Immunological and Molecular Diagnostic Tests for Cestodes and Metacestodes: Review

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Abstract: The diagnosis of cestode parasites of major economic and medical importance are mainly relies on detection of the adult parasite or their metacestodes. However, due to the chronic nature of the disease caused by these parasites and the location of the larval stage in various tissues, accurate and timely detection of the presence of the problem is often difficult. ELISA detection of serum antibodies produced in response to the hydatid cyst fluid protein, protoscolex and oncosphere of E. granulosus infected sheep shows diagnostic sensitivity from 51.4%-62.7% and specificities from 95.8 to 99.5%. Enzyme linked immunotransfer blot (EITB) shows high sensitivity (90-97.5%) and specificity (100%) than ELISA for antibody detection in porcine cysticercosis. Similarly, Nested PCR on mitochondrial 12S rRNA gene shows 100% specificity when it was tested against E. multilocularis and E. granulosus isolates. PCR methods using primers targeting the Mitochondrial (Mit) cox1 gene and a repetitive element (Rep) from genome of E. granulosus were able to detect E. granulosus during both prepatent and patent periods. Multiplex PCR with cytochrome c oxidase subunit 1 gene yielded evident differential products unique for T. saginata, T. asiatica and T. solium. Detecting the presence of metacestodes in animals is challenged by absence of circulating antigens or DNA products when the cysts are intact. There should be an effort towards improvements in sensitivities and specificities as well as commercialization of diagnostic tests.

Key words: Cestodes • Immunological Tests • Metacestodes • Molecular Tests • Veterinary Importance

INTRODUCTION

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. Timely and accurate diagnosis of the problem is essential in the control and prevention of the diseases caused by the parasites. Such diagnosis could be both in the final and intermediate hosts. In the final hosts, the adult parasite, its segments or eggs could be identified in live patients whereas in the intermediate host where the cysts are embedded in tissues, it is often difficult to see the parasite unless the animal is killed or slaughtered. As some of them are commonly hidden in soft tissues such as muscles, post-mortem examination often misses majority of the infections although the level of detection depends on the skill of the inspector [1]. This results in continued transmission and maintenance of the infections and failure to control or prevent the problem. Moreover, the best test should be one which could detect early on the complete resolution of the disease after medical or surgical treatment.

Bovine cysticercosis, caused by the intermediate stage (syn Cysticercus bovis) of the human tapeworm Taenia saginata, has both aesthetic and food safety implications to consumers of beef. Diagnostic challenges occur at every stage of the control cycle, from recognition and confirmation of the parasite at slaughter to identification of the source of infection [2]. Despite its many limitations, visual inspection of carcasses remains the most common method of diagnosing T. saginata cysticercosis. Studies showed failure rate of detection during meat inspection as few as 27% and as high as 85% [3]. T. solium cysticercosis causes considerable morbidity in humans and economic losses in pig populations in developing countries. T. solium cysticercosis is now recognized as an important (re-) emerging zoonosis.
and research priorities include the development of more sensitive and specific diagnostic tests [3]. When recovered from the abattoir, a suspect lesion requires laboratory confirmation particularly if it contains a dead cyst [4]. The limitations of the current meat inspection procedures pose significant challenges for regulators and diagnosticians tasked with preventing zoonotic transmission of the parasite. This could be addressed if a reliable serological test, e.g., ELISA, was available for use on live animals [5].

To overcome the above diagnostic problem, 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 serological tests for cysticercosis and hydatidosis have prevented the development of any practical test for ante-mortem diagnosis of infection [1].

In comparison with the extensive investigations in humans, relatively little research has been directed toward the development of immunodiagnostic techniques for *E. granulosus* infection in domesticated animals such as sheep and cattle. Currently, diagnosis of cystic echinococcosis (CE) in intermediate hosts is based mainly on necropsy procedures. However, up to 37% of animals classified as positive at necropsy may be actually false positives caused by unspecific granulomas, pseudo-tuberculosis, fatty degeneration, abscesses, caseous lymphadenitis and larval stage of *T. hydatigena*, whereas false negative diagnoses may be due to small intraparenchyma cysts [6]. The diagnosis of echinococcosis in dogs or other carnivores requires the demonstration of the adult cestodes of *Echinococcus* spp. in their faeces or the small intestine or the detection of specific coproantigens or copro-DNA [7]. For diagnostic purposes, PCR shows the best features, providing rapid amplification of parasite-specific DNA (or RNA) sequences and thereby greatly increasing the sensitivity of the assay [8].

The antemortem diagnosis, based on clinical signs is usually not possible because clinical symptoms are not well defined in animals. Clinical manifestation is noticeable only when the cysticerci get lodged in the eye or brain of pig when nervous symptoms are exhibited [9]. The current method for detecting infected pigs in the field is tongue inspection. In tongue inspection, the pigs are considered positive for infection if cyst-like nodules are either seen or felt on palpation. In vivo examination of tongue shows high specificity although its sensitivity is low, which is not always desirable [10]. Palpable lingual cysts have been used as indicator to estimate the prevalence of porcine cysticercosis and to study the potential risk factors associated with porcine cysticercosis [11].

Molecular biological techniques have evolved rapidly, resulting in technical innovations with potential applications to diagnostic parasitology. The identification of parasite species nucleic acid sequences has resulted in the development of DNA probes useful for hybridization to DNA from diagnostic samples. Diagnostic polymerase chain reaction (PCR) is a molecular method used to amplify and thus optimize detection of specific nucleotide sequences. It relies on the availability of appropriate target nucleic acid sequences that flank regions of interest (“primers”), the synthesis of these oligonucleotide primers and a suitable DNA isolation (extraction) technique for test samples. Molecular diagnosis based on PCR test assumed significance due to its high specificity and sensitivity and can be used as simple presence / absence assay to detect *Taeniid* spp. cysticerci [12].

DNA approaches are now being used routinely for accurate identification of *Echinococcus* and *Taenia* spp., subspecies and strains and in molecular epidemiological surveys of echinococcosis/taeniasis in different geographical settings and host assemblages. The publication of the complete sequences of the mitochondrial genomes of *E. granulosus*, *E. multilocularis*, *T. solium* and Asian *Taenia* and the availability of mtDNA sequences for a number of other taeniid genotypes, has provided additional genetic information that can be used for more in depth phylogenetic and taxonomic studies of these parasites [13]. Moreover, with the availability of complete mtDNAs of *T. multiceps*, *T. hydatigena* and *T. pisiformis*, numerous alternative gene regions are likely to capture greater nucleotide variation from which new PCR primers can be developed so as can be used for molecular ecology, population genetics and diagnostics [14]. Based on the existing information the current review is aimed at exploring developments in the immunological and molecular diagnostic tests for cestodes and metacestodes of veterinary importance.

**Cestodes and Metacestodes of Veterinary Importance:**

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 or hydatidosis, respectively [1].

**Taenia Solium / Cysticercus Cellulosae:** The transmission of *T. solium* eggs to pigs, the essential link in the pig-man-pig cycle, requires that pigs have access to human feces and that people consume improperly cooked infected intermediate host (pork) [15]. The uterus of a mature proglottid carries up to 55,000 eggs. The mature proglottid or strobilar fragments, consisting of five-six proglottids in a chain, detach from the strobila and are usually expelled passively with the host’s feces. Coprophagy is a normal activity of all free ranging and scavenging pigs. Indeed, in some parts of the world, pigs may be kept for the purpose of removing human feces and in others they may be fed feces deliberately as a cheap feed. Consequently, pigs may ingest whole proglottids along with a massive numbers of eggs [16].

With modern intensive pig husbandry practices, which involve controlled feeding of grain-based feeds, epidemic cysticercosis or ‘feedlot’ type infection, as seen in bovine cysticercosis, is unlikely to occur in countries where small holder pig farming predominates, restraint of pigs (to prevent free ranging and scavenging) can be very effective in interrupting the transmission of *T. solium* to pigs [15]. Concern is growing in East and Southern Africa that the rapid expansion of pig farming and pork consumption will exacerbate the problems with *T. solium* cysticercosis because, since 1961, the pig population in Uganda, Tanzania, Kenya, Zambia, Zimbabwe and Mozambique has increased nearly threefold (in Uganda over six-fold). The lack of adequate slaughterhouse facilities and the reliance on extensive and free-ranging rearing practices throughout the region represent serious obstacles to reducing the risk of this zoonosis [17].

**Taenia Saginata/ Cysticercus Bovis:** *T. saginata* cysticercosis represents the parasitic larval stage of the beef tapeworm belonging to the *Taeniidae* family of tapeworms (Cestoda). Humans are the final and bovines the intermediate host to this infection. Human taeniosis, or infection with the adult *T. saginata* worm, is characterized by the presence of between 3.5 to 20m-long worm in the small intestine of the infected person, who may pass millions of eggs daily. These eggs may survive for months in an appropriate environment [18].

Transmission to cattle can occur by the contamination of pasture, fodder or water with eggs. The direct transmission of eggs can also occur when a human carrier with contaminated hands raises suckling calves. Oncospheres have been found in fingernail dirt and water used to wash hands and underwear. Infected farm workers and herdsmen represent major risk factors in the epidemiology of bovine cysticercosis. In developed countries, the activities and movement of people in the form of camping and tourism provides an important opportunity for the spread of proglottids and feces to cattle raising areas. Uncontrolled defecation and inadequate destruction of viable *taenid* eggs in sewage also play important roles in the spreading of *T. saginata* infection. Most conventional sewage treatment plants do not effectively remove taenid eggs. The distribution of bovine cysticercosis is related, of course, to that of taeniosis in humans. The rates vary from very low, (0.03%) in North America and Europe, to very high in Africa and Latin America (10%- 80%). Information on prevalence’s of bovine cysticercosis within African countries is limited, but rates as high as 80% (Ethiopia) occur and are reported to be increasing in Botswana and Nigeria [15].

**Echinococcus/ Hydatid Cyst:** Hydatid cyst is produced by the larval cestodes found in canids, called *E. granulosus*. It is one of the most important parasitic zoonoses universally found in different parts of the world including Europe, Central Asia, China, Australia, Africa, America and Middle East. The adult worm is abundantly found in the small intestine of canids (the definitive host) which contain large numbers of ruptured eggs in the host intestine to set the eggs free. Intermediate hosts (herbivores) and humans may accidentally come into contact with the eggs through vegetables and water where by the eggs finds their way into their host digestive system. The larval tapeworm then burrows through the intestinal wall and travels to different body organs via the blood. The hydatid cyst develops then in the organ where it resides [19]. Hydatid cysts of *E. granulosus* develop in internal organs (mainly the liver and lungs) of humans and intermediate hosts (herbivores including: sheep, horses, cattle, pigs, goats and camels) as unilocular fluid-filled bladders [20].
Taenia Hydatigera/Cysticercus Tenuicollis: C. tenuicollis is the metacestode of canine tapeworm T. hydatigena, which has been reported in domestic and wild ruminants, pigs, squirrels and monkeys. Metacestodes are found attached to the omentum, mesentery and occasionally on the liver surface; however, unusual locations of C. tenuicollis have been described as lungs, kidneys, brain, ovaries, uterine tubes, uterus, cervix and vagina. An aberrant location of C. tenuicollis vesicle inside the chorioallantoic membrane of a goat foetus was reported. Pathogenicity of adult parasites is not high for definitive hosts. However, large numbers of developing cysticerci migrate contemporaneously in the liver of intermediate hosts, producing “hepatitis cysticercoasa”, a condition whose gross pathology resembles acute fasciolosis and which is often fatal [21].

Taenia Multiceps / Coenurus Cerebralitis: T. multiceps is a taeniid cestode that in its adult stage lives in the small intestine of dogs and other canids. The metacestode, C. cerebralis, is usually found in the nervous system including brain and spinal cord in sheep and other ruminants. Eggs could contaminate the environment and waters and resist for 15 days under dry conditions, or 30 days with high levels of humidity. At high temperatures, they died in a few hours. When ingested by ruminants, in the small intestine the oncospheres spread from eggs and through the blood circulation they reach various locations, but only in the CNS they could develop into mature Coenurus cysts. The disease has been reported in Europe, Asia, Middle East and Africa including Ethiopia with prevalence ranging from 2.3% to 4.5%. Coenurosis affects sheep during their first year of age, mainly when small lambs of 3-4 months are left in the grass at the beginning of the spring season, when their immune system and rumen activity are not yet well developed. The disease could be revealed as an acute or chronic gid. The first form is produced by the penetration and migration of the oncospheres in the tissues and particularly in the CNS. Symptoms are caused mostly by an acute inflammatory response due to a toxic and allergic reaction than by a mechanical action of the larval cestode [22].

Immunodiagnosis of Cestodes and Metacestodes Echinococcus/hydatid Cyst: Almost all available immunodiagnostic techniques, including methods for detecting specific antibodies and circulating parasite antigens in serum or other body fluids, have been applied for diagnosing echinococcosis not hydatidosis. However, all the tools developed to date are generally applicable for laboratory research purposes only. None of the available diagnostic tools, kits, or methods are generally accepted by clinical physicians. Nevertheless, such serological tools are potentially important for epidemiological studies, confirmation of infection status and treatment and the monitoring of control programs and efforts should continue so that new assays for improved, practical diagnosis of echinococcosis are developed [23].

Diagnosis in the Final Host Detection of Antibodies: Craig and Nelson [24] developed immunological diagnostic approach to identify the generic origin of taenid eggs by using monoclonal antioncosphere antibodies. Gasser et al. [25] demonstrated stage-specific antioncospheral humoral immune responses, which strongly suggested that oncospheres from Echinococcus eggs actually hatch in the intestines of the specific definitive hosts. In this respect, serum antibodies against the metacestode stage-specific Em2 antigen could be demonstrated in dogs and foxes infected with adult-stage E. multilocularis. The corresponding Em2 ELISA was evaluated for assessing fox populations with E. multilocularis infection. The species specificity of the test was demonstrated by the absence of cross-reactions with antibodies from carnivores infected with intestinal or tissue-dwelling non-Echinococcus cestodes or nematodes. ELISA-based methods for detection of circulating antibodies against E. granulosus and E. Multilocularis infection in definitive hosts have poor sensitivity, specificity is unclear and there is no correlation with worm burden [20].

Detection of Parasite Antigens: Much more success has been achieved using immunological approach for the diagnosis of Echinococcus infection in the definitive host by detection of adult worm products in faeces using sandwich ELISA methodology and this technique has been widely used with successful detection of E. granulosus and E. multilocularis [26]. The antigens are large molecular weight molecules that may be derived from the carbohydrate-rich surface glycocalyx of adult worms and are shed, released or secreted during the life-span of the tapeworm [27].

The application of a monoclonal antibody which is specific for an oncosphere antigen of E. granulosus has been described for the species-specific identification of E. granulosus eggs. 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 taken 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 and results showed 100% specificity and 73% sensitivity for *E. granulosus*. Despite the test being highly specific, it has been described as cumbersome and impractical for testing large numbers of dogs and it relies on perianal contamination and egg hatchability [29].

Coproantigens can be detected prior to release of eggs by *Echinococcus* worms and therefore are not related to egg antigen(s). This has the advantage of detection of prepatent infections. Furthermore, coproantigen levels return to the preinfection baseline within 5 days of anthelminthic treatment of infected dogs. A coproantigen ELISA assay was developed to target *E. granulosus* and *E. multilocularis* coproantigens in dogs, dingoes and foxes showed diagnostic specificity and sensitivity of 98% and 87% respectively for animals harbouring >200 worms [29].

When testing for genus-specific *Echinococcus* coproantigens (against necropsy as a gold standard), specificity is around 98% and overall sensitivity approximately 70%; however, when mean worm burdens are >50-100, sensitivity approaches 100% [28]. When the capture ELISA uses either anti-ES or anti-somatic proglottid antibodies to *E. granulosus*, the sensitivity for *E. multilocularis* infection may be reduced, though genus specificity remains intact. Polyclonal-or monoclonal-antibody-based ELISAs for coproantigens exhibit high sensitivity and specificity to *E. granulosus* (~80%), even though they were developed for *E. multilocularis*. However, for low worm burdens (<50), the sensitivity of the *E. multilocularis* coproantigen ELISA is below that of the mucosal smear method at necropsy [30].

In another study into the development of a coproantigen ELISA, the authors reported an unexpectedly low diagnostic probability of 37.5% in the detection of canine echinococcosis in 59 dogs in Uruguay. It was suggested that it may be due to excess antigen presentation in the host or there could be antigenic differences of genetic variants of the parasite in Uruguay. Moreover faecal antigen-detection antibody sandwich enzyme-linked immunosorbent assay (ELISA) can detect coproantigens shortly after infection (10-14 days) and the level declines rapidly following expulsion of the worms. The sensitivity and specificity of the test have been estimated at 70% and 98%, respectively [31].

Huang et al. [32] describe a double-sandwich coproantigen ELISA assay that has been designed whereby the capture and detection antibodies were produced to recognise specifically the carbohydrate portion of the parasite tegument. The author reported that the assay was used in the surveillance of Tibetan dogs and that it detected both *E. granulosus* and *E. multilocularis* coproantigens alike but was unable to distinguish between the species.

**Diagnosis in Intermediate Host:** Antigen 5 is a taeniid cestode specific antigen present in *Taenia* spp. and *Echinococcus* spp. and which induces specific antibody in cysticercosis and hydatidosis. Antibodies are produced to this antigen in sheep infected mono-specifically with *T. ovis* or *T. hydatigena*. Thus 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. 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 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 [33].

Serological tests for the diagnosis of hydatid infection have been assessed with sera from infected goats, cattle, pigs, Indian buffaloes and camels. 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 [1]. Sheep are capable of mounting a specific immunological response during a natural *E. granulosus* infection but that the response does not lead to raised serum antibody levels (may be because the antigens are concealed in the protective cyst) in many animals or they are not maintained throughout the course of the infection [34].
Kittelberger et al. [35] used an ELISA with antigen comprising either a purified 8 kDa hydatid cyst fluid protein (8kDaELISA), a recombinant EG95 oncosphere protein (OncELISA) or a crude protoscolex preparation (ProtELISA) to develop an immunological method for the identification of sheep infected with *E. granulosus*. The authors used sera obtained from sheep infected either naturally or experimentally with *E. granulosus* and from non-infected sheep. The highest diagnostic sensitivity was obtained using the ProtELISA at 62.7% and 51.4%. Assay sensitivities were lower for the 8kDaELISA and the OncELISA. Diagnostic specificities were high among the three tests (95.8 to 99.5%) although a small number of sera from sheep infected with *T. hydatigena* and *T. ovis* were recorded as positive. Western immunoblot analysis revealed that the dominant antigenic components in the crude protoscolex antigen preparation were macromolecules of about 70-150 kDa, most likely representing polysaccharides. This study demonstrated that the ProtELISA was the most effective immunological method for those assessed for detection of infection with *E. granulosus* in sheep. Because of its limited diagnostic sensitivity of about 50-60%, it could be useful for the detection of the presence of infected sheep on a flock basis but cannot be used for reliable identification of individual animals infected with *E. granulosus*.

Simsek and Koroglu [36] investigated the antigenic characteristics of hydatid cyst fluid in sheep by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to evaluate the sensitivity and specificity of hydatid cyst fluid (HCF)-ELISA and immunoblotting for diagnosis of sheep hydatidosis. One band with a molecular weight of 116 kDa showed 88% sensitivity and 84% specificity in the immunoblot assay. Sensitivity (60%) was less but specificity was higher (94%) with the HCF-ELISA.

Specific circulating antibodies against the parasite antigens Em2, Em2 (G11) and II / 3-10 have been detected in many intermediate hosts of *E. multilocularis*. Thus, detection of antibodies against the Em2 antigen has been shown to be a useful approach for detecting the parasite in cynomolgus monkeys [37]. In addition, Em2 (G11) and the somatic Em10CH antigen have been used successfully to monitor the early development of *E. multilocularis* lesions in pigs infected experimentally with parasite eggs [38].

Abuseir et al. [18] used crude *E. granulosus* cysts antigen from sheep and cattle to evaluate the reactivity with *E. granulosus* and *T. saginata* cysticerci positive serum by immunoblot test. All protein bands reacted with positive *E. granulosus* sera except the 260- and 35- kDa bands which were considered as hidden antigens. Except 260- kDa, 60- kDa and 35-kDa proteins all protein bands reacted with positive *T. saginata* sera, suggesting the potential for the differential diagnosis of hydatidosis.

**Cysticercosis**

**Cysticercus Bovis:** The development of a reliable antemortem test for bovine *T. saginata* cysticercosis should ensure that control strategies are focussed on only the infected animals. The test could be used in the feedlot and with herds where an exposure to the parasite is suspected. It can also be used in herds with history of *T. saginata* to determine which animals could be sent to slaughter. Because most serological tests will not correctly classify all infected animals due to low sensitivity, other measures such as an enhanced meat inspection of specific tissue sites could be used in conjunction with the test to prevent zoonotic transmission [5].

**Detection of Antibody:** Ogunremi and Benjamin [5] developed Antibody detection enzyme-linked immunosorbent assay (Ab-ELISA) which detects bovine anti-*T. saginata* immunoglobulin G1 antibodies produced in response to the excretory-secretory antigens of *T. saginata*. The ES antigen was used as the coating antigen in an indirect ELISA to detect anti-*T. saginata* IgG1 antibodies in bovine sera. Sheep anti-bovine IgG1 antibody labelled with horseradish peroxidase was used as detect antibody. ELISA results of *T. saginata* showed 90.6% specificity and test sensitivity was 92.9% (26 out of 28) on an experimentally infected population which had a parasite load similar to a naturally infected population. All animals inoculated with high doses of *T. saginata* eggs (5000-10,000) were more readily detected by ELISA as shown by their faster and more sustained seroconversion when compared to cattle receiving fewer eggs.

**Detection of Antigen:** A significant advance has been made in the diagnosis of *T. saginata* cysticercosis in cattle based on the detection 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 *C. bovis*. 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 *Fasciola hepatica*. The test is positive only when cattle have more than 200 living cysticerci. Antigen detecting ELISA (double antibody sandwich ELISAs) using monoclonal antibodies (mAbs) against the surface and/or excretory-secretory products of *T. saginata* metacestodes for better correlation with the presence of viable, infectious cysticerci was developed [39].

Serum derived from 40 calves infected experimentally with different doses of *T. saginata* eggs and from groups of three cattle infected experimentally with *Fasciola hepatica*, *Osartagia ostertagi* or the protozoan parasites *Babesia bigemina*, *Anaplasma marginale* and *Theileria parva* and from 15 sheep infected experimentally with *Echinococcus granulosus* were used for the assay. Serum samples taken before infection or within the first month after infection remained negative. Antigens were detected in only 4 of the 11 calves (36.4%) in which fewer than 100 living cysticerci were found post mortem, but they were detected in 21 of the 24 cattle harbouring more than 100 living cysts (87.5%). The animals harbouring no cysts or only dead cysts, gave negative results. No cross-reactions were observed with the sera from cattle infected with *F. hepatica*, *O. ostertagi* or the protozoan parasites. Two of the 15 sheep infected with *E. granulosus* cyst gave positive reactions.

Avidin-biotin complex (ABC) immunostaining method was developed for the diagnosis of formalin-fixed *T. saginata* cysticerci. The assay uses a monoclonal (Mab) IgG1 antibody to an excretory/secretory (ES) tegumental protein of cultured *T. saginata* cysticerci developed by Draelants et al. [40]. Preliminary evaluation demonstrated this assay to be 100% sensitive in the identification viable and degenerated cysts and 100% specific when tested against *Sarcocystis*, *Actinobacillus* and a variety of normal bovine tissues (hemal lymph nodes, lymph nodes, adipose tissue, skeletal and cardiac muscle). IgM monoclonal antibodies generated against bovine cysticercus ES antigen have demonstrated cross-reactivity with serum antigens of *T. solium*, *T. ovis* and *E. granulosus*. It has been demonstrated to be 98.7% specific when used in a serological ELISA to test cattle infected with a variety of common helminths and hemoparasites [4].

Scandrett et al. [41] evaluated a monoclonal antibody (monoclonal IgG1 against excretory/secretory (ES) antigens of *T. saginata* cysticerci based immunohistochemical (IHC) assay and standard histological method used for diagnosis of *C. bovis*. The IHC assay identified significantly more known positive bovine cysticerci than the histological method, 91.7% and 38.5%, respectively. There was no evidence of cross-reactive IHC staining of the known-negative specimens with MAb other than the metacestode larvae of *T. ovis* and *E. granulosus*. The authors indicated the effect of Positive IHC staining occurred on sections from other cestode species on diagnostic specificity of this assay for bovine cysticercosis can be rule out by considering different host and/or tissue preferences amongst these parasites.

Recently Abuseir et al. [18] used immunoblot test for the evaluation of *T. saginata* cysticerci crude antigen prepared from protein extracts of mature *T. saginata* cysts. All protein bands of *T. saginata* reacted with *T. saginata*-positive sera obtained from experimentally infected cattle. Positive *E. granulosus* sera reacted with all *T. saginata* protein bands except the 18 and 14-kDa proteins. Serum samples from animals infected with *F. hepatica* showed no antigenic reactions. The authors identified two (18 and 14-kDa) *T. saginata* protein bands as potential serodiagnostic antigens.

**Cysticercosis Cellulosae**: Crude and purified whole cyst antigen, cyst fluid antigen, scolex and its fractionated antigens, membrane antigens, antigen B, excretory and secretory products of metacestode (E/S) of *T. solium* were used with different sensitivity and specificity in different diagnostic methods. Cyst fluid antigens are more sensitive than other components of *T. solium* metacestode as they are enriched with sensitive diagnostic glycoproteins. Crude antigens of *T. solium* metacestode serologically cross react with other helminth parasites of pig [42]. Immunoperoxidase and indirect immunofluorescence studies showed distribution of cross reacting antigens mainly on the tegument of *T. solium* metacestode. No cross reactions were observed with serum samples from pigs infected by other parasites tested using antibody enzyme linked immunosorbent assay (Ab-ELISA-F3) to detect the purified cyst fluid of *T. solium* fraction (F3) antigen [43].

**Detection of Antibody**: Counter immunoelectrophoresis (CIEP) test showed sensitivity and specificity of 84.5 to 86.6 and 88.5 to 94.2 % with scolex and its fractionated antigens in diagnosis of porcine cysticercosis and could be used as field test in antemortem diagnosis [44]. Enzyme linked immunotransfer blot (EITB) is highly sensitive (90-97.5%) and specific (100%) test than ELISA for antibody detection in pigs [45]. The EITB test has been extensively used for the diagnosis of human and porcine cysticercosis and is commercially available. The assay, however, has some drawbacks. It depends on infected
pigs for supplying the source material and, nowadays, strict regulations are in place for (international) transport of biological specimens. Preparation of the antigen and performance of the western blot require considerable technical expertise [46].

Indirect ELISA for the detection of antibodies taken from the serum of pig infected with swine cysticercosis was found to be 100% sensitive. Cross-reactivity was observed with trichinellosis and toxoplasmosis showing 73% specificity. Best results were obtained during acute clinical cysticercosis [47].

Detection of Antigen: It is difficult to determine cysticercosis positive pigs only by antibody detection methods, because antibodies might continue to be present even after cure. The specific antibodies can be detected only after 1 week of post infection and reach the peak after 6-7 weeks where as circulating antigens exist very early and will disappear as soon as parasite is killed. So, infected animals can be detected at the early stage of infection from the level of larval circulating antigens [48].

Identification of infected pigs with viable larvae is achieved through detection of their secretory and excretory products using a monoclonal antibody-based capture assay [49]. Sensitivity and specificity of Ag-ELISA (86.7% and 94.7%) is more than Ab-ELISA (35.8% and 91.7%) in estimating the prevalence of porcine specificity (nearly 100%) and sensitivity (at least 89%). Sensitivity and specificity of metacestode. In the final host, PCR allows the detection excretory products using a monoclonal antibody-based viability and growth activity of the achieved through detection of their secretory and PCR has shown good potential for determining the infection from the level of larval circulating antigens [48].

Cysticercosis Tenuicollis: The application of a monoclonal antibody which is specific for an oncosphere antigen of T. hydatigena has been described for the species-specific identification of T. hydatigena eggs. 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. T. hydatigena oncospheres can then be identified by incubation with the monoclonal antibody and examination using fluorescence microscopy. Western blot analyses of T. hydatigena cyst fluid revealed an immuno-reactive 36 kDa antigen. However, no data on sensitivity or specificity with respect to this antigen when being used in a serological test were provided [52].

Coenurosis: Serodiagnosis through indirect ELISA was successfully used in experimental Coenurosis infection in sheep, however, sero-positivity was observed only at the beginning of the 35th day postinfection in serum samples [53].

Molecular Diagnosis of Cestodes and Metacestodes

**Echinococcus/hydatid Cyst:** For diagnostic purposes, PCR shows the best features, providing rapid amplification of parasite-specific DNA (or RNA) sequences and there by greatly increasing the sensitivity of the assay. However, the use of PCR for routine diagnostic or large-scale epidemiological studies is limited by its high cost and complexity. In addition, this methodology requires highly purified nucleic acids to avoid the inhibitory effect of uncharacterized substances. Thus, PCR is generally used for confirmation of positive or suspected positive results obtained with other diagnostic tests. This approach, which has been shown to be E. multilocularis specific, has also been used successfully for diagnostic purposes in other intermediate hosts, including voles and wild boars [8].

PCR has shown good potential for determining the viability and growth activity of the E. multilocularis metacestode. In the final host, PCR allows the detection of parasite DNA in faecal material with high specificity (nearly 100%) and sensitivity (at least 89%). Copro-diagnosis by PCR has been used for the diagnosis of E. multilocularis infection in foxes, dogs and wolves on the basis of an E. multilocularis DNA probe, pAL1, the respective nucleic acid sequence can be analyzed in order to obtain oligonucleotide primers suitable for use in PCR amplification of specific target sequences from diagnostic Echinococcus genomic DNA. Two designed E. multilocularis oligonucleotides, BG1 and BG2, defined a 2.6-kbp fragment in the genome of E. multilocularis [8].

Dinkel et al. [54] developed a nested PCR to allow the sensitive and specific diagnosis of E. multilocularis infections directly from diluted fecal samples from foxes. The target sequence for amplification was part of the E. multilocularis mitochondrial 12S rRNA gene. The specificity of the method was 100% when it was tested against E. multilocularis isolates (metacestodes and adult worms) and E. granulosus. The presence of one egg was sufficient to give a specific signal. Necropsy and PCR of rectal contents were compared for the sensitivity test, in that PCR ranged from 100% (>1,000 gravid worms) to 70% (<10 nongravid worms) where as necropsy method was found to be not higher than 76%.
Several PCR-based protocols have been developed that allow identification of *Echinococcus* DNA from eggs or from adult parasites. These provide a highly complementary approach for positive and highly specific diagnosis of canines infected with *E. granulosus* and *E. multilocularis* and environmental detection of *Echinococcus* eggs in soil samples. PCR methods using primers targeting the mitochondrial (Mit) cytochrome c oxidase subunit 1 (cox1) gene (Mit-PCR) and a repetitive element (Rep) - PCR from the genome of *E. granulosus* were able to detect *E. granulosus* during both prepatent and patent periods, even when microscopic observation of eggs in faecal samples was negative. The Mit-PCR produced the same amplification pattern for all the parasite genotypes tested while the amplification patterns with the Rep-PCR differed among groups of strains [55].

**Bovine and Porcine Cysticercosis:** The prevalence of porcine cysticercosis is underestimated due to poor efficiency of visual meat inspection, which was vindicated by many serological tests [56]. In contrast, its prevalence may also be overestimated through misdiagnosis of other morphological alterations in affected muscles, as the meat safety system is based only on conventional post-mortem inspection at slaughter house. Therefore, molecular diagnostics have been considered for validation of macroscopic diagnosis of ambiguous lesions as these tests were reported to be highly specific and sensitive [57].

PCR based techniques are being employed to study genetic variability, for species-specific identification of *Taenid* spp. cysticerci and to validate meat inspection results in porcine cysticercosis, which is an appropriate postmortem test that could be applied on meat samples in suspected cases. Suspected lesion from the liver that resembled milk spot was also confirmed by PCR. Milk spots in the liver of the pigs infected experimentally with *T. solium* eggs are confirmed by histology, ruling out *Ascaris* infection. Though, PCR based techniques are not difficult and have high sensitivity and specificity, demand expensive infrastructure and is not suitable as rapid on-site diagnostic test preventing the general use of this methods [12].

Identification of *T. solium* cysticerci from the infected pig carcasses based on amplification of its DNA with specific primers targeting large subunit rRNA gene (TBR) and Cox1 proven to be valuable in the diagnosis of swine cysticercosis. All samples visually identified as *C. cellulosae* at meat inspection were also confirmed in PCR test using these two sets of primers. In addition suspected *C. cellulosae* lesions collected. The PCR test with TBR primers detected *C. cellulosae* DNA at a lower concentration than with Cox1 primers [58].

PCR technique using specific primers for the amplification of fragments of the *C. bovis* gene LSU RNR can be used for the diagnosis of vesicular and colloidal vesicular stages of *C. bovis* with higher sensitivity (>90%) than granular-nodular and calcified-nodular stages (approximately 75%) [59].

A multiplex PCR with primers based on the sequence of the HDP2 probe, which specifically amplified *T. saginata*, *T. solium* and *E. granulosus* DNA sequences, were developed. The test gave different amplification patterns of *T. saginata*, *T. solium* and *E. granulosus*, while it was negative for other taenids. Use of the PCR assays for the positive identification of the parasites in dubious cysts, lesions, or cyst residues in domestic animals at the slaughterhouse would aid in the appropriate treatment of the carcasses and in the control of these parasites in domestic livestock [60].

Multiplex PCR with cox1 gene was established for differential diagnosis of taeniasis and cysticercosis. The technique yielded evident differential products unique for *T. saginata*, *T. asiatica* and *T. solium* with lower test sensitivity in samples stored for long period of time. Moreover, diagnostic results obtained by multiplex PCR were same as those based on the nucleotide sequences of cox1 from samples examined (taenia, cysticerci and egg), indicating that the multiplex PCR can be used with a high degree of accuracy [61,62].

PCR detection of swine cysticercosis genomic DNA extracted from swine sera using different protocols showed a higher specificity (100%) with no cross-reaction to trichinellosis and toxoplasmosis. But sensitivity was lower Cox1 PCR (23%), T3/T4 PCR (32%) and Nested PCR (64%) than ELISA based detection of antibody from serum in the same study [47].

Using primer designed to hybridize with region of the 18S and 28S ribosomal gene of DNA taken from *T. solium* (eggs, cysts, immature and mature worms) and *T. saginata* (eggs and mature worms), Mayta et al. [63] demonstrated unique identification pattern for each cestode after subjected to restriction enzymes Alul, DdeI, or MboI. All of the specimens histologically identified as *T. solium* and *T. saginata* were also identified as *T. solium* and *T. saginata* by PCR-restriction enzyme analysis (REA).
CONCLUSION

With the advancement of proteomics and genomics a lot of immunological and molecular techniques for the diagnosis of cestodes and metacestodes parasites of veterinary importance have been developed. Immunological diagnosis of infection in animals is difficult due to multiple infections with different species and antigenic cross reactivity between related parasites. Moreover many of immunological diagnostic tests show less sensitivity and are not generally accepted by clinical physicians. As compared to immunological techniques, most of molecular methods have higher sensitivity and specificity but due to the relative higher cost few are commercially available. Most of the immunological and molecular diagnostic tests developed to date are generally applicable for laboratory research purposes. The developments in genomic and proteomic analysis should be used for further understanding of parasite-animal host interaction with the objective of finding additional targets for diagnosis.

REFERENCES


