Serodiagnosis of fasciolosis in ruminants

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Summary: Various serological techniques including immunoprecipitation, indirect haemagglutination and the indirect fluorescence antibody test have been successfully applied for the detection of fasciolosis, mainly in experimental infections. Recently, the most frequently used assay is the ELISA technique with somatic or excretory-secretory antigens obtained from adult flukes. Further purification of such antigens has considerably increased both the sensitivity and specificity of the system. However, most data indicate that serodiagnosis is reliable in groups of naturally-infected cattle and sheep but cannot yet be applied for the detection of individually infected animals.

KEYWORDS: Cattle - ELISA - Fasciola gigantica - Fasciola hepatica - Serodiagnosis - Sheep.

INTRODUCTION

Fasciolosis occurs world-wide as a result of infection with the helminth parasites Fasciola spp. and causes substantial economic losses in animal production (5, 14, 19, 28). The animal species most often affected are cattle and sheep; goats are less frequently infected. Depending on their feeding habits, infections in other mammals and in man also occur and may play a role in fluke endemic areas (2, 3, 4).

Clinically, fasciolosis is often seen as a chronic wasting disease. In cattle, subacute or acute outbreaks occasionally occur, whereas in sheep, acute and subacute diseases are more frequent (4). For various reasons, including the long prepatent period of Fasciola spp. infections, a faecal examination is often unsatisfactory and allows detection only after a very long time. As a consequence, serodiagnostic methods have been introduced and are now widely used for the detection of fasciolosis in man and experimental animals (1, 23, 35, 43). However, such results have mostly been achieved under well-defined conditions, and large-scale application of serological techniques for the detection of naturally-acquired fasciolosis has often been unsatisfactory.

The aim of this review is to present and to discuss various currently available serodiagnostic methods with regard to their application for the detection of natural infections. Since only very limited work has been published on the serodiagnosis of F. gigantica infections or fasciolosis in other animals, this paper deals primarily with the detection of F. hepatica infections in cattle and sheep. Also, technical details will not be discussed.

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Immunological methods such as immunodiffusion were already being used for the detection of fasciolosis in the 1950's, but the results were not altogether convincing with regard to a field application (37, 39). Amongst the many techniques performed thereafter, a promising result for a broader application was first achieved by the introduction of an indirect haemagglutination test (IHA) using antigens extracted from adult worms (40) and of an indirect fluorescence antibody test (IFAT) with 25-day-old juvenile flukes as antigen (20). This technique allowed the detection of an infection as early as three weeks p.i.; the specificity was considered to be satisfactory at the time but the results were variable. Despite a high sensitivity (87%) and the earliest possible detection p.i. (22), the IFAT never became a routinely used assay for detecting *F. hepatica* infections, in particular because it is time-consuming, expensive and, in addition, difficult to read.

After the introduction of the ELISA technique for the diagnosis of parasitic diseases (42), a micro-ELISA was successfully applied for the detection of experimental fasciolosis in cattle. In addition to high sensitivity when using a crude *F. hepatica* extract antigen, Burden and Hammet (9) did not discover any relevant cross-reactions with the gastro-intestinal nematode *O. ostertagi* and Farrell et al. (13) demonstrated a high degree of repeatability. For these reasons, and also because of its simplicity and easy automation, the ELISA became the most frequently used serological assay for the detection of fasciolosis, particularly in experimental infections (8, 15, 16, 21, 29, 30, 31, 34, 44, 45). However, the use of whole worm extracts — mostly originating from adult *F. hepatica* — did not entirely satisfy the requirements for specificity and sensitivity when used to diagnose natural infections. The question thus arose as to whether a further purification of somatic antigens and/or the use of excretory-secretory (ES) products of *F. hepatica* in an ELISA would improve the serodiagnosis. When fractionating crude adult *F. hepatica* antigen by column chromatography, Oldham (26) found in a Sephadex G-200 elution the Fl fraction to be the most antigenic and used it for the development of a standardised and reproducible assay which allowed him to detect *F. hepatica* antibodies in experimentally-infected calves by three weeks p.i. The use of a Sephadex G-200 F3 antigen fraction in a large-scale application allowed for the differentiation between naturally-acquired *F. hepatica* infections and negative controls or naturally-acquired *Dicrocoelium dendriticum* infections (27). However, the enormous variation of the ELISA values in infected and uninfected cattle and the high background levels in the latter made a routine application of this antigen preparation of limited value. After fractionating antigens on Sephadex G-100, Rhee et al. (31) discovered and subsequently tested a 26,000 MW fraction which provided 100% specificity and sensitivity and which gave 8.4 fold higher ELISA values in infected cattle than in negative controls. A field evaluation has not been performed, nor have cross-reactions been tested.

Another improvement of the serodiagnostic possibilities is offered by the adaptation of a kinetics-ELISA technique to a microplate system which allows not only an earlier detection of *F. hepatica* antibodies, but which can also detect quantitative differences in relation to different intensities of infection (46). Again, the assay has been developed for experimental fasciolosis and no results are presented for natural infections. Since cross-reactions in this assay may in part be due to residue host antibodies coating the surfaces of adult flukes, Pfister et al. (29) eluted these
host antibodies with NaSCN. Thereafter, the flukes were homogenised, particulate antigens were sedimented by centrifugation, and the supernatant soluble antigens used directly or fractionated by Sephacryl S-200; the resultant major protein fraction was taken as antigen for an ELISA. Using these antigens, the sensitivity in cattle of varying age naturally infected with *F. hepatica* was about 85%, with a considerably lower variation of the ELISA values for the chromatographed antigen. Due to the simple means of preparation, these antigens are easy to produce and a recent large-scale application in cattle from an abattoir survey (Fig. 1) has confirmed its high degree of specificity and sensitivity (10). However, there is still considerable between-animal variation.

![Graph showing ELISA values](image)

**FIG. 1**

**ELISA values (mean ± SD) of tested bovine sera per group**

Figures in brackets indicate number of cattle (10)

In order to improve the detection rate further, some workers have used ES products (11, 27, 34, 36) or tegumental antigens (16, 38) from adult *F. hepatica*. Although immunoprecipitation methods including counterimmunoelectrophoresis with ES...
antigens revealed sensitivities of up to 90% or more in cattle (11), the ELISA technique appears to be used more frequently and ES products allowed the detection of an experimental bovine infection two weeks p.i. (34). Furthermore, they were found to be superior to a soluble adult fluke extract for detecting experimental infections and also in a survey in areas with high and low incidence of fasciolosis (36). Nevertheless, ES antigens have not been used for a large-scale application under field conditions, possibly because of the difficulty of preparing sufficient material.

The various assays mentioned above have already provided a considerable degree of specificity and sensitivity. However, it must be concluded that for natural infections these methods are suitable for herd diagnosis, but are of limited value for diagnosis of fasciolosis in individual animals (29, 44, 45). Moreover, Welch et al. (44) stated that for herd diagnosis a minimum of 5-15 samples/ herd — according to the size — should be tested in order to obtain a sufficient degree of certainty. These authors also showed that the test was more valuable when used in herds of young cattle with primary infections than when used in adult cattle previously infected with F. hepatica.

By the application of an ELISA technique, using somatic antigen, for diagnosis in pooled milk samples and individual serum samples, Boulard et al. (8) demonstrated a satisfactory correlation between the frequency of fasciolosis in slaughtered cattle and the corresponding serodiagnostic test. Consequently, these authors suggested a herd diagnosis in dairy cattle could be based on the examination of pooled milk samples.

**SERODIAGNOSIS OF FASCIOLOSIS IN SHEEP**

Fasciolosis in sheep generally occurs as an acute, subacute or chronic infection and causes considerable losses, particularly in endemic areas (4, 41). Early diagnosis, i.e. during the migratory phase of the parasite, has therefore long been recommended. A promising serodagnosis was developed in 1976 by van Tiggele and Over (40) who found that the introduction of an IHA and counterimmunoelctrophoresis preceded the presence of parasite eggs in the faeces by at least 50 days.

Since then, Zimmerman et al. (47) have developed an ELISA which allows a diagnostic detection of experimental ovine fasciolosis by 6-8 weeks p.i., and Santiago and Hillyer (34) were able to detect ELISA antibodies to adult ES and somatic antigens by four weeks p.i. The superiority and practicability of this ELISA method were successfully demonstrated in a sero-epidemiological study of a sheep population in Ethiopia (25).

Zimmerman et al. (48) and Arriaga de Morilla et al. (6) have introduced a DOT-ELISA against ES antigens as a rapid microdiagnostic test which allows the detection of F. hepatica infections by four weeks and which is highly sensitive. Most recently, in a comparative study using ES antigens, Bautista-Garcias et al. (7) demonstrated a 100% sensitivity and specificity in natural and experimental fasciolosis with a diffusion in gel (DIG)-ELISA whereas the sensitivity obtained by simultaneously using an IHA was only 68%.
Although serological tests for human *F. gigantica* infections are available (24), there is little information on the serological detection of the infection in animals. Recently, Fagbemi and Obarisiagbon (12) have successfully developed an ELISA method for cattle using a crude adult *F. gigantica* extract as antigen. The ELISA values were significantly different between infected animals and negative controls which were subsequently compared by a post-mortem liver examination and bile and faecal sedimentation.

**PERSPECTIVES**

Despite the numerous above-mentioned assays, the serodiagnosis of naturally-acquired fasciolosis in ruminants — in contrast to experimental infections — is not yet entirely satisfactory and often rather limited. Recent research efforts have concentrated on the isolation of *F. hepatica* antigens by elution from polyacrylamide gels (32, 33, 34), and on the isolation and translation of messenger RNA from adult *F. hepatica* (17). In 1989, Hillyer also described the expression of *F. hepatica* antigen after fusion of parasite cells with cells from a murine myeloma line (18).

Future investigations will show whether continued development of *F. hepatica* antigens by molecular biology techniques can lead to an improved, widely applicable and economical assay for the serodiagnosis of naturally acquired fasciolosis.

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**DIAGNOSTIC SÉROLOGIQUE DE LA FASCIOLOSE CHEZ LES RUMINANTS.** — K. Pfister.

*Résumé*: Diverses méthodes sérologiques, notamment les épreuves d’immunoprécipitation, d’hémagglutination indirecte et la méthode de détection des anticorps par fluorescence indirecte ont été utilisées avec succès pour décéler la fasciolose, principalement dans le cas d’infections expérimentales. Actuellement, la méthode la plus fréquemment utilisée est la technique ELISA réalisée avec des antigènes somatiques ou des produits d’excrétion ou de sécrétion provenant de douves adultes. En purifiant ces antigènes, on a augmenté notablement la sensibilité et la spécificité de ces épreuves. Cependant, les données recueillies montrent pour la plupart qu’une méthode de diagnostic sérologique appliquée aux bovins et aux ovins infectés naturellement, n’est fiable que lorsqu’elle est pratiquée à l’échelle d’un troupeau et que, par conséquent, elle ne peut être encore utilisée pour détecter la fasciolose chez un sujet isolé.

**MOTS-CLÉS** : Bovins - ELISA - Fasciola gigantica - Fasciola hepatica - Ovins - Sérodiagnostic.

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DIAGNÓSTICO SEROLÓGICO DE LA FASCIOLIASIS EN LOS RUMIANTES. - K. Pfister.

Resumen: Diversas pruebas serológicas, en particular las de inmunoprecipitación, de hemaglutinación indirecta y la prueba de detección de los anticuerpos por fluorescencia indirecta, han demostrado ser eficaces para determinar la presencia de fascioliasis, principalmente en el caso de infecciones experimentales. El método más usado en la actualidad es la prueba ELISA realizada con antígenos somáticos o productos de excreción o de secreción provenientes de trematodos adultos. Al purificar estos antígenos, se aumentó notablemente la sensibilidad y la especificidad de las pruebas. Sin embargo, la mayoría de los resultados obtenidos hasta el momento indican que un método de diagnóstico serológico que se aplique a bovinos y ovinos infectados naturalmente sólo es fiable si se practica a nivel del rebaño y que, en consecuencia, no puede usarse todavía para detectar la enfermedad en animales aislados.

PALABRAS CLAVE: Bovinos - Diagnóstico serológico - ELISA - Fasciola gigantica - Fasciola hepatica - Ovinos.

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REFERENCES


