

Detection of antibodies against *Fasciola hepatica* in cattle of Ulleung island, Korea - short communication

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ABSTRACT

We performed a cross-sectional study to estimate the seroprevalence of *Fasciola hepatica* in herds of cattle on Ulleung island, Korea. Blood samples were collected from randomly selected cattle and the sera were separated and analysed with an ELISA to detect antibodies against *F. hepatica*. The positive samples were classified as mildly, moderately or strongly positive. Out of 405 cattle sera assessed, 38 (9.4%) were seropositive for antibodies against *F. hepatica*. From these, 2.5% each were moderately or strongly positive and 4.4% were mildly positive. A significantly higher seroprevalence ($P < 0.05$) was observed in young animals (<2 y old, 15.3%) compared to adults (≥ 2 y old, 5.4%), while no significant difference in seropositivity was found between male and female animals. This is the the first report of *F. hepatica* seroprevalence in cattle herds in Korea. These findings may be used to establish a base-line of information for future investigations focused on the significance of *F. hepatica* in Korea.

Key words: *Fasciola hepatica*, seroprevalence, ELISA, cattle, Korea

Introduction

Fasciola hepatica, also known as the common liver fluke or sheep liver fluke, is a causative agent of fasciolosis. This parasite is a global public health concern that

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causes severe economic losses in cattle, sheep and goats production systems worldwide (SOULSBY, 1982; CARNEVALE et al., 2001; MAS-COMA et al., 2005). *F. hepatica* is endemic in Europe, Africa, Asia, the United States and Oceania (MAS-COMA and BARGUES, 1997). The distribution of *F. hepatica* infection is primarily determined by patterns of climatic conditions favourable for the snail intermediate hosts (of the genus *Galba*) and free-living stages of the parasite (BORAY et al., 1969; McCANN et al., 2010). The epidemiology of this parasite is also influenced by the grazing habits and feeding systems of the host animals. Animals grazing in wet, marshy or swampy areas, favoured by the intermediate host, are most likely to become infected (BORAY et al., 1969).

A wet, marshy environment, especially in Korean islands, is hospitable for fluke eggs, host snails and larval flukes. Although the cattle on these islands are not allowed to graze, the farmers use a cut-and-carry system in which animals are fed vegetation from wet marshy areas, thereby creating an opportunity for *Fasciola* infection. However, there is no recent epidemiological information for *F. hepatica* infection in Korea, except a report on the prevalence (36.5% by intradermal serological tests and 45% by condemnation) by the Food and Agriculture Organization published two decades ago (OVER et al., 1992).

Besides the traditional coproscopic examination, serological techniques such as ELISA have been utilized recently for diagnostic and prevalence studies of *F. hepatica* in live animals for the earlier detection of circulating antibodies against *F. hepatica* antigen (SALIMI-BEJESTANI et al., 2005). The purpose of this study was to estimate the seroprevalence of *F. hepatica* in cattle raised in Ulleung island of Korea using an ELISA.

Material and methods

The study was conducted on Ulleung island located off the far eastern coast of Korea. The island is composed of three regions: Buk-myeon, Seo-myeon and Ulleung-eup. The study population included 762 cattle raised on intensive beef farms with limited or no grazing. The entire study area is located between 37°30'0" North latitude and 130°52'0" East longitude. The mean annual temperature is 12°C and mean annual precipitation is 142.6 mm.

Blood samples were collected following a simple stratified random sampling design, according to THRUSTFIELD (2005). The sample size was determined with an expected prevalence of 50%, an accepted error of 5% and a confidence level of 95%, with a simple random sampling design. The resulting sample size was proportionally allocated to the strata (regions). A total of 405 animals were selected, and blood samples were collected by jugular vein puncture from September to November 2011. Serum was separated according to standard procedures and stored at -20°C until use.

The sera samples were analysed with an ELISA test kit (IDEXX, Montpellier, France) according to the manufacturer's instructions to detect antibodies against the

“f2” antigens of *F. hepatica*. The sensitivity and specificity of the test kit were 98% and 100%, respectively (REICHEL, 2002). The positive results were classified into mildly, moderately and strongly positive, as per instructions, based on the color intensity, which is proportional to the concentration of bound antigen. A cut-off value of 30% of sample to positive (S/P) percentage was used. Samples with S/P percentage less than or equal to 30% were considered negative for the presence of antibodies against *F. hepatica* antigen. While samples with S/P percentage greater than 30% and less than or equal to 80% were considered mildly positive, samples with S/P percentage greater than 80% and less than 150% are considered moderately positive and samples with S/P percentage greater than 150% were considered strongly positive for the presence of antibodies against *F. hepatica* antigen.

A chi-square test was used to analyse the significant differences among the proportions; where P-values <0.05 were regarded as statistically significant. The analytical software package GraphPad Prism version 5.04 (GraphPad Software Inc., La Jolla, USA) was used for statistical analysis.

Results and discussion

A diagnosis of *F. hepatica* by traditional sedimentation techniques or faecal egg counts can be made only after the period of prepatency (8-10 wks after infection) when most of the pathological lesions have developed (CARNEVALE et al., 2001). Additionally, these methods are not sufficiently sensitive to detect infections where the parasite burden is low or when the host is harbouring immature flukes in the liver parenchyma or bile ducts (HAPPICH and BORAY, 1969). However, immunodiagnostic methods, such as ELISA, have been shown to possess adequate sensitivity and specificity for overcoming these factors (PHIRI et al., 2005; SALIMI-BEJESTANI et al., 2008; ARIAS et al., 2012).

In the current study, we used a commercially available IDEXX ELISA kit for detecting *F. hepatica* in cattle. Out of the 405 animals we tested, 38 were positive for antibodies against *F. hepatica* antigen (Table 1). Aside from detecting antibodies directed against *F. hepatica*, the IDEXX ELISA kit, as prescribed by the manufacturer, can be used to determine the level of infection, which is directly proportional to the antibody concentration. Hence, the 38 seropositive animals were categorized as mildly (18), moderately (10) and strongly (10) positive. Assays that can discriminate between strong and mild infections would be useful for developing cost-effective treatment programmes, by targeting only severely infected animals for treatment. Furthermore, lower quantities of drugs would be administered, and the risk of drug resistance developing within fluke populations would be reduced (SALIMI-BEJESTANI et al., 2008). The small number of strongly positive animals we observed compared to those that were moderately positives indicates that the infections could be controlled in a cost-effective manner. Additionally,

understanding the environmental circumstances of Ulleung island, appropriate farm management practices could lead to complete control and/or possible eradication of *F. hepatica* infection on the island. Our results were further analysed according to variables such as age, gender and region (Table 2). The prevalence of *F. hepatica* infection in young cattle (<2 y old) was significantly higher than in adults (≥ 2 y old) ($P < 0.05$). However, no significant differences associated with gender or region were found.

Table 1. Seroprevalence of *Fasciola hepatica* in 405 cattle reared on Ulleung Island

Degree of seropositivity	No. positive	Prevalence (%)	95% CI ¹
Mildly positive	18	4.4	2.8-6.9
Moderately positive	10	2.5	1.4-4.5
Strongly positive	10	2.5	1.4-4.5
Total	38	9.4	6.9-12.6

¹CI, confidence interval

Table 2. Seroprevalence of *Fasciola hepatica* according to age, sex and region

Variable		No. tested	No. (%) positive	95% CI ¹
Age	Young (<2 yrs old)	163	25 (15.3)*	10.6-21.7
	Adult (≥ 2 yrs old)	242	13 (5.4)	3.2-9.0
Gender	Male	174	16 (9.2)	5.8-14.4
	Female	231	22 (9.5)	6.4-14.0
Region	Buk-myeon	69	9 (13.0)	7.1-23.0
	Seo-myeon	121	11 (9.1)	5.2-15.6
	Ulleung-eup	215	18 (8.4)	5.4-12.8
Total		405	38 (9.4)	6.9-12.6

¹CI, confidence interval; *Significant difference in each group ($P < 0.05$).

The overall *F. hepatica* seroprevalence observed in the present study was lower compared to the results of similar investigations (RAPSCH et al., 2008; SALIMI-BEJESTANI et al., 2008) conducted in England (64.2%) and Switzerland (18%). These differences observed from other countries might be due to the difference in climatic conditions resulting from their geographic location, while the reduction from the past studies (OVER et al., 1992) could be attributed to the fast development of the country, which allowed installation of modern farm facilities and a complete change of the farm management practices. However, ours is the first report from Korea and these data could serve as a basis for future studies. The findings from the present study also indicate the need for further investigation of *F. hepatica* infections throughout the country. The significantly higher seroprevalence in younger cattle observed in our study might be due to a low

resistance against parasitic infection. Fasciolosis has been reported most frequently in young parasite-naive calves (ARMOUR, 1975; KAPLAN, 2001), which is in agreement with the results of our study. Likewise, the lower seroprevalence rates we observed in older cattle might be due to the development of partially protective immune response against *F. hepatica* during previous exposure to the parasite.

In an experimentally infected cattle study of antibody kinetics, all experimentally infected cattle showed seroconversion within 2 weeks of infection, and the sera remained serologically positive until patency of infection was reached (REICHEL, 2002). However, any past treatment could have an effect on seropositivity, as previous studies (CASTRO et al., 2000; LEVIEUX et al., 1992) showed that antibodies against *F. hepatica* antigens become undetectable after an elapsed time of 4-6 months of treatment by dot-ELISA and hemagglutination technique in individual animals. This suggests an alternative explanation for the observed low seroprevalence in the adults, although we have no information on the anthelmintic treatment (de-worming practices of the farms) of the animals in this study. The degree of parasite establishment and the pathological impact of the infection are determined by interaction of factors, such as age, innate resistance, previous host exposure and the current level of parasite exposure (KAPLAN, 2001).

In conclusion, the present study is the first to report the seroprevalence of *F. hepatica* infection in cattle herds in Korea. These findings may be used to establish a base-line of information for future investigations focused on the significance of *F. hepatica* in Korea.

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SAŽETAK

Provedeno je presječno istraživanje radi procjene seroprevalencije invadiranosti velikim metiljem *Fasciola hepatica* u stadima goveda na otoku Ulleung u Koreji. Uzorci krvi bili su uzeti od nasumce odabranih goveda, a odvojeni uzorci seruma bili su pretraženi imunoenzimnim testom na prisutnost protutijela specifičnih za velikog metilja. Pozitivni uzorci bili su svrstani u skupine: slabo, umjereno i jako pozitivni. Od 405 pretraženih uzoraka seruma, 38 (9,4%) je bilo pozitivnih na protutijela specifična za metilj *F. hepatica*. Od toga je 2,5% uzoraka bilo umjereno ili jako pozitivno, a 4,4% slabo pozitivno. Značajno veća seroprevalencija ($P < 0,05$) ustanovljena je u mladim životinjama (u dobi manjoj od dvije godine, 15,3%) u usporedbi s odraslima (≥ 2 godine, 5,4%), dok nije ustanovljena značajna razlika u seropozitivnosti između mužjaka i ženki. Ovo je prvo izvješće o seroprevalenciji velikog metilja u stadima goveda u Koreji. Nalazi mogu biti od koristi za buduća istraživanja o značenju velikog metilja *F. hepatica* u Koreji.

Ključne riječi: *Fasciola hepatica*, seroprevalencija, imunoenzimni test, govedo, Koreja
