CHAPTER 2.1.16.

TRICHINELLOSIS

SUMMARY

Trichinellosis in humans is caused by eating raw or undercooked meat from Trichinella-infected domestic animals or game. The adult worms survive less than 2 months in the small intestine of host species including humans, pigs, rats, bears, walruses, occasionally in horses and many other flesh-eating mammals, and birds and reptiles. Trichinella larvae occur in the muscles of their hosts and susceptible individuals become infected by ingestion of tissue containing these larvae.

Identification of the agent: Diagnostic tests for trichinellosis fall into two categories: 1) direct detection of first-stage larvae encysted or free in striated muscle tissue, and 2) indirect detection of infection by tests for specific antibodies.

Tissue digestion and tissue compression methods have been used for the direct detection of Trichinella larvae in tissues. Trichinella larvae usually localise in preferred muscle sites, particularly in low level infections, and these sites may vary by host species. It is important that preferred sites be sampled to maximise test sensitivity. For example, in pigs, the diaphragm (crus), tongue, masseter and abdominal muscles are preferred sites, whereas in horses, the tongue and masseter harbour the most worms, followed by the diaphragm and neck muscles.

The artificial digestion methods involve the enzymatic digestion of individual or pooled muscle tissue samples and incorporate mechanical homogenisation or grinding, stirring, and incubation. This is followed by filtration and sedimentation procedures to recover and concentrate any larvae that are released from muscle during digestion. Samples processed by these methods are examined under a stereomicroscope for the presence of larvae. Digestion tests can detect <1 larva per gram (lpg) of tissue, but at these low levels of infection, uneven distribution of larvae within tissues is a limiting factor. This is compensated for by testing larger samples per carcass, such as a minimum of 1–5 g for pigs and 5–10 g for horses and game. Digestion methods are recommended for the inspection of individual carcass of food animals such as pigs, horses and game.

The compression method is less sensitive than artificial digestion and is not recommended as a reliable test for inspection of carcasses. Although now outdated, this method has been used widely in the past and is mentioned here for completeness. It involves visual inspection of compressed pieces of muscle tissue for the presence of larvae in situ. This method can be performed with a stereomicroscope, or a specialised microscope, (trichinoscope) which has an estimated efficiency of detecting as few as three larvae/g of tissue. It has the disadvantage of requiring considerable time for the inspection of multiple samples from each carcass. It is also very difficult to detect the non-encapsulated Trichinella larvae (T. pseudospiralis, T. papuae and T. zimbabwensis). Compression techniques are only useful for detecting medium to high infections when few animals require examination and facilities are not available for testing by artificial digestion.

Serological tests: Serological assays are the most common tests used for indirect detection. The sensitivity and specificity of serological methods are mainly dependent upon the type and quality of antigen used. Most serological test performance (validation) data are from pigs. False negative serological results may occur 3 weeks or longer after muscle larvae become infective in pigs with light or moderate infections. A low rate of false-positive results has also been reported for serological tests. For the purposes of individual carcass inspection, only direct methods can be recommended. For surveillance or verification of Trichinella-free herds or regions, serological methods are acceptable. Pigs harbouring as few as one larva/100 g of tissue have been detected. The specificity of enzyme-linked immunosorbent assays (ELISA) for Trichinella infection is directly linked to the type and quality of the antigen employed in the test. Secretory antigens collected by short-term (18–20 hours) maintenance of T. spiralis muscle larvae in vitro and synthetic
carbohydrate antigens currently provide the most specific and economical source, although a low rate of false-positive results has been obtained in some studies. It is critical that appropriate positive and negative control sera be used to ensure that ELISAs are performing at a minimum acceptable level of sensitivity and specificity. The digestion of 100 g or more of tissue is recommended as a confirmatory test for serologically positive animals.

Requirements for vaccines and diagnostic biologicals: There are no suitable vaccines for Trichinella infection in food animals. For indirect (serological) detection methods, appropriate antigens must be used to ensure adequate test specificity and sensitivity. These antigens may be obtained from the secretory products of muscle larvae maintained in-vitro. There is a critical need for an international bank of reference sera to provide a common standard for Trichinella serological assays.

A. INTRODUCTION

Trichinellosis in humans is caused by eating raw or undercooked meat from Trichinella-infected food animals or game (10). The short-lived adult worms live in the small intestine of host species including humans, pigs, rats, bears, walruses, horses, many other flesh-eating mammals, and some birds and reptiles. The parasite has a direct life cycle. Within hours following consumption of infected muscle by a suitable host, first stage muscle larvae (L1) are released by digestion and burrow into the villi of the small intestine. They develop rapidly into adults (males up to 1.8 mm long, females up to 3.7 mm long) and survive for less than 2 months. During this time, copulation takes place and the ovo-viviparous females release new-born larvae (NBL), which migrate via venules and lymphatics into the general circulation. NBL are distributed throughout the body where they invade striated muscles, showing predilection for specific muscle groups. For example, in pigs, the tongue usually contains the highest concentration of larvae, followed by the diaphragm, and in horses, the tongue followed by masseter muscle. Predilection sites vary by host species, but in general, tongue, masseter and diaphragm are optimal sites for sampling. Current knowledge on predilection sites is available for several host species (22). In cases of severe infection most voluntary muscles contain high numbers of larvae. The larvae of most Trichinella species become encapsulated in collagen in host musculature where they remain infective for years.

Within the genus Trichinella eleven genotypes have been identified, eight of which have been designated species status (10, 21, 27). Trichinella spiralis (T-1) is distributed in temperate regions world-wide and is commonly associated with domestic pigs. It is highly infective for domestic and sylvatic swine, mice and rats, but it can be also be detected in other mammalian carnivores. Trichinella nativa (T-2) occurs in mammalian carnivores of arctic and sub-arctic regions of North America, Europe and Asia. Trichinella britovi (T-3) is found predominantly in wild animals, and occasionally in pigs or horses. It occurs in temperate regions of Europe, Asia, and in Northern and Western Africa. Trichinella pseudospiralis (T-4) is cosmopolitan in distribution and has been recovered from raptorial birds, wild carnivores and omnivores, including rats and marsupials in Asia, North America, Europe and Australia. Unlike most other Trichinella genotypes, T-4 is not enclosed within a collagen capsule in muscle. Trichinella murrelli (T-5) is found in mammalian carnivores of North America. It has low infectivity for domestic pigs, but poses a risk to humans who eat game meats. Trichinella T-6 is cold-climate-adapted and appears to be closely associated with T. nativa in northern North America (27). Both T. nativa and T-6 are highly resistant to freezing. They have limited infectivity for pigs. Trichinella nelsoni (T-7) has been isolated from mammalian carnivores and sporadically from wild pigs in Eastern Africa. Trichinella T-8 has been detected in mammalian carnivores in Namibia and South Africa and Trichinella T-9 in mammalian carnivores in Japan (27). T-8 and T-9 have some intermediate characteristics with T. britovi and T. murrelli, respectively. Like T. pseudospiralis, T. papuae (T-10) and T. zimbabwensis (T-11) are non-encapsulated muscle parasites. Trichinella papuae has been reported from wild and domestic pigs, farmed crocodiles and humans in Papua New Guinea. Trichinella zimbabwensis has been described in farmed and wild crocodiles in Zimbabwe, Ethiopia and Mozambique and in monitor lizards in Zimbabwe. Experimentally, it shows a high infectivity for a wide spectrum of mammalian hosts including pigs and rats (27). All species and genotypes of Trichinella cause disease in humans.

Human trichinellosis can be a debilitating disease and may result in death. The short-lived adult worms in the intestine can cause transient gastroenteritis, but the most severe signs and symptoms result from the migration and presence of the larvae in voluntary muscle. The disease is transmitted by eating infected meat that has not been sufficiently cooked (or otherwise made safe). Prevention of human infection is accomplished by meat inspection, by processing (cooking, freezing, or curing of meat), and by preventing the exposure of food animals to infected meat including uncooked food waste, rodents and other wildlife (10, 12, 13.). Game meats should always be considered a potential source of infection, and should be tested or properly cooked. Trichinella found in game meats (mainly T. nativa, T-6 and to a lesser degree T. britovi) may be resistant to freezing and therefore untested, frozen game poses a public health risk.
Testing methods for the detection of Trichinella infection in pigs and other species either: (a) directly demonstrate the parasite in tissue samples; or (b) indirectly demonstrate the parasite by using immunological methods to detect specific antibodies to Trichinella spp. in blood, serum or tissue fluid samples.

B. DIAGNOSTIC TECHNIQUES

1. Identification of the agent (the prescribed test for international trade)

The only recommended procedures for the detection of Trichinella larvae in meat are digestion assays. A number of digestion assays are officially recognised in various countries for trade purposes. The International Commission on Trichinellosis (ICT) recommends several of these assays, which are documented standards in the EU, Canada or the USA. However, a number of other official methods not currently used routinely are not recommended because of their lack of efficiency or reliability. Modern diagnostic assays should meet internationally accepted standards of quality assurance, which include scientifically derived validation data and a design that allows routine monitoring and documentation of critical control points. Although there is general consensus that the digestion assay is the best procedure, a universally accepted digestion test protocol for trade and food safety purposes is not yet available. The digestion assay recommended here is based on desirable innovations inherent in some digestion assays that are accepted for international trade purposes.

a) Recommended direct procedure for testing meat

Sensitivity: the sensitivity of direct testing methods depends on the amount of tissue examined and the site from which the sample was obtained. Direct methods will identify infected pigs, horses or other animals infected with T. spiralis 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. To insure the viability of the trichinae, tissue samples must not be kept for long periods of time or frozen before testing. Current methods for testing by artificial digestion employing a 1-g sample have a sensitivity of approximately three larvae/g of tissue, and testing of a 5-g sample increases sensitivity to 1 larva/g of tissue (13, 22). Where large amounts of tissue (up to 100 g) are available for digestion, the sensitivity of this test is further increased.

Sampling: tests are usually conducted on carcass samples collected post-mortem. Muscle samples are taken from predilection sites, usually the diaphragm pillars or tongue of pigs, or tongue or masseter muscles of horses. Sample sizes can vary; individual samples of 100 g may be taken from one animal, or multiple samples of lesser amounts may be collected from a number of animals to make a 100-g pool. The size of the samples that make up the pool will determine the sensitivity of the method. The ICT recommends 5-g samples per pig for testing in endemic areas. For testing horsemeat, a minimum of 5 g per carcass is required. For horses originating from endemic areas, a 10-g sample is recommended.

o Digestion and detection
  i) Determine the volume of digestive solution required for the digestion (2000 ml of the solution for 100 g of meat, and 1000 ml for 50 g or less).
  ii) Digestive solution: Prepare the appropriate volume of 37% HCl/water solution (0.55% v/v 37% HCl) by combining the HCl with tap water (e.g. 11 ml of 37% HCl with 1989 ml water). Do not add pepsin to the solution at this time. This solution should be preheated to 45°C before use.
  iii) Remove as much fat and fascia as possible from each sample of meat.
  iv) Weigh the appropriate amount of trimmed meat from each sample. Cut each sample into 1–2 g pieces and pool with other samples into a 100-g amount.
  v) Place the pooled meat sample into a blender. Add 50–100 ml of the water/HCl solution for a 100 g sample pool.
  vi) Chop the meat in a blender until it is homogeneous (no chunks of meat should be present; the sample should be the consistency of pureed baby food). This is usually achieved with several 1–3-second pulses. Add approximately 100 ml of the prepared water/HCl solution and blend until the mixture is uniformly liquid. This may take 5–10 seconds (additional solution may be needed).
  vii) Sprinkle 10 g of pepsin (1:10,000 NF/1:12500 BP/2000 FIP, granular preferred) on to the homogenate, add about 200 ml of water/HCl solution, and blend for about 5 seconds.
  viii) Transfer the homogenised sample to a 3-litre beaker containing a stir bar. Add the remainder of the 2 litres of water/HCl solution by pouring the water/HCl into the blender and rinsing all residual homogenate into the 3-litre beaker. Rinse any adhering material from the blender lid into the beaker using 10–20 ml of digestive solution from a squirt bottle.
ix) Place the beaker on a preheated magnetic stirrer hotplate or in an incubation chamber set at 45±2°C. Cover the beaker with aluminium foil. Activate the stirrer at a sufficiently high speed to create a deep vortex without splashing. Note: If the digest temperature at the beginning of digestion is not 45±2°C, the sample should be allowed to warm to this temperature before the timing of the digestion is started.

x) Allow the digestion to proceed for 30 minutes. If the temperature of the digest has fallen below 45±2°C, additional digestion time may be required to complete the digestion. This can be determined by observing the digestion mixture. If pieces of undigested muscle tissue are present, the digestion should be continued for an additional 30 minutes or until the pieces are digested. Care should be taken to ensure that the digestion temperature range is not exceeded. Alternatively, the digestion may be performed at 37°C for a longer period of time.

xi) Within 5 minutes of removal from the magnetic stirrer hotplate pour the digestion fluid through a 177–180-µm sieve and into a 2-litre separatory funnel. Rinse the beaker with room temperature tap water from a squirt bottle and pour this through the sieve into the 2-litre separatory funnel.

xii) Rinse the sieve into the 2-litre separatory funnel by squirting a small volume of room temperature tap water through the top of the sieve. There should be no undigested pieces of muscle remaining on the sieve, although small remnants of fat, fascia and other tissues may be present. Allow the fluid in the separatory funnel to settle undisturbed for 30 minutes.

xiii) Drain 40 ml of digestion fluid from the separatory funnel into a 50 ml conical tube or measuring cylinder (Pilsner flask) and allow to stand for 10 minutes.

xiv) At the end of 10 minutes use a pipette to remove 30 ml of the upper part of the fluid (supernatant), leaving the bottom 10 ml in the tube (do not pour off the upper 30 ml, as this will disturb the sediment).

xv) Gently swirl the remaining 10 ml of fluid and quickly transfer it into a gridded Petri dish or larval-counting basin. Rinse the tube or cylinder into the Petri dish twice using 5 ml of tap water each time. The layer of fluid in the petri dish should not be more than a few millimetres deep.

xvi) Wait a minimum of 1 minute to allow larvae to settle to the bottom, then use a stereomicroscope at ×10–16 magnification to systematically examine each grid of the Petri dish for the presence of *Trichinella* larvae. The detection of any suspect larvae on the systematic examination must be confirmed by the identification of morphological details at a higher magnification such as ×40. If the sediment is cloudy or otherwise difficult to examine, it will require further clarification as described below.

xvii) Digests should be examined soon after they are ready. Under no circumstance should examination of digestes be postponed until the following day.

xviii) If digestes are not examined within 30 minutes of their preparation they may require clarification as described below.

xix) Sample clarification: transfer the contents of the Petri dish into a 50 ml conical tube using a pipette. Rinse the Petri dish thoroughly with tap water, adding the rinse water to the conical tube. Add additional tap water to bring the volume to 45 ml. Let the tube settle undisturbed for 10 minutes.

At the end of 10 minutes use a pipette to withdraw the supernatant, leaving the bottom 10 ml (do not pour off the supernatant, as this will disturb the sediment). Save the removed fluid for disposal or decontamination after the sample has been read.

Repeat steps xv and xvi.

xx) In the event of a positive or doubtful result, a further sample should be collected from each carcass making up the pooled sample. These should be tested individually or in successive smaller pools until the individual infected animals are identified.

**Identification of the larvae:** first stage larvae, digested free from muscle cells, are approximately 1 mm in length and 0.03 mm in width. The most distinguishing feature of *Trichinella* larvae is the stichosome, which consists of a series of discoid cells lining the oesophagus and occupying the anterior half of the worm’s body. *Trichinella* larvae may appear coiled (when cold), motile (when warm) or C-shaped (when dead). In case of doubt, larvae should be viewed at higher magnification and further tissues should be examined. If the counts are high, appropriate dilutions must first be made.

**Quality assurance:** laboratories using artificial digestion methods should maintain a suitable quality assurance system to ensure test sensitivity. Components of a quality assurance system for digestion testing are described by the ICT (13) and elsewhere (9) and should include regular use of proficiency testing (7, 8).

b) Other tests

- Other direct detection methods
i) The double separatory funnel method: this assay is recommended as an alternative to the commonly used digestion procedure described above, and is approved by the EU for export use. The method was designed to operate under strict conditions of quality control, minimize technical error, and has been extensively validated for use on pork and horse meat (5). It includes a spin-bar digestion technique and sequential separatory funnels for sedimentation of the larvae. The procedure has fewer steps, requires less time and seldom needs further clarification steps. An incubation chamber equipped with transparent glass doors and set at 45°C is used to perform the digestion. The digestion is conducted in 3 litres of digest fluid on a magnetic stirrer. Following digestion the suspension is poured into a 4-litre separatory funnel through a 177–180-µm sieve, which is rinsed thoroughly into the separatory funnel with tap water. The suspension is allowed to settle for 30 minutes and 125 ml is drained into a 500-ml separatory funnel. The volume is increased to 500 ml by adding 375 ml of tap water, and the resultant suspension is allowed to settle for an additional 10 minutes. Finally, 22–27 ml of sediment is drained into a Petri dish and observed for larvae as previously described.

ii) The mechanically assisted pooled sample digestion method/sedimentation technique (Method 4: 84/319/EEC): this method uses a heated Stomacher blender for the digestion phase, and a separatory funnel for sedimentation of the larvae (3).

iii) Polymerase chain reaction: limited studies have shown that PCR can be used to detect larvae in the nucleic acid of larvae in the musculature of infected animals. However, this method lacks sensitivity and is not practical for routine testing of food animals. Identification of the species or genotype of *Trichinella* recovered from muscle tissue is useful in understanding the epidemiology of the parasite in animals, in assessing the relative risk of human exposure and to trace back the infection to the farm of origin. Specific primers have been developed that allow the identification of larvae collected from muscle tissues at the species and genotype by PCR (25). Requests for speciation or genotyping of *Trichinella* larvae can be made through the OIE Reference Laboratories in Rome, Italy or Saskatoon, Canada (see Table given in Part 3 of this *Terrestrial Manual*; and www.iss.it/Trichinella/index.asp).

o Direct detection methods not recommended for meat inspection

i) Trichinoscopy: This method involves the compression of multiple 2 × 10 mm pieces of muscle tissue between two glass plates (compressorium) until they become translucent, followed by examination using a microscopic technique (2). Although this method has been in use for many decades, it is labour intensive and there are good comparative data available indicating that it is not as sensitive as digestion assays (6). Increasing the sample size to compensate is not practical for testing large numbers of animals, and the non-encapsulated *Trichinella* spp. (*T. pseudospiralis, T. papuae and T. zimbabwensis*) may occur uncoiled outside of muscle cells, making them difficult to detect using trichinoscopy. Because of these limitations, trichinoscopy and similar compression methods are not recommended for the routine examination of carcasses.

ii) Trichomatic 35: this method involves an automated digestion chamber and a membrane filter for the recovery and examination of larvae. The critical steps of the digestion and recovery process are difficult to monitor in this system, and test capacity is only 35 g.

o Immunological methods

A variety of immunological assays have been described for the diagnosis of *trichinellosis* in domestic and wild animals (17). Methods include immunofluorescence assay (IFA), immuno-electrotransfer blot (IEBT), western blot, enzyme immunohistochemical assays, and enzyme-linked immunosorbent assays (ELISA). Except for the ELISA, these tests have not been standardised, and reagents are not available for routine use. Nevertheless, the ICT has provided a uniform set of recommendations for the development and use of serological tests for the detection of circulating antibodies (17). The ELISA is the only immunological assay endorsed by the ICT. It is only approved as an epidemiological surveillance tool to detect anti-*Trichinella* antibodies in pigs; it is not reliable for the detection of *Trichinella* infection in individual animals.

2. Serological tests

Although other serological tests may have some practical applications, the ELISA is generally acknowledged as the test of choice based on economy, reliability, adaptability to good quality assurance practices, increasing body of validation data and good sensitivity and specificity when conducted under appropriate conditions. It is a useful tool for testing populations and is routinely used for surveillance programmes and disease outbreak investigations. Nevertheless, for reasons given below, the ELISA is not recommended for the testing of individual pigs for food safety purposes.

a) Enzyme-linked immunosorbent assay (ELISA)

o Sensitivity and specificity
Infection levels as low as one larva/100 g of tissue are detectable by ELISA in pigs (17). This high level of sensitivity makes serological testing by ELISA a useful method for detecting ongoing transmission of Trichinella infection at the farm or for more broadly based surveillance programmes. A disadvantage of serology for the detection of trichinellosis is the low rate of false-negative results observed in infected animals. This is primarily due to the lag time of the immune response following the ingestion of infective larvae. Detectable levels of antibody are not usually present in pigs until 3–5 weeks or more following exposure (11, 14). For this reason, serological methods are not recommended for individual carcass testing. Serological responses in pigs persist for a long time after infection with no decline in titre, however, antibody has been reported to decline in horses within a few months following infection (22). Serological tests may be of little practical use in horses as antibody titres eventually drop below diagnostic levels despite the presence of infective larvae in muscle (19, 26). Little is known of antibody responses to Trichinella infection in game animals, but high quality serum samples should be obtained to reduce the likelihood of false positive reactions.

- **Samples**

The use of ELISA to detect the presence of parasite-specific antibodies provides a rapid method that can be performed on serum, blood or tissue fluid collected before or after slaughter (15). The dilution used is different for serum than for tissue fluid (23).

- **Antigens**

The specificity and sensitivity of ELISA is largely dependent on the quality of the antigen used in the test. Antigens that are specifically secreted from the stichocyte cells of living L1 larvae and bear the TSL-1 carbohydrate epitope are recognised by Trichinella-infected animals. The antigens recognised in worm ES products consist of a group of structurally related glycoproteins with molecular weights of 45–55 kDa (24). A synthetic carbohydrate antigen (Tyvelose) has also been used in ELISA. Studies in swine indicate that Tyvelose may be as good as ES antigen for surveillance testing in pigs however, the sensibility of the ELISA using this synthetic antigen is lower than that using ES antigens (4, 18). Antigen preparations have been developed that provide a high degree of specificity for Trichinella infection in pigs (16). The T. spiralis ES antigens used in the ELISA are conserved in all species and genotypes of Trichinella (24), and therefore infection may be detected in pigs or other animals harbouring any of the eight species.

- **Antigen production**

Diagnosis of Trichinella infection by ELISA can be accomplished by using stichosome antigens collected from the ES products of Trichinella larvae in culture (16). For purposes of standardisation, it is recommended that T. spiralis be used for antigen production for food animal testing. However, it has been demonstrated that antigen prepared from any of the Trichinella species can be used for detection of antibodies in infected animals regardless of the infecting species (20). Parasites to be used for antigen preparation may be maintained by serial passage in mice or rats.

To prepare antigen for use in the ELISA (16), T. spiralis (T-1) muscle-stage larvae are recovered from skinned, eviscerated, ground mouse or rat carcasses by digestion in 1% pepsin with 1% HCl for 30 minutes at 37°C (as described above). These larvae are washed (three times for 20 minutes each) in Dulbecco's modified Eagle's medium (DMEM) with penicillin (500 units/ml) and streptomycin (500 units/ml), and then placed (at a density of 5000 L1/ml) into DMEM supplemented with HEPES (N-2-hydroxyethylpiperazine, N-2-ethanesulphonic acid) (10 mM), glutamine (2 mM), pyruvate (1 mM), and penicillin (250 units/ml)/ streptomycin (250 µg/ml) (complete DMEM) at 37°C in 10% CO2 in air. Culture medium is recovered after 18–20 hours, worms are removed by filtration, and the fluid is concentrated under pressure with a 5000 Da molecular weight retention membrane. ES antigens thus recovered may be stored frozen for short periods at −20°C or for longer at −70°C; they consist of approximately 25 protein components as determined by SDS/PAGE (sodium dodecyl sulphate/polyacrylamide gel electrophoresis), many of which bear the diagnostic TSL-1 carbohydrate antigen epitope.

Antigen purity is critical to the specificity of the ELISA. Steps should be taken to monitor growth of bacteria either visually, by phase microscopy, or by plating a sample of media. Cultures showing any bacterial growth should be discarded. Larvae should not be maintained longer than 18 hours; worm deterioration after this time contributes to leaking of somatic antigens that reduce test specificity. Antigen, produced as described, should have a 280:260 nm absorbance ratio of >1.0. The antigens obtained from in-vitro maintenance of Trichinella larvae, should be tested against a panel of known negative and positive sera before use.

- **Test procedure**

An example of an ELISA for detecting Trichinella infection in pigs is given below. It is essential that all reagents used in the assay be standardised for optimal concentration to obtain reliable results. Typical values are indicated in the example.
Coat 96-well microtitre plates with 100 µl/well of *T. spiralis* ES antigens diluted to 5 µg/ml in coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6). Coating is performed for 60 minutes at 37°C or overnight at 4°C.

Wash antigen-coated wells three times with wash buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 5.0% non-fat milk powder and 1.0% Triton X-100. Following each washing, plates are blotted dry.

Dilute pig sera 1/50 or 1/100 in wash buffer. Alternative sources of antibodies that may be used in place of sera include whole blood or tissue fluids at the dilution of 1/5 or 1/10 (23). Add 100 µl of diluted sera to antigen-coated wells. A known positive and known negative serum sample should be used on each plate at the same dilution as the test sera. Incubate at room temperature for 30 minutes.

Wash wells three times as in step ii.

Add 100 µl/well of an affinity-purified rabbit anti-swine IgG–peroxidase conjugate at an appropriate dilution in wash buffer, e.g. a 1/1000 dilution of rabbit anti-swine IgG (0.1 mg/ml) produced by Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA. Following the addition of the second antibody, incubate the plates for 30 minutes at room temperature.

Wash wells three times as in step ii. Rinse once with distilled water.

Add 100 µl of a suitable peroxidase substrate (e.g. 5'-aminosalicylic acid [0.8 mg/ml] with 0.005% hydrogen peroxide, pH 5.6–6.0).

After 5–15 minutes, read plates for colour density at 450 nm on an automated microplate reader. Values obtained in the ELISA four times that of normal serum pool controls are considered to be positive. Values three times higher than normal are classified as suspect.

Commercial adaptations of the ELISA are available. The manufacturer must validate the kit prior to licensure and the user should also evaluate the performance of the kit, prior to use, by using selected negative and positive reference samples.

The test should be conducted within an environment in which internationally accepted standards of quality management, such as ISO 17025, have been implemented.

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

There are no vaccines for *trichinellosis* in food animals or game. There are no biological reagents required for direct observation of the parasite. For applicable immunological methods, TSL-1 antigens are recommended to maximise test specificity. These antigens may be obtained as ES products recovered from in-vitro maintenance of muscle larvae as described above.

REFERENCES


NB: There are OIE Reference Laboratories for Trichinellosis (see Table in Part 3 of this Terrestrial Manual or consult the OIE Web site for the most up-to-date list: www.oie.int).