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Review

Comparative analysis of different immunological techniques for diagnosing fasciolosis in sheep: A review

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Fasciolosis is a worldwide zoonotic infection caused by liver flukes of the genus Fasciola, of which *Fasciola hepatica* and a larger species, *Fasciola* gigantica are the most common representatives. These two food-borne trematodes usually infect domestic ruminants and cause important economic losses to sheep, goats and cattle. In commercial herds, fasciolosis is of great economic significance worldwide with losses estimated to exceed 2000 million dollars yearly, affecting more than 600 million animals, in articles reported a decade ago. In addition, *F. hepatica* causes an estimated loss of $3 billion worldwide per annum through livestock mortality, especially in sheep, and by decreased productivity via reduction of milk and meat yields in cattle. The parasitological diagnosis of fasciolosis is often unreliable because the parasite's eggs are not found during the prepatent period. Even when the worms have matured, the diagnosis may still be difficult since eggs are only intermittently released. Repeated examinations of stools are usually required to increase the accuracy of the diagnosis. Early diagnosis of fasciolosis is necessary for institution of prompt treatment before irreparable damage of the liver occurs. For these reasons, serology is the most dependable method for diagnosing fasciolosis. Attempts have been made to diagnose fasciolosis by detecting antibodies in the serum of sheep suspected of being infected with the flukes. Advances in immunodiagnosis have focused on detection of *Fasciola* antigens in host body fluid; these tests have an advantage over antibody detection because antigenemia implies recent and active infection. Similarly, somatic and excretory secretory (E/S) antigens of *Fasciola* sp. or their partially purified component are the commonest source of antigens used in protection trials and serodiagnosis. Thus, the aim of the present review is to encourage more young researchers to initiate work on this aspect of these economically cosmopolitan parasites.

Key words: Fasciolosis, Antigenemia, E/S antigens, serodiagnosis, immunoassay, *Fasciola* spp., zoonotic disease.

INTRODUCTION

Livestock infection by the liver flukes like *Fasciola hepatica* and *Fasciola gigantica* causes major economic losses worldwide. Mostly, the infection by members of the genus *Fasciola*, commonly known as liver flukes, may be

responsible for morbidity and mortality in most mammal species, but have particular importance in sheep and cattle to livestock producers. Infection with *F. hepatica* and *F. gigantica* is regarded as one of the most common single helminth infection of ruminants in Asia and Africa (Hammond and Sewell, 1990). *Fasciola* spp. parasitizes a wide spectrum of domestic and wild animals (e.g., sheep, cattle, buffaloes and deer) and it causes a huge economic loss of $3 billion annually to the agriculture sector worldwide through losses of milk and meat yields (Mas-Coma et al., 2005; Robinson and Dalton, 2009). The immature flukes after penetrating the liver capsule migrate into the liver hepatic tissue. This migration usually cause trauma with hemorrhages, necrosis and subsequent granulation end by liver cirrhosis (Ozer et al., 2003). It has been reported that sheep and cattle do not develop strong immunity to infection by *Fasciola* species, or to re-infections, and this lack of resistance in ruminants is believed to be associated with the inability of their macrophages to produce nitric oxide. The parasitological diagnosis of fasciolosis is often unreliable because the parasite eggs are not found during the prepatent period (Noureldin et al., 2004). Even when the worms have matured, the diagnosis may still be difficult since eggs are only intermittently released. Repeated examinations of stools are usually required to increase the accuracy of the diagnosis. Early diagnosis of liver fluke infection is necessary for institution of prompt treatment before irreparable damage of the liver occurs (Rokni et al., 2004). For these reasons, serology is the most dependable diagnostic method. Attempts have been made to diagnose fasciolosis by detecting antibodies in the serum patient suspected of being infected with the flukes (Maleewong et al., 1999). Advances in immuno-diagnosis have focused on detection of parasite antigens in host body fluid; these tests have an advantage over antibody detection because antigenemia implies recent and active infection (Cornelissen et al., 1999). The somatic and E/S antigens of *Fasciola* spp. or their partially purified component are the commonest source of antigens used in protection trials and serodiagnosis (G'nen et al., 2004). Immunodiagnosis of parasitic disease is mainly based on antibody detection (Fagbemi et al, 1999) and revealed both recent and current infections with early diagnosis. To obtain reliable diagnostic method or to identify crude antigens, many authors prepared antigens from whole worm (Hillyer et al., 1987) or from tegument (Charmy et al., 1997) also coproantigen (Allan et al., 1996), egg antigen (Khalil et al., 1989 and Abdel-Rahman and Abdel Mageed, 2000), and excretory secretory products (Espino et al., 1994). Currently, haemaglutination (HA), indirect fluorescence antibody test (IFAT), immunoperoxydase (IP), counter-electrophoresis (CEP) and enzyme linked immunosorbent assay (ELISA) are used in the early diagnosis of this disease, but they have some disadvantages such as cross reactions with other trematodes leading to false positive results. Therefore, the reliability of these tests is not high. In recent years, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting procedures have initiated a new era in immunodiagnosis which greatly reduced cross-reactions. Enzyme-linked immunetrotransfer blot analysis (EITB) or Western blotting is evaluated in some research centers and encompasses remarkable sensitivity and specificity in diagnoses of the fasciolosis. Evaluation of sandwich ELISA and Dot ELISA as an immunological assay is used for detecting *Fasciola* copro-antigen and serum antigens in infected sheep, thus presenting an experimental trial that could be of value in providing a tool that may help in immunodiagnosis of fasciolosis.

OBJECTIVES OF IMMUNODIAGNOSIS

The objective of research in immunodiagnosis of fasciolosis is to develop rapid, cheap and technically easy tests that can be used in epidemiological surveys to evaluate the effects of various national or international schemes of control in areas where these infections are endemic. It should provide tests that have a high degree of sensitivity and that are specific for each infection, thus enabling their employment in immunodiagnosis even when few parasites are available for direct parasitological examinations. This is an important consideration in epidemiological surveys since it is recognized that in endemic areas, only a portion of the people carrying an infection may present clinical symptoms. Research in immune-assays also needs to provide tools that assess the effectiveness of chemotherapy or other curative measures, and thereby permit monitoring of treatment. Finally, it should provide tests that identify those individuals or animals that develop immunity to the infection. Such tests will be valuable in assessing the efficacy of vaccine programmes that can be expected in the future when anti-parasite vaccination becomes available.

NEW DEVELOPMENTS ENCOURAGED

Progress in the development of RIA, ELISA and related procedures has not yet, however, been so extensive as to replace conventional techniques. The main pitfalls have been the lack of commercial pressure to develop test kits, and the lack of good reagents. The specificity and sensitivity of the immunoassay depends on the

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Table 1. Immunodiagnosis: Antigen-antibody detection.

S/N	Antigen catalogue	Techniques	Agents	Sensitivity (%)	Specificity (%)	References
1.	Somatic antigen	ELISA	Sheep serum		95	Cornelissen et al. (1992)
1a.	Somatic antigen	IHA	Sheep serum		86	Cornelissen et al. (1992)
2.	Crude excretory-secretory products (ES)	ELISA	Sheep serum		95	Cornelissen et al. (1992)
2a.	Crude excretory-secretory products (ES)	IHA	Sheep serum		86	Cornelissen et al. (1992)
3	28 kDa antigen (purified from ES)	ELISA		100		Dixit et al. (2002)
3a.	28 kDa antigen (purified from ES)	Western blot		100		Dixit et al. (2002)
3b.	28 kDa antigen (purified from ES)	Dipstick-ELISA		100		Dixit et al. (2002)
4.	mAb MM3	Copro ELISA	Sheep stool sample	0.3 ng/ml of F. hepatica ES antigen (100% with 1 fluke)	100	Mezo et al. (2004, 2007)

technology and on the reagents used. Limitations are now set by the lack of well-defined reagents and the false positive and false negative reactions that are seen as a consequence of this. The importance of the need for improved immunodiagnostic tests to use in individual or epidemiological studies is widely recognized and reflected in the priorities established by the Special Programme on Research and Training in Tropical Diseases of the UNDP, World Bank, and WHO, as well as by the IAEA's subprogramme component on parasitic diseases. Recent advances in separation techniques, and the production of antigens and antibodies using genetic engineering and biotechnology, augur well with development of improved serodiagnostic immunoassays using radionuclide and other tracers. This development is being encouraged by both WHO and IAEA.

TECHNIQUES USED FOR SERODIAGNOSIS OF FASCIOLOSIS IN SHEEP

Immunodiagnosis: Antibody detection

Infection with *Fasciola* spp. results in a specific antibody response. These antibodies can be detected in either serum or milk (Charlier et al., 2007). Several techniques have been described for the detection of antibodies against *Fasciola* spp. infection in sheep, such as the indirect hemagglutination test (IHA) (Levieux et al., 1992), indirect immunofluorescence assay (IFA) (Hanna and Jura, 1977), ELISA and the Western immunoblot (Hillyer and Soler de Galanes, 1988), Dot- ELISA (Shaheen et al., 1989) and Micro-ELISA (Carnevale et al., 2001).

Antibodies to *Fasciola* spp. in infected hosts can be detected by ELISA (Ab ELISA) as early as one to two weeks post-infection (Hillyer et al., 1992), while eggs of flukes are found in faeces only after 12 - 14 weeks of infection (Burger, 1992). The Ab ELISAs have sensitivities and specificities of 87-100 and 86-100%, respectively. However, cross-reactions were seen with serum samples obtained from patients with hydatidosis and toxocariasis (Rokni et al., 2004) when using crude excretory secretory products (ES) of adult worms as the antigen. To improve the sensitivity and specificity of Ab ELISA, antigens purified from crude ES of flukes, recombinant antigens, or synthetic protein antigens should be used (Cornelissen et al., 2001; Silva et al., 2004; Yokanath et al., 2005) (Table 1).

Immunodiagnosis: Antigen detection

Active infection by *Fasciola* spp. can be demonstrated by the detection of metabolic products of flukes in the circulation. Such a test can also be used to confirm the efficacy of chemotherapy. Several assays have been developed to detect *Fasciola* spp. antigen in serum and faeces using monoclonal antibodies (Espino et al., 1994; Fagbemi et al., 1997; Mezo et al., 2004).

The antigen in blood can be detected by ELISA from one week post-infection onwards. However, Ag-ELISA has not been further developed because antigenaemia only develops when immature flukes are actively migrating through the liver parenchyma during 1-3 weeks post-infection and circulating antigens cannot be detected anymore once the flukes are established and mature to adult worms (Langley and Hillyer, 1989).

Copro Ag-ELISA has been applied to detect ES productions of *Fasciola* in stool samples by using monoclonal antibodies. The antigen can be detected as early as 3-4 weeks post-infection when the flukes reside in the host liver (Fagbenmi et al., 1997). In addition, a strong correlation between copro-antigen levels and the numbers of flukes was seen (Abdel-Rahman et al., 1998). Circulating antigens were detected in 100% of sheep with 1 fluke and in 100% of cattle with 2 flukes, from five weeks post-infection (wpi) onwards (Mezo et al., 2004). The copro-antigen became undetectable from 1 - 3 weeks after treatment with a flukicide in sheep and cattle (Mezo et al., 2004) and from 2 months post-treatment in 78.6% of patients (Espino et al., 1994). The copro Ag-ELISA was demonstrated to have a sensitivity and specificity close to 100% (Table 1).

CONCLUSIONS

Although, coprological techniques based on the demonstration of eggs in faeces of the definitive host can be seen as a “gold-standard”, these technique are not always adequate, especially for diagnosis of human fasciolosis, because during the long prepatent period immature flukes do not lay eggs, and in the case of ectopic migration of flukes, “false” positive results were seen in some cases of humans following eating from bovine livers harboring fluke eggs (Hillyer, 1999). Immunological techniques provide the advantage of being applicable during all stages of the liver fluke lifecycle. These are reliable detection approaches, especially during the invasive or acute phases.

In spite of that, parasitological and immunological techniques are useful tools in epidemiological studies to estimate the prevalence and to map the presence of human and animal fasciolosis (Hillyer, 1999). Several serological techniques have also proved to be excellent methods for monitoring post-treatment evolution (Mas-Coma et al., 2005). The need to find and establish a new sensitive and specific method and to decrease as much as possible the cases of cross-reactions made us to evaluate the EITB test in this regard.

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 and on the isolation and translation of messenger RNA from adult *F. hepatica*.

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.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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