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Coproantigens in taeniasis and echinococcosis

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Abstract

The application of modern immunodiagnostic or molecular diagnostic techniques has improved the diagnosis of the taeniid cestode infections, echinococcosis and taeniasis. One particularly promising approach is the detection of parasite-specific antigens in faeces (coproantigens). This approach has been applied to both *Echinoccocus* and *Taenia* species and it has gained increasingly widespread use. Taeniid coproantigen tests are based on either monoclonal or polyclonal antibodies raised against adult tapeworm antigens. These tests have the following common characteristics; they are largely genus-specific, specificity is high (>95%), parasite antigen can be detected in faeces weeks prior to patency, levels of coproantigen are independent of egg output, coproantigen is stable for days at a range of temperatures (− 80 °C to 35 °C), for several months in formalin-fixed faecal samples, and coproantigen levels drop rapidly (1 – 5 days) following successful treatment. In the genus *Taenia*, most work has been done on *Taenia solium* and coproantigen tests have reliably detected many more tapeworm carriers than microscopy. For *Echinococcus* species, there is a broad positive correlation between test sensitivity and worm burden with a reliable threshold level for the test of >50 worms. Characterisation of taeniid coproantigens in order to further improve the tests is ongoing. Studies indicate taeniid coproantigens to include high molecular weight (>150 kDa), heavily glycosylated molecules with carbohydrate moieties contributing substantially to the levels of antigen detected in faeces. Application of the existing coproantigen tests in epidemiological and control programmes for *Echinococcus* and *Taenia* species infection has begun to contribute to an improved understanding of transmission and of surveillance of these important zoonotic cestodes.

Keywords: *Echinococcus*; *Taenia*; Diagnosis; Coproantigen

1. Parasitological diagnosis of taeniid cestodes

Broadly speaking the definitive host range for the zoonotic taeniid species is more restricted than the intermediate host range of the same parasite. For example, humans represent the sole natural definitive host for both *Taenia solium* and *Taenia saginata*. This therefore leaves these two species theoretically susceptible to interventions designed at reducing the rate of humans carrying the intestinal stage. Thus, from a control perspective, the diagnosis of the adult stages of these parasites is more important than that of the cystic stages. Indeed accurate diagnosis of the intestinal stages of *Echinococcus* and *Taenia* species parasites has been recognized as an important contributor in their control [1,2].

Traditional parasitological methods for the diagnosis of *Taenia* and *Echinococcus* species tapeworms range from microscopic detection of eggs in faeces to detection of proglottides and/or scoleces passed by the host either naturally or following treatment or purgation, or necropsy in the case of echinococcosis. Some of the problems with these approaches can be summarised as follows:

- Sensitivity: eggs are often absent from faeces for long periods during infection. Purgation of dogs with compounds such as arecoline hydrobromide and treatment of humans or dogs to recover tapeworm material passed in the faeces similarly does not always result in recovery of material from infected animals. During necropsy, very low infections of *Echinococcus* maybe missed.
- Specificity: taeniid eggs appear identical under the light microscope. This represents a problem when trying to differentiate between the three *Taenia* species infecting man or the more than ten species of *Taenia* and *Echinococcus* that infect canids.
- Biohazard: working with proglottides passed during infection or following treatment or purgation, or during necropsy can expose individuals to potentially infective oncospheres.

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- Estimation of worm burden: microscopic detection of taeniid eggs does not allow an estimate of infection intensity to be made. This is particularly relevant in understanding the epidemiology of *Echinococcus* species. Techniques like treatment and purgation to recover strobilate material and/or scoleces premortem are more appropriate but are more labour intensive and are still not entirely accurate.

For parasites of the genus *Echinococcus*, postmortem diagnosis of definitive hosts has been used to detect and count these parasites in the intestines of their canid hosts. The standard approach for postmortem diagnosis of *Echinococcus multilocularis* in definitive hosts is based on sedimentation and counting of worms from the intestine [3]. Whilst this approach arguably deals with issues of sensitivity, specificity and determination of worm burden it still leaves the biohazard issue and can introduce ethical and sample size problems related to getting access to animals for necropsy.

2. Alternatives to parasitological diagnosis

The application of alternative means to the diagnosis of these cestodes is not new. Immunodiagnosis of intestinal *Taenia* was first attempted in the early years of the 20th century and a number of immunological and latterly molecular approaches have been applied to the diagnosis of intestinal stages of both *Echinococcus* and *Taenia* in the intervening years [4,5]. One of these approaches will be reviewed here.

3. Coproantigen-based diagnosis

The diagnosis of intestinal infections by detection of pathogen-/parasite-specific antigens in faeces (coproantigens) is an approach now widely applied to a broad range of infectious organisms. If these organisms release products of metabolism into the intestine, these should be amenable to immunological detection. Theoretically, if these antigens are not directly associated with parasite reproduction, they should be present when reproductive material (like taeniid eggs) is absent from the faeces and should disappear shortly after successful treatment of the infection.

The first report of the detection of taeniid-specific antigens in the faeces of infected hosts was made with *Echinococcus granulosus* in infected dogs [6]. This study demonstrated *Echinococcus* antigens in infected dog faeces prior to patency, and thus in the absence of eggs. The test format used an immunoprecipitation in agar test with hyperimmune rabbit serum raised against larval worm antigens. Cross-reaction with antigens in human *Taenia*-infected faeces did, however, occur. The authors suggested that this approach would allow for the development of improved control measures for both canine echinococcosis and human taeniasis. Unfortunately, this work was not followed up for many years.

In the late 1980s, work on *Hymenolepis diminuta* infection in experimental rats indicated that it was possible to reliably detect cestode antigens in faeces using immunoassays based on antibodies raised by hyperimmunisation of rabbits against adult worm somatic or surface antigens [7,8]. Coproantigen could be detected prior to patency and specificity of the resulting tests was to the genus level.

Subsequent coproantigen-based immunodiagnostic studies for *Taenia* and *Echinococcus* species in humans and dogs have largely been based on variants of the antigen capture ELISA format. The antibodies used in these tests have varied from rabbit polyclonal or chicken egg yolk-derived antibodies to mouse monoclonal antibodies. The rabbits, chickens or mice were hyperimmunised either with adult worm somatic, surface or excretory–secretory antigens or purified cestode coproantigens. These tests have been developed for *T. solium* and *T. saginata* in humans [9–17], *E. granulosus* in dogs [10,18–21] and *E. multilocularis* in dogs and foxes [19,22–27]. They have all been used to detect antigen in detergent solubilised faecal samples.

4. Time course infection

Babos and Nemeth [6] first showed antigen to be present in the faeces of *E. granulosus*-infected dogs prior to the onset of egg production. Follow-up on work with taeniid cestodes in time course infections demonstrated that antigen is present in the faeces of *Taenia hydatigena*-, *Taenia pisiformis*- and *E. granulosus*-infected dogs before patency, apparently reaching a plateau several weeks before onset of egg production and is independent of egg output thereafter [10,13,19,28]. Where research has not been carried out in the natural definitive host, studies in rodent model systems have shown that taeniid antigens can be detected in faeces prior to patency in *T. solium* and *Echinococcus vogeli* infections [9,29].

Taeniid coproantigens have been demonstrated to disappear from faeces within approximately 1 week of successful treatment of the infection [9,13,28]. This is a useful characteristic in a number of regards; it allows rapid assessment of treatment efficacy and also means that, unlike antibody-based methods of immunodiagnosis, diagnostic results based on parasite antigen in faeces are closely tied to current, active infection.

5. Sensitivity/Worm burden determination

Of the *Taenia* species infecting humans, most coproantigen application has been carried out on *T. solium*. These studies have utilised either a microplate format ELISA test [9] or a dipstick format test [11]. Several studies involving follow-up on coproantigen positive results to determine firstly whether the diagnostic result could be parasitologically confirmed, and secondly the species of tapeworm involved have indicated that coproantigen testing by microplate ELISA detects over twice as many cases as microscopy. Testing in a less sensitive dipstick ELISA format [11] also detected significantly more cases than microscopy. False negative results did, however, occur with both test types (Table 1). Similar large-scale studies have not yet been published on the other human taeniids, thus the differential sensitivity of microscopy and coproantigen testing for these species has not been established. However,