IMMUNOLOGY AND MOLECULAR BIOLOGY OF ECHINOCOCCUS INFECTIONS

M. W. LIGHTOWLERS

University of Melbourne, Veterinary Clinical Centre, Princes Highway, Werribee, Victoria 3030, Australia

Abstract—LIGHTOWLERS M. W. 1990. Immunology and molecular biology of Echinococcus infections. International Journal for Parasitology 20: 471–478. Echinococcus spp. are the etiological agents of hydatid disease in man and other intermediate hosts. Many questions regarding the factors which determine susceptibility/resistance to hydatid disease, and the factors which influence the viability and fertility of hydatid cysts, remain to be answered. Recent research into the effects of hydatid infection on the immune system of the host has provided some insights into the host–parasite relationships. Immunochemical and recombinant DNA techniques are being applied to improve diagnosis of hydatidosis in man and E. granulosus infection in dogs, and also in the development of vaccines against infection with taeniid cestode larvae. The successes which have been achieved in these areas are likely to provide valuable tools for the control of cystic hydatidosis in man. These recent studies in the areas of immunobiology, serological diagnosis and vaccination are reviewed.

INDEX KEY WORDS: Echinococcus; hydatid; immunology; molecular biology; diagnosis; vaccination.

INTRODUCTION

HYDATID disease in the intermediate hosts of Echinococcus spp. is typically a chronic parasitic infection with viable cysts persisting, in many instances, for the life of the host. The factors which influence initial susceptibility to infection and the characteristics of the disease in infected individuals are unclear. Do all individuals of a susceptible host species which ingest viable Echinococcus eggs develop hydatid cysts? What determines the growth rate of the parasite and whether the cysts are fertile or sterile? The answers to these questions remain largely unknown, but recent studies on the effects of hydatid disease on the immune system of the host provide some insight into the host–parasite relationship. Current research on the application of recombinant DNA methods to the production of antigens for diagnosis of infection and for vaccination against infection may soon provide valuable practical tools for the control of cystic hydatid disease.

IMMUNE RESPONSES TO INFECTION AND IMMUNOPATHOLOGY IN THE INTERMEDIATE HOST

Few studies have been undertaken to determine the factors which regulate innate susceptibility to egg-induced infection with E. granulosus or E. multilocularis. Work with other taeniid species has indicated that host age, sex, strain and physiological state all have marked influences in determining innate susceptibility/resistance to infection (reviewed by Rickard & Williams, 1982). These factors are also likely to affect egg-induced infection with Echinococcus. Secondary hydatidosis can be established and passaged through laboratory animals by inoculation of E. granulosus protoscoleces or part of the E. multilocularis cyst mass intraperitoneally or subcutaneously. These infections have provided laboratory models for the study of hydatidosis and have established that host age, sex and strain influence susceptibility/resistance to hydatid infection (reviewed by Heath, 1986).

The intermediate hosts of E. granulosus and E. multilocularis develop specific humoral and cellular responses to the parasites (reviewed by Heath, 1986) and they confer a significant level of resistance to reinfection (Sweatman,Williams, Moriarty & Henshall, 1963; Yarulin, 1968; De Rycke & Pennoit-De Cooman, 1973; Rau & Tanner, 1973; Aminzhanov, 1976). The contribution of these immune responses to the destruction of the parasite after initial establishment is less clear. A proportion of hydatid cysts in man (Rausch, Wilson, Schantz & McMahon, 1987) and animals (Gemmell, Lawson & Roberts, 1986) die at some point after initial establishment of the parasite such that calcified lesions can be observed macroscopically. It is not known whether the host's immune response is responsible for the death of these parasites. Both dead and viable, fertile cysts can occur in the same host, and in sheep the death of cysts occurs predominantly in the first 2 years of infection (Gemmell et al., 1986). In host species and strains refractory to infection with E. multilocularis, there is evidence that the host's inflammatory response is associated with restricting the growth and metastasis of the cyst mass (Ali-Khan & Siboo, 1980). Thymectomy and treatment with anti-thymocyte serum also influence the growth and dissemination of...
**E. multilocularis** in mice (Baron & Tanner, 1976). Despite the development of specific immune responses which can be readily demonstrated in *in vitro* assays, viable hydatid cysts frequently persist for long periods (Gemmell et al., 1986; Rausch et al., 1987). In addition, protoscoleces of *E. granulosus*, released through accidental rupture of a cyst or during surgery, are capable of generating secondary cysts. This indicates that in some circumstances the immune responses to the primary infection do not protect against subsequent infection.

The mechanisms by which established hydatid cysts survive in hosts resistant to re-infection and which have antibody, and cells capable of killing the parasite *in vitro* (Baron & Tanner, 1977), remain unknown. Evidence exists for the contribution of a variety of mechanisms to the successful evasion of the protective effects of the host's immune response against the larval stages of *Taenia* species. These include masking of surface antigens by host-derived or host-like antigens, immunosuppression, antigen sequestration and direct interference with the effector mechanisms of the host's defence system (reviewed by Rickard & Williams, 1982). In hydatidosis, recent studies have focused attention on the influence of the parasite on the specific and non-specific immune responses of the host. In experimental animals, *E. granulosus* and *E. multilocularis* infection induce pathological alterations in the architecture of lymphoid organs (Ali-Khan, 1978a; Riley, Dixon, Kelly & Cox, 1985). These gross alterations are associated with alterations in some immunological responses. During the proliferative growth phase of the *E. multilocularis* larval cyst mass in permissive mice, cell-mediated immune responses against parasite antigens are suppressed (Ali-Khan, 1978b) and leucocyte chemotaxis is inhibited (Alkarmi & Behbehani, 1989). These alterations reflect complex changes in the host responses rather than generalized immunosuppression, since cell-mediated immune responses to non-specific antigens are not suppressed (Ali-Khan, 1978b). Alveolar hydatidosis in man is associated with impaired T cell function in *in vitro* assays, a reduction in the number and percentage of circulating B lymphocytes and a reduction in the number of peripheral blood lymphocytes (Vuitton, Lasségue, Miguet, Hervé, Barale, Seillés & Capron, 1984). Reduced reactivity to lymphocyte mitogens *in vitro* (Riley, Dixon, Jenkins & Ross, 1986) and suppression of antibody responses to sheep red blood cell (SRBC) antigens (Allan, Jenkins, Connor & Dixon, 1981) also occur in mice infected with *E. granulosus*. The suppression of anti-SRBC reactivity was shown by Allan et al. (1981) to be associated with a population of suppressor T cells which were highly suppressive to anti-SRBC responses of syngeneic recipient normal mice in adoptive transfer experiments.

Another immunopathological correlate of hydatid infection is the detection of auto-antibodies to a wide spectrum of antigens in patients infected with *E. granulosus* or *E. multilocularis* (Pini, Pastore & Valesini, 1983; Ben Izhak & Tatarsky, 1985; Ameglio, Saba, Bitti, Aceti, Tanigaki, Sorrentino, Dolei & Tosi, 1987; Mori, Wernli, Weiss & Franklin, 1986) (see Table 1). Particularly interesting is the discovery of auto-antibodies to major histocompatibility complex antigens (Ameglio et al., 1987) because of the pivotal role these molecules play in antigen presentation to T cells in the initiation of immune responses. It may be relevant that a role has been postulated for alloreactive autoimmune T cells in the survival of *E. granulosus* cysts in mice (Allan et al., 1981). *E. granulosus* protoscoleces have been found to express a protein with a high degree of amino acid sequence homology with a human protein termed cyclophilin (Lightowlers, Haralambous & Rickard, 1989). In man, the binding of an undecapeptide of fungal origin (cyclosporin A) to cyclophilin has profound suppressive effects on T cell function due in part to the suppression of the early events in T cell activation (Hess, Esa & Colombani, 1988). Cyclophilin has been found to be a peptidyl prolyl cis-trans isomerase (Takahashi, Hayano & Suzuki, 1989). The degree of sequence conservation in this protein between a

---

**TABLE 1—ANTIGENS TO WHICH AUTOANTIBODIES HAVE BEEN DETECTED IN THE SERA OF PATIENTS INFECTED WITH Echinococcus granulosus OR E. multilocularis**

<table>
<thead>
<tr>
<th>Parasite infection</th>
<th>Antigen</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. granulosus</em></td>
<td>Smooth muscle</td>
<td>Pini et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Erythrocyte</td>
<td>Ben Izhak &amp; Tatarsky (1985)</td>
</tr>
<tr>
<td></td>
<td>MHC* Class I</td>
<td>Ameglio et al. (1987)</td>
</tr>
<tr>
<td></td>
<td>MHC* Class II</td>
<td>Ameglio et al. (1987)</td>
</tr>
<tr>
<td><em>E. granulosus</em>/</td>
<td>Double-stranded DNA</td>
<td>Mori et al. (1986)</td>
</tr>
<tr>
<td><em>E. multilocularis</em></td>
<td>Histone</td>
<td>Mori et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Actin</td>
<td>Mori et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Desmin</td>
<td>Mori et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Vinventin</td>
<td>Mori et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Keratin</td>
<td>Mori et al. (1986)</td>
</tr>
</tbody>
</table>

* MHC, Major Histocompatibility Complex; antibodies found to several different Class I and Class II antigens.
cestode parasite and man suggests that the molecule plays an important biological role. The association between the mammalian molecule and immunosuppression, and the existence of a very similar molecule produced by a chronic, tissue-dwelling parasite, may be merely coincidental. If, however, the parasite were also to produce a ligand for the cyclophilin-like molecule, which had some of the suppressive activities of cyclosporin A, this ligand could have a role to play in the long-term survival of hydatid cysts in infected hosts. It is interesting to note that cyclosporin A has been found to interfere with the deletion of autoreactive T cells in the thymus leading to the appearance of autoimmune disease (Shi, Sahai & Green, 1989).

**SERODIAGNOSIS**

*Man*

Hydatidosis in man leads to the production of readily detectable levels of specific antibody in the majority of infected individuals which allows successful serodiagnosis of infection (reviewed by Rickard & Lightowlers, 1986; Schantz & Gottstein, 1986). Not all infected patients are positive in assays for specific serum antibody, and cross-reactivity occurs between Echinococcus antigen preparations and antibodies in the sera from patients with other helminth infections. Cross-reactivity is particularly a problem in differentiating between infections with E. granulosus, E. multilocularis and T. solium. Difficulties with wide-scale application of serodiagnosis occur due to limitations in supply of parasite antigens for tests and in quality control of antigen preparations derived from different batches of hydatid cysts. These areas—sensitivity, specificity and antigen supply—are the major topics currently being investigated in research towards improving diagnostic tests for human hydatidosis. Assays based on detection of circulating parasite antigen in the sera of patients infected with E. granulosus are useful for diagnosis of a proportion of cases negative in other serological tests (Craig & Nelson, 1984; Gottstein, 1984; Craig, 1986).

Immunochromatographic analysis of the diagnostic antigens and the development of monoclonal antibodies against the two major lipoprotein antigens of hydatid cyst fluid (Antigen 5 and Antigen B) have provided valuable information on the sensitivity of detection and specificity of individual antigens (Di Felice, Pini, Afferni & Vicari, 1986; Shepherd & McManus, 1987; Lightowlers, Liu, Haralambous & Rickard, 1989). The antigenic subunits of the major diagnostic antigen, Antigen 5, which forms Arc 5 in immunoelectrophoresis (IEP) (Capron, Yarzábal, Vernes & Fruit, 1970; Yarzábal, Schantz & López-Lemes, 1975), are immunoprecipitated strongly by most sera from patients with either E. granulosus or E. multilocularis infection (Lightowlers, Liu, Haralambous & Rickard, 1989). They are also non-specifically precipitated by sera from patients with no known parasitic disease (Shepherd & McManus, 1987; Lightowlers, Liu, Haralambous & Rickard, 1989). This contrasts with the specificity of the antigen in IEP, where Arc 5 has not been found except in patients infected with taenid cestodes (see Rickard & Lightowlers, 1986). Shepherd & McManus (1987) found that Antigen 5 contained phosphorylcholine epitopes and that addition of 10 mmol-l⁻¹ phosphorylcholine to the serum diluent reduced non-specific reactivity with cyst fluid antigens in ELISA. The lower molecular weight subunits of Antigen B were found by Shepherd & McManus (1987) to be specific for E. granulosus. A pool of sera from four patients with E. multilocularis infection and another pool from two patients with T. solium cysticercosis did not show reactivity with these subunits in immunoprecipitation analysis. More detailed investigations using larger numbers of sera individually have, however, shown unequivocally that these antigens are not species specific for E. granulosus (Lightowlers, Liu, Haralambous & Rickard, 1989).

Production of antigens for immunodiagnosis using recombinant DNA techniques has the potential to improve the specificity and sensitivity of diagnosis as well as alleviating problems with quality control and supply of antigens derived directly from hydatid cysts. A cDNA library has been prepared from E. granulosus protoscolex mRNA in λgt11 and clones expressing potentially useful diagnostic antigens have been identified using sera from Australian patients with cystic hydatidosis (Lightowlers, unpublished). Two clones in particular show high sensitivity for diagnosis in plaque immunoassay with sera from patients prior to any surgical or chemotherapeutic treatment for hydatid infection (initial diagnosis). One of these clones has been tested extensively in ELISA as a glutathione S-transferase (GST) (Smith & Johnson, 1988) fusion protein and gives a positive reaction with 50% of initial diagnosis sera. A difficulty with this test based on use of GST fusion protein is background reactivity of normal human sera with the fusion protein partner, GST (Lightowlers & Mitchell, 1989). Continuing experiments are investigating the use of this protein expressed using the pGEX2 plasmid vector (Smith & Johnson, 1988) and cleaved from GST at the thrombin cleavage site created by the polylinker sequence in this vector. Other cloned antigens are also being evaluated in the ELISA to improve the sensitivity of the test. These antigens show no cross-reactivity with sera from patients with protozoan, nematode or trematode infections, but they do cross-react with sera from patients infected with E. multilocularis and T. solium. The clones are being investigated for the possible presence of multiple epitopes, some of which may show specificity of E. granulosus and could be subcloned with the cross-reacting epitopes excised.

Immunochromatographic analyses and antibody affinity chromatography techniques (Rickard, Honey. Brumley & Mitchell, 1984) have not been successful to date in identifying an antigen preparation or individual antigens specific for E. granulosus. In contrast, Gottstein and his colleagues have been successful in identifying an antigen specific for E. multilocularis. Rabbit
antibodies against *E. granulosus* were used to affinity deplete a crude *E. multilocularis* antigen preparation and the depleted antigen, termed Em2, shown to have high specificity for discriminating between sera from *E. multilocularis* and *E. granulosus*-infected patients (Gottstein, Eckert & Fey, 1983). The specific component Em2a was purified and found to be a molecule of molecular weight 54,000, pI 4.8 (Gottstein, 1983, 1985). In an international study (Gottstein, Schantz, Todorov, Saimot & Jacquier, 1986), sera from 50 *E. granulosus* and 32 *E. multilocularis*-infected patients were tested and only three *E. granulosus* were incorrectly assigned as being *E. multilocularis* on the basis of the reactivity of the sera in Em2 ELISA. Sera from 12,000 blood donors from an *E. multilocularis* endemic region in Switzerland were screened in Em2 ELISA and five found positive, one of which was confirmed to have been from a patient infected with the parasite (Gottstein, Lengeler, Bachmann, Hagemann, Kocher, Brossard, Witassek & Eckert, 1987).

Using recombinant DNA techniques, a clone expressing an antigen specific for *E. multilocularis* has been identified, although it does not appear to be related to the Em2 antigen(s) (Vogel, Gottstein, Müller & Seebeck, 1988). The clone produces an unstable fusion protein. However subclones from the initial cDNA clone identified in ggll have been successful in improving the yield of parasite antigen (Müller, Gottstein, Vogel, Flury & Seebeck, 1989; Müller, Vogel, Gottstein, Scholle & Seebeck, 1989). ELISA with the stabilized protein has been found to have a diagnostic sensitivity of 90% and a specificity of 99%; cross-reaction was detected with only one of 108 *E. granulosus* sera and two of 15 *T. solium* sera (Müller, Gottstein, Vogel, Flury & Seebeck, 1989). Recombinant *E. multilocularis* antigens with potential for use in serodiagnosis have also been produced by another group (Hemmings & McManus, 1989).

### Animal intermediate hosts

Seraological diagnosis of *E. granulosus* infection in domestic livestock has not been successful. The principal problems stem from the production of very low or undetectable levels of specific antibody in many animals with fertile cysts and in cross-reactivity between antibodies raised against other parasites, particularly taeniid cestodes, and hydatid antigens (reviewed by Rickard & Lightowlers, 1986). Some success has been achieved in distinguishing flocks of infected sheep (Lightowlers, Rickard, Honey, Obendorf & Mitchell, 1984; Ris, Hamel & Mackle, 1987), however, reliable diagnosis of individual infected animals has not been achieved. The low level of specific antibody production contrasts with the situation in man where *E. granulosus* typically leads to the production of readily detectable levels of specific antibody. Infected sheep, however, are capable of responding to parenteral immunization with cyst fluid antigens, including fluid released from an animal's own cysts (Lightowlers, Rickard & Honey, 1986). This suggests that antigen sequestration in sheep is more likely to be the cause of the low levels of specific antibody rather than antigen-specific or generalized immunosuppression. The finding that some sheep with low levels of detectable specific antibody have circulating hydatid antigens in serum (Judson, Dixon, Clarkson & Pritchard, 1985) appears to contradict the sequestration hypothesis. On the basis of the results of studies published to date, there is little prospect for the development of a diagnostic test for hydatidosis in domestic livestock based on detection of antibody in serum.

### Diagnosis of *E. granulosus* in dogs

Dogs infected with *E. granulosus* have been found to produce specific circulating antibodies against the parasite (Chordi, Gonzalez-Castro & Tormo, 1962; Movsesian & Mladenovic, 1971; Jenkins & Rickard, 1986; Gasser, Lightowlers, Obendorf, Jenkins & Rickard, 1988). In a carefully controlled experiment, Jenkins & Rickard (1986) followed the course of production of specific antibodies in experimentally infected dogs through to patency and showed that the protoscolex antigens used in the test did not cross-react with sera from infected with *Taenia* species or nematode parasites. A diagnostic test for *E. granulosus* infection in dogs would be of value in studies on the epidemiology of infection and in hydatid control campaigns. The widespread availability of the highly cestocidal drug praziquantel has limited the use of arecoline purging for diagnosis of infection. The potential for a diagnostic test has been shown by Gasser and his colleagues (Gasser et al., 1988; Gasser, Lightowlers, Rickard, Lyford & Dawkins, in press) using ELISA with protoscolex antigens and sera from dogs with naturally acquired infection. A major limitation in practical application of the test is in the limited supply of suitable antigen. Here again, recombinant DNA techniques have been successful in isolating diagnostic cloned antigens. Initially, immunochemical analyses were used to identify specific diagnostic components of protoscoleces (Gasser, Lightowlers & Rickard, 1989). Antibodies from naturally infected dog sera were affinity purified on Western blots and used to isolate cDNA clones expressing the antigens (Gasser, Lightowlers & Rickard, unpublished). Specificity of one cloned protein tested in ELISA was found to be 100%, but sensitivity was low. Continuing studies aim at improving the sensitivity of the test by combined application of other cloned antigens. Other possible approaches to diagnosis of infection in dogs are the specific identification of *E. granulosus* eggs in faeces with monoclonal antibodies (Craig, Macpherson & Nelson, 1986; Craig, Macpherson, Watson-Jones & Nelson, 1988) and detection of copro-antigens (Deplazes, Gottstein, Stingelin & Eckert, 1989).
**VACCINATION**

Two features common to taeniid cestode infections in their intermediate hosts are the natural development of highly protective immune responses against re-infection and the spectacular success achieved in experimental vaccination against infection (reviewed by Rickard & Williams, 1982). High levels of protection have been achieved in vaccination trials against *E. granulosus* infection in sheep. Gemmell (1966) used viable *E. granulosus* eggs or activated oncospheres injected intramuscularly into sheep and demonstrated protection against the establishment of hydatid cysts from a challenge infection (91.2 and 92.0%, respectively) and also protection against post-encystment survival of cysts (99.6 and 100%, respectively). Heath, Parmeter, Osborn & Lawrence (1981) achieved even greater levels of protection following subcutaneous injection of activated *E. granulosus* oncospheres on two or more occasions. Of 25 vaccinated sheep only two animals had any cysts and only a single viable cyst was found. High levels of protection against *Taenia* infection have been achieved using non-living antigen preparations (Rickard & Williams, 1982). Osborn & Heath (1982) have shown that non-living antigens can also be used against *E. granulosus* in sheep. Medium from *in vitro* culture of *E. granulosus* oncospheres was filtered through a 0.2 \( \mu \text{m} \) membrane. Following intramuscular injection into sheep it was shown to stimulate 99.4% protection against the establishment of hydatid cysts compared with controls. Only one of eight vaccinated sheep developed cysts.

The major obstacle in practical application of vaccination against *E. granulosus* infection in domestic livestock is supply of antigen, particularly since antigens from oncospheres have achieved higher levels of protection compared with antigens from other life cycle stages (Turner, Dennis & Berberian, 1937; Dada & Belino, 1981). The recent development of the first highly successful recombinant vaccine against a parasite (Johnson, Harrison, Lightowlers, O'Hoy, Cougle, Dempster, Lawrence, Vinton, Heath & Rickard, 1989) represents a major advancement in overcoming difficulties with supply of parasite antigens for vaccination. The vaccine, which is being developed commercially, protects sheep against infection with *Taenia ovis*. Messenger RNA was isolated from activated *T. ovis* oncospheres and cDNA cloned into the bacteriophage expression vector *Ag*ll. The recombinant plaques were screened for reactivity with affinity-purified antibodies prepared against a pair of native oncosphere antigens. These native antigens had been found to be associated with protection in a series of vaccination trials in sheep using fractionated oncosphere antigens. The parasite-encoded protein was isolated from *Escherichia coli* as fusion proteins with the bacterial enzyme \( \beta \)-galactosidase (\( \beta \)-gal). In vaccination trials, these \( \beta \)-gal fusion proteins failed to protect sheep against challenge infection with *T. ovis*. However, they did raise antibodies specific for the two native oncosphere antigens, for which there was strong evidence to indicate they were likely to be host-protective. A second expression vector was used with the cloned insert DNAs. This vector was a fore-runner of the pGEX series of vectors (Smith & Johnson, 1988) and expressed the *T. ovis* antigens as fusion proteins with GST. One of the advantages of this vector system was that the GST fusion allows single step purification of the soluble fusion protein from *E. coli* lysates by affinity chromatography on glutathione agarose under non-denaturing conditions. Antigen purified in this way from one of the clones, designated 45W, achieved 94% protection against *T. ovis* infection in sheep when given as a total of 50 \( \mu \text{g} \) fusion protein over three injections with saponin.

It is possible to achieve a substantial degree of protection against infection with *Taenia* species and *E. granulosus* using antigens derived from an heterologous taeniid species (reviewed by Lightowlers, Mitchell & Rickard, in press). Indeed antigens from *T. ovis* have been found to cross-protect against *E. granulosus* infection in sheep (Gemmell, 1966; Heath, Lawrence & Yong, 1979). It is possible that the *T. ovis* 45W antigen may be used directly to vaccinate against *E. granulosus*. Protection with antigens from an heterologous species has typically been less than that achieved with homologous antigens. Apparently, the native equivalent of the 45W clone protein is exposed on the surface of the *T. ovis* oncosphere, or performs some important function in its invasion/establishment in the host, since immune responses against this specific molecule are able to prevent infection. Oncospheres of other taeniid species may contain a protein homologous to the *T. ovis* 45W clone protein which occupies a similar vulnerable position or performs a similar biological function and may also be susceptible to host immune attack. Nucleotide sequence homology between the *T. ovis* gene and the equivalent genes in other species would allow rapid identification of clones encoding these genes in recombinant cDNA libraries from oncospheres of these other taeniid species. In this way, the *T. ovis* vaccine may pave the way towards the rapid development of practical vaccines against infection with other species, including *E. granulosus*. The recent development by Dempster and his colleagues (unpublished), of a mouse model system for egg-derived infection with *E. granulosus*, will provide a valuable model system in research towards development of a vaccine against cystic hydatidosis.

**REFERENCES**


AlI-KHAN Z. & SIIBO R. 1980. Pathogenesis and host


Craig P. S. 1986. Detection of specific circulating antigen, immune complexes and antibodies in human hydatidosis from Turkana (Kenya) and Great Britain, by enzyme-immunoassay. Parasite Immunology 8: 171–188.


Turner E. L., Dennis E. W. & Berberian D. A. 1937. The


