grass fattening. The grazing herds are generally fed on natural pastures, characterised mainly by annual grass species, and most of them are only brought into the paddocks during the night. This livestock system allows the possibility of infection and challenge by several parasites as the liver-fluke *Fasciola hepatica*, especially in areas with frequent rain and mild temperatures.[1]

In a previous investigation, the usefulness of a 2.9 kDa *F hepatica*-recombinant protein for the early detection of active fasciolosis in sheep kept under field condi-

tions has been demonstrated.[2] In the present paper, we report the results of a cross-sectional study designed to evaluate several factors affecting the presence of *F hepatica* in pastured cattle. For this purpose, the prevalence of fasciolosis was estimated by means of an enzyme-linked immunosorbent assay (ELISA) test and the 2.9-kDa recombinant protein.

MATERIAL AND METHODS

Blood samples were randomly collected from 296 female Rubia Gallega autochthonous breed cattle in Lugo (NW Spain) (42° 20' – 43° 45' N, 6° 49' – 8° 00' W). The grazing herd is generally fed on natural pastures, characterised mainly by annual grass species.

To select the animals, the SPSS statistical package (version 12) was employed. For this purpose, we obtained a list with the cattle farms in Lugo provided by a government quota department, where farmers are obligated to list all their cattle. For each farm, this directory includes farmer private data and animal data.

According to their age, cattle were divided into 3 groups: G-1 (0-2 years), G-2 (3-8 years), and G-3 (>8 years) (Table 1). Three groups were also defined by considering the altitude where the farms were located: G-A (0-500 m), G-B (501-1000 m), and G-C (>1000 m). Finally, we considered 2 rainfall categories: <1000 mm and >1000 mm.

ELISA

The humoral IgG response against rFhAPS was evaluated on serum samples by using polystyrene plates. This antigen was diluted to 3 µg/mL in PBS (phosphate buffered saline, pH 7.4), sera (tested in duplicate) at 1:50 in PBS-0.3% Tween 20 and 10% skimmed milk, and horseradish peroxidase-conjugated rabbit anti-bovine IgG (H&L chains, Nordic Immunology Laboratories, Tilburg, The Netherlands) at 1:1000. After adding the substrate, the enzymatic reaction was stopped with 100 µL per well of 3N sulphuric acid, and absorbances were read using a spectrophotometer (Titertek Multiskan) at 492 nm.

In order to establish the cut-off point, positive values were the mean optical density (OD) of 15 negative sera plus 3 standard deviations.[3] Thus, positive values were OD ≥ 0.256.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA). The unit of analysis was the herd's cattle-level. Statistical significance was considered when $P < 0.05$. All the estimations were done by using the SPSS statistical package (version 11.1).

To obtain the values of sensitivity and specificity, faecal and serum samples from 95 naturally infected cattle were obtained. The faeces were analyzed by the coprological sedimentation technique[2] and the sera by using an ELISA and the recombinant protein. The levels of sensitivity of the ELISA + FhAPS were 86% and 88%, respectively.

Cattle Owner's Survey

We solicited answers from the farm owners in a questionnaire which included 20 questions at the moment of sampling, on descriptors of the farm, questions about the procedures for control of bovine parasites, number of treatments/year, time of treatment, anthelmintic used, herd anthelmintic-treatment frequency, use of quarantine drenching and dosage calculation (Table 1). Cooperation with the farmers was fully satisfactory, and all of them participated in the survey. This questionnaire was easily filled out in 20 minutes.

Fourteen of the questions were semi-closed. The questionnaire was pilot tested by eight farmers who were not surveyed, but reproducibility of answers was not tested directly.

RESULTS AND DISCUSSION

Survey

Table 1 reflects that the only practice used for control of *F hepatica* infections was chemotherapy. Albendazole was the most used fasciolicide (94%), and treatment was mainly administered 2 times/year (Table 1). Sixty-nine percent of the farmers adminis-

Table 1. Responses of 36 Cattle Farmers to a Questionnaire Regarding Fasciola hepatica-Control Procedures in Rubia Gallega Autochthonous Cattle (Lugo, Galicia, NW Spain) (April–November 2003).

Factor	% Yes	95% CI
Routine coprological examinations to ascertain the presence of parasites	0	
Presence of other animals grazing on the farm		
Do you operate pasture rotation/mixed grazing?	0	
F hepatica-control procedures		
Animal management conditions	0	
Chemotherapy	100	
Pastures management	0	
Fasciolicides used regularly		
Albendazole	94	86-100
Albendazole + triclabendazole	6	1-17
All the cattle are dewormed at one time	100	
Do you rotate the drugs used?	0	
Frequency of treatment/year		
1	31	15-47
2	69	53-85
Time of treatment		
Autumn	31	15-47
Spring + autumn	69	53-85
Coprological examination prior to treatment	0	
Coprological examination after treatment	0	
Treatment of animals before they first enter the farm	0	
Do you know the procedures for the control of the intermediate host?	0	
Could you establish the main risk zones for *F hepatica* infection?	0	

CI = confidence interval.

tered the fasciolicides in spring and autumn, and 31% in autumn only. Echevarria et al[4] and Claxton et al[5] demonstrated that the double treatment regimen per year did not significantly reduce the overall parasite burden, but did control them to a level similar to that achieved by traditional programmes averaging 3-t treatments/year.

None of the farmers surveyed asked for a parasitological analysis either before or after the treatment. The animals were not weighed before drenching on any of the farms in the survey, and their weight was calculated only by visual appraisal.

Farmers did not routinely use coprological examinations to ascertain the presence of parasites or deworm new animals before introducing them onto the farm.

The responses suggest lack of knowledge about *F hepatica*-control strategies, the use of anthelmintics, and unwillingness to apply such knowledge. The only control measure applied was chemotherapy, but its value is questionable as farmers administer the anthelmintics without any laboratory support.

ELISA

Results of the ELISA test indicated that 71% (95% confidence interval [CI], 66-76) of the cattle were positive to fasciolosis. Seroprevalence ranged from 59% (95% CI, 53-64) in the youngest animals to 76% (95% CI, 71-81) in the oldest ones, and these differences were found to be significant ($F = 6.380$, $P = 0.041$).

Ninety percent of the farms had cattle positive by the ELISA test. The highest percentages were observed in the farms above 1000 m (100%) and the lowest in those at 0-500 m (92%). These differences were not significant.

All the farms with less than 15 cattle or with more than 25 were positive to the ELISA test and significant differences with respect to the number of animals/farm were observed ($F = 6.763$, $P = 0.046$).

The odds ratio (OR) estimation showed that the highest risk for the presence of antibodies against *F hepatica* was found among the oldest animals. The herd-level analysis demonstrated that farms with more than 25 animals, located above 1000 m altitude with <1000 mm rainfall and 0%-13% slope had the greatest risk for the presence of positive IgG values against the rFhAPS (Table 2). The highest OR value was recorded in the farms treating 2 times/year using albendazole.

Current efforts for the control of fasciolosis need diagnostic technique support that allows the early detection of active infections.[1] Egg elimination indicates the presence of mature adult flukes in the bile ducts and gall-bladder, but a period longer than 10 weeks after infection is required, so most of the pathological damage has already occurred. In a previous report, the suitability of the FhrAPS recombinant protein for the detection of current active fasciolosis in sheep was demonstrated.[2]

The sensitivity of most serological assays by using *F hepatica*-crude antigens is satisfactory. Nevertheless, collection of these antigens is very hard work, involving a visit to the abattoir, collection of adult fasciolae, and incubation into suitable media. Most of the applications of molecular biology have been focused on searching an efficient vaccine to prevent fasciolosis[6] and very few attempts to obtain recombinant proteins for detecting cattle fasciolosis.[7]

Epidemiological studies are essential because more reliable information about the distribution and determinants of a parasitic disease can be achieved. These studies are also needed to get an overview of the global actions to protect the health of the animals, for preventing the possibility of F hepatica infection, or reducing its frequency. Our results led us to conclude that there is an elevated prevalence of fasciolosis in bovine maintained under field conditions in Galicia (NW Spain). We also conclude that the use of FhrAPS-ELISA provides an excellent tool to obtain knowledge about the real parasitic

Table 2. Analysis of the Risk Factors Involved in the Fasciola hepatica Infection in Rubia Gallega Autochthonous Cattle in Lugo (Galicia, NW Spain) (April–November 2003).

Risk Factor	OR	
	rFHAPS	EPG
Fasciolicides used regularly		
Albendazole	7.5	
Albendazole + triclabendazole		1.9
Frequency of treatment/year		
1		1.5
2	1.6	
Time of treatment		
Autumn	1.2	1.2
Spring + autumn		
Number of cattle/farm		
1-15		
16-25		
>25	1.3	2.9
Altitude farms and pastures (m)		
0-500		
501-1000		
>1000	1.7	1.7
Slope pastures (%)		
0-13	1.5	1.3
14-25		
Rainfall (mm)		
0-1000	2.8	1.3
>1000		
Age of the cattle (years)		
0-2		1.5
3-8		
>8	1.5	

EPG = eggs per gram.

status of the animals in any region around the world. Because both tests, the coprological sedimentation and the FhrAPS-ELISA, demonstrate current fasciolosis, we suggest their application to epidemiological surveys focused to establish the prevalence of this economically important parasitic infection.

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