Detection of parasites in food

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SUMMARY

The importance of foodborne parasitic zoonoses remains high in many regions of the world. Although control efforts have been exerted for quite some time, overall progress has not been satisfactory, even in many well developed countries. An important drawback in control programmes for parasites such as *Trichinella*, *Toxoplasma* and *Taenia* has been the absence of rapid, accurate and sensitive diagnostic tests for these meatborne parasites. However, the rapid advances in the molecular biology of these organisms has yielded concomitant gains in precision of detection. This review highlights these advances and their impact or potential application to the control of foodborne parasites.

Key words: Parasites, zoonoses, foodborne diseases.

INTRODUCTION

Foodborne parasitic zoonoses remain an important cause of global illness and economic loss (WHO, 1984; Roberts, Murrell & Marks, 1994; Murrell, 1995). Of particular importance are toxoplasmosis, cysticercosis and trichinosis (trichinellosis), although fishborne parasites are a problem in some regions of the world. The rise in general public concern over food safety has helped to focus more attention on these parasites. Although efforts to control these zoonoses have persisted for quite some time, overall progress has not been satisfactory, even in well-developed countries (WHO, 1992). Coincident, however, with the tremendous advances in immunology and molecular biology since the 1970s, the development of valuable new tools for parasite control are offering new opportunities to make great improvements in the control of these parasites. Among these, immunodiagnostic tests and molecular (DNA) probes stand out. The application of these techniques has been relatively rapid and as we stand on the threshold of the new millennium, more effective control strategies and programs are highly likely. This review will highlight these advances in detection technologies for foodborne parasites, their relevance to treatment and control, and the research gaps remaining to be filled before we can fully realize the potential of these tools.

TOXOPLASMA GONDII

The protozoan parasite *Toxoplasma gondii* is found in a wide range of warm-blooded hosts. It is transmitted by ingestion of the environmentally resistant oocyst stage, shed by felids which serve as the definitive host for this parasite, or by ingestion of cysts found in the musculature of infected animals. Most species of livestock including sheep, goats and pigs, have been shown to be infected with *Toxoplasma gondii* at varying rates of prevalence (Dubey, 1994). It is not possible to determine the relative role of cats (oocyst transmitted) and meat as a source of toxoplasmosis in humans; however, among the meat animals, pigs are thought to be the most important source of infection (Dubey, 1986). Prevalence rates in pigs vary, but generally exceed 10–20% in most countries (Dubey, 1986; Dubey, 1994). Infection rates are higher in breeding populations compared with market pigs, reflecting that time of exposure is a factor in acquiring toxoplasmosis. *Toxoplasma gondii* in humans causes birth defects including mental retardation and loss of vision in congenitally-infected children. Although it is not normally a clinical disease in healthy adults, a serious, and often fatal encephalitis can develop in immunosuppressed or immunocompromised individuals. An estimated 3–20% of AIDS patients die from toxoplasmosis (Luft, Haffler, & Korzun, 1993).

There are currently no requirements for the inspection of pigs or other food animals for *Toxoplasma* at slaughter in any country. Several laboratory methods exist for the detection of this parasite, but these methods are either not adaptable to slaughter testing (in the case of direct detection methods) or have not been sufficiently tested in food animal species (in the case of serology tests).

Direct detection methods

It is not practical to detect the presence of tissue cysts in food animal tissues by visual means since these stages are microscopic, randomly distributed and may be present at a low level. The most reliable method for testing for *T. gondii* is direct demonstration of the parasites by biological assay. Methods

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for biological assay for *Toxoplasma* in meat have been described by Fayer *et al*. (1989). Briefly, meat is ground and digested in an acidified pepsin solution, then sedimented and inoculated into mice. After 4 weeks, the serum of mice is tested for antibodies to *T. gondii* and impression smears are made of brain or lung tissue to identify tissue cysts. Recent improvements in the pepsin digestion method have been described by Dubey (1998). Bioassay testing of food animals for *Toxoplasma* infection has been used in prevalence studies (Dubey *et al*. 1995*a*), but is not suitable where information is needed in a short time frame (inspection of animals or meat at slaughter).

Immunological tests

Serological assays, such as the Sabin–Feldman dye test (Sabin & Feldman, 1948; Feldman & Lamb, 1966), have been widely used for the diagnosis of toxoplasmosis in humans but have not been studied extensively for detection of infection in livestock species. Due to the high degree of technical skill involved in performing the dye test, it is considered unsuitable for routine testing of animals. Other serological assays which have been tested in food animal species include various forms of agglutination tests and ELISA. Dubey *et al*. (1985) compared the Sabin–Feldman dye test (DT) with other agglutination assays, including indirect haemagglutination (IHA), latex agglutination (LA) and modified agglutination (MAT), for their ability to detect infection in cattle. The DT was not effective in detecting *Toxoplasma*, while the MAT, using formalized tachyzoites as described by Desmonts & Remington (1980), was superior to the other agglutination methods. Prickett *et al*. (1985) found the indirect fluorescent antibody test (IFAT), using air dried tachyzoites, to be useful for detecting antibodies in experimentally inoculated pigs. Uggla & Hjort (1984) used the same test to study antibodies to *Toxoplasma* in sheep, pigs and cattle in Sweden; however, the parasitological status of seropositive animals was not determined. Dubey *et al*. (1995) compared MAT, LA and IHA with parasitological status as determined by bioassay in 1000 naturally exposed pigs. The MAT was superior overall with a sensitivity of 82·9% and a specificity of 90·3%. Dubey (1997) confirmed the specificity of the MAT, showing the lack of cross reactivity with sera from pigs infected with *Sarcocystis miescheriana*, *Ascaris suum*, *Trichuris suis*, *Trichinella spiralis* and a number of swine viruses. Despite relatively good performance in detecting *Toxoplasma* infection in pigs, the MAT is not suitable for use in the slaughterhouse or for field use by veterinary personnel because it requires large numbers of intact tachyzoites. The availability of an ELISA with these same levels of sensitivity and specificity would enable wider testing for toxoplasmosis. The ELISA has been compared by various authors to other serology tests for the detection of *Toxoplasma* infection in food animals.

Waltman *et al*. (1984) and Takahashi & Konishi (1986) used an ELISA to test swine and found good correlation with the DT, IFA, IHA and LA. The latter authors suggested the need for an IgG specific anti-swine reagent to increase test specificity. O'Donoghue, Riley & Clarke (1987) compared ELISA methods for detecting IgM or IgG with an IHA test for *Toxoplasma* infection in sheep. Good correlation of results was obtained with IHA and the IgG ELISA; the IgM ELISA produced more than twice the number of positive results, suggesting a high rate of false positives. Zimmerman *et al*. (1990) and Berends *et al*. (1991) used an ELISA to estimate prevalence of antibodies to *Toxoplasma* in pigs from Iowa (USA) and the Netherlands; however, no parasitological data were obtained to support the efficacy of the test. Dubey *et al*. (1995) found the ELISA for *Toxoplasma* in pigs to have a specificity of 85·9% and a sensitivity of 72·9% when compared with isolation of parasites by bioassay. These results support the validity of prevalence data gathered in previous studies on pigs. Lind *et al*. (1997) compared several variations of the ELISA, using a crude tachyzoite lysate, for detecting *T. gondii* in experimentally infected pigs. They found that detection of anti-*Toxoplasma* IgG was a reliable method for identifying infected animals and correlated well with dye test results. Detection of IgM gave a positive result in infected animals slightly earlier, but IgM was transitory during the first 2 weeks of infection. In contrast, the anti-IgG ELISA remained positive for 18 weeks. These authors found cross-reactivity with pigs harbouring *Sarcocystis* infection, but not other swine parasites or bacterial infections. Wingstrand *et al*. (1997) extended these studies using an IgG ELISA showing that the test had a sensitivity of 95·1% when compared with recovery of parasites by bioassay. These authors also demonstrated the use of meat juice (tissue fluids) as an alternative to serum for use in the ELISA.

Most ELISA studies in food animals have used a crude soluble extract from tachyzoites as antigen. However, in order to standardize testing, suitable diagnostic antigens need to be identified which can reliably detect antibodies in *Toxoplasma*-infected food animals. For example, the P30 surface protein from tachyzoites has been shown to be quite useful for detecting acute and chronic infections in humans (Santoro *et al*. 1985). The identification of antigens should also facilitate further cloning of diagnostic antigens. Several recombinant antigens have been identified. Johnson, Roberts & Tenter (1992) and Tenter, Vietmeyer & Johnson (1992) used two recombinant gene fragments (H4 and H11) in an ELISA to detect infection in humans, cats and

sheep. Compared with the traditional ELISA, the use of recombinant antigen gave a sensitivity of 79 % and a specificity of 100 % using sera from naturally exposed sheep. Antibody responses in two experimentally infected sheep remained high for up to 6 months post-inoculation. Andrews *et al*. (1997) evaluated these same antigens in an ELISA for *Toxoplasma* infection in pigs. Pigs, experimentally inoculated with *T. gondii*, showed strong reactivity with the H4 and H11 recombinant antigens during the acute phase of infection (4 weeks post-inoculation) but antibodies fell to near background levels as the infection became chronic (11–42 weeks post-inoculation). In contrast, a strong response, throughout the course of the experiment, was detected using native tachyzoite antigen in the ELISA. This problem points to the need to identify other antigens which are recognized by sera in chronically infected animals.

Detection of circulating antigens

Raizman & Neva (1975) first reported the presence of circulating *Toxoplasma* antigens in heavily infected mice and rabbits using antibody precipitation assays. van Knapen & Panggabean (1977, 1981) used an antigen capture ELISA to detect *Toxoplasma* antigen in human sera and mouse tissues. These authors concluded that sufficient antigen was available for detection only for a short time, during the acute phase of infection. Ise *et al*. (1985) found the sensitivity of antigen detection to be approximately 4 ng ml^{-1} while Brooks, Sharma & Remington (1985) was able to detect as little as 0·3 ng ml^{-1}. Based on the need to detect both acute and chronic infections, the use of antigen detection methods in food animals does not appear to be a reasonable approach.

DNA probes

Savva & Holliman (1989, 1990) reported on the use of PCR using primers contained within the P30 gene for detection of *Toxoplasma* in tissues of sheep and humans. The test was reported to be capable of detecting one tachyzoite among 10^6 host cells. Burg *et al*. (1989) used the polymerase chain reaction (PCR) to detect a repetitive gene fragment designated B1; this test was reported to have a sensitivity of 10 tachyzoites/10^5 leucocytes. Cazenave *et al*. (1991) took advantage of the abundance of ribosomal DNA to identify PCR primers which could be used for the specific identification of *Toxoplasma*. This test was shown to be superior to amniotic fluid culture or foetal blood testing; when combined with PCR using primers for the P30 gene, this test was highly sensitive and specific. Muller *et al*. (1996) combined PCR with a subsequent hybridization step to improve assay specificity. Using the *Toxoplasma* B1 gene fragment as the basis for PCR, these authors were able to reduce potential cross reactivity with samples containing DNA from *Sarcocystis* spp., *Hammondia* and *Neospora*. Based on the studies described, detection of DNA in animal tissues or meat samples could be a useful method of testing for *Toxoplasma*. However, the use of this method as a tool for slaughter inspection of food animals would require a high degree of automation at a minimal cost.

CYSTICERCOSIS

The term cysticercosis refers to infection with larval taeniid cestodes. The importance of cysticercosis lies in the fact that these parasites are meatborne (beef or pork) and develop to the adult stage in the intestine of humans (taeniasis). The species of public health concern are *Taenia solium* (pork tapeworm) and *T. saginata* (beef tapeworm). *Taenia solium* is of particular concern because, in contrast to *T. saginata*, humans may also serve as the intermediate host for the metacestode or cysticercus stage if the parasite's eggs are accidentally ingested. Although hard data on the public health and economic costs of animal and human infections are lacking, there is sufficient information available to regard these two parasites as significant public health and economic problems (Pawlowski, 1994; Roberts *et al*. 1994; Tsang & Wilson, 1995). For a general overview of the biology, clinical and epidemiological aspects of taeniasis and cysticercosis see Pawlowski (1994).

Direct detection methods

The current and long-practised method for meat inspection is the organoleptic procedure. Meat inspection personnel employ specific procedures to detect lesions of *T. saginata* during post-mortem inspection of beef carcasses (Snyder & Murrell, 1986). These procedures involve the incision of muscle tissue and palpation of other tissues. The tongue is palpated from tip to base to detect embedded cysts in this organ. If cysts are suspected in the tongue, it is then incised. The cysts are usually found embedded in the cut surfaces and rarely on the external or internal surfaces of the heart. When a *T. saginata* cyst is detected in a carcass, all carcasses represented by that lot are subjected to additional incisions. Inspection for *T. solium* cysticerci generally follows a similar procedure but may vary from visual to invasive and is quite dependent upon the importance of this parasite in the region. Inspection and slices are generally made on tongue, diaphragm and shoulder. Although *T. solium* cysticerci can be detected before slaughter by examining the underside of the tongue, this appears to be sensitive only in heavy infections (Schantz & Sarti-Gutierrez, 1989; Gonzalez *et al*. 1990).

Because the accuracy of these organoleptic procedures for meatborne cysticerci is not considered high (Viljoen, 1937; Dewhurst, Cramer & Sheldon, 1967; Kyvsgaard *et al*. 1990) and because these methods are laborious and time consuming, considerable effort has been expended in recent years to develop more rapid and sensitive detection technologies. The development of immunodiagnostic tests for food animals has benefited greatly from the rapid advances made in detection methods for human cysticercosis.

Antibody or antigen detection

In the past, immunodiagnostic tests for cysticercosis, whether human or animal, have suffered from low specificity and sensitivity. Great improvement, however, has resulted from advances in the identification and separation of specific, highly reactive antigens (Gottstein, 1994; Tsang & Wilson, 1995). Crude antigens are usually derived from both adult and larval stages of *T. solium*, including whole worm extracts, protoscoleces cyst fluid, and excretory-secretory (ES) products. Not surprisingly, crude extracts of these materials yield very complex antigenic preparations. For example, Grogl *et al*. (1985) reported 21 antigens in whole metacestode extracts. While Espinoza *et al*. (1986) observed that crude extracts had a sensitivity of 80% for human cysticercosis serum samples and 60–90% for cerebral spinal fluid (CSF), others have reported that these crude antigen extracts contain many components which cross-react with antibodies induced by other helminths, such as *Echinococcus* and filarial nematodes (Gottstein, Tsang & Schwartz, 1986; Schantz, Shanks & Wilson, 1980). A more specific test for human cysticercosis was developed by Gottstein *et al*. (1986) using the Western blot technique that identified two species-specific polypeptides (26 kDa and 8 kDa). These authors obtained confirmation of infection in 92% of infected patients with serum samples and 100% with CSF samples, with no cross-reaction with other helminth infections. Subsequently, Tsang, Brand & Boyer (1989) introduced an enzyme-linked immunoelectrotransfer blot (EITB) assay for human cysticercosis that employed lentil-lectin affinity purified glycoproteins. In immunoblots, 7 diagnostic bands are recognized with either immune serum or CSF from infection confirmed patients. The test was positive in 98% of infected patients sera and yielded no cross reactions with persons harbouring other infections.

Clinical and field comparisons of the EITB with ELISA using crude extracts of *T. solium* cysticerci have consistently demonstrated the superiority of the EITB (Feldman *et al*. 1990; Diaz *et al*. 1992), although the specificity and sensitivity of the ELISA can be greatly improved if cyst fluid is used as the antigen (Sloan, Schneider & Rosenblatt, 1995). Currently, the EITB with purified glycoprotein antigens is the method of choice for the serologic diagnosis of human cysticercosis and strips with the antigens are commercially available (Tsang & Wilson, 1995). However, Ito *et al*. (1998) have reported a simplified ELISA for neurocysticercosis using low molecular weight *T. solium* antigens that appears quite effective.

A satisfactory immunodiagnostic test for swine cysticercosis for epidemiologic research and control programmes and for slaughterhouse testing is crucial to the eventual control of human infections (Gonzales *et al*. 1990; Murrell, 1995). Kumar *et al*. (1989) compared the sensitivity and specificity of several immunological tests using both crude and partially purified *T. solium* larval antigens. They observed that the ELISA coupled with a partially purified antigen fraction, achieved a high sensitivity and a specificity for both experimentally and naturally infected pigs. They proposed that while other immunodiagnostic methods using crude antigen extracts could be useful for initial screening in a field test, the ELISA should be employed as a laboratory confirmatory test. Because the EITB has proved so successful in the immunodiagnosis of human cysticercosis, there has been strong interest in its suitability as an ante-mortem test in pigs. In Peru, the EITB proved to have 100% specificity and sensitivity with naturally infected swine sera (Gonzales *et al*. 1990). Similar results were reported by Pathak *et al*. (1994).

In a study designed to differentiate immunoglobulin responses during infection (Tsang *et al*. 1991) it was observed that the IgM and IgG responses identified different antigens in the EITB. The authors suggested that these isotype differences could be useful in differentiating infection histories in epidemiological investigations. This was confirmed by Aluja *et al*. (1996) who also observed, in a group of experimentally infected pigs, that the number of glycoproteins recognized increased up to about 7 weeks, then decreased. However, they also observed considerable individual variation in responses, perhaps not surprising given the genetic heterogeneity of commercially-bred swine; therefore, caution should be exercised in interpreting these isotype responses. The EITB or ELISA, using purified antigens, will require much more extensive testing on naturally infected pigs before their value as slaughterhouse tests can be finally decided. It is likely that a significant percentage of pigs in endemic areas are lightly infected, which may present any post-mortem test method with a severe challenge. For example, pigs with one detectable cyst yielded only 60–80% sensitivity with the EITB method (Tsang *et al*. 1989).

An important limitation to more rapid development of immunodiagnostic technologies for slaughterhouse testing for both porcine and bovine

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cysticercosis is the availability of antigen. Large-scale ante-mortem and post-mortem testing will demand quantities of antigen that are unlikely to be met through recovery of cysts from experimentally or naturally-infected pigs and cattle. An obvious drawback is the necessity of obtaining eggs for animal infection from infected humans who are always a difficult and unreliable source. Efforts to overcome this drawback have focused upon three approaches: use of heterologous cestode antigens; genetic engineering of diagnostic antigens and detection of circulating parasite antigens.

Heterologous antigens were first investigated for the detection of bovine cysticercosis. Craig (1979) and Craig & Rickard (1980) reported cross-reactions between *T. saginata* and *T. hydatigena*. Geerts *et al.* (1981*a*, *b*) reported that the immunodominant antigen of *T. saginata* cross-reacted also with antibodies to *T. crassiceps*. Subsequently, Rhoads *et al.* (1985) observed that *T. saginata*-infected cattle produced antibodies that react with lipoprotein antigens recovered from *T. hydatigena* cyst fluid; this antigen (ThFAS) had low reactivity with anti-*Fasciola hepatica* antibodies (which are notorious for cross-reactions with crude *T. saginata* extracts). This antigen preparation has also proved effective in detecting pig infections with 'Taiwan *Taenia*' – a pig-infecting subspecies of *T. saginata* (Rhoads *et al.* 1989).

Larralde *et al.* (1990) observed that an antigen extract of *T. crassiceps* performed as well as similar extracts from *T. solium* cysts in an ELISA using infected human CSF. In a subsequent study in pigs naturally infected with *T. solium*, *T. crassiceps* antigens achieved 97% specificity and 100% sensitivity (Biondi *et al.* 1996). These results are highly encouraging and should stimulate further studies on the suitability of heterologous cestode antigens for the serological detection of porcine cysticercosis. *T. crassiceps* is a particularly attractive source of heterologous antigen because it can be readily propagated in mice.

Another approach that has received limited study, but which also appears promising, is the detection of circulating *T. solium* parasite antigens in the blood or tissue fluids of infected hosts. Rodriguez-del-Rosal, Correa & Flisser (1989), using monoclonal antibodies generated against metacestode extracts, were able to detect circulating antigens in the sera of 79% of infected pigs, although the specificity was 97%. Similar results have been reported by Sciutto *et al.* (1998). These results are encouraging but further research is needed to determine the threshold of infection level at which the test will perform.

There have been only a few efforts to produce *T. solium* antigen by recombinant methods. Mantoucharian *et al.* (1996) succeeded in cloning five antigens that could be used to detect porcine antibodies, although the applicability of these antigens for practical diagnosis remains to be determined. Following an alternative approach, Zarlenga, Rhoads & Al-Yarnan (1994) succeeded in producing a recombinant antigen from a *T. crassiceps* cDNA sequence (TcA2-MBP) that is specific for *T. saginata* and is capable of detecting infections in cattle. Importantly, the recombinant shares antigenic epitopes with the low molecular weight lipoprotein of *T. crassiceps* that cross-reacts with *T. saginata* antigen (Hayunga *et al.* 1991*b*). Because *T. crassiceps* antigens also cross-react with those of *T. solium*, the possibility that TcA2-MBP might prove useful for the diagnosis of porcine cysticercosis deserves investigation. Recently, Sciutto *et al.* (1998) reported good results in detecting low levels of antibody in pigs with a 66 kDa antigen (HP10) in *T. crassiseps* ES materials. Benitez *et al.* (1996) succeeded in cloning a *T. saginata* oncospheral antigen (18 kDa) for vaccine study; this antigen too should be evaluated for its value for diagnosing *T. solium*. In a very different approach, Gevorkian *et al.* (1996) prepared synthetic peptides of *T. crassiceps* antigens that were effective in detecting mouse and human *T. solium* infections. Again, it will be interesting to see whether these antigens are effective for pig infections.

The effort to develop an immunodiagnostic antigen for bovine cysticercosis has received considerable effort with good results. Beginning in the 1960s with the introduction of new methods for separating proteins (e.g. electrophoresis), a number of investigators undertook the challenge to develop an immunodiagnostic test for bovine cysticercosis. For a concise and thoughtful review of the progress in this field up to the mid 1980s, the reader is referred to Harrison & Parkhouse (1985).

Currently, the ELISA is considered the most suitable diagnostic system for bovine cysticercosis. However, as with porcine cysticercosis, test specificity and sensitivity is dependent upon the antigens employed. False positives are particularly an issue in cattle because of shared antigens between *T. saginata* cysticerci and other cattle cestodes (e.g. *T. hydatigena*) and with flukes (e.g. *Fasciola hepatica* and *F. gigantica*) (see Kyvsgaard *et al.* 1991). Kamanga-Sollo & Lindquist (1991) were able to improve sensitivity greatly and reduce cross-reactions in the ELISA by fractionating crude cyst extracts with affinity-chromatography. Other antigen preparations that have been investigated are excretory-secretory antigens (Harrison & Sewell, 1981; Joshua, Harrison & Sewell, 1988) and detergent extracts (Gibbens, Harrison & Parkhouse, 1986; Harrison *et al.* 1989). However, the latter preparations are not entirely satisfactory from the standpoint of specificity. Kyvsgaard *et al.* (1991), using a detergent extract of *T. saginata* cysts, reported that although sensitivity of the ELISA was high, specificity was a problem with older cattle. As with *T. solium*, immuno-

diagnostic tests for low prevalence infections, such as bovine cysticercosis, result in poor test predictability because of non-specificity problems.

Recent efforts to develop a test for bovine cysticercosis applicable to large-scale testing have also searched for more readily sourced or propagated parasite antigens. The most promising results have come from the utilization of *T. hydatigena* and *T. crassiceps* metacestodes (Rhoads *et al*. 1985; Kamanga-Sollo, Rhoads & Murrell, 1987; Hayunga & Sumner, 1991; Hayunga *et al*. 1991*a*). The hydrophobic antigens from *T. hydatigena* have yielded excellent specificity in ELISA tests, although sensitivity in low level infections may be troublesome. Bogh *et al*. (1995) initially reported that sensitivity was increased when the hydrophobic antigen fractions were used in a dot blot system although, in a subsequent comparison, these investigators did not observe a higher sensitivity for the dot blot method (Bogh *et al*. 1996). Importantly, it was observed that regardless of whether calves received a single large egg dose or a dozen smaller exposures (trickle infection), the antibody levels declined after about 10 weeks post infection and viable cysts were not recoverable after 22 weeks. In contrast, Kyvsgaard *et al*. (1991), in a long-term study, found that antibody levels remained detectable 2·5 years after initial infection. This is an important issue, and the relationship between cyst number, their viability and detectable antibody (or antigen) deserves far more evaluation than it has received. A slaughterhouse antibody test must have a high reliability for identifying carcasses with low numbers of viable cysts because the number of cysts in most infected animals may be quite low (Kyvsgaard *et al*. 1990). It is not well-documented that the presence of antibody indicates the presence of viable metacestodes.

The prospect for direct detection of circulating *T. saginata* metacestode antigens in the blood of infected cattle has also attracted the attention of investigators. The excretory-secretory (ES) products of cultured *T. saginata* cysts can be used to detect antibodies in infected cattle (Harrison & Sewell, 1981; Joshua *et al*. 1988). Harrison *et al*. (1989) reported that monoclonal antibody against such glycoproteins, whether secreted or shed from the metacestode surface, performed well in an ELISA-antigen capture assay. The system was able to detect antigen in cattle with as few as 200 live cysticerci. Subsequently, Brandt *et al*. (1992) in a similar approach using monoclonal antibodies with specificity for different epitopes were able to detect circulating antigen in calves harbouring as few as 88 living cysticerci. In a somewhat different approach, Draelants *et al*. (1995) developed a monoclonal antibody-based dot-ELISA that performed as well as the standard sandwich ELISA. This system could detect animals infected with less than 100 live cysticerci and demonstrated a sensitivity of 88 % and a specificity of 94 %. The applicability of this test case for swine cysticercosis requires further evaluation since, in these studies, the monoclonals cross-reacted with *T. solium* antigen.

Variability is also a problem for the direct detection of circulating antigen. For example, although antigen can be detected in cattle with very low numbers of viable cysts, calves with much heavier infections may be negative (Brandt *et al*. 1992; Draelants *et al*. 1995; Bogh *et al*. 1996). The relationship between infection level, status of the immune response and cyst viability must be assessed before this approach can be considered for a slaughterhouse test. There have been only a few attempts to explore the applicability of intradermal tests to detect animals infected with cysticercosis (see Kumar *et al*. 1989). No tests reported to date have had sufficient specificity and sensitivity to challenge the antibody detection methods described above.

The impact of DNA probes for the diagnosis of cysticercosis has, so far, been limited to specific differentiation of recovered cestode tissues. Recent questions such as the taxonomic resolution of the so-called 'Taiwan *Taenia*' (Fan, 1992), and the genetic heterogeneity of different isolates of *T. solium* (Rabiela, Rivas & Flisser, 1989; Rishi & McManus, 1988) have provided opportunities to utilize these methods. In the case of the Taiwan *Taenia* from Southeast Asia, its current taxonomic assignment as a subspecies of *T. saginata* (*T. s. asiatica*) is based on DNA-based molecular systematics (Zarlenga *et al*. 1991; MacManus, 1997). Harrison, Delgado & Parkhouse (1990) developed DNA probes for *T. saginata* and *T. solium* that, when used in combination, could provide a positive identification of *T. saginata* proglottids from faecal samples; this approach should have value in clinical applications.

It is likely that as DNA-based methods come into wider use, new questions will arise concerning variation in these cestodes, their evolution and genetics. We can hope that greater facility with molecular concepts and tools will also stimulate efforts to find new approaches to the diagnosis of cysticercosis.

TRICHINELLA

Trichinellosis in man is caused by ingesting raw or undercooked meat containing infective larvae of the nematode parasite *Trichinella* spp. *Trichinella* is found in many species of warm blooded carnivores and omnivores, but human infection is most commonly associated with eating pork, bear meat and horse meat. Although there are several species and types of *Trichinella*, *T. spiralis* is the species most commonly found in pigs and is therefore of most concern to public health. Other species (*T. nativa*, *T. britovi* and *T. pseudospiralis*) and other types of

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Trichinella (T-5 and T-6 in the United States, T-3 in Europe and Asia, and T-7 in Africa) found in bears, foxes and other mammals also cause human disease (McLean, Poirier & Gyorkos, 1992; Zarnke *et al*. 1997; Pozio, 1998). Because of its direct life history, *Trichinella* infection can only be acquired by ingestion of an infected animal, animal carcass or waste food containing infective stages.

Many countries place high priority on inspection of swine and horse carcasses for *Trichinella* muscle larvae (trichinae) at slaughter. It is estimated that the European Union (EU) spends $570 million each year on testing for *Trichinella* (Pozio, 1998).

Regulations governing testing requirements often serve as a trade barrier for countries where inspection is not performed. Long-term programmes of testing have proven useful, as many countries with inspection programmes have effectively eliminated *Trichinella* from their domestic pork supply (Pozio, 1998). However, in these countries, trichinae continue to pose a risk since the infection persists in wildlife. In countries and regions where trichinae control is not strictly enforced and where pig husbandry supports transmission of trichinae, pigs are a regular source of human infection (Gamble, 1997).

Direct detection methods

Regulations mandating meat inspection require direct testing for the parasite by several different methods. The regulations defined by the EU are the most specific concerning the performance of trichinae testing (Commission Directive 77/96/EEC, Official Journal of the European Communities, 26, 67-77; Commission Directive 84/319/EEC, Official Journal of the European Communities, 167, 34–43). Two general methods, compression or digestion of muscle tissue, are used for the direct detection of *Trichinella* infection. Both methods determine the presence of parasites in tissues where infection is heaviest, these being in descending order of incidence in pigs, the diaphragm (crus), tongue, massester and abdominal muscles, although this partially depends on the degree of infection (Kotula *et al*. 1989; Gamble, 1996*a*). In horses, muscles of the tongue and masseter harbour the most worms, followed by the diaphragm and muscles in the neck (Gamble *et al*. 1996*b*). These direct methods of testing will identify infected pigs as early as 17 days after exposure, coincident with the time that muscle larvae become infective for a new host. Direct methods remain effective as long as muscle larvae remain viable. The compression method, using a specialized microscope called the trichinoscope, is time consuming and relatively insensitive due to the limited amount of muscle which can be examined (0·5–1·0 g). Compression testing is not recommended for use in routine meat inspection; however, for inspection of a few animals, where facilities for digestion methods are unavailable, testing by compression is acceptable for detecting animals harbouring infections of >3 larva per g tissue (Ruitenberg, van Knappen & Vermeulen, 1978).

Digestion of muscle tissue with artificial digestive fluid releases live trichinae from the muscle cysts. This is currently the most accepted method for slaughter inspection of pigs for trichinae. Four digestion procedures are recommended within the EU, and EU Directives should be consulted for more complete details. The procedures include: (1) the artificial digestion method for individual or pooled samples; (2) the mechanically assisted pooled sample digestion sedimentation technique; (3) the mechanically assisted pooled sample digestion method on filter isolation technique; and (4) the magnetic stirrer method for pooled samples.

The magnetic stirrer method for pooled samples can be employed in a variety of circumstances with a minimum of equipment. Details of these procedures can be found in the EU Directives, or the OIE Manual of Standards for Diagnostic Tests and Vaccines (Gamble, 1996*b*). Muscle samples to be tested should be taken from the diaphragm pillars or tongue of pigs, or tongue or masseter muscle of horses; other sites generally have lower numbers of larvae. Sample sizes can vary; individual samples of 100 g may be taken from one animal, or multiple samples may be collected from a number of animals to make a 100 g pool. The size of the samples which make up the latter will determine the sensitivity of the method. The sensitivity limit achieved by testing a 1 g sample is an infection level of >3 larvae per g tissue (Ruitenberg *et al*. 1978; Gamble, 1996*a*; Gamble, Solomon & Gajadhar, 1996). Testing a 5 g sample increases this sensitivity to approximately 1 larvae per g of tissue. EU Directives require 1 g samples in 100 g pools for testing pig carcasses. For testing horse meat, the EU requires 5 g samples. In the U.S., 5 g samples are required for pigs (USDA, Code of Federal Regulations). For endemic areas, a minimum sample size of 5 g is recommended, with 20 animals involved in each 100 g pool, based on the assumption that infections >1 larvae per g are sufficient to cause human disease.

Immunological tests

Serological tests, detecting antibodies to *Trichinella* in infected animals, offer an alternative to direct methods of testing and are most useful for epidemiological studies or herd surveillance. They also provide an alternative for screening animals at slaughter. The ELISA, first used by Ruitenberg and colleagues for detection of *Trichinella* infection in pigs (Ruitenberg *et al*. 1976; van Knapen *et al*. 1980) is the best method for ante-mortem diagnosis. It is comparable in sensitivity to the best direct methods.

Infection levels as low as one larva per 100 g tissue have been detected (Gamble *et al*. 1983; Smith & Snowdon, 1989; Gamble, 1996*a*). The specificity of ELISA for trichinellosis is directly linked to the type and quality of the antigen employed in the test. The only antigen which can be used in ELISA at the present time is excretory secretory (ES) antigen collected either by short-term *in vitro* maintenance of infective *T. spiralis* larvae (Gamble *et al*. 1983) or purification using biochemical methods (Seawright *et al*. 1983; Arriaga *et al*. 1991). Other antigens, including crude extracts of muscle larvae, have been shown to cause a high rate of false positive results in the ELISA (Gamble *et al*. 1983, 1988; Arriaga *et al*. 1993; Perez-Martin *et al*. 1993). Methods for preparation of ES antigen and performance of the ELISA can be found in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Gamble, 1996*b*). Commercial adaptations of the ELISA are available in a short format, taking <1 h for completion.

Trichinella spiralis ES antigens, consisting of a group of structurally-related glycoproteins with molecular weights of 45–55 kDa (Ortega-Pierres *et al*. 1996), are specifically secreted from the stichocyte cells of living first stage larvae. They bear the TSL-1 carbohydrate epitope which is the predominant antigen recognised by *Trichinella*-infected animals. These antigens are found in all species/isolates of *Trichinella* (Gamble & Murrell, 1986) and, therefore, when used in ELISA can detect infections with any of the *Trichinella* species.

A low rate of false-positive results has been obtained in some studies using ELISA (Oliver *et al*. 1989; Vitoorakool *et al*. 1993). False-positive results may be attributable to the quality of the antigen used. Cultivation of larvae for longer than 24 h produces an ES antigen which contains cross-reactive antigens (Gamble *et al*. 1988). It has also been demonstrated that pigs infected with *Metastrongylus apri* can produce a false positive ELISA for *Trichinella* infection (Ko & Yeung, 1989). In abattoir testing, the ELISA yielded less than 0·3 % false-positive results and was nearly 100 % sensitive in detecting infected hogs with more than one larva/gram of tissue (Oliver, Singh & Allison, 1988). Overall estimates of the efficacy of ELISA for *Trichinella* infection are 93·1–99·3 % sensitivity and 90·6–99·0 % specificity (Murrell *et al*. 1986; Oliver, Singh & Allison, 1988; Oliver *et al*. 1989; van der Leek *et al*. 1992; Gamble, Wisnewski & Wassom, 1997). When serology is used for testing animals at slaughter, the digestion of up to 100 g tissue from seropositive pigs is useful as a confirmatory test and to provide information on intensity of infection and parasite species.

Some investigators have used antigens purified by monoclonal antibody affinity chromatography for detection of antibodies to *Trichinella* in pigs (Gamble & Graham, 1984; Su & Prestwood, 1991; Homan, Derksen & van Knapen, 1992). No significant improvements were observed over use of ES products from *T. spiralis* muscle larvae. Western blots have been used as a secondary method for detecting *Trichinella* infection following initial screening by ELISA (Shin *et al*. 1997). These authors used a series of three ELISA tests to reduce the number of putative *Trichinella* positive samples, then tested these by Western blot to show specific reactivity to TSL-1 antigens. Parasitological infection was confirmed in only 6 of 49 (12·2 %) of pigs positive by Western blot and ELISA suggesting other factors affected the specificity of the test.

Recent studies have shown that meat juice can serve as a useful source of antibodies for testing by ELISA (Gamble & Patrascu, 1996). Collection of a 5–10 g sample of the crus muscle is sufficient to collect tissue fluids following flash freezing and thawing. This method has the advantage that tissue is available for confirmatory digestion testing if necessary.

A disadvantage of serology for detection of *Trichinella* infection is the occurrence of a low rate of false-negative results in the case of infected pigs. Such results are due to a lag in the kinetics of antibody responses in animals lightly or moderately infected with *T. spiralis* (Perez-Martin *et al*. 1993; Gamble, 1996*a*) or possibly, infection with sylvatic *Trichinella* species (Navarrete *et al*. 1989). This slow rate of antibody production means that pigs harbouring infective larvae in muscle tissue might not be detected for several weeks. Once positive, serological responses in pigs persist for at least 6 months post-infection with no decline.

Recombinant and synthetic antigens

Due to the need to recover live parasites from rodents for preparation of diagnostic antigens, several investigators have attempted to produce recombinant antigens useful for diagnosis. Zarlenga & Gamble (1990) prepared a recombinant antigen, homologous with the 53 kDa antigen found in *T. spiralis* ES products and found it useful for detection of infection in mice. Su, Prestwood & McGraw (1991) produced a recombinant antigen homologous with the 49 kDa antigen from *T. spiralis* which was recognized by sera from infected pigs. Yepez-Mulia *et al*. (1993) identified recombinant antigens using antisera to surface antigens of *T. spiralis* muscle larvae. One antigen (Ts-3) was used in an ELISA and detected 65 % of positive animals.

The major problem associated with the use of recombinant antigens for the serodiagnosis of *Trichinella* infection is the inability to reproduce the glycoprotein structure of the immunodominant antigens. These antigens (TSL-1) consist of a group of related glycoproteins with molecular weights of

43–68 kDa containing N- and O-linked oligosaccharides (Denkers, Wassom & Hayes, 1990). Wisnewski *et al*. (1993) and Reason *et al*. (1994) found that the immunodominant antigens are glycoconjugates containing the sugars fucose and tyvelose. A synthetic neoglycan with the antigenic structure found on *Trichinella* glycoproteins was synthesized (Wisnewski *et al*. 1998) and used in an ELISA (Gamble *et al*. 1997). The synthetic carbohydrate performed as well as native ES antigens when testing sera from experimentally and naturally infected pigs. This synthetic antigen shows promise as an alternative to parasite derived ES products in ELISA, although further evaluation is required.

Detection of circulating antigens

Several attempts have been made to detect antigen in animals infected with *Trichinella* (Arriaga *et al*. 1993; Li & Ko, 1993). Since antigenaemia was detected in only 56 % of animals tested (Arriaga *et al*. 1995), it does not appear that antigen detection is reliable for routine diagnosis in food animals. However, as an adjunct to serology testing, antigen detection could be useful.

DNA probes

Of considerable importance in the case of detection of *Trichinella* in food animal species is the identity of the infecting species. This information has public health implications because of the relative pathology of different species/types to humans and can aid in identifying the source of infection.

Most studies differentiating species or types of *Trichinella* are conducted to establish phylogenetic relationships rather than to address diagnostic needs. Original methods developed for differentiating of *Trichinella* spp. by isoenzyme analysis (Pozio, 1987; Fukumoto *et al*. 1988) have been replaced with methods using repetitive DNA probes, restriction fragment length polymorphisms and PCR (Zarlenga *et al*. 1991; Soulé *et al*. 1993; Mincheila, Eddings & Neel, 1994; Appleyard *et al*. 1998, 1999). A system of randomly primed PCR reactions (RAPD) has been used to differentiate the 8 accepted groups of *Trichinella* (Dupouy-Camet *et al*. 1993; Bandi *et al*. 1993). This method generates multiple bands for each species or type of *Trichinella* with a characteristic pattern. Other efforts have been made to identify specific primers for polymorphic regions of ribosomal DNA which are species or type specific (Zarlenga, Aschenbrenner & Lichtenfels, 1996; Appleyard *et al*. 1998*a*, *b*). This method can make use of as few as four primer sets in PCR to identity all species or types of *Trichinella*. Molecular tools such as these, which can verify species from just a few parasites, can play an important role in determining the epidemiology of human or animal infections.

FISHBORNE PARASITES

There are a number of zoonotic fishborne parasites that, although not widely distributed, may be of significance for certain regions of the world. It is estimated by WHO (1995) that there are about 40 million people infected with fishborne parasites. The anisakid parasites, *Anisakis simplex* and *Pseudoterranova decipiens* are of major significance; over 80 % of Pacific salmon and red snapper are infected with the larvae of these species (McKerrow, Sakanara & Deardorff, 1988). As a consequence, inspection for this fishborne parasite has been instituted in some countries (Deardorff, 1991). Adams, Murrell & Cross (1997) describe in detail the procedures for direct examination of fish for anisakid larvae. The adoption of an immunodiagnostic test to replace direct inspection does not appear to be likely in the near future, although an antigen-based ELISA test has been reported (Yagihashi *et al*. 1990).

CONCLUSIONS

Considerable work needs to be undertaken on the development and implementation of testing methods for zoonotic parasites transmissible to man through food animals. Insensitive organoleptic methods should be replaced by rapid and specific assays for cysticercosis in beef and swine and available technology should be used to the fullest extent in testing for trichinae in pigs. More needs to be learned of the importance of *Toxoplasma* in food animals before decisions can be made on the need to test at slaughter or on the farm. It is clear that high rates of infection can be found in some food animal species and this is likely to contribute to human infection. It is very likely that greater attention will be drawn to the role of food animals in the transmission of toxoplasmosis to humans and the veterinary public health community should have the tools to address these very legitimate concerns.

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