REVIEW OF LITERATURE
2.0 REVIEW OF LITERATURE

The literature pertaining to immunodiagnosis, copro-antigen detection and molecular diagnosis of Echinococcosis in dogs with special reference to Enzyme Linked Immunosorbent Assay (ELISA), Sodium do-decyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting and Polymerase chain reaction based assays have been reviewed.

2.1 Immunodiagnosis of echinococcosis

Studies conducted by Gasser et al. (1988) demonstrated that 25–60 percent of the sera from dogs infected with *Echinococcus granulosus* did not show significant levels of specific antibody, and revealed cross-reactivity with other parasite species.

Jenkins et al. (1990) evaluated somatic antigen of *Echinococcus granulosus* for serodiagnostic purpose and found variable diagnostic sensitivity and high cross-reactivity with antigens from other parasite species. They demonstrated that 25-60 percent of the sera from dogs infected with *Echinococcus granulosus* did not show significant levels of specific antibody and cross-reactivity with other parasite species occurred.

The diagnosis of current intestinal *Echinococcus granulosus* infection on an individual dog was not always reliable by serology as observed by Gasser et al. (1993). They reported that overall specificities ranged between 97.3 and
100 per cent and sensitivities ranged between 73 and 84 per cent for IgG, IgA and IgE ELISA.

Ersfeld et al. (1997) analyzed adult worm extracts of *Echinococcus granulosus* by ELISA and showed a sensitivity of 83 per cent for cystic echinococcosis. They showed that *Echinococcus granulosus* adult worms could provide an alternative source to metacestode antigens (native and recombinant) for the serodiagnosis of cystic echinococcosis.

Zhang et al. (2003) found poor sensitivity and specificity in ELISA based methods for detection of circulating antibodies in canines. There was no correlation of ELISA results with worm burden.

Carmena et al. (2005a) demonstrated the presence of antibodies that recognized excretory-secretory antigens of *Echinococcus granulosus* protoscoleces in sera from dogs infected with *Echinococcus granulosus* and other helminths. They indicated that excretory-secretory products contained potential diagnostic antigens that could be used in the immunodiagnosis of canine echinococcosis. The protein of 89 kDa was found to be most promising.

Reiterova et al. (2005) found that the detection of circulating antibodies was unsuitable for estimating the actual parasite prevalence in definitive hosts because of insufficient correlation between the serum antibodies and the presence of intestinal worm burden.
Carmena et al. (2006) observed that somatic extracts from protoscoleces were the suitable source of antigens for the immunodetection of *Echinococcus granulosus* infection in dogs and other canids. The initial immunological responses to *Echinococcus granulosus* in the definitive host were said to be directed against infective protoscoleces and later against the adult parasite.

Zhang and McManus (2006) used polypeptides encoded by egM4 and egM123 for serodiagnosis of *Echinococcus granulosus* in definitive hosts besides their encouraging results in pilot vaccine/challenge experiments in dogs which showed reduced egg production, suggesting their value as potential vaccine candidates.

### 2.2 Copro-antigen Detection

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 *Echinococcus 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.

Kohno (1991) used sandwich ELISA for detecting the coproantigens in faeces and circulating antigens in sera of experimentally infected dogs. *Echinococcus spp.* coproantigens were detected in 3 of the 4 infected dogs as early as 4 to 7 days postinfection in the prepatent period.
Allan et al. (1992) developed genus specific coproantigen detection ELISA incorporating polyclonal antibodies against somatic and excretory secretory antigens of *Echinococcus granulosus*. They reported high specificity (over 96 per cent) with good sensitivity (87.5 per cent) and could detect infection when the post mortem burden is as low as 15-30. They reported that coproantigen tests were genus specific and had a specificity of approximately 97 per cent (when worm burdens are more than 50–100 worms). Coproantigen tests had facilitated large-scale screening of definitive hosts; however the overall average test sensitivity was approximately 60 per cent for natural canine *Echinococcus granulosus* infection.

Deplazes et al. (1992) developed an ELISA test based on a parasite specific layer of capture IgG antibodies that retain antigens from faecal supernatants. They obtained sensitivity up to 93% and specificity up to 99 percent for this assay and reported it as one of the most useful ways for collecting prevalence data in large communities. They observed that the accuracy of coproantigen detection in canine echinococcosis was dependent on the parasite burden as they detected only one in eight dogs infected with less than 100 worms, using polyclonal antibodies produced against E/S products from adult tapeworms.

Gottstein (1992) used affinity-purified polyclonal antibodies raised against excretory secretory antigens of adult-stage tapeworms to develop an ELISA for the detection of coproantigens from *Echinococcus granulosus* in dog
or fox faecal samples as alternative to the coprological or serological diagnosis of adult-stage *Echinococcus* infections in definitive hosts.

Sakashita (1992) identified coproantigen detection as a more effective diagnostic method than detection of circulating antibodies or fecal examination for *Echinococcus granulosus*. Copro ELISA facilitated diagnosis during the early stages of infection and provided a good estimate of the parasite burden in the host. Freezing and subsequent heating or formalin treatment of the feces as a safety precaution had not affected the sensitivity of the ELISA and it also reduced the biohazard involved in routine diagnosis.

Craig *et al.* (1995) observed only a minimal difference in prevalence of *Echinococcus granulosus* during field studies with copro-ELISA test and arecoline test in Uruguay (22.7 per cent and 23.6 per cent, respectively). However, they reported that the detection of current infection of individual dogs with *Echinococcus granulosus* by coproantigen ELISA had the potential to replace arecoline purgation, while specific serum antibody detection should be useful in assessing *Echinococcus* exposure in dog populations.

Nonaka *et al.* (1996) developed an assay to detect heat resistant coproantigen in heat sterilized fecal samples and found it to be a useful method for the diagnosis of *Echinococcus spp.* in definitive host in natural infection and also for monitoring parasite development and change in parasite burden during an experimental infection. The detection limit of the assay was estimated to be around 100 worms.
Accurate diagnosis of *Echinococcus* infection in definitive hosts had always been an important component for establishing epidemiological parameters of echinococcosis and preventing human and livestock infection. Sakai (1996) observed that coproantigen detection, which recognized excretory/secretory (ES) products of parasites in faeces, corresponded well to the presence of worms in the intestine and hence was a good tool for diagnosis of infected definitive hosts.

Fraser and Craig (1997) developed a simple method of coproantigen ELISA which involved a pure IgG capture antibody layer isolated from rabbit hyperimmune sera directed against a crude somatic extract of immature adult proglottids. They reported high genus specificity with polyclonal antibodies, even when crude somatic proglottid extracts were used. The very stable carbohydrate rich molecules in coproantigens retained the antigenicity in the gut and fecal environment.

Furusawa (1997) developed a Dot-ELISA, suitable for field surveys in which a nitrocellulose membrane coated with biotinylated capture antibody as primary antibody and streptavidin biotinylated horseradish peroxidase (HRP) complex were used to visualize the presence of the coproantigens for 'on the spot' diagnosis.

Malgor *et al.* (1997) developed a sandwich ELISA for the detection of *Echinococcus granulosus* coproantigen in formalin and heat-treated faecal supernatants of dogs. The assay used affinity-purified polyclonal antibodies
obtained from rabbits hyperimmunised with *Echinococcus granulosus* excretory/secretory antigens and biotinylated monoclonal antibody EmA9 produced against adult *Echinococcus multilocularis* somatic extract.

Ahmad and Nizami (1998) found that CCIEP was a simple, inexpensive and rapid test where results could be determined approximately within 45-60 min indicating its potential for wider application in the coprological diagnosis. They developed an indirect ELISA for detection of coproantigens of *Echinococcus granulosus* which permitted the detection of the parasite during the prepatent period.

Cohen *et al.* (1998) conducted a survey among 117 dogs in a Uruguayan community and showed that almost 20 per cent were found infected with *Echinococcus granulosus* after purge examination. The dogs with mean number of 67 worms and other eight dogs that were purge negative were found to be positive. The coproantigen ELISA for *Echinococcus* was shown to be highly specific (>95 per cent) and very sensitive (100 per cent) when purge worm counts were greater than 20. They reported the reliability of immunodiagnostic tests for canine echinococcosis based on serum antibody detection and particularly coproantigen detection which could provide a more effective methodology for screening and identification of dogs currently infected with *Echinococcus granulosus* tapeworms.

Dinkel *et al.* (1998) observed poor correlation between antibody titers and the presence of worms in the intestine and reported unsuitability of
serological screening for the diagnosis of infections with *Echinococcus spp.* in definitive hosts. They reported the reliability of coproantigen enzyme-linked immunosorbent assays in the detection of heavy infections which were responsible for the bulk of environmental contamination and thus its suitability for epidemiological purposes.

Nonaka *et al.* (1998) analysed the coproantigens and reported that they contained parasite specific products such as breakdown products of proglottids or excretory/secretory antigens in the host faeces. They concluded that coproantigen detection assays would be a practical tool for epidemiological studies of the definitive hosts of *Echinococcus*.

Tanaka (1998) compared serum IgG, IgA and IgM, and copro IgA against somatic and excretory/secretory antigens of adult tapeworms and protoscolices of *Echinococcus multilocularis* in dogs experimentally infected with *Echinococcus multilocularis* by ELISA. Positive results were obtained in all except copro IgA.

Konapur *et al.* (1999) used CIEP for determining the prevalence of cystic echinococcosis in cattle. They observed a sensitivity and specificity of 90.7 and 88.1 per cent, respectively for the assay.

The frequency of canine echinococcosis in central Peruvian Andes was 46 (23/50) and 32 percent (16/50) respectively by the coproantigen ELISA test and arecoline purging in a study conducted by Moro *et al.* (1999). The
sensitivity of the coproantigen ELISA was 50 percent (8/16), compared to arecoline purge as ‘gold standard’. They recommended both coproantigen ELISA and arecoline purging for the study of canine echinococcosis.

Guarnera et al. (2000) estimated that the antigens of *Echinococcus spp.* persisted for upto 20 days within canine feces under rural conditions in the north of Argentina and found that coproantigens could be preserved within such samples for as much as 13 months in closed containers at 4°C. They devised an ELISA using canine faeces collected from the ground to detect dogs infected with *Echinococcus granulosus*, thus determining sheep farms with active transmission.

Jenkins et al. (2000) reported high stability of coproantigen to variable environmental conditions for up to 6 days. They demonstrated positive test results after storage of fecal samples collected from environment at -20°C for 1 year.

Shimizu (2000) developed a simple and rapid latex agglutination test for the detection of *Echinococcus* coproantigens in the definitive host.

Eckert et al. (2001a) were of the opinion that coproantigen ELISA was the best overall laboratory based test for ante mortem diagnosis of canine or vulpine echinococcosis caused by *Echinococcus granulosus* or *E.multilocularis* species.
The results of the two *Echinococcus multilocularis* specific ELISA tests by Raoul *et al.* (2001) showed no difference in the detection of specific coproantigens collected from humid, medium or dry faeces. They analysed coproantigen measurement data by the comparison of the whole distributions of ELISA OD values and reported that the results were more reliable compared with the use of coproantigen prevalence based on arbitrary cut-off value.

Zoljargal *et al.* (2001) compared coproantigen detection with fecal egg examination in Japan and showed that only 6 samples were positive by both methods. Of the taenid egg positive samples 6 were coproantigen negative, and eggs were not found from 11 coproantigen positive faecal samples. This could indicate the possibility of infection with taenid species other than *Echinococcus spp.* or *Taenia hydatigena*. It was observed that the coproantigen detection assay had higher sensitivity when compared with fecal examination of eggs.

Commercial copro-ELISA kit (Genzyme Virotech) had a sensitivity and specificity of 76 and 95 per cent, respectively, while Bommeli Diagnostics claimed 90.9 per cent sensitivity and 98.8 percent specificity. Christofi *et al.* (2002) observed the sensitivity and specificity of the Bommeli Diagnostics ELISA kit to be 83 and 98 percent in areas of Cyprus where the prevalence of *Echinococcus granulosus* infection in dogs was 0.2 percent, although the specificity was reduced to 80 per cent in a group of dogs infected
simultaneously with *Taenia* spp. They attempted two options for dealing with the relatively small percentage of coproantigen-positive dogs, re-examination by arecoline purging with subsequent treatment of those animals infected with *Echinococcus granulosus* or treatment of all coproantigen ELISA positive dogs with praziquantel without re-examination. The latter option was found to be cheaper and more practical.

Svobodova and Lenska (2002) reported 8.1 per cent prevalence of echinococcosis in dogs in the Czech Republic by coproantigen ELISA.

Abbasi *et al.* (2003) observed that the diagnosis of *Echinococcus granulosus* infection based on parasitologic detection of adult worms at necropsy or after arecoline purgation were difficult to use in large scale epidemiologic studies because they were laborious, bio-hazardous, and lacked sensitivity. It was reported that coproantigen detection assays were more suitable and practical for this purpose.

Allan *et al.* (2003) defined parasite coproantigens as parasite specific products in the faeces of the host that were amenable to immunological detection and were associated with parasite metabolism. They should be present independent of parasite reproductive material (i.e. *Taenia* eggs or proglottids) and should disappear from faeces shortly after removal of the intestinal infection. They evaluated coproantigen ELISA for the diagnosis of *Echinococcus granulosus* in dogs and reported that coproantigen detection was possible prior to patency and the levels were independent of the presence of
worms or number of eggs. Coproantigens were no longer detectable within a week of treatment of dogs with praziquantel. Coproantigens were stable for many 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.

Detection of coproantigens of *Echinococcus granulosus* by ELISA showed higher sensitivities between 84 and 95 per cent, combined with very high specificity of more than 96 percent (Eckert, 2003).

Elayoubi et al. (2003) found the presence of significant levels of carbohydrate in the *Echinococcus granulosus* coproantigens. Periodate treatment of coproantigen positive faecal supernatants resulted in a significant reduction (53 per cent) in ELISA activity and established carbohydrates in the antigenic structure of *Echinococcus granulosus* coproantigens. Protease treatment of antigenic molecules resulted in an 11 per cent reduction in absorbance in ELISA, and indicated that protein components were also present. They subsequently used lectin-binding assay and specific enzyme treatments and showed that α–mannose, α–glucose, β–galactose and N-acetyl-β-glucosamine residues were important components of *Echinococcus granulosus* adult antigens present in infected dog faeces. They suggested that the coproantigen molecule was probably very large (>100 kDa) and may contain a peptide core, as indicated by a degree of susceptibility to
protease cleavage and related to glycocalyx molecules from the tegument of the adult worm.

A zero prevalence of *Echinococcus granulosus* in dogs was reported by Koski *et al.* (2003) in Finland by coproantigen ELISA using two commercial coproantigen ELISA kits of Genzyme Virotech and Bommeli Diagnostics.

A survey was conducted by Lopera *et al.* (2003) in Peru and an overall prevalence of 79 per cent (84/106) was found in canines with ELISA and the frequency of canine echinococcosis was found to be 82 per cent (61/74) and 34 per cent (25/74) by the coproantigen ELISA and arecoline purging, respectively. They used dogs from middle-class owners from Lima city in Peru as negative controls as these had received anti-parasitic treatment and lived in areas where *Echinococcus granulosus* was virtually non-existent. The sensitivity and specificity of the coproantigen ELISA was 88 and 95 percent, respectively. They reported that an important limitation of coproantigen ELISA could be its application in areas of low endemicity where the predictive positive value would be expected to be low and where potential cross-reactions with other *Taenia* spp. could occur.

Coproantigen detection of *Echinococcus granulosus* using faeces collected from the environment by Raoul *et al.* (2003) allowed the estimation of relative infection in faeces and thus the level of infection pressure in the area, rather than an estimate of the parasite prevalence in individual animals.
Deplazes et al. (2004) indicated the detection of *Echinococcus* specific coproantigens by enzyme-linked immunosorbent assay (ELISA) as a novel approach for diagnosis of intestinal *Echinococcus* infection and reported that this method had proved its value for the diagnosis in both live and dead animals. The authors also pointed out that coproantigen detection was the method of choice for mass screening because it was sensitive, fast and cheap.

Elayoubi and Craig (2004) fractionated supernatants prepared from *Echinococcus granulosus* infected dog faecal samples by size-exclusion fast protein liquid chromatography (FPLC) and showed that the antigens were large molecular weight molecules that might be derived from the carbohydrate-rich surface glycocalyx of adult worms, and were shed, released or secreted during the life-span of the tapeworm.

Benito and Carmena (2005) determined an overall sensitivity of 78.4 per cent and specificity of 93.3 per cent, to sandwich ELISA in Argentina for the detection of *Echinococcus* coproantigens. Positive and negative predictive values were 72 and 95 per cent respectively, and the diagnostic efficiency was 90.5 per cent. In addition, the sandwich ELISA detection limit was estimated to be 5.12 ng/ml of protoscolex excretory/secretory antigen.

Buishi et al. (2005) used coproantigen ELISAs successfully under field conditions in a high endemic region of northwest Libya and reported a prevalence of *Echinococcus granulosus* of 25.8 per cent (15/58) in stray dogs by necropsy and of 21.6 per cent (72/334) by coproantigen ELISA in owned
dogs and reported it as a useful tool for epidemiological studies and for surveillance of screening programs. They observed a specificity of 98.1 per cent and sensitivity of 100 per cent for coproantigen ELISA based on a comparison with necropsy findings. Dogs that were reported to eat offal were nearly seven times more likely to test positive for *Echinococcus granulosus* coproantigen. Deworming of dogs and owner knowledge about hydatid disease were associated with a decreased risk of a coproantigen positive dog.

Casaravilla *et al.* (2005) developed a coproantigen capture ELISA using a rabbit polyclonal antibody against E/S products from adult tapeworms as capture antibodies. They found that the detection limit of the coproantigen assay using coproantigen capture ELISA with affinity-purified polyclonal IgG anti *Echinococcus granulosus* somatic extract was related to the biomass and the antigen production capacity of growing parasites. They obtained false negative results from dogs with less than 20 worms detected at purge. Although the test showed a very high sensitivity (100 per cent) in naturally and experimentally infected animals, cases of cross-reactivity with *Taenia hydatigena* were observed.

Cavagion *et al.* (2005) screened dog faecal samples to evaluate the prevalence of *Echinococcus granulosus* infection in sheep farms of the Argentine Patagonia. A total of 1042 samples were obtained from 352 sheep farms of which 26 (7.3 per cent) were found positive by coproantigen ELISA. They reported that identification of parasitized dogs was an essential activity
upon which rested the strategy of control and surveillance. Coproantigen test with fecal matter obtained directly from the dog contributed information on individual prevalence, while the use of coproantigens detected in samples collected from the ground was of greater epidemiological value.

Moro et al. (2005) undertook diagnosis of canine echinococcosis using the coproantigen ELISA in areas of Peru where no other diagnostic techniques are available and found an overall prevalence of 51 per cent (31/61) of canine echinococcosis in endemic areas using the coproantigen ELISA. The interpretation of results regarding sensitivity of the coproantigen ELISA varied according to the number of tapeworms in the dog intestine and dogs infected with a low tapeworm burden (<20 worms) could be mistaken as negative resulting in underestimation of the prevalence.

Reiterova et al. (2005) studied the correlation between the number of detected fecal samples and the value of optical density of coproantigen ELISA test in Slovakia. It was observed that when worm burdens were low (1–25 worms), the sensitivity of the method was 31.3 +/- 8.64 per cent and when worm burdens were more than 50 it was 81.8 +/- 0.66 per cent, and with high worm burdens of more than 1000 worms the sensitivity reached 100 +/- 0.34 per cent. They also found coproantigen ELISA to provide a highly complementary approach for specific diagnosis of canines infected with *Echinococcus granulosus* and *Echinococcus multilocularis*. 
Wei et al. (2005) evaluated the effect of slow releasing praziquantel medicated bars by coproantigen ELISA in north Xinjiang, China. Coproantigen of *Echinococcus granulosus* was found in 41.2 per cent of the dogs at the start of experiment. In the second and third year after medicated-bar implantation, coproantigen could not be detected in all the dogs examined but 3.0 per cent of dogs were positive at the end of the fourth year. They concluded that slow releasing praziquantel medicated bars efficiently blocked reinfection in dogs at least for 2 years.

Allan and Craig (2006) found positive correlation between coproantigen test sensitivity and worm burden with a reliable threshold level of greater than 50 worms for *Echinococcus spp*. They reported that application of the existing coproantigen tests in epidemiological and control programmes for *Echinococcus* and *Taenia* species infection had improved understanding of transmission and surveillance of these important zoonotic cestodes.

Benito et al. (2006) detected *Echinococcus* worms in 14.0 per cent of 721 dog faecal supernatants by coproantigen ELISA and in 9.1 per cent of 754 dog serum samples by serum antibody ELISA. In addition *Taenid* eggs were detected in 10.3 per cent of 726 faecal samples examined by coproparasitological (flotation and sedimentation) tests. They found no significant correlation between coproantigen ELISA OD values and age of dogs. This suggested that there were no differences in the prevalence of *Echinococcus granulosus* between old and young dogs.
Prathiush (2007) evaluated three different immunodiagnostic tests viz, CIEP, indirect ELISA and sandwich ELISA for detection of coproantigen of *E.granulosus* in dogs. The sensitivity and specificity of CIEP and indirect ELISA was found to be 50 & 100 and 100 & 71.11 per cent, respectively. The sandwich ELISA was found to be 100 per cent sensitive and 96.94 per cent specific.

### 2.3 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Laemmli (1970) demonstrated cleavage of structural proteins during the assembly of the head of bacteriophage T₄ using improved method of disk electrophoresis in sodium dodecyl sulphate (SDS) which combines the high resolution power of disk-electrophoresis with the capability of SDS to break down proteins into their individual polypeptide chains and proteins which separated according to their molecular weight.

Gasser *et al.* (1992) characterized excretory / secretory antigen of adult *Echinococcus granulosus* by SDS-PAGE under reducing conditions at 200 V for 1.5hrs using 15 per cent linear gels, but did not report their separated protein molecular weights.

Guarnera *et al.* (2000) prepared three types of antigen Viz, coproantigen from dry feces, fresh feces and adult worm antigen. They separated proteins of all the three antigens by SDS-PAGE, but did not indicate the molecular weights of the proteins.
Elayoubi and Craig (2004) prepared coproantigen and worm extracts of *E. granulosus* and subjected them to fast protein liquid chromatography on a superpose-6 column. Then the three eluted fractions were characterized by SDS-PAGE with 4 per cent stacking gel and 12 per cent linear separating gel with the molecular weight marker ranging from 4 to 250 kDa.

Casaravilla *et al.* (2005) used somatic extract and E/S products of adult *E. granulosus* for protein separation by SDS-PAGE in 10 per cent linear gel under reducing conditions.

### 2.4 Enzyme Immuno Tranfer Blot (EITB)

Towbin *et al.* (1979) devised a method for the electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets which resulted in quantitative transfer of ribosomal proteins from gels containing urea but the transfer was not quantitative with sodium dodecyl sulphate gels. The immobilized proteins were detectable by immunological procedures.

Western blot analysis of worm excretory secretory antigen and protoscolex somatic antigen of *E. granulosus* were studied by Gasser *et al.* (1992) using sera from infected and uninfected dogs. The results revealed that the relative molecular mass of antigenic components of worm excretory secretory antigen ranged between 94 to 39 kDa and these were specific for *Echinococcus granulosus* and were not identified in protoscolex somatic antigen.

Guarnera *et al.* (2000) prepared three types of antigen viz, coproantigen from dry feces, fresh feces and adult worm antigen. Copro-western blot assay
was performed and the sensitivity and specificity was found to be 70 per cent and 100 per cent, respectively.

Dhanalakshmi (2003) used EITB for the sero-diagnosis of *Taenia solium* cysticercosis in pigs and found that the sensitivity varied from 40-53, 57.4-91.4 per cent and 85-100 per cent with whole cyst antigen, somatic and excretory secretory antigen, respectively whereas their specificity was 100 per cent.

Elayoubi and Craig (2004) reported that the immunoblotting assay of fractionated faecal supernatant of *E.granulosus* revealed the molecular weight to be more than 670 kDa and ranged from 146 to 440 kDa. The *E.granulosus* worm extract detected a molecule with an approximate molecular weight of 155 kDa.

Casaravilla *et al.* (2005) produced two IgM murine monoclonal antibodies EgC1 and EgC3 against the excretory/secretory products of *Echinococcus granulosus* adult worms. Immunoblotting of somatic extract and E/S products of *E.granulosus* adult worm antigens were predominantly recognized as 50 kDa and 85 kDa, respectively.

Carmena *et al.* (2005 a) used somatic and E/S antigen of protoscoleces of *E.granulosus* for diagnosis of intestinal echinococciosis in dogs. The immunoblotting assay was performed by using sera from dogs infected with *E.granulosus* and other helminths. The assay showed four cross reacting proteins of 65, 61, 54 and 45-46 with E/S-antigen. The antigens with apparent masses 89 and 50 kDa in E/S-antigen and 130 and 67 kDa in
somatic antigen were identified by sera of dogs infected with *E. granulosus* only, whereas protein of 41-43 kDa was recognized by the majority of the sera from dogs with other infections.

Carmena *et al.* (2005 b) used hydatid cyst fluid (HCF), somatic (S-antigen) and E/S-antigens of *Echinococcus granulosus* protoscoleces for immunodiagnosis of canine echinococcosis by immunoblotting. The immunoblot assay revealed the shared antigenic components of hydatid cyst fluid antigen (HCF), somatic antigen (S-Ag) and excretory secretory antigen (E/S Ag) with molecular masses of 4-6, 20-24, 52-80 and 100-104 kDa, including doublets of 41/45, 54/57 and 65/68 kDa. The non-shared polypeptides of each antigenic extract of *E. granulosus* were identified as 108 and 78 kDa for HCF, 124, 94, 83 and 75 kDa for S- Ag and 89, 42, 39, 37 and 35 kDa for E/S-Ag.

Moreno *et al.* (2004) studied the IgG antibody response in dogs infected with *E. granulosus* by western blot analysis using serum prior to infection and 28 days after infection. The western blot analysis showed specific recognition of several bands in the range of 97-37 kDa.

**2.5 Copro-Polymerase Chain Reaction (c-PCR)**

Bretagne *et al.* (1993) used copro-PCR to identify *E. multilocularis* DNA in fox faeces for epidemiological purpose. Twenty nine faecal samples collected from foxes in East France were tested. Out of 10 samples in which no *E. multilocularis* adult worms could be observed after necropsy, 7 were positive. The PCR test was found to be more sensitive than microscopical
examination. Out of 19 samples from foxes harbouring E.multilocularis adult worms, 18 were PCR positive. The remaining PCR-negative samples could be either due to the misidentification of species of adult worm E.granulosus and E.multilocularis, or to DNA variation between different isolates of E.multilocularis.

Monnier et al. (1996) compared traditional microscopic examination and PCR method for the diagnosis of E.multilocularis in foxes. Finally they concluded that the PCR based diagnosis was found to be 82.3% sensitive and 95.5% specific compared to traditional microscopic examination.

Mathis et al. (1996) isolated DNA of the Taeniid eggs after alkaline lysis and PCR was performed using E.multilocularis species-specific primers. It was compared with the parasitological examination of the small intestines of the foxes and the specificity of the PCR was 100 per cent and the sensitivity was 94 per cent.

Dinkel et al. (1998) developed nested PCR and an improved method for DNA extraction to allow sensitive and specific diagnosis of E.multilocularis infection directly from diluted faecal samples from foxes. The specificity of the method was 100 per cent when it was tested against 18 isolates of 11 cestode species, including E.granulosus. The sensitivity of the method was evaluated by adding egg suspensions and individual eggs to samples of diluted faeces from uninfected foxes. The presence of one egg was sufficient to give a specific signal. To confirm the PCR results, an internal probe which hybridized only with E.multilocularis amplification products but not with the DNA of other
cestodes was constructed. Finally they concluded PCR system as an alterative method for the routine diagnosis of *E. multilocularis* in carnivores.

Cabrera et al. (2002) developed *E. granulosus*-specific PCR that utilized the mitochondrial CO1 gene with reported sensitivity equivalent to the detection of 200 eggs and no cross-reactions were observed with DNA from *E. multilocularis, Taenia hydatigena, Taenia ovis* or *Dipylidium caninum* cestodes.

Abbasi et al. (2003) reported that copro-PCR assay was found to be 100% specific and also detected all necropsy positive *E.granulosus* infected dogs. They also suggested that this copro-PCR assay has the potential for pre-mortem diagnosis of *E.granulosus* infection in areas where *E.granulosus* and *E.multilocularis* are co-endemic. To provide a more specific and sensitive diagnosis they developed a target repeated sequence newly identified in the genome of the common sheep strain of *E.granulosus*. They suggested that the corresponding primer used in PCR could easily detect a single egg with no cross amplification of DNA from closely related cestodes, including *E.multilocularis* and *Taenia* spp.

Stefanic et al. (2004) developed highly sensitive and specific copro-PCR assays for the detection of *E.granulosus* infection in dogs.

Shaikenov et al. (2004) screened 120 soil samples from 30 gardens in southern Kazakhstan for the presence of taenid eggs by concentration methods. It was observed that 21 samples were contaminated with taeniid eggs. These isolated taenid eggs were further analysed using a polymerase chain reaction specific for G1 (sheep) strain of the tapeworm *E.granulosus* and
five were shown to be positive. They concluded that the PCR method was feasible for identifying echinococcus eggs in the environmental samples such as soil. This technique was found useful in documenting the extent of environmental pollution with *E.granulosus* eggs in epidemiological studies.

Naidich *et al.* (2006) reported the efficacy of Mit-PCR and Rep-PCR based methods to detect patent and pre-patent infection in dogs experimentally infected with *E.granulosus*. Both PCR methods could detect *E.granulosus* in pre-patent and patent periods, even when microscopical examinations for eggs were negative in faecal samples. The faecal samples collected from dogs of an endemic area were diagnosed with more sensitivity than arecoline hydrobromide purgation. They also reported that these molecular methods could be applied in the confirmation of coproantigen-positive faecal samples and to verify the success of control programme.

Mathis and Deplazes (2006) reported that PCR was a proven method for diagnosis of taenid cestodes in animal definitive hosts, although only few specific tests are available at the moment. PCR was found to be a technically demanding and expensive technique. The DNA isolation from faecal specimens remains a laborious task because of the presence of PCR inhibitory substances and special precautions need to be taken to avoid false-positive results due to cross-contamination of amplification reactions. PCR is therefore, mainly used for confirmative purposes of coproantigen-positive samples or for identification of taenid eggs recovered from faecal specimens or from environmental samples.
Zhang et al. (2006) examined 30 dogs by copro-PCR with Eg 1f, Eg 1r primer in Hejing County, and found that 17 dogs were infected with *E.granulosus* which was confirmed to belong to the sheep strain or the G1 genotype by sequencing. They also suggested that the corresponding primer used in PCR could easily detect a single egg with no cross amplification of DNA from closely related cestodes, including *E.multilocularis* and *Taenia* spp.