Cestode infections in animals: immunological diagnosis and vaccination

M.W. LIGHTOWLERS *

Summary: Cestode infections in animals are important because several species are zoonotic, causing cysticercosis and hydatidosis in man, and because of the economic losses incurred due to infections in livestock. Information on immunological diagnosis of and vaccination against cestode infection is restricted almost exclusively to the taeniid cestodes in which two groups of mammalian hosts are concerned: the intermediate host infected with the larval parasite and the definitive host infected with the adult tapeworm parasite. Research towards developing serological tests for the diagnosis of larval cestode infection in animals has been largely unsuccessful. Substantial problems remain, due to the frequent existence of multiple infections with different taeniid species and antigenic cross-reactivity between these related parasites, and the low level of specific antibody response to infection. Problems with poor specificity and sensitivity of traditional serological tests for cysticercosis and hydatidosis have prevented the development of any practical test for ante-mortem diagnosis of infection. A recent new approach to the diagnosis of Taenia saginata infection by detecting circulating parasite antigen offers some prospect for the development of a practical diagnostic test for cysticercosis in cattle.

The effectiveness of the arecoline purge for detection of Echinococcus granulosus in dogs has been reduced by the widespread availability of praziquantel. A serological method for diagnosis of E. granulosus in dogs has been developed which offers equivalent or superior diagnostic sensitivity compared with arecoline purge. This test should provide a valuable tool in hydatid control campaigns for the diagnosis of existing or recent past infections in dogs.

Substantial progress has been made towards developing a practical vaccine for the prevention of T. ovis infection in sheep. An antigen derived from the parasite egg has been identified and produced in Escherichia coli using recombinant DNA techniques. The vaccine, which protects sheep against challenge infection with T. ovis, is the first highly effective defined antigen vaccine against any parasite infection of man or animals. Commercial development of this vaccine is in progress. The success achieved with the T. ovis vaccine augurs well for the rapid development of other recombinant vaccines against cysticercosis caused by other taeniid species and against hydatidosis in animals.

KEYWORDS: Cestode - Cysticercosis - Diagnosis - Echinococcosis - Hydatidosis - Review - Taeniasis - Vaccination.

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INTRODUCTION

The cestode parasites of major economic and medical importance are members of the family Taeniidae, genera Taenia and Echinococcus. These parasites all have indirect life cycles involving a carnivorous or omnivorous definitive host, in which the tapeworm stage develops in the small intestine, and an intermediate host in which the larval (metacestode) form develops in the host tissues. Taenia and Echinococcus species cause taeniasis/cysticercosis and echinococcosis/hydatidosis, respectively. In addition to the economically and medically important species, other naturally occurring taeniid host/parasite systems have been used extensively in experimental investigations of the immunobiology of the host/parasite relationship in this group of parasites. The general characteristics of these parasites are summarised in Table I. The older literature concerning the larval stages of these parasites uses genus and species names (e.g. Cysticercus bovis for the larval stage of Taenia saginata in cattle) which were rendered obsolete following the discovery of the life cycles of these parasites. Some of these names continue today as colloquial terms for the larvae, for example "tenui", "ovis", "bovis", etc.

**TABLE I**

<table>
<thead>
<tr>
<th>Species</th>
<th>Obsolete synonyms</th>
<th>Principal intermediate hosts</th>
<th>Metacestode type</th>
<th>Principal definitive host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinococcus granulosus</td>
<td></td>
<td>Sheep, goats, cattle, pigs and other herbivores (Man)</td>
<td>Unilocular hydatid cyst</td>
<td>Dog</td>
</tr>
<tr>
<td>E. multilocularis</td>
<td></td>
<td>Microtine rodents (Man)</td>
<td>Multilocular hydatid cyst</td>
<td>Fox</td>
</tr>
<tr>
<td>Taenia solium</td>
<td>Cysticercus cellulosae</td>
<td>Pigs (Man)</td>
<td>Cysticercus</td>
<td>Man</td>
</tr>
<tr>
<td>T. saginata</td>
<td>C. bovis</td>
<td>Cattle</td>
<td>Cysticercus</td>
<td>Man</td>
</tr>
<tr>
<td>T. hydatigena</td>
<td>C. tenuicollis</td>
<td>Sheep</td>
<td>Cysticercus</td>
<td>Dog</td>
</tr>
<tr>
<td>T. ovis</td>
<td>C. ovis</td>
<td>Sheep</td>
<td>Cysticercus</td>
<td>Dog</td>
</tr>
<tr>
<td>T. multiceps</td>
<td>Multiceps multiceps</td>
<td>Sheep (Man)</td>
<td>Coenurus</td>
<td>Dog</td>
</tr>
<tr>
<td>T. pisiformis</td>
<td></td>
<td>Rabbit</td>
<td>Cysticercus</td>
<td>Dog</td>
</tr>
<tr>
<td>T. taeniaeformis</td>
<td></td>
<td>Rodents</td>
<td>Strobilocercus</td>
<td>Cat</td>
</tr>
</tbody>
</table>

The taeniid species have been the subject of intensive research interest, particularly during the 1970’s and 1980’s, which has led to a substantial understanding of the parasites' epidemiology and immunobiology (45, 46, 80, 130, reviewed in 88, 127). Although a substantial degree of success has been achieved in controlling cystic hydatid...
disease in certain countries or areas in which control campaigns have been undertaken, hydatidosis and cysticercosis continue to be important parasitic diseases of livestock and man in many parts of the world (47). Crucial to the control and eradication of these parasites are tests for sensitive and specific diagnosis of infection and vaccines to prevent infection. Research towards providing these tools has culminated recently in a publication (69) describing the first highly effective defined antigen vaccine against any parasite. The recombinant T. ovis peptide vaccine protects sheep against infection with T. ovis and is being developed commercially.

This article reviews the recent progress which has been made in diagnosis of and vaccination against cestode infections. Emphasis is placed on veterinary rather than medical health aspects, drawing relevant information from the medical literature where appropriate. Limitations imposed on the size of the review necessitate a selective rather than comprehensive coverage of the literature. For more detailed coverage of the topics, the reader is referred to other review articles cited in the text and a recent broad review of the immunology and molecular biology of cestode infections (88).

IMMUNOLOGICAL DIAGNOSIS OF CESTODE INFECTIONS

General considerations

Ante-mortem diagnosis of specific larval cestode infections in animals is currently not possible. Despite a substantial amount of research having been undertaken in attempts to develop immunodiagnostic tests, there are few instances in which diagnosis has been applied in a practical situation. The major problem with developing such tests lies in the almost ubiquitous presence of infection with multiple species of antigenically cross-reacting platyhelminth parasites. Mixed infections with taeniid species are common in many livestock species and, in situations where one species predominates, for example T. saginata in cattle, the environment may be contaminated with the eggs of other species, e.g. T. hydatigena, which may raise cross-reacting antibodies following penetration of ingested oncospheres. Claims made in some publications of high sensitivity and specificity of diagnostic techniques must be interpreted with care. When serum samples are collected from infected and non-infected animals at abattoirs, consideration must be made of the prevalence, intensity and viability of other cross-reactive helminth infections. It is only when these factors have been considered that differences in serological testing can be ascribed to infection with the particular parasite of interest. Ideally, testing of new diagnostic techniques should be performed initially with experimentally infected animals where controls have the same spectrum of exposure to potentially cross-reactive parasites, or using animals raised helminth-free. Follow-up studies should then take place with specimens from naturally infected stock, taking into consideration the details of infection with cross-reacting parasites as well as other potentially important factors such as age, breed and sex of the animals involved. Claims made for successful diagnosis of infection with a specific cestode species are likely to be meaningful only if such factors have been considered. Unfortunately, there are many reports in the literature where these matters have not been assessed, and the exceptionally high test sensitivity and specificity claimed in some cases cannot be justified on the basis of the data provided.
HYDATIDOSIS

Two *Echinococcus* species are the principal aetiological agents of hydatidosis in animals and man. *E. granulosus* is distributed widely in many countries of the world and causes unilocular or cystic hydatidosis, with many animal species acting as intermediate hosts. *E. multilocularis* causes multilocular or alveolar hydatid disease, has principally a holarctic distribution and involves voles as intermediate hosts. Serological diagnosis of hydatidosis in animals concerns the diagnosis of infection with *E. granulosus*.

A marked contrast exists between the apparent immunogenicity of *E. granulosus* in animals compared with man. Typically, human hydatid patients are found to have readily detectable levels of specific antibody against *E. granulosus* antigens (reviewed by 128 and 137) while the level of specific antibody detectable in animals is frequently low or not detectable at all. Some reports of high titre antibody responses to hydatid infection in animals have been published, although in some cases it is difficult to assess the adequacy of non-infected controls in relation to the meaningful level of 'background' reactivity determined in the test. Carefully controlled experimental infections have demonstrated unequivocally that infected animals do produce specific antibody following infection with *E. granulosus*. In addition, hydatid-infected sheep which show no serological reactivity with hydatid antigens do respond to parenteral immunisation with small quantities of cyst fluid antigen, rapidly producing specific antibody (83). Indeed, fluid surgically released from an animal's own cysts into the peritoneal cavity also stimulates a vigorous anamnestic immune response (83). These experiments indicate that sheep are not unable to respond to the parasite, but suggest that the antigen is effectively sequestered from the immune system in sheep (and perhaps also in other animal species). Cysts in man may be more leaky and hence likely to induce an antibody response. An alternative explanation may be that most cases of human hydatidosis which come to the attention of medical practitioners are those in which the cyst is causing clinical problems. It is possible that a greater proportion of human patients might have low levels of specific antibody if more cases were examined where the cysts were asymptomatic.

**Antigens**

Hydatid cyst fluid from fertile cysts and protoscoleces are the most convenient and frequently used sources of antigen for diagnosis of hydatid disease in animals. Only two antigenic molecules are defined with some precision, and the majority of the research performed with these antigens has been in relation to diagnosis of infection in man (128). Rickard and Lightowlers (128) have proposed a standardised nomenclature for these antigens to overcome confusion in the literature resulting from there being three separate published nomenclatures. The standardised nomenclature has been adopted in recent publications in the field (87, 89, 138).

Antigen 5 [synonyms — Antigen 4 of Chordi and Kagan (16); Antigen A of Oriol et al. (106)] is a dominant heat-labile (10, 32, 79, 106) lipoprotein (106) antigen which forms the diagnostic 'Arc 5' in immuno-electrophoresis with sera from the majority of humans infected with *E. granulosus* (14). In cyst fluid, Antigen 5 has a high molecular weight, estimated to be several hundred thousand kDa (113, 149), breaking down in the presence of the detergent sodium dodecyl sulphate (SDS) into subunits whose molecular weights have been estimated in several studies as being between 56 and 69 kDa (10, 31, 32, 87, 114). The subunit appears to be a heterodimer comprised
of 24 and 38 kDa molecules linked by disulphide-bonds (87). Antigen 5 is a taeniid cestode specific antigen present in *Taenia* spp. and *Echinococcus* spp. and which induces specific antibody in cysticercosis and hydatidosis (reviewed in 88 and 128). Antibodies are produced to this antigen in sheep infected monospecifically with *T. ovis* or *T. hydatigena* (155). Thus, currently available diagnostic techniques based on detection of antibody to Antigen 5 are not suitable for specific diagnosis of ovine hydatidosis.

Antigen B, also a lipoprotein, is present in approximately a ten-fold excess compared to Antigen 5 in sheep hydatid cyst fluid (101). Antigen B is heat stable (79, 106), withstanding long periods of boiling, has a molecular weight estimated between 120 and 160 kDa in cyst fluid (106, 107, 113) and breaks down into subunits in the presence of SDS, the sizes of which are not affected by reducing agents (87, 114). Immunochemical analyses using monoclonal antibodies against Antigen B have identified a curious characteristic of the antigen in SDS (87). The antigen appears to be comprised of an 8 kDa subunit with additional subunits whose molecular weights suggest that they may be made of multiple copies of the 8 kDa monomer. The relative abundance of the subunits decreases asymptotically with increasing size from 8 kDa.

There is substantial debate at present concerning the specificity of Antigen B for *E. granulosus*. Bout *et al.* (10) used antisera raised in rabbits against several helminth parasite species and found that an antigen termed 'lipoprotéine ubiquitaire', almost certainly Antigen B, was identified in sheep hydatid cyst fluid with many of these antisera. This does not mean that the different helminths would raise antibodies against this antigen during natural infection.

Shepherd and McManus (138) found that the lower molecular weight subunits (estimated by them to be 12 kDa and 16 kDa) were specific for *E. granulosus* and did not cross-react with *E. multilocularis*. More thorough analysis has shown that this is not the case and that a large proportion of sera from humans infected with *E. multilocularis* detect the lower molecular weight subunits of Antigen B (87). At present there is some evidence that Antigen B (87) and an 8 kDa antigen (89), likely to be the Antigen B subunit, are *Echinococcus* genus-specific. Kagiko, Njeruh and their colleagues (39, 72, 73, 102-105) have purified a heat stable antigen from cattle hydatid cyst fluid which they designate Antigen 880 and which they believe to be Antigen B. Their serological assays with cattle, sheep, goat and human sera suggest that the antigen may be specific for *E. granulosus*. More extensive testing is required to validate this conclusion, with particular attention being paid to the intensity of infection with other related parasites in the experimental animals. The antigen was found to be present in extracts of several gastro-intestinal cestodes and nematodes (104).

Additional information regarding the tissue localisation of Antigen 5 and Antigen B, the identity of other hydatid antigens and the presence of host serum proteins in hydatid cysts has been reviewed previously (128).

**Hydatid diagnosis in sheep**

Sheep are important intermediate hosts in the natural transmission of hydatidosis throughout the world. Early investigations into the serological diagnosis of hydatidosis in sheep applied techniques such as the Casoni test for immediate-type hypersensitivity, indirect haemagglutination, complement fixation and latex agglutination, which were being used successfully at the time for diagnosis of human hydatidosis. It became
clear that unlike diagnosis in man, negative reactions occurred commonly in infected sheep and that false positive reactions were common, particularly in aged sheep infected also with *Taenia* spp. (9, 49, 111, 112, 132, 135, 136 and literature cited therein, 143). Other more recent reports have come to similar conclusions or have provided insufficient information regarding infection rates with other taeniid cestodes to allow critical evaluation of more successful results (6, 33, 71, 90). Large-scale serological surveys for hydatid infection have been undertaken in the Soviet Union involving tens of thousands of sheep (7, 134); however, it is difficult to determine the value of the results considering the probable imperfections in specificity and sensitivity of the diagnostic tests employed.

Problems with false positive and false negative results in serological tests for ovine hydatidosis could not be overcome by application of tests for detection of antibody to Antigen 5 (Arc 5). In man, the value of immunoelectrophoresis or double diffusion tests for Arc 5 relies upon the availability of additional clinical details which support the diagnosis. Detection of antibody to Antigen 5 is particularly valuable where the patient is unlikely to have been exposed to infection with other larval cestodes. In sheep, infection with *Taenia* spp. is ubiquitous and the presence of *T. hydatigena* could be expected to be coincident with hydatid transmission. *T. hydatigena* cyst fluid contains an antigen which forms Arc 5 in immunoelectrophoresis and sera from sheep infected with *T. hydatigena* or *T. ovis* produce Arc 5 in immunoelectrophoresis with hydatid cyst fluid antigens (115). A proportion of sheep infected with *E. granulosus* also develop antibodies which react to produce Arc 5. However, the detection of these specific antibodies cannot be used for diagnostic purposes on individual animals, even in the absence of infection with other taeniids (18, 91, 155).

Controlled studies on lambs experimentally infected with *E. granulosus* have proved unequivocally that sheep do respond to hydatid infection by producing specific antibodies (18, 143, 154, 155). Responses are typically of low titre, and in some individual experimental animals antibody test results can be negative despite the presence of viable, fertile cysts. Monospecific experimental infections have also confirmed the cross-reactivity between antibodies produced in response to *E. granulosus* and antibodies to *T. hydatigena, T. ovis* and *F. hepatica* (20, 143, 154, 155).

Investigations with experimentally infected sheep which have shown that a specific antibody response to *E. granulosus* does occur in sheep, albeit of low titre, have encouraged attempts to improve the specificity of diagnosis by comparative analysis of results with different antigen preparations or application of purified antigen preparations. The results of this substantial effort have been disappointing. No test has achieved sufficient specificity or sensitivity for diagnosis of infection on an individual animal basis. One technique which has achieved an improvement in the specificity for diagnosis of *E. granulosus* has been to compare the titres of sera using antigens from homologous and heterologous species. Yong *et al.* (154) found that indirect haemagglutination titres of sera from sheep with *E. granulosus* infection gave the highest titre in the test when hydatid fluid antigen was used, compared with results obtained using *T. hydatigena* or *T. ovis* antigens. Sheep infected with *Taenia* species, on the other hand, gave a higher titre using homologous antigen compared with results using hydatid antigens. These authors extended this approach using the enzyme-linked immunosorbent assay (ELISA) (156); however, none of the antigens tested detected specific infections and the antibody titres were not statistically related to the number of cysts present in the animals. The test could be used only for non-specific detection of larval cestode infection.
Craig and Rickard (22) used antibody affinity chromatography to purify antigenic components from hydatid cyst fluid or protoscolex extract. These purified antigens did improve the specificity for diagnosis of *E. granulosus* infection compared with results using the unprocessed antigen. Another approach was also taken by Craig et al. (21) who generated a murine monoclonal antibody against an antigenic determinant of hydatid cyst fluid which cross-reacted with antibodies in the sera of sheep infected with *Taenia* spp. or *F. hepatica*. This monoclonal antibody was used to affinity deplete crude hydatid cyst fluid antigen of the cross-reacting antigen. The depleted antigen preparation was found to have improved specificity for sera from sheep with experimental *E. granulosus* infection. The promising results of Craig et al. were extended by Lightowlers et al. (82) using sera from naturally infected sheep. Sera were obtained from groups of sheep whose exposure to, and infection status with, taeniid cestodes were carefully defined. These groups included aged sheep reared on an island without contact with dogs, sheep with naturally acquired heavy burdens of fertile hydatid cysts and fifty sheep from a flock known to have a proportion of the animals infected with *E. granulosus*. The latter group was slaughtered, all the meat boned out and the entire musculature, lung and liver finely sliced and the viscera searched thoroughly in order to count and assess the viability of all cestode larvae. Crude sheep hydatid fluid as well as the same antigen depleted of cross-reacting antigens using the monoclonal antibody of Craig et al. (21), were tested in ELISA using these groups of sheep sera. However, the tests were unable to reliably differentiate even the heavily infected sheep from the cestode-free sheep on an individual animal basis. The major difficulty lay in the low titre of specific antibody detectable in sheep infected with *E. granulosus*.

Other researchers have applied antibody affinity purified antigens (94) or fractions purified from crude cyst fluid or parasite extracts (53, 104, 129, 144). Of these, the antigen that offers the most promise is a polysaccharide which migrates cathodically in immunoelectrophoresis (53, 129). These various preparations showed improved specificity compared with crude antigen, but none provided specific diagnosis for individual animals.

**Hydatid diagnosis in other intermediate host species**

Serological tests for the diagnosis of hydatid infection have been assessed with sera from infected goats (39, 73, 90, 104), cattle (1, 2, 39, 72, 78, 90, 104), pigs (2, 64, 90, 111, 142), Indian buffaloes (118) and camels (27, 33, 133). The results of these studies suggest that these host species are similar to sheep in that they produce a weak antibody response to hydatid infection. As in sheep, non-specificity is a major problem due to the occurrence of mixed infections with *Taenia* spp. and other cross-reacting parasite species. The results of three studies stand out as being unusual. Dada et al. (27) found that a high proportion (75%) of camels with hydatid cysts produce antibodies to Antigen 5 detected in double diffusion and that there were no false positives in the non-infected camels tested. This specificity is not reflected in indirect haemagglutination assays with crude cyst fluid antigen from camel hydatid cysts using the same sera (27). Kagiko et al. (72) and Njeruh and Gathuma (104) used a precipitated antigen extract from bovine hydatid cysts and found high sensitivity and specificity for diagnosis of hydatid infection in Kenyan cattle. However, insufficient information is provided concerning the relative prevalence and intensity of infection with other potentially cross-reactive parasite species. For this reason, it is not possible to assess the true significance of their results.
Specific and sensitive diagnosis of *T. solium* cysticercosis in pigs and *T. saginata* cysticercosis in cattle would be a major advance on the relatively insensitive method of carcass meat inspection. Tests for ante-mortem diagnosis of cysticercosis in animals would also be valuable for studying the epidemiology of the *Taenia* spp. parasites involved, particularly in the monitoring of infection rates during parasite control campaigns.

The difficulties which have been described above for diagnosis of hydatid infection apply equally to diagnosis of cysticercosis in animals. A relatively low specific-antibody titre and cross-reactivity with antibodies produced in response to other helminth infections have combined to thwart attempts to produce effective diagnostic tests for cysticercosis in cattle (19, 40, 43, 61), pigs (29, 109) and sheep (9, 22, 23, 29, 52, 154, 156). Antibody affinity purification or depletion of antigen has achieved a limited degree of success in improving the specificity of ELISA for diagnosis of cysticercosis in cattle and sheep, although these steps resulted in a reduced sensitivity for diagnosis (22). Kumar and Gaur (77) reported a test sensitivity and specificity of greater than 90% for diagnosis of *T. solium* cysticercosis in pigs using chromatographic fractions of *Cysticercus* scolex antigens. However, no data is provided to indicate the parasite burdens in the experimental animals which would allow an assessment of the minimal level of parasitism which can be detected using their test.

In addition to problems with antigenic cross-reactivity between parasite species and low levels of antibody, there are also problems with the supply of parasite material which limits the widespread application of tests for diagnosis of cysticercosis in animals. Antigens from heterologous, more readily obtained cestode species have been used for diagnosis of *T. saginata* infection in cattle (41, 42, 74, 119, 120) although this could be expected to exacerbate problems with specificity.

Immunochromatographic analysis of *Cysticercus* antigens is providing valuable information and may identify specific diagnostic components (34, 70). This approach has been applied with outstanding success to the diagnosis of *T. solium* cysticercosis in man (50). The immunoblotting technique used is not applicable to large-scale use for diagnosis of animal cysticercosis, but the information gained provides the basis for research towards the production of these particular diagnostic components using recombinant DNA techniques.

A significant advance has been made in the diagnosis of *T. saginata* cysticercosis in cattle with the discovery by L.J.S. Harrison and her colleagues (54) of parasite antigens in the blood of infected animals. Detection of these antigens was achieved using a monoclonal antibody against a carbohydrate antigenic epitope of the cysticercus. Most importantly, the assay was found to be positive in the presence of infection with viable cysticerci only and significant cross-reactions did not occur due to infection with *T. hydatigena*, *E. granulosus* or *F. hepatica*. It is hoped that further refinement of the test will improve the present sensitivity of the test which is positive only when cattle have more than 200 living cysticerci. A similar assay has since been applied successfully for the detection of circulating parasite antigen in *T. solium*-infected pigs (131).

**ECHINOCOCCOSIS/TAENIASIS**

Diagnosis of taeniid cestode infection in the definitive host is most conveniently performed by identification of the tapeworm segments or of the characteristic eggs
in the faeces. These simple methods do not enable the species of taeniid parasite to be identified readily. Expert analysis of the gravid segment morphology may identify the parasites to species level but the eggs themselves are morphologically indistinguishable between any of the taeniid species. Differentiation between the species is particularly important in order to distinguish between *T. solium* and *T. saginata* infection in man and between *Taenia* spp. and *E. granulosus* in dogs. Species-specific diagnosis in dogs has played a crucial role in several campaigns for the control of cystic hydatid disease. *Echinococcus* segments are difficult to detect in faeces due to their small size. Diagnosis has been performed by administration of an arecoline purgative and examination of purge contents for the presence of *E. granulosus*. Infectivity of the purge material for the personnel involved, poor sensitivity in light infections, incomplete purgation and adverse reactions to the drug in some dogs are disadvantages of this method.

A new method has been described for the species-specific identification of *E. granulosus* eggs (25). The technique involves the application of a monoclonal antibody which is specific for an oncosphere antigen of *E. granulosus*. Eggs are either treated with artificial gastric and intestinal fluids in order to hatch and activate the oncospheres, or treated with sodium hypochlorite and sodium deoxycholate. *E. granulosus* oncospheres can then be identified by incubation with the monoclonal antibody and examination using fluorescence microscopy. The method has been used successfully for detection of *E. granulosus* eggs in 'sellotape samples' from the perianal region of infected dogs and in faecal material and samples obtained from contaminated environmental sites in the Turkana region of northern Kenya (26). Similar techniques with a different monoclonal antibody can be used to differentiate oncospheres of *T. hydatigena* and *T. pisiformis* (24). The technique and equipment involved are not readily applicable to field use in parasite control campaigns. The method is not applicable to the diagnosis of pre-patent infection.

The value of the various methods discussed above for the diagnosis of *E. granulosus* infections in hydatid control campaigns has been greatly reduced by the introduction and ready availability of praziquantel. Dog owners who had failed to prevent their animals from eating hydatid cysts could avoid punitive action by hydatid control authorities by dosing their dogs with praziquantel prior to examination by control officers. This possibility has stimulated research towards the development of a serological test for diagnosis of existing or recent past infection with *E. granulosus* in dogs. Several early reports described the presence of antibodies (15, 99) or immediate-type hypersensitivity (146, 152) to hydatid antigen in dogs infected with *E. granulosus* and more recent studies have confirmed the immunogenicity of *E. granulosus* for its definitive host (3, 18, 139, 140). The specificity of these antibodies for *E. granulosus* was subsequently proved in carefully controlled experimental infections by Jenkins and Rickard (66-68). An ELISA method was developed using excretory-secretory antigens derived from protoscoleces cultured *in vitro*. Seroconversion occurred in the experimentally infected dogs within 5 days post infection, approximately 40 days prior to the first detection of eggs in the faeces. Sera from dogs infected with other cestode or nematode parasites did not cross-react in the assay. The method has been used with sera from naturally infected dogs (37), and the sensitivity obtained (72.7%) was superior to that achieved in previous studies using arecoline purge. A potential limitation on the wide-scale application of this test would be the availability of a sufficient supply of parasite antigen for the test. Gasser and his colleagues (38) identified the specific antigenic components of
protoscoleces and have been successful in producing one diagnostic antigen in *Escherichia coli* using recombinant DNA techniques (Gasser, Lightowlers and Rickard, submitted for publication). The antigen involved is specific; however, its low sensitivity in ELISA (20%) is insufficient for practical application. Hopefully the application of additional cloned antigens will improve the sensitivity of the assay.

Specific antibodies have also been demonstrated in the sera of dogs infected with *Taenia* species (56, 58, 66, 68). In New Zealand, the ongoing campaign to control hydatidosis and cysticercosis in sheep has adopted a serological test for the diagnosis of *T. ovis* infection in dogs which will largely replace the six weekly dosing of dogs with praziquantel (5).

**VACCINATION AGAINST CESTODE INFECTIONS**

**General perspective**

Vaccines for prevention of infection with cestode parasites would be valuable in reducing the economic losses due to infections with these parasites in livestock and also in breaking the life cycle of the medically important species. However, in common with the paucity of vaccines against infection with other parasites, no vaccines have been available for the prevention of infection with cestodes. Many aspects of the immunological relationship between taeniid cestodes and their intermediate hosts favour this group as targets for the development of defined antigen vaccines. This goal was achieved with the recently published report of a highly effective vaccine using a defined antigen against *T. ovis* in sheep (69). Vaccination against cestode infection has been reviewed elsewhere (88, 127). Detailed referencing on the topic can be obtained from these reviews and only selected or more recent literature is cited herein.

Research on vaccination has been restricted almost entirely to attempts to prevent infection with the larval stages of taeniid cestodes. Several general aspects of the immunobiology of taeniid cestode infection in the intermediate host are common to the group and provide the framework upon which research and optimism for success are based. These are:

1. Immunity plays a central role in the natural regulation of transmission. Infected individuals exhibit a high degree of immunity to re-infection (concomitant immunity).

2. Colostral antibody transfers protection from infected dam to offspring. Immunity can also be transferred to naive recipients with sera or purified antibody from infected animals.

3. Vaccination with crude parasite extracts achieves very high levels of protection against challenge infection.

4. Colostral antibody transfer from vaccinated animals protects recipients against challenge infection.

**Vaccination against cysticercosis**

Much of the early information on vaccination against cysticercosis was provided by detailed studies using *T. taeniaeformis* (13, 75) and this species has continued to
provide a useful model for studies on vaccination against this group of parasites (11, 12, 81, 84, 85, 115-117). Rickard and his colleagues have also provided much valuable practical information concerning the use of vaccines to prevent infection with *T. ovis* in sheep or *T. saginata* in cattle (see 127). In this respect it was found that:

a) A single vaccination affords protection against infection for a full year (124).

b) Colostral antibody from the vaccinated dam protects the young against infection after birth (123, 124).

c) Offspring can be successfully vaccinated in the presence of protective colostral antibody (123).

d) Field trials of the vaccines have achieved significant levels of protection against naturally acquired infection (122, 126).

The major limitation to practical application of vaccines to control these parasites is in the supply of antigen. The most potent source of antigen for vaccination is the oncosphere. It is impractical to consider preparation of sufficient antigen from oncospheres for anything but experimental trials, especially for *T. solium* or *T. saginata* where man is the obligate definitive host. Limited success has been claimed for the establishment of taeniid parasite-derived cell lines (35, see also 63) but these experiments have not shown promise for the production of antigens for vaccination. The only practical proposition is to produce the parasite molecules *in vitro* using recombinant DNA techniques. With this in mind Bowtell *et al.* (11) constructed a cDNA library for *T. taeniaeformis*, the first against a helminth parasite, and isolated cloned antigens for vaccination trials in mice. Subsequently, a tripartite research project was established between the University of Melbourne, Coopers Animal Health New Zealand Ltd. and the New Zealand Ministry of Agriculture and Fisheries with the aim of producing a recombinant vaccine against *T. ovis* infection in sheep. The project achieved the production of a highly effective vaccine which is currently being developed for commercial release.

The development of the vaccine is detailed in Johnson *et al.* (69). Initially, immunochemical methods were used to investigate the antigenic molecules in *T. ovis* oncospheres. Investigations using sera from immune and control sheep identified a pair of oncosphere antigens of 47 kDa and 52 kDa as being associated with immunity. Subsequent trials using the 47/52 kDa antigens purified in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) induced a high level of protection. A cDNA library was prepared from *T. ovis* oncospheres which had been hatched and activated prior to mRNA extraction. The library was prepared in the bacteriophage vector γgt11 which expressed the cloned parasite antigens in *E. coli* as fusion proteins with the bacterial enzyme β-galactosidase. Antibodies specific for the 47/52 kDa region were purified by transferring the whole oncospheral antigen extract separated in SDS PAGE to nitrocellulose, excising a narrow membrane strip corresponding to 45/52 kDa, reacting this with antibodies against the whole oncosphere preparation and finally eluting the specific antibodies from the membrane strip using low pH buffer. The purified antibodies were used to screen the *T. ovis* library for clones expressing the parasite antigen. Two groups of clones were detected and designated 45S and 45W. These antigens were purified as β-galactosidase fusion proteins and used in vaccination trials in sheep. The vaccination procedure with 45S or 45W antigens produced antibody specific for the 47/52 kDa native oncosphere antigen, however, no protection was achieved against challenge infection with *T. ovis*. The 45S and 45W clones were
subcloned into a plasmid expression vector in which the parasite antigens are produced in \textit{E. coli} as a fusion protein with the enzyme glutathione S-transferase (GST). In this system, soluble fusion protein can be purified under non-denaturing conditions. Vaccination trials with the 45S- and 45W-GST fusion proteins found that the 45W-GST antigen achieved high levels of protection against challenge infection with \textit{T. ovis} in sheep. A total of 50 µg of purified protein per sheep using saponin as adjuvant induced 94% protection against challenge infection.

Similar research is likely to follow for the other \textit{Taenia} species which cause economically and medically important cysticercoses. Progress to date on vaccination with antigen derived directly from the parasites for vaccination against \textit{T. saginata} in cattle (see 127) and \textit{T. solium} in pigs (76, 95, 110; see also 96) has been encouraging.

**Vaccination against hydatidosis**

Relatively few studies have investigated vaccination against egg infection with \textit{E. granulosus}. The risk of human infection posed by rearing dogs with patent worms and purifying the eggs for challenge infection and the long period of time required after challenge before the infection can be assessed (more than six months) are major difficulties encountered by those working in this area. Many of the immunological characteristics of the host-parasite relationships between \textit{E. granulosus} and its intermediate hosts (reviewed in 57 and 127) are similar to those of the other taeniid species. Passive transfer of protection with colostrum does not occur between infected sheep and their lambs (Heath and Yong, unpublished observations, cited in 57) to the extent that it does with the other taeniid species, perhaps reflecting the low titre of serum antibodies raised against the hydatid parasite in sheep (82).

Vaccination with oncospheres (55) or their \textit{in vitro} culture products (108) have achieved very high levels of protection in sheep against challenge infection with eggs. Antigens derived from cyst fluid or protoscoleces have been less successful (28, 148). Heath et al. (55) found that two or more immunisations subcutaneously with activated live oncospheres of \textit{E. granulosus} stimulated almost complete protection against challenge infection. The immunisations resulted in the development of masses of cysts at the site of the initial injection but no cysts at the site into which subsequent immunisations were made. Several lines of evidence led Heath et al. (55) to conclude that resistance was stimulated by the activated oncosphere or a stage in the development of the parasite prior to 14 days of age. In a follow-up experiment, Osborn and Heath (108) showed that it was not necessary to use living parasites for immunisation, with high levels of protection being achieved using the \textit{in vitro} culture products from activated \textit{E. granulosus} oncospheres.

The challenge to researchers in developing a practical vaccine to prevent hydatidosis in livestock is to devise a method for supplying adequate quantities of the appropriate antigen(s). Limited success has been achieved with culture of \textit{E. granulosus} cells \textit{in vitro} (35, see also 63) or \textit{Echinococcus} hybridomas (30); however, the techniques most likely to be effective for antigen production are recombinant DNA methods. The success achieved with \textit{T. ovis} in this respect will provide the model on which to base research towards achieving a vaccine against hydatidosis.

**Vaccination against echinococcosis/taeniasis**

While host protective immunity against infection with taeniid cestodes is a prominent feature of the host-parasite relationship in intermediate hosts, evidence
for the existence of protective immune responses against the adult worms in definitive hosts is less clear. Protective immunity against the hymenolepid cestodes has been established beyond doubt (reviewed in 62, 65). Against the taeniids, however, immune mechanisms have tended to induce only partial protection at best. Nevertheless, a sufficient number of experiments have shown that a degree of apparently immunologically mediated protection can occur against establishment/survival/fecundity of *E. granulosus* worms in dogs.

Resistance to re-infection with *E. granulosus* in dogs was examined in a series of experiments by Gemmell *et al.* (45). In one experiment, sixteen dogs were repeatedly infected on eight or nine occasions, with each infection being terminated and assessed by arecoline purge between 5 and 12 weeks post-infection. The number of prior infections with the parasite and the time after infection at which the challenge infections were assessed and were examined for effects on the number of worms present as well as their growth rate and/or oogenesis. Repeated challenge infection had no effect on the proportion of worms which were patent, and there was a large variation in the worm numbers between and within individual dogs at different challenge infections. Rather than a continuous decline in the infectivity of the dogs, each animal remained susceptible for a varying number of infections after which they became less susceptible. Five of the sixteen dogs did not show a reduction in infectivity over the length of the trial. However, the other dogs developed significant resistance to infection with 50% of the dogs showing resistance by the sixth infection. The size of the worm burden and longevity of the infection had no influence on susceptibility. Extrapolation of the data suggested that 99.9% of dogs would be expected to become resistant by the twelfth infection. Previous workers had achieved partial resistance to *E. granulosus* in dogs by oral administration of irradiated protoscoleces prior to challenge infection (4, 97, 98). Little or no immunity against re-infection has been found against *Taenia* spp. in dogs and cats (17, 93, 125, 151, 153).

Vaccination trials using non-living parasite extracts to immunise dogs against challenge infection with *E. granulosus* or *Taenia* spp. have led to widely varying levels of immunity. Turner *et al.* (145, 147) achieved high levels of protection against *E. granulosus* following multiple immunisations with antigens derived from protoscoleces or cyst membranes. Other researchers have also achieved a degree of protection against *E. granulosus* following parenteral immunisation with antigens (4, 36, 44, 48, 60). Immunisation of dogs with antigens from heterologous taeniid species have been less successful in inducing immunity against *E. granulosus* (121, 141), although Gemmell *et al.* (45) achieved a short-acting immune response by parenteral injection of activated oncospheres of *E. granulosus*, *T. hydatigena*, *T. ovis*, *T. multiceps*, *T.pisiformis* or *T. serialis*. The immune response affected either the number of *E. granulosus* worms establishing from a challenge infection or their growth or oogenesis or all three parameters.

The reports which describe some success in vaccination against *E. granulosus* in dogs encourage further work towards improving the results achieved thus far. Immunochemical methods for characterisation of *E. granulosus* components which are antigenic in dogs (38) and immunisation with defined recombinant antigens (51) are comparatively new techniques which are beginning to be applied in this area.
CONCLUDING REMARKS

The recent success of research towards the development of a vaccine against *T. ovis* infection in sheep should provide a foundation for the rapid development of vaccines against other cysticercoses and hydatidosis. Many of the host-parasite immunological relationships in the taeniid cestode parasites predispose these parasites towards the likely success of vaccine production. Despite this, the *T. ovis* vaccine clearly indicates that it is possible to achieve very high levels of protection against infection with a parasite using a single defined protein antigen. The *T. ovis* vaccine antigen renders the invading oncosphere, or early developing parasite, susceptible to immune attack by the host. It is hoped that the other closely related parasites, particularly *E. granulosus, T. solium* and *T. saginata*, will have homologous oncosphere antigens which also render these parasites susceptible to immune attack. Nucleic acid sequence homology between the antigen-encoding mRNA’s and the cloned *T. ovis* gene should allow rapid identification of homologous cDNA clones in libraries prepared from oncosphere mRNA from these other parasite species.

The outlook for the development of sensitive and specific diagnostic tests for hydatidosis and cysticercosis is not so optimistic. Recombinant DNA techniques have been used successfully for the production of *Echinococcus* antigens (51, 59, 86, 100, 150) although no results have been reported for the effectiveness of these antigens for diagnosis of hydatidosis in animals. Approaches such as the detection of circulating antigens or antigen-specific T cells are alternatives to standard serological techniques which may be effective for diagnosis of larval cestodes and deserve further investigation.

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CESTODOSES ANIMALES : DIAGNOSTIC IMMUNOLOGIQUE ET VACCINATION.
— M.W. Lightowlers.

Résumé: Les cestodoses animales sont des maladies importantes car plusieurs d’entre elles sont transmissibles à l’homme, chez lequel elles peuvent provoquer la cysticercose et l’hydatidose ; elles ont, en outre, de graves répercussions économiques sur la production animale. Les informations disponibles en matière de diagnostic immunologique et de vaccination concernent presque exclusivement les Taenidés pour lesquels il existe deux types d’hôtes (des mammifères dans les deux cas) : l’hôte intermédiaire infesté par la forme larvaire du parasite et l’hôte final infesté par la forme adulte. Les recherches visant la mise au point d’épreuves sérologiques destinées au diagnostic des cestodoses transmises par la forme larvaire du parasite ont échoué en grande partie. Des difficultés considérables subsistent, du fait de l’existence fréquente d’infestations multiples
Las cestodiasis animales son enfermedades importantes. Por una parte, varias de ellas son transmisibles al hombre, en quien pueden provocar cisticercosis e hidatidosis; por otra, pueden significar pérdidas graves en la producción animal. Las informaciones con que se cuenta en materia de diagnóstico inmunológico y vacunación se refieren casi exclusivamente a los cestodos Taeniidae. Estos tienen dos tipos de huéspedes, que son mamíferos: el huésped intermediario, infestado por la forma larval del parasito y el huésped final, infestado por su forma adulta. Las investigaciones para obtener pruebas serológicas que permitan diagnosticar cestodiasis transmitidas por la forma larval del parasito han fracasado en una gran medida. Se interponen dificultades considerables: la frecuencia de infestaciones múltiples provocadas por diferentes especies de Taeniidae, el fenómeno de reacción cruzada entre parásitos emparentados, y el bajo grado de especificidad de la respuesta inmunitaria a la infestación. La especificidad y sensibilidad insuficientes de las pruebas serológicas clásicas para detectar cisticercosis e hidatidosis no han permitido obtener pruebas eficaces para el diagnóstico ante mortem. En cambio, un intento reciente de diagnosticar la infestación por T. saginata a partir de la búsqueda...
de los antígenos circulantes del parásito parece permitir posibilidades de desarrollar un método de diagnóstico eficaz de la cisticercosis en bovinos.

La eficacia de la prueba de arecolina para la detección de Echinococcus granulosus en los perros presenta en la actualidad un interés reducido debido a la gran cantidad de praziquantel de que se dispone. En efecto, se ha puesto a punto un método de diagnóstico serológico de E. granulosus en los perros cuya sensibilidad es igual cuando no superior a la de la prueba de arecolina. Esta debería ser un medio eficaz para diagnosticar infestaciones existentes o recientes en perros durante campañas de lucha contra la hidatidosis.

Se observan importantes progresos en las investigaciones para obtener una vacuna eficaz que pueda prevenir la infestación por T. ovis en ovinos. Se identificó un antígeno proveniente del huevo del parásito y se lo cultivó en Escherichia coli mediante técnicas de ADN recombinante. Esta vacuna, que protege a los ovinos de toda nueva infestación por T. ovis, es la primera vacuna antígenica específica de gran eficacia tanto en el hombre como en los animales. Se está preparando su comercialización. Este logro permite confiar en un desarrollo rápido de otras vacunas recombinantes contra la hidatidosis en los animales y contra la cisticercosis provocada por otras especies de Taeniidae.


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REFERENCES


