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Review article

Immunodiagnostic tools for taeniasis

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Abstract

Most diagnostic work conducted on the *Taenia* species zoonoses has been carried out on the larval stage of *Taenia solium* in man, reflecting the relative severity of the pathology caused by this stage of that organism. This review will, however, concentrate on the immunodiagnosis of the adult intestinal stages of these parasites in humans. Diagnosis of *T. solium* will be examined in most detail because of the relative importance of this parasite but relevant work from other cestodes of man and animals will also be discussed. In addition both classical and molecular approaches to diagnosis will be briefly covered. There have been a number of advances in immunodiagnosis of taeniasis over recent years that have improved both diagnostic sensitivity and specificity. Techniques for the detection of *Taenia* specific coproantigens in human taeniasis infections have been shown to more than double the numbers of *T. solium* cases accurately diagnosed in epidemiological studies. More recently, work on the serological diagnosis of *T. solium* have led to the development of a sensitive and specific enzyme linked immuno-transfer blot for the detection of species and stage specific circulating antibodies to adult worm excretory–secretory antigens. Work is ongoing to further improve these assays.

Keywords: Taeniasis; Immunodiagnosis; Coproantigens; Antibodies

1. Introduction

The detection of the human carriers of adult *Taenia solium* and *T. saginata* was identified as one of the keys to the implementation of viable control programmes for these diseases (WHO, 1983). The techniques classically employed are dependent on the detection of parasite material (proglottides or eggs) in the faeces. These techniques are known to have both low sensitivity and specificity. No single technique will always detect infection (Pawlowski and Schultz, 1972). The intermittent nature of egg excretion leads to underestimation of the prevalence of taeniasis (Hall et al., 1981; Allan et al., 1996a). If destrobilisation has led to a massive discharge of eggs these may be absent from the faeces for up to several weeks thereafter (WHO, 1983). Furthermore the eggs of *T. saginata* and *T. solium* appear identical under the light microscope leading to problems with diagnostic specificity, which is particularly

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important given the risks associated with *T. solium* infection.

Identification of human intestinal taeniids to species level classically relies on the recovery of mature proglottides or scoleces. With the use of modern cestocidal drugs the identification of the latter is difficult as this causes disintegration of the proximal end of the worm and hence loss of the scolex (WHO, 1983). More recent approaches include identification of cestode material by enzyme electrophoresis (Le Riche and Sewell, 1978) or biochemical analysis of the total protein by electrophoresis (Bursey et al., 1980). These techniques have not been put into routine use. More recently still DNA based technologies have been shown to be capable of differentiating these parasites with a high degree of certainty and based on the recovery of relatively small amounts of parasite material (Harrison et al., 1990; Chapman et al., 1995; Mayta et al., 2000; Gonzalez et al., 2002).

This review will concentrate on the immunodiagnosis of these infections. Recent advances in this area offer the potential to considerably improve the diagnosis of taeniasis, addressing both issues of sensitivity and specificity.

2. Early approaches to immunodiagnosis in humans and work in canine taeniasis

Intradermal tests for the diagnosis of human taeniasis, using a variety of antigens, were shown to elicit high levels of false positive and false negative results (Ramsdell, 1927; Brunner, 1928; Podyapolskaya and Kamalova, 1942; Machinicka Roguska and Zweirz, 1971). These tests also continued to give positive results for long periods after successful treatment of the infection (Machinicka Roguska and Zweirz, 1971). Slusarski and Zapart (1971), however, reported a relatively low rate of false positives (3–7%) and a sensitivity of over 70%. Despite this the technique has never been applied on a large scale.

Raised levels of parasite specific antibodies in human taeniasis have been demonstrated (Machinicka Roguska and Zweirz, 1964, Machinicka Roguska and Zweirz, 1966). Increases in non-specific serum IgA and IgE have also been described (Nepote et al., 1974).

Application of the indirect haemagglutination test to the diagnosis of *Taenia saginata* infections showed low sensitivity and once again continued to give positive results after removal of the infection (Machinicka Roguska and Zweirz, 1971) with some patients remaining positive for up to 18 months following treatment. Furthermore some previously negative patients became positive after treatment. Later studies indicated 44% false negative and 1.35% false positive rates and indicated limited diagnostic applicability for the approach (Flentje and Padelt, 1981).

Work on the immunodiagnosis of canine taeniids indicated that the detection of parasite specific antibodies, on a basis reliable enough for diagnostic purposes, was possible. Antibodies to scolex excretory secretory antigens from *T. hydatigena*, *T. pisiformis*, and *E. granulosus* were demonstrated in the serum of infected dogs prior to patency (Jenkins and Rickard, 1985; Heath et al., 1985; Jenkins and Rickard, 1986). Although no cross-reactions were shown with sera from uninfected animals, cross-reactions were demonstrated between the sera from different taeniid infections. Application of these antigens in field studies on canine echinococcosis demonstrated problems with specificity (Gasser et al., 1988). Antibodies to oncospheres, however, proved to have a higher degree of specificity but were present from only about 1 week after patency whilst titres dropped rapidly following treatment (Gasser et al., 1988). A recombinant *E. granulosus* oncosphere antigen appears 100% specific for *E. granulosus* in dogs but with low sensitivity (Gasser et al., 1990). The presence of parasite specific antibody in the saliva of dogs infected with *T. pisiformis* has also been demonstrated (Kinder et al., 1992).

3. Immunodiagnostic differentiation of taeniid eggs

The specific identification of hatched oncospheres of the canine taeniids *E. granulosus*, *T. hydatigena*, and *T. pisiformis* was demonstrated using either monoclonal or affinity purified polyclonal antibodies against the oncosphere in an

immunofluorescent assay (Craig, 1983; Craig et al., 1986, 1988). This technique has been successfully applied to the identification of *T. solium* oncospheres using a species-specific monoclonal antibody (Montenegro et al., 1996). These techniques rely on the ability to recover oncospheres, which leaves the existing issue of test sensitivity unresolved.

4. Coproantigen detection for *Taenia solium*

The detection of parasite specific antigens in host faeces, a technique that is now widely used in microbiology and virology, was first reported for canine *E. granulosus* by Babos and Nemeth (1962). They demonstrated the presence of *Echinococcus* antigen in the faeces of dogs prior to patency using an immunoprecipitation test with hyperimmune rabbit serum raised against larval worm antigens. Cross-reaction with *Taenia* antigens in human patient faeces did, however, occur. These authors suggested that this approach would allow for the development of improved control measures for both canine echinococcosis and human taeniasis. Unfortunately this report, in Hungarian, seems to have been almost entirely overlooked for many years. Twenty years later the World Health Organization in its guidelines on the diagnosis of echinococcosis (WHO, 1984) suggested that if it were possible to detect *Echinococcus* antigen in dog faeces this might constitute an improvement on current diagnostic techniques. A similar recommendation was made for the diagnosis of human taeniasis (Flisser, 1985).

Parasite coproantigens constitute parasite specific products in the faeces of the host that are amenable to immunological detection. If these products are associated with parasite metabolism they should be present independently of parasite reproductive material (i.e. taeniid eggs or proglottides) and should disappear from faeces shortly after removal of the intestinal infection.

Further studies in a variety of cestodes have demonstrated the utility of this approach. The first of these, by Machnicka and Krawczuk (1988) detected *Hymenolepis diminuta* specific antigen in rat faeces using a hyperimmune rabbit serum raised against worm surface antigen using both double diffusion and two site radio-immunoassay. Antigen was detected prior to patency (thus independently of egg excretion) in all rats. The test was genus specific with cross-reactions occurring with faeces from *Hymenolepis nana* infection. Broadly similar results were seen in an antigen capture ELISA based on rabbit antibodies to *H. diminuta*, with detection of antigen prior to patency and demonstrating that eggs are not the primary source of antigens detected in infected rat faeces (Allan and Craig, 1989, 1994).

Subsequent coproantigen based immunodiagnostic studies for *Taenia* in dogs or humans have all employed antigen capture ELISA assays using sera from rabbits hyperimmunised with either adult worm somatic or excretory–secretory products (Allan et al., 1990, 1992, 1993, 1996b; Deplazes et al., 1990, 1991; Maass et al., 1991; Machnicka et al., 1996a,b). They have been used to detect antigen in detergent sollubilised faecal samples. Consistent across these studies have been the following features:

- Antigen detection is genus specific with *T. saginata* and *T. solium* both reacting in the assay but with no cross-reactions with faeces from other parasite infections including *Hymenolepis* cestodes
- Coproantigen detection is possible prior to patency (several weeks prior in animal models) and the levels are independent of the presence or number of eggs
- Coproantigens are no longer detectable within a week of treatment
- Coproantigens are stable for days in unfixed faecal samples held at room temperature and very long periods (months to years) either in frozen sample or in samples fixed in formalin and kept at room temperature.

The levels of sensitivity of these assays are dependent on the assay format (both microplate and dipstick formats have been used to date) and the quality of the rabbit sera used in their production (high titre sera being better). In terms of their field application, the assays have been used most extensively in the diagnosis of *T. solium* and

it is these results that will be reviewed here. It is important to note that, given its greater fecundity and the active expulsion of proglottides, *T. saginata* is probably easier to diagnose by classical means than *T. solium* and this should be borne in mind when the comparative performance of coproantigen tests and classical diagnosis are discussed here.

The most extensively tested coproantigen formats have been based on hyperimmune rabbit sera raised against *T. solium* adult worm somatic antigens (Allan et al., 1990). Briefly, worms were recovered from hamsters immunosuppressed with methylprednisolone acetate and infected orally with *T. solium* cysts (Depomedrol, Upjohn) (Allan et al., 1991). Rabbits were immunised with a saline extract prepared by homogenisation and centrifugation of the tapeworm material. This antigen was administered with Freund's complete and incomplete adjuvants, respectively. The IgG fraction from the sera was purified and approximately half of this conjugated to horseradish peroxidase. The unconjugated IgG was used to coat microtitre plates (the most consistent results being obtained with Dynatech Immulon 4 plates). This layer of antibody is used to capture any antigen present in a detergent sollubilised faecal extract (best results being obtained if foetal calf serum is added to the extract in the microtitre wells to a concentration of approximately 50% to protect the capture antibody layer from factors in the faecal extract that sollubilise and/or destroy it). After washing the plate, the enzyme-conjugated antibody is added and after an incubation and further washing a chromogenic substrate added. The extent of the colour reaction indicates the degree of antigen binding.

The use of this test in relatively large field studies has been demonstrated to increase the detection of parasitologically proven intestinal *T. solium* cases by a factor of at least 2.6 times in comparison to microscopy (Allan et al., 1996b). No cross-reactions have been seen with any other parasite species including *Ascaris, Trichuris, H. nana, H. diminuta,* hookworm or parasitic protozoa. The test is not, however, 100% sensitive; 98% of all cases diagnosed in one field study were diagnosed by the test (55/56) in comparison to only 38% diagnosis by microscopy (21/56) (Allan et al., 1996b). True coproantigen test sensitivity is likely to be greater than 90% in the microplate format. However, the test does produce some false positives; test specificity of at least 99.2% was seen in one large study (Allan et al., 1996b).

Coproantigen testing for *T. solium* has also been carried out using a dipstick ELISA format (Allan et al., 1993). This format allowed testing of samples in rural villages directly after collection and it proved faster but less sensitive than the microplate assay. Two probable reasons for this was that, in order for visual interpretation to be unambiguous (i.e. any colour reaction to indicate a positive result) some sensitivity was sacrificed for specificity. The lack of foetal calf serum meant that the detergent sollubilised faecal material of some samples, containing high levels of factors that interfere with the test, yielded results that were uninterpretable. Nonetheless, testing by this format detected 31 out of a total of 41 (76%) clinically diagnosed cases in studies in Mexico and Guatemala compared to only 23 (56%) cases diagnosed by microscopy. Test specificity was 99.9%.

The utility of coproantigen testing by either dipstick or microplate formats is probably best illustrated in data from four Guatemalan communities where epidemiological studies were conducted on *T. solium.* The dipstick format was employed in two of the villages and the microplate test used in the other two villages. Use of the tests considerably increased the numbers of *T. solium* cases diagnosed in comparison to microscopy alone (Table 1).

Further work has been carried out to characterise taeniid coproantigens with a view to improving test performance (Maass et al., 1992; Kohno et al., 1995; Machnicka et al., 1996b; Fraser et al., 2002). Determination of the nature of these antigens may allow the development of a species-specific test capable of differentiating *T. solium* from *T. saginata.* Analysis has been carried out by Western blot, FLPC and a variety of other biochemical techniques to determine the nature of the taeniid antigens present in the host faeces. Recent work indicated that these are predominately of large molecular weight ($\approx$150–600 kDa) with carbohy-

Table 1
Effects of inclusion of coproantigen tests in a field epidemiological programme on *T. solium* in Guatemala: diagnosis of taeniasis by microscopy and either a dipstick or microtitre plate type ELISA for *Taenia* coproantigens

Test type	Number of samples	Coproantigen positive	Microscopy positive	Total number of cases diagnosed[a]	Cases diagnosed by coproantigen only[b]
Microtitre plate	1582	55	21	56	35 (63%)
Dipstick	1710	25	18	33	15 (45%)

[a] Of the worms recovered 54 cases were *T. solium*, one case was *T. saginata* and in 34 cases it was not possible to determine the species present. These results were reported as part of a study on the epidemiology of taeniasis in Guatemala (Allan et al., 1996b). In the dipstick study a small number of additional samples (107) were tested by microscopy only (Garcia Noval et al., 1996).
[b] Samples negative for eggs by microscopy but positive for coproantigen.

drate moieties apparently key to their antigenicity (Fraser et al., 2002).

5. Serological diagnosis of intestinal *Taenia solium* infection

Recently the possibility of diagnosing *T. solium* taeniasis by the detection of species-specific circulating antibodies has been demonstrated by EITB (Wilkins et al., 1999). Using excretory secretory derived antigens from non-gravid *T. solium* tapeworms recovered from immunosuppressed hamsters and cultured in serum free medium containing 1% glucose, molecules ranging from 32.7 to 42.1 kDa appeared to be specific for *T. solium* taeniasis infections when screened with a pool of sera from taeniasis cases and separate pool of sera from *T. solium* cysticercosis cases.

Further analysis indicated that these antigens were detected by sera from 95% (69/73) of individuals infected with *T. solium* taeniasis from Guatemala, Peru and Indonesia whilst sera from individuals with other parasitic infections were completely negative. These included *Echinococcus, H. nana, Ascaris,* filariasis and schistosomiasis cases. Most significantly sera from a small group of *T. saginata* carriers (eight individuals) was also completely negative. One serum sample, from a patient with *T. solium* neurocysticercosis was positive in the *T. solium* taeniasis EITB. Given the increased risk of cysticercosis associated with infection with the intestinal stage (Garcia Noval et al., 1996) it was considered likely that this individual had harboured an undiagnosed intestinal infection. Test specificity was therefore considered to be 100%.

This serological test offers the possibility to overcome some of the problems that remain with coproantigen testing; it provides species-specific diagnosis and avoids the potential biohazard of collecting faeces. It also offers the potential, in combination with other immunodiagnostic techniques for diagnosis of cysticercosis (such as that of Tsang et al., 1989), for diagnosis of both *T. solium* stages infecting man from a single serum sample.

One area that remains to be investigated is the rate at which sera, following removal of the intestinal infection, become negative for circulating antibodies to the diagnostic antigens. As discussed previously this has been a problem with some serological approaches to diagnosis of both human and canine taeniasis. Work is ongoing on this issue, including sequencing of antigens for cloning to allow easier production of the diagnostic antigens.

6. Conclusions

New and improved immunodiagnostic tools for intestinal taeniasis now exist. Some of these tools have already demonstrated their applicability in field studies and have improved the quality of epidemiological data obtained from them. Further work in the future offers the potential to improve these tests still further. To date, whilst some tests, particularly of the coproantigen type, have been

applied successfully as part of field research programmes in endemic countries, issues such as cost and accessibility remain to be addressed if these tests are to be used on a routine basis in endemic countries. Work is ongoing to improve the performance of these tests and to adapt their format to make them easier to use and interpret. If these tests become more readily available and show significant cost benefits over existing approaches to the diagnosis of taeniasis they may be able to contribute substantially to the control of these parasites.

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