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INTERNATIONAL COMMISSION ON TRICHINELLOSIS: RECOMMENDATIONS ON THE USE OF SEROLOGICAL TESTS FOR THE DETECTION OF *TRICHINELLA* INFECTION IN ANIMALS AND MAN

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Summary:

The use of serological tests to detect *Trichinella* infection in domestic and wild animals and in humans has not been standardised yet. This review provides an uniform set of recommendations for the development and use of serological tests to detect circulating antibodies in serum samples. The recommendations are based on the best scientific published information and on the unpublished data from laboratories with a great expertise in this field and represent the official position of the International Commission on Trichinellosis regarding acceptable methods and the evaluation of the sensitivity and specificity. These recommendations are subject to change as new scientific information becomes available.

KEY WORDS : *Trichinella*, trichinellosis, serodiagnosis, animals, humans, antigens, specificity, ELISA, sensibility.

Résumé : COMMISSION INTERNATIONALE SUR LA TRICHINELLOSE: RECOMMANDATIONS SUR L'UTILISATION DES TESTS SÉROLOGIQUES POUR LA DÉTECTION DE L'INFECTION À *TRICHINELLA* CHEZ LES ANIMAUX ET L'HOMME

L'utilisation de tests sérologiques pour le diagnostic de l'infection à Trichinella *chez les animaux domestiques et sauvages et chez l'homme n'a pas été standardisée jusqu'à présent. Cette revue fournit un ensemble de recommandations pour le développement et l'utilisation de tests sérologiques pour détecter les anticorps circulants dans les échantillons de sérum. Les recommandations sont basées sur les meilleures informations scientifiques publiées et sur des données non publiées issues des laboratoires qui possèdent une grande expérience dans ce domaine. Elles représentent la position officielle de la Commission internationale sur la trichinellose à propos des méthodes acceptables et de l'évaluation de leur spécificité et sensibilité. Ces recommandations sont susceptibles d'être modifiées en fonction de nouvelles données scientifiques.*

MOTS CLÉS : Trichinella, *trichinellose, diagnostic sérologique, animaux, homme, antigènes, ELISA, spécificité.*

INTRODUCTION

According to the International Commission on Trichinellosis (ICT) (Gamble *et al.*, 2000; www.med.unipi.it/ict/welcome.htm) indirect (serological) methods are not recommended for the testing of individual carcasses of food animals at slaughter. This recommendation is consistent with practical legislation of many governmental bodies, under which meat inspection programs for *Trichinella* in pork, horse and game meats are performed using direct methods including trichinoscopy and artificial digestion (see Gamble *et al.*, 2000; Nöckler *et al.*, 2000; European Commission, 2001). Legislation for inspection of meat and meat products for the presence of *Trichinella* includes direct tests only, which if performed properly, have the sensitivity and specificity sufficient to detect all carcasses with larval burdens > 1 per gram of meat, the minimum level of infection considered to be of public health concern. No serological methods should be included in legislation for meat inspection because these methods do not meet the required standards for protection of public health.

Although serological methods for detection of *Trichinella* infection are not considered to be suitable for the purpose of meat inspection, there are important uses for these tests including: 1) surveillance of infection and epidemiological investigations in animal populations, particularly where the prevalence is high; and, 2) presumptive or retrospective diagnosis and surveillance of human infection. Due to the various methods that have been employed for serological detection of *Trichinella* infection, the ICT has compiled a series of recommendations for the use and interpretation of these tests.

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SEROLOGICAL METHODS

TYPES OF ASSAYS

Many types of serological assays have been, and continue to be, used for the detection of *Trichinella* infections in animals and man. For animals, serological assays include, but are not limited to: 1) indirect immunofluorescence assay (IFA) employing cryostat sections of infected rodent muscle, frozen sections of free muscle larvae or formalin fixed whole larvae; 2) immuno-electrotransfer blot assay (IETB), or western blot, using muscle larvae excretory-secretory products as antigen; 3) enzyme immunohistochemical technique (EIH) employing cryostat sections of infected rodent muscles (e.g., diaphragm) or frozen sections of free muscle larvae; and 4) enzyme-linked immunosorbent assay (ELISA) employing the excretory-secretory (ES) products of the muscle larvae (Gamble *et al.*, 1983; 1988) or synthetic tyvelose as antigen (Gamble *et al.*, 1997; Kapel & Gamble, 2000; Pozio *et al.*, 2002a). For detection of human infection, indirect hemagglutination (IHA), bentonite flocculation, IFA, latex agglutination and the ELISA are the more commonly used tests, the last being the most sensitive (Murrell & Bruschi, 1994; Dupouy-Camet *et al.*, 2002). Competitive inhibition assay (CIA) and IETB (Dupouy-Camet *et al.*, 1988; Ivanoska *et al.*, 1989; Homan *et al.*, 1992; Pozio *et al.*, 1993, 1997, 2002a; Owen *et al.*, 2001; Pinelli *et al.*, 2001) have also been used for the diagnosis of trichinellosis in humans. These tests are considered confirmatory tests, rather than primary screening tests.

The ELISA is the most commonly used method for the detection of *Trichinella* infection in both animals and man because it is economical, reliable, readily standardized and provides an acceptable balance of sensitivity and specificity. It is the only serological method recommended by the Office International des Epizooties (OIE, 2000) for testing domestic pigs. For these reasons, the ELISA will be the primary focus of these recommendations. However, other types of tests can have practical applications; therefore, the principles for use of the ELISA (requirements for performance, suitability for particular species, etc.) should be points for consideration in selecting any serological test for detection of *Trichinella* infection. For the ELISA, and for any serological test, independent validation should be performed using sera from infected and *Trichinella*-free animals or humans representative of the local population where the test is being used. The cut-off value of the ELISA, for example, can change based on host genotype and exposure to antigens in the local environment and in food. These are important considerations when testing either animals or humans (Navarrete *et al.*, 1989). Control positive and negative sera are required on every ELISA plate to assure the test is performing properly (OIE, 2002).

ANTIGENS

In the 1970s somatic antigens prepared from whole body extracts of muscle larvae were used in the ELISA for detection of *Trichinella* antibodies in pigs (Ruitenberg *et al.*, 1974; Ruitenberg & van Knapen, 1977). This crude larval extract, produced from first stage muscle larvae collected by artificial digestion, should not be used for serological tests of either animals or humans due to a high probability of cross-reactions with other pathogens. For example, cross-reactions occur with serum samples from humans infected with *Loa loa*, *Toxocara* sp., and *Anisakis* (J. Dupouy-Camet, personal communication; E. Pozio, unpublished data). During the 1980s, the specificity of the ELISA was improved by utilizing excretory-secretory (ES) antigens obtained from the *in vitro* incubation of *Trichinella* muscle larvae (Gamble *et al.*, 1983). These antigens, originating from larval secretions, consist of a group of structurally related glycoproteins. Information regarding *Trichinella* antigens has been summarized by Appleton *et al.* (1991) and Ortega-Pierres *et al.* (1996). The predominant antigen epitope recognized by animals and humans infected with *Trichinella spiralis*, or any of the other species of *Trichinella* currently known, is the so-called TSL-1 group. TSL-1 antigens are found in the stichocyte cells and on the surface of the parasite's cuticle; they are actively secreted by first-stage larvae in the muscle. These antigens are produced for diagnostic use by *in vitro* cultivation of isolated muscle larvae or by biochemical methods of recovery from this same parasite stage. Methods for the preparation of these antigens have been published (Gamble *et al.*, 1983; Seawright *et al.*, 1983; Grencis *et al.*, 1986; OIE, 2000) and the reader is referred to these articles for a detailed description of the methods.

As noted, the TSL-1 antigen epitopes recognized by antibodies in *Trichinella*-infected animals and humans are common to all encapsulated and non-encapsulated species (Appleton *et al.*, 1991). Consequently, *Trichinella* antigen can be routinely prepared from *T. spiralis* muscle larvae, because this species is readily maintained in laboratory mice, and used for ELISA to detect infection with any *Trichinella* species.

TSL-1 antigens share a common carbohydrate epitope (tyvelose), which has been synthesized (Reason *et al.*, 1994; Wisnewski *et al.*, 1993). Synthetic tyvelose is available commercially (Safe-Path Laboratories, LLC, Carlsbad, CA), and has been evaluated in several research studies, where its performance compared favorably with ES antigens for testing humans, pigs and other animals (Gamble *et al.*, 1997; Bruschi *et al.*, 2001; Owen *et al.*, 2001; Pozio *et al.*, 2002a). The synthetic

carbohydrate antigen offers the advantages of stability and standardization. It has been shown to provide greater specificity, in many species, but may sacrifice sensitivity. Recent reports of possible cross-reactions obtained using the tyvelose antigen and evidence of tyvelose-containing glycans in other nematode eggs will necessitate further studies to better define its specificity (Dea-Ayuela *et al.*, 2001; Pozio *et al.*, 2002a).

REAGENTS

Conjugates - Better specificity is obtained in the ELISA and IETB using a species-specific anti-IgG reagent as compared with a Protein A conjugate. It is strongly recommended that a species-specific anti-IgG reagent be used even if it is necessary to have it custom made.

SAMPLE COLLECTION

Serum is the preferred sample for conducting serological tests for *Trichinella*. Other samples that have been used include plasma, whole blood, and tissue fluids (Gamble & Patrascu, 1996). After collection, blood samples should be clotted and the sera frozen at – 20° C as soon as possible; samples frozen at – 20° C may be used for several months. It is important to avoid repeated freezing and thawing of samples, since serum titers will decline as a result; therefore, samples that will be used frequently should be made into aliquots. For periods of storage greater than three months, serum samples should be frozen at – 80° C or lyophilized. If freezing is not possible, 1 % merthiolate, or another suitable preservative, should be added to each serum sample at a dilution of 1:10,000.

Another alternative method for sample collection and storage is blood spots on filter paper (Owen *et al.*, 2001). This method is useful when there are no facilities to collect blood using a syringe; blood spots may be stored at room temperature.

For tests performed using animal carcasses, where blood or serum is not available, tissue fluids or meat juice samples are an alternative. Several studies have reported on the use of tissue fluids for detection of *Trichinella* infection (Gamble & Patrascu, 1996; Kapel *et al.*, 1998). Generally samples of tissue fluids are used at a lower dilution (higher concentration) in serological assays. If meat samples are used for the extraction of tissue fluids it is recommended to wash the tissue, cut it into small pieces, freeze and thaw it and use these new extracts instead of muscle fluids found in an original sampling, which is often mixed with blood and/or other surface contaminants.

VALIDATION AND QUALITY CONTROL

An acceptable serological assay is one that is properly standardized and validated for its intended purpose. All components of the test that are critical for maintaining suitable performance (critical control points) should be identified and appropriately controlled, and the test should be conducted within a laboratory quality system (Gajadhar & Forbes, 2002; OIE, 2002). In particular, each batch or lot of antigen should be checked by checkerboard titration against a standardized positive control serum.

Commercially available serological tests such as the ELISA often must meet specific standards of sensitivity and specificity established by an individual country's licensing authority. Users of commercial tests should verify that the test has been adequately evaluated using international reference standards and has received the approval of any relevant regulatory authorities. Commercial test kits that are not regulated often yield results that include high numbers of false positive (quite frequently) and false negative (less frequently) results. The user of any test should always conduct an independent evaluation of test performance using panels of defined positive and negative sera that are representative of the population to be tested. The performance of the test relative to specificity and sensitivity should meet the purpose of the test.

When intended for use in epidemiological studies, the sensitivity and specificity of the ELISA, and other serological tests, must be considered when interpreting results. Estimates of sensitivity and specificity can be obtained by testing adequate numbers of known positive and known negative animals or humans. The negative control group should be representative of the local population and positives should represent various stages and levels of infection. For humans, blood donors often represent a good source of reference serum samples. The Office International des Epizooties (OIE, 2000) recommends a minimum of 300 known positive and 1,000 known negative samples to establish sensitivity and specificity.

For a comprehensive discussion of requirements for the validation of a serological test, the reader is referred to the OIE (2000; 2002)

(www.oie.int/eng/normes/mmanual/A_00013.htm).

RECOMMENDED USES

SURVEILLANCE IN FOOD ANIMALS

1. Swine

• Suitability of test

The ELISA test, using ES or tyvelose antigens, is useful for surveillance systems to estimate the prevalence of infection in a domestic pig population. When possible, serologically positive animals should be examined by artificial digestion to confirm infection, to estimate the

worm burdens, and to identify the species of *Trichinella*. Artificial digestion using an appropriate amount of tissue (*i.e.*, 100 grams or more) should be performed according to ICT Recommendations (Gamble *et al.*, 2000; www.med.unipi.it/ict/welcome.htm).
Serological methods, including the ELISA, should not be used for the detection of *Trichinella* infection in individual carcasses at the slaughterhouse, for the purpose of protecting human health.

• Sensitivity and specificity
In pigs, the sensitivity of the ELISA, using an ES antigen, has been reported to range from 93.1-99.2 %, provided sufficient time has elapsed for infected animals to develop an antibody response (see Interpretation of Results, below). The specificity of the ES ELISA in pigs has been reported to range from 90.6-99.4 % (Murrell *et al.*, 1986; Oliver *et al.*, 1989; van der Leek *et al.*, 1992). Infections of as few as 1 larva per 100 g of tissue can be detected by ELISA (Gamble *et al.*, 1983; Kapel & Gamble, 2000).
The use of a somatic extract of *Trichinella* (e.g., Melcher's antigen) in the ELISA results in false positive reactions with sera from pigs infected with other parasites and/or microbes. Several studies have demonstrated that both ES and tyvelose antigens reduce cross-reactions that occur when using a crude *Trichinella* antigen in the ELISA (Gamble *et al.*, 1983, 1988, 1997). The quality of ES antigen depends on adhering to proper methods for the cultivation of *Trichinella* muscle larvae and proper purification of the antigen (Gamble *et al.*, 1988).

• Methodologies
A general method for conducting an ELISA test in pigs is described in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Chapter 2.2.9) (Gamble, 2000) (www.oie.int/eng/normes/mmanual/A_00013.htm).
By special arrangements, standard antigens, reference sera and scientific consultation can be obtained from ICT members' laboratories (www.med.unipi.it/ict/welcome.htm) and the OIE Reference Laboratories (www.oie.int/eng/oie/organisation/en_listeLR.htm).

• Interpretation of results
The level of infection of pigs with *Trichinella* larvae (worm burden) is directly correlated with the time required for antibodies to appear in the blood. For light infections (< 1 larva per gram of tissue), antibodies might not be detected by ELISA for 4-7 weeks or more following exposure (Gamble *et al.*, 1983; Gamble, 1996, 1998), while antibodies might be detected from 2.5-3 weeks in pigs with higher numbers of muscle larvae (Smith & Snowdon, 1989; Kapel & Gamble, 2000). Thus, there is a risk of obtaining false-negative results during the early stages of infection, and for this reason, the ELISA and other tests measuring a serological response cannot be used for individual carcass testing.
On the other hand, there is no correlation between the ultimate worm burden (larvae per gram of tissue) and the result of the ELISA test once an animal has become serologically positive. Therefore, artificial digestion of tissue is an important adjunct to ELISA to determine the public health risk associated with infected animals. *Trichinella* antibodies may persist in pigs for a long time (Nöckler *et al.*, 1995). It can be assumed that in slaughter pigs, which have a live weight of 90 to 100 kg at an age of 25 to 30 weeks, it is unlikely that a false-negative result will result from declining antibody titer (Nöckler *et al.*, 2000).

2. Horses

• Suitability of the test
Studies in horses have shown that serological responses to *Trichinella* infection in this species are less consistent than responses observed in pigs, rodents and humans (Smith & Snowdon, 1987; van Knapen *et al.*, 1987; Polidori *et al.*, 1989; Soule *et al.*, 1989; Gamble *et al.*, 1996; Voigt *et al.*, 1997; Yepez-Mulia *et al.*, 1999; Sofronic-Milosavjevic *et al.*, 2001; Pozio *et al.*, 2002a). Studies in horses experimentally infected with 5,000 *Trichinella spiralis* larvae reported a decline in antibody titer at week 15 post-infection, at which time infective larvae were still present in the muscle tissue (Voigt *et al.*, 1997).
Low specificity with ES antigen and tyvelose antigen and low sensitivity due to a decreasing antibody titer, even in the presence of high numbers of larvae in the musculature, have been reported using ELISA and IETB (Soule *et al.*, 1989; Pozio *et al.*, 1997, 2002a).
Considering the present state of knowledge, the ICT cannot recommend serological methods for use in horses either for detection of single infections or for reliable surveillance. However, further studies are needed, especially in endemic regions, to determine if serological methods might have some value.

• Special considerations
A recent report (Pozio *et al.*, 2002a) suggested the possibility that *Trichinella* has antigens in common with some plant parasitic nematodes, including tyvelose, which is believed to be highly specific (Spiegel & McClure, 1995; Lopez de Mendoza *et al.*, 1999). If this report is substantiated in further studies, it will pose further problems for serological detection of *Trichinella* infection in the horse and more generally in herbivores.

3. Game and other animals intended for human consumption

• Suitability of the test
Relatively few studies have used serological methods for estimating *Trichinella* infection in game animals

intended for human consumption. There have been a few field studies estimating *Trichinella* infection in wild boars using an ES antigen in an ELISA. From the Netherlands, 31 of 458 samples (6.8 %) were positive (van der Giessen *et al.*, 2001) and from Germany, 234 of 16,888 samples were positive, corresponding with a seropositivity of 1.4 % (Nöckler *et al.*, 1999). This seroprevalence is much higher (up to 100 times) when compared with the prevalence of *Trichinella* in wild boars as determined by artificial digestion (between 0.001 and 0.01 % in Germany). These findings might be interpreted as resulting from the higher sensitivity of the ELISA or a lower specificity of the ELISA due to cross-reactions. Based on these aforementioned studies, insufficient information is available on the performance of the ELISA in game animals to recommend this method for individual carcass inspection or for epidemiological studies for the purpose of estimating true prevalence. Further studies are required to determine the influence of cross-reactions and quality of blood samples on the specificity of the ELISA and also to determine whether very low level infections may be responsible for some of the positive serological results obtained.
Game animals intended for human consumption should be examined by artificial digestion, according to approved methods (Gamble *et al.*, 2000; www.med.unipi.it/ict/welcome.htm).

SURVEILLANCE IN OTHER ANIMAL SPECIES

- Suitability of the test

Due to the high number of parasitic, bacterial, fungal and viral infections, which can be present in wildlife, the risk of cross-reactions with non-specific antibodies is very high.
Therefore, the use of serological methods for determining prevalence of *Trichinella* infection in wildlife must be preceded by a thorough evaluation of test sensitivity and specificity in the species and population being tested. The ICT discourages the use of serological methods for the detection and surveillance of *Trichinella* infection in wildlife, without such an evaluation. Any evaluation should include digestion of a minimum of 100 grams of tissue.
It is likely that some reports of serologically positive animals are the result of false positive reactions. This caution on the use of serological methods for testing wild animals is suggested by studies that reported a high serological prevalence for *Trichinella* in animals without larvae in muscles from regions where *Trichinella* infection was never observed (Vercammen *et al.*, 2002) or the prevalence rate detected by artificial digestion was 100 times lower than that recorded using serological methods (Nöckler & Voigt, 1997a; Wacker *et al.*, 1999). However, the much greater sensitivity of ELISA might also be a factor when testing wildlife populations where worm burdens may be very low (only a few larvae per kilogram of tissue). Additional studies are required to assess the occurrence of false positive reactions and the detection of very low level infections by serological methods in wildlife populations.
To assure prevention of human disease, any meat-eating game animals intended for human consumption should be examined by tissue digestion.

- Sensitivity and specificity

Sensitivity and specificity should be determined by testing panels of sera from a sufficient number of known positive and known negative animals representing the species and population, prior to using a test. Since this is often not possible with wildlife species, the limitations should be clearly understood and explained when reporting or interpreting results.

- Methodologies

Wild boar
In one study, an antibody response was detectable by ELISA three to four weeks after experimental infection of wild boars with different encapsulated *Trichinella* species (Kapel, 2001), however, seroconversion was delayed in infections with non-encapsulated species. The antibody level remained stable in wild boars infected with *T. spiralis, T. britovi* and *T. nelsoni*, but the rapid decrease of antibodies directed against *T. nativa* and *Trichinella* T6 was apparently associated with the rapid disappearance of larvae. A few epidemiological studies have used ES antigens for the evaluation of prevalence in wild boar populations (van der Giessen *et al.*, 2001), but these prevalence results have in general been much higher as compared to results obtained by artificial digestion. In contrast, the experimental studies of Kapel (2001) have shown a good correlation between larval burden and antibody response. Such differences are probably related to cross reactions resulting from infection with other tissue-migrating nematodes (*Toxocara*, *Ascaris*, *Spirura*), but no studies have been conducted to evaluate this phenomenon in wild boar.

Bear
Several studies have reported the use of ELISA to detect infection in bears (*Ursus arctos*, *Ursus americanus*) (Zarnke *et al.*, 1997; Nutter *et al.*, 1998); however, confirmatory digestion testing did not correlate well with serological results. Better specificity in the ELISA can be obtained by using a customized anti-bear IgG reagent as compared with the Protein A conjugate in the ELISA test (H.R. Gamble, unpublished).

Fox
Experimental studies in silver foxes (*Vulpes vulpes*) showed that use of an ES antigen in an ELISA is a sui-

table method for serological detection of *Trichinella* infection in this species (Kapel, 2000). Specific antibodies were detectable in all foxes infected with 500 or 2,000 larvae between two and six weeks post infection, and these antibodies persisted throughout the experiment (30 and 76 weeks post-infection) with mean larval recovery rate at post mortem ranging from 4.3 to 11.3 larvae per gram of tissue (Nöckler & Voigt, 1997b). Anti-dog-IgG peroxidase conjugate is suitable as an alternative to the species-specific anti-fox antibody system since there is a close phylogenetic relationship between foxes and dogs. Results from field studies demonstrated a 100 times higher *Trichinella* seroprevalence in the red fox population (7.7 %) compared to a prevalence of 0.07 % in foxes examined by artificial digestion (Wacker *et al.*, 1999). As in wild boars the huge difference between seroprevalence and actual detection of parasites may be due, in part, to the higher sensitivity of ELISA (0.01 larvae per g) and the limited ability to detect muscle larvae in small samples of muscle tissue. It should be considered that the larval recovery rate in the majority of foxes might be very low, i.e. about or less than 0.1 larvae per g of musculature, as published by Enemark *et al.* (2000) and van der Giessen *et al.* (2001).

• Special considerations

The variability of collection methods for game meat samples often creates problems in conducting serological tests. Samples are frequently contaminated by bacteria or fungi, or they may be hemolysed; these problems can cause false positive results. Filtration of contaminated samples with a 0.22 µm filter is useful to reduce the background, but is not always entirely effective.

• Interpretation of results

False negative results have been observed in wild animals (e.g., foxes and wild boars) with long-term low-level infections with sylvatic species, (e.g., *T. britovi*) (E. Pozio, unpublished data).

Use in diagnosis of human trichinellosis

According to Ljungström (1983) there are three objectives in the immunodiagnosis of human trichinellosis: a) recognizing the acute infection to allow early anthelminthic treatment; b) making a retrospective diagnosis; and c) adding information to the epidemiology of the infection.

• Suitability of test

Many serological tests are available for human diagnosis (Despommier, 1986; Murrell & Bruschi, 1994; Kociecka *et al.*, 2001; Dupouy-Camet *et al.*, 2002). These tests include, for example, ELISA, IFA, CIA, IETB and counterimmunoelectrophoresis (CIE). Diagnostic laboratories might use more than one test for screening; for example, screening by ELISA to detect the response against a soluble antigen and by IFA to detect antibodies that react with antigens on the larva's cuticle. When a single preliminary serological test is positive, another test, such as an IETB or CIA might be performed for confirmation. As a general rule, however, the ELISA with ES or tyvelose antigen can be used as the primary diagnostic of choice to detect infections in humans.

• Sensitivity and specificity

Absolute sensitivity (100 %) has been obtained in humans infected with *T. spiralis* by ELISA (measuring IgG) using both a crude larval extract or ES antigens (van Knapen *et al.*, 1982, Bruschi *et al.*, 2001). This high rate of sensitivity, observed 50 days after infection, declined to about 80 % after two years. However, parasite-specific IgM was detected for up to 11 years after infection. Serological tests measuring other classes of antibodies (IgA, IgE) resulted on lower sensitivity rates (Murrell & Bruschi, 1994). The study of the humoral response against stage-specific antigens has not resulted in an improvement in serodiagnosis; however, the search for newborn larvae specific IgA has shown promise for use in detection of the early phase of the infection (more than 80 % of positivity after three weeks of infection) (Mendez-Loredo *et al.*, 2001). The sensitivity of ELISA was reported to be higher with ES antigen than with tyvelose for detecting circulating antibodies early in the infection (E. Pozio, unpublished data). However, in another report, asymptomatic infections are detected using the tyvelose antigen (Owen *et al.*, 2001). Additional comparative studies are needed with these two antigens. High sensitivity (up to 100 %) was also obtained with other tests, including IFA (Pozio *et al.*, 1988) and CIA (Ivanoska *et al.*, 1989). Specificity depends primarily on the type of antigen used and the cut-off value established. Using the ELISA (Engvall & Ljungström, 1975), ES antigens are preferable to crude extracts of *T. spiralis* muscle larvae, since the former resulted in a higher specificity. This is particularly important in regions where other human helminth infections (e.g., *Ascaris, Trichuris*, filariae) are common and cross-reactions with these parasites could give false positive results (Au *et al.*, 1983; Mahannop *et al.*, 1995). In industrialized countries, the risk of cross-reactions using ES antigen is low, but cross-reactions do occur with human larval migrans of unknown species. In sera from Greenlanders, false-positive reactions were suspected to be due to infection with *Anisakis* or *Pseudoterranova* from ingestion of marine fish (C. Kapel, unpublished data).

The use of the synthetic tyvelose antigen in the ELISA has resulted in even greater specificity (Bruschi *et al.*,

2001; Owen *et al.*, 2001), although some exceptions have been noted (Dea-Ayuela *et al.*, 2001). Further study on this synthetic antigen is required.

Western blots have shown promise for discriminating trichinellosis patients from patients with other helminth infection, although possible cross-reactions may occur with cases of anisakiasis (Yera *et al.*, 2003) and schistosomiasis (Dupouy-Camet J., unpublished data).

• Methodologies

Indirect hemagglutination (IHA), bentonite flocculation (BFT), IFA, latex agglutination (LA), and ELISA are the more commonly used tests, the last being the most sensitive (Murrell & Bruschi, 1994; Kociecka, 2000). CIA (Ivanoska *et al.*, 1989) and IETB (Dupouy-Camet *et al.*, 1988; Pozio *et al.*, 1993; Owen *et al.*, 2001) have also been used. The ELISA for human infection is performed essentially as described for pigs, with the substitution of an anti-human IgG reagent.

IFA can be carried out either using frozen sections of infected tissue (Ljungström, 1974) or formalin fixed whole larvae (Brzosko *et al.*, 1965; Pozio *et al.*, 1988), as antigens, the former being more sensitive. With this test, all relevant immunoglobulins can be detected. Cross-reactions with *Trichinella* antigens were observed in persons with autoimmune diseases (Robert *et al.*, 1996). Persons reading the fluorescent sections should be aware that only sections with a uniform fluorescence should be considered as positive, whereas those, which show a non-uniform fluorescence along the cuticle, should be considered as false-positive. Counter staining with Evans Blue reduces false positive results and facilitates reading of slides (Boireau *et al.*, 1997).

IETB can be used as a primary or confirmatory test and when ES antigens are used it is quite specific and useful for follow-up studies (Andrews *et al.*, 1995). The presence of antibodies specific for the TSL-1 antigen family (40-70 kDa in the reduced form) should be considered diagnostic.

• Interpretation of results

For humans, as for animals, it is imperative to evaluate the cut-off value on the basis of a panel of serum samples (at least 100-200 sera), which are representative of the human population for which the test will be used. The human genotype, food habits and environmental characteristics can influence the background of a serological test. This preliminary evaluation should be done for both commercial kits and laboratory-developed tests. It is also important that the cut-off value be confirmed, every time the antigen, reagents or materials (e.g., ELISA plate) are modified or substituted.

Antibody levels do not correlate with the severity or the clinical course of trichinellosis in humans (Murrell & Bruschi, 1994). Seroconversion usually occurs between the second and fifth week of infection and the time required for seroconversion is inversely correlated with the infective dose. Serum may remain positive up to one year or more (19 years has been reported) after the end of the acute phase of infection (Pozio *et al.*, 1993). In *T. britovi* human infections, seroconversion has been documented up to two months post infection. Serological testing performed in a large outbreak of human trichinellosis due to *T. nativa* revealed a seropositive rate of 45 % and 87 % at 3-4 and 10-11 weeks post-infection among confirmed cases. However, seroconversion from acute to convalescent samples was 55 % (Schellenberg *et al.*, 2003).

An evaluation of the antibody kinetics in infected persons should be conducted every three months to follow the success of chemotherapy. In persons infected with *T. britovi*, circulating antibodies disappear (in about one half of patients) within six months and within three years all persons are seronegative (Pozio *et al.*, 1993). In *T. spiralis* infected persons, who received a delayed or ineffective therapy, specific antibodies can be detected for years (Pozio *et al.*, 2001), whereas in those who received effective treatment in the first two weeks after infection specific antibodies can disappear in a shorter period of time.

The IFA has proved to be the most reliable method for discriminating between patients with biopsy-confirmed infection and those patients suspected of exposure, but biopsy negative (Murrell & Bruschi, 1994). Due to the fact that IFA is based on cuticle surface antigens of muscle larvae cross-reactions may occur with *Onchocerca* spp. and *Schistosoma mansoni* (Robert *et al.*, 1996).

SPECIES IDENTIFICATION

According to current taxonomy, the genus *Trichinella* is composed of eight distinct species, all of which can infect humans (Pozio, 2001; Pozio *et al.*, 2002b). Of these eight species, five have an encapsulated larva in host muscles and infect only mammals (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, and *T. nelsoni*) and three show non-encapsulated larvae in host muscles and infect both mammals and birds (*T. pseudospiralis*) or mammals and reptiles (*T. papuae* and *T. zimbabwensis*) (Pozio *et al.*, 2004). Furthermore, other genotypes strictly related to the above reported species have been described. Specifically: *Trichinella* T6 is related to *T. nativa*, *Trichinella* T8 and *Trichinella* T9 are related to *T. britovi* (Pozio, 2001). All of these species and genotypes can be easily identified by a multiplex-PCR and PCR-RFLP (Wu *et al.*, 1999; Zarlenga *et al.*, 1999; Pozio & La Rosa, 2003).

Serological methods cannot be used to differentiate species. Although minor differences in the immune res-

ponse against different *Trichinella* species have been described (Pozio *et al.*, 1993; Bruschi *et al.*, 1999; Kapel & Gamble, 2000) these differences were generally related to the kinetics of the antibody response. The antibody response is dependent on the rate of production of newborn larvae, which is generally higher in *T. spiralis* as compared with other species.
Differences have been observed in ELISA optical density values or IETB patterns obtained using serum samples of animals (Kapel & Gamble, 2000; Kapel, 2001) and humans (Pozio *et al.*, 1993) infected with a *Trichinella* species which is the same or different from that which was used to prepare the antigen. However, these differences are of limited value for diagnostic purposes.

CONCLUSIONS

This guideline is based on the best scientific published information and on the unpublished data from laboratories with a great expertise in this field and represent the official position of the International Commission on Trichinellosis regarding acceptable methods and the evaluation of the sensitivity and specificity. These recommendations are subject to change as new scientific information becomes available.

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REFERENCES

ANDREWS J.R.H., BANDI C., POZIO E., GOMEZ-MORALES M.A., AINSWORTH R. & ABERNETHY D. Identification of *Trichinella pseudospiralis* from a human case using random amplified polymorphic DNA. *American Journal of Tropical Medicine & Hygiene*, 1995, *53*, 185-188.

APPLETON J.A., BELL R.G., HOMAN W. & VAN KNAPEN F. Consensus on *Trichinella spiralis* antigens and antibodies. *Parasitology Today*, 1991, *7*, 190-192.

AU A.C.S., KO R.C., SIMON J.W., RIDELL N.J., WONG F.W.F. & TEMPLER M.J. Study of acute trichinosis in Ghurkas: specificity and sensitivity of enzyme-linked immunosorbent assay for IgM and IgE antibodies to *Trichinella* larvae antigens in diagnosis. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 1983, *77*, 412-415.

BOIREAU P., FABIEN J.F., LE GUERHIER F., VAYSSIER M., PERRET C. & SOULÉ C. Detailed antigenic analysis of *Trichinella* T5 and T1 using monoclonal antibodies. Ortega Pierres G., Gamble R., van Knapen F. & Wakelin D. (Eds.) Trichinellosis. Germar Press, Nonoalco Tlateloco, Mexico, 1997, 211-217.

BRUSCHI F., POZIO E., WATANABE N., GOMEZ MORALES M.A., ITO M., HUANG Y. & BINAGHI R. Anaphylactic response to parasite antigens: IgE and IgG1 independently induce death in *Trichinella*-infected mice. *International Archives of Allergy and Immunology*, 1999, *119*, 291-296.

BRUSCHI F., MORETTI A., WASSOM D. & PIERGILI-FIORETTI D. The use of a synthetic antigen for the serological diagnosis of human trichinellosis. *Parasite*, 2001, *8*, S141-S143.

BRZOSKO W., GANCARZ Z. & NOWOSLAWSKI A. Immunofluorescence in the serological diagnosis of *Trichinella spiralis* infection. *Experimental Medicine and Microbiology*, 1965, *17*, 355-365.

DEA-AYUELA M.A., ROMARIS F., UBERIRA F.M., RAMA-INIGUEZ S., MARTINEZ-FERNANDEZ A. & BOLAS F. Possible presence of common tyvelose-containing glycans in *Trichinella* L_1 larvae and embryonated eggs of several helminths. *Parasite*, 2001, *8*, S120-S122.

DESPOMMIER D.D. Trichinellosis. Walls K.W., Schantz P.M. (Eds). Immunodiagnosis of Parasitic Diseases, Vol. 1. Helminthic Diseases. New York, Academic Press, 1986, 163-181.

DUPOUY-CAMET J., BOUGNOUX M.E., ANCELLE T., FAGARD R. & LAPIERRE J. Antigenic characteristics of two strains of *Trichinella spiralis* isolated during the horsemeat-related outbreaks of 1985 in France. *Parasitolology Research*, 1988, *75*, 79-80.

DUPOUY-CAMET J., KOCIECKA W., BRUSCHI F., BOLAS-FERNANDEZ F. & POZIO E. Opinion on the diagnosis and treatment of human trichinellosis. *Expert Opinion on Pharmacotherapy*, 2002, *3*, 1117-1130.

ENGVALL E. & LJUNGSTRÖM I. Detection of human antibodies to *Trichinella spiralis* by enzyme-linked immunosorbent assay (ELISA). *Acta Pathologica Microbiologica Scandinavica*, 1975, *83*, 231-237.

ENEMARK H.L., BJORN H., HENRIKSEN S.A. & NIELSEN B. Screening for infection of *Trichinella* in red fox (*Vulpes vulpes*) in Denmark. *Veterinary Parasitology*, 2000, *88*, 229-237.

EUROPEAN COMMISSION. Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on trichinellosis, epidemiology, methods of detection and *Trichinella*-free pig production. Adopted on 21-22 November 2001 in Brussels.

GAJADHAR A.A. & FORBES L.B. An internationally recognized quality assurance system for diagnostic parasitology in animal health and food safety, with example data on trichinellosis. *Veterinary Parasitology*, 2002, *103*, 133-140.

GAMBLE H.R. Detection of trichinellosis in pigs by artificial digestion and enzyme immunoassay. *Journal of Food Protection*, 1996, *59*, 295-298.

GAMBLE H.R. Sensitivity of artificial digestion and enzyme immunoassay methods for inspection of trichinellosis in pigs. *Journal of Food Protection*, 1998, *61*, 339-343.

GAMBLE H.R., ANDERSON W.R., GRAHAM C.E. & MURRELL K.D. Diagnosis of swine trichinosis by enzyme-linked immu-

nosorbent assay (ELISA) using an excretory-secretory antigen. *Veterinary Parasitology*, 1983, *13*, 349-361.

GAMBLE H.R., BESSONOV A.S., CUPERLOVIC K., GAJADHAR A.A., KNAPEN F. VAN, NÖCKLER K., SCHENONE H. & ZHU X. International Commission on Trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. *Veterinary Parasitology*, 2000, *93*, 393-408.

GAMBLE H.R. & PATRASCU I.V. Whole blood, serum and tissue fluids in an EIA for swine trichinellosis. *Journal of Food Protection*, 1996, *59*, 1213-1217.

GAMBLE H.R., RAPIC D., MARINCULIC A. & MURRELL K.D. Evaluation of excretory-secretory antigens for the serodiagnosis of swine trichinellosis. *Veterinary Parasitology*, 1988, *30*, 131-137.

GAMBLE H.R., SOLOMON M.B. & GAJADHAR A.A. Detection methods for trichinellosis in horses. *Journal of Food Protection*, 1996, *59*, 420-425.

GAMBLE H.R., WISNEWSKI N. & WASSOM D. Detection of trichinellosis in swine by enzyme immunoassay using a synthetic glycan antigen. *American Journal of Veterinary Research*, 1997, *58*, 417-421.

GRENCIS R.K., CRAWFORD C., PRITCHARD D.I., BEHNKE J.M. & WAKELIN D. Immunization of mice with surface antigens from the muscle larvae of *Trichinella spiralis*. *Parasite Immunology*, 1986, *8*, 587-596.

HOMAN W.L., DERKSEN A.C. & VAN KNAPEN F. Identification of diagnostic antigens from *Trichinella spiralis*. *Parasitology Research*, 1992, *78*, 112-119.

IVANOSKA D., CUPERLOVIC K., GAMBLE H.R. & MURRELL K.D. Comparative efficacy of antigen and antibody detection tests for human trichinellosis. *Journal of Parasitolology*, 1989, *75*, 38-41.

KAPEL C.M.O. Host diversity and biological characteristics of the *Trichinella* genotypes and their effect on transmission. *Veterinary Parasitology* 2000, *93*, 263-278.

KAPEL C.M.O. Sylvatic and domestic *Trichinella* spp. in wild boars; infectivity, muscle larvae distribution, and antibody response. *Journal of Parasitology*, 2001, *87*, 309-314.

KAPEL C.M.O. & GAMBLE H.R. Infectivity, persistence, and antibody response to domestic and sylvatic *Trichinella* spp. in experimentally infected pigs. *International Journal for Parasitology*, 2000, *30*, 215-221.

KAPEL C.M.O., WEBSTER P., LIND P., POZIO E., HENRIKSEN S-AA., MURRELL K.D. & NANSEN P. *Trichinella spiralis, Trichinella britovi* and *Trichinella nativa*. Infectivity, muscle larvae distribution and antibody response after experimental infection of pigs. *Parasitology Research*, 1998, *84*, 264-271.

KOCIECKA W. Trichinellosis: human disease, diagnosis and treatment. *Veterinary Parasitolology*, 2000, *93*, 365-383.

KOCIECKA W., BRUSCHI F., MARINI C., MROZEWIZ B. & PIELOK L. Clinical appraisal of patients and detection of serum antibodies by ELISA and CIA tests in late periods of *Trichinella* sp. invasion. *Parasite*, 2001, *8*, S147-S151.

LJUNGSTRÖM I. Antibody response to *Trichinella spiralis*. Kim C. (Ed.) Trichinellosis. New York, Intext, 1974, 449-460.

LJUNGSTRÖM I. Immunodiagnosis in man. Campbell W.C. (Ed.) *Trichinella* and Trichinosis. New York, Plenum Press, 1983, 403-424.

LOPEZ DE MENDOZA M.E., CURTIS R.H.C. & GOWEN S. Identification and characterization of excreted-secreted products and surface coat antigens of animal and plant-parasitic nematodes. *Parasitology*, 1999, *118*, 397-405.

MAHANNOP P., SETASUBAN P., MOKAROTE N., TAPCHAISRI P. & CHAICUMPA W. Immunodiagnosis of human trichinellosis and identification of specific antigen for *Trichinella spiralis*. *International Journal for Parasitology*, 1995, *25*, 87-94.

MENDEZ-LOREDO B., MARTINEZ Y., ZAMORA R., CHAPA-RUIZ R. & SALINAS-TOBON R. Class specific antibody responses to newborn larva antigens during *Trichinella spiralis* human infection. *Parasite*, 2001, *8*, 152-157.

MURRELL K.D. & BRUSCHI F. Clinical trichinellosis. Sun T. (Ed.) Progress in Clinical Parasitology. Boca Raton, Fla, CRC Press, 1994, 117-150.

MURRELL K.D., ANDERSON W.R., SCHAD G.A., HANBURY R.D., KAZACOS K.R., BROWN J. & GAMBLE H.R. Field evaluation of the ELISA test for swine trichinosis: efficacy of the excretory-secretory antigen. *American Journal of Veterinary Research*, 1986, *47*, 1046-1049.

NAVARRETE I., SERRANO F., REINA D., MARTINEZ FERNANDEZ A., ALBARRAN E., HABELA M. & NIETO C. Standardization of the enzyme-linked immunosorbent assay for detection of swine trichinellosis. Tanner C.E., Martinez Fernandez A.R. & Bolas Fernandez F. (Eds.), *Trichinellosis*, Consejo Superior de Investigaciones Cientificas Press, Madrid, Spain, 1989, 223-230.

NÖCKLER K., VOIGT W.P., PROTZ D., MIKO A. & ZIEDLER K. Intravitale Diagnostik der Trichinellose beim Schwein mit dem indirekten ELISA (Indirect ELISA for the diagnosis of trichinellosis in living pigs). *Berliner and Münchener Tierärztliche Wochenschrift*, 1995, *108*, 167-174.

NÖCKLER K. & VOIGT W.P. Comparison of methods for the diagnosis of trichinellosis. Ortega-Pierres G., Gamble H.R., van Knapen F. & Wakelin D. (Eds.) *Trichinellosis*. Germar Press, Nonoalco Tlateloco, Mexico, 1997a, 653-658.

NÖCKLER K. & VOIGT W.P. Experimental *Trichinella spiralis* infection in the silver fox (*Vulpes vulpes fulva*). Ortega-Pierres G., Gamble H.R., van Knapen F. & Wakelin D. (Eds.) *Trichinellosis*. Germar Press, Nonoalco Tlateloco, Mexico, 1997b, 319-323.

NÖCKLER K., PALLY R., DEDEK J., VOIGT W.P., MIKO A. & SCHUSTER R. Untersuchungen zum Vorkommen von *Trichinella* spp. beim Schwarzwild in Mecklenburg-Vorpommern mit dem indirekten ELISA und der Verdauungsmethode (Investigations on *Trichinella* prevalence in wild boars in Mecklenburg-Vorpommern using indirect ELISA and digestion method). Tagungsband der DVG, Fachgruppe "Parasitologie und parasitäre Krankheiten", Thema: Neuere Methoden und Ergebnisse zur Epidemiologie von Parasitosen, 10.-12.03.99 in Hannover, 1999, 102-112.

NÖCKLER K., POZIO E., VOIGT W.P. & HEIDRICH J. Detection of *Trichinella* infection in food animals. *Veterinary Parasitology*, 2000, *93*, 335-350.

NUTTER F.B., LEVINE J.F., STOSKOPF M.K., GAMBLE H.R. & DUBEY J.P. Seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in North Carolina black bears (*Ursus americanus*). *Journal of Parasitology*, 1998, *84*, 1048-1050.

OFFICE INTERNATIONAL DES EPIZOOTIES. Manual of standards for diagnostic tests and vaccines. Paris, France, 2000, 322-327.

OFFICE INTERNATIONAL DES EPIZOOTIES. Quality standards and guidelines for veterinary laboratories: Infectious diseases. Paris, France, 2002, 1-63.

OLIVER D.G., SINGH P., ALLISON D.E., MURRELL K.D. & GAMBLE H.R. Field evaluation of an enzyme immunoassay for detection of hogs in a high volume North Carolina abattoir. Tanner C.E. (Ed.) *Trichinellosis*. Consejo Superior de Investigaciones Cientificas Press, Madrid, Spain, 1989, 439-444.

ORTEGA-PIERRES M.G., YEPEZ-MULIA L., HOMAN W., GAMBLE H.R., LIM P., TAKAHASHI Y., WASSOM D.L. & APPLETON J.A. Workshop on a detailed characterization of *Trichinella spiralis* antigens: A platform for future studies on antigens and antibodies to this parasite. *Parasite Immunology*, 1996, *18*, 273-284.

OWEN I.L., POZIO E., TAMBURRINI A., DANAYA R.T., BRUSCHI F. & GOMEZ MORALES M.A. Focus of human trichinellosis in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene*, 2001, *65*, 553-557.

PINELLI E., VAN DER LUGT G., HOMAN W., VAN DER GIESSEN J., & KORTBEEK L.M. Antigen recognition by IgG4 antibodies in human trichinellosis. *Parasite*, 2001, *8*, S168-S171.

POLIDORI G.A., GRAMENZI F., PIERGILI FIORETTI D., FERRI N., RANUCCI S., MORETTI A., SCACCHIA M., BELLELLI C. & BALDELLI B. Experimental trichinellosis in horses. Tanner C.E., Martinez-Fernandez A.R. and Bolas Fernandez F. (Eds.), *Trichinellosis*. Consejo Superior de Investigaciones Cientificas Press, Madrid, Spain, 1989, 268-274.

POZIO E. New patterns of *Trichinella* infections. *Veterinary Parasitology*, 2001, *98*, 133-148.

POZIO E., CAPPELLI O., MARCHESI L., VALERI P. & ROSSI P. Third outbreak of trichinellosis caused by consumption of horse meat in Italy. *Annales de Parasitologie Humaine et Comparée*, 1988, *63*, 48-53.

POZIO E., FOGGIN C.M., MARUCCI G., LA ROSA G., SACCHI L., CORONA S., ROSSI P. & MUKARATIRWA S. *Trichinella zimbabwensis* n. sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. *International Journal for Parasitology*, 2002b, *32*, 1787-1799.

POZIO E. & LA ROSA G. PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. *Methods in Molecular Biology*, 2003, *216*, 299-309.

POZIO E., MARUCCI G., CASULLI A., SACCHI L., MUKARATIRWA S., FOGGIN C.M. & LA ROSA G. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. *Parasitology*, 2004, *128* (3) (in press).

POZIO E., SACCHINI D., SACCHI L., TAMBURRINI A. & ALBERICI F. Failure of mebendazole in treating *Trichinella spiralis* infection in humans at the stage of encapsulating larvae. *Clinical Infection Diseases*, 2001, *32*, 638-642.

POZIO E., SOFRONIC-MILOSAVLJEVIC L., GOMEZ MORALES M.A., BOIREAU P. & NÖCKLER K. Evaluation of ELISA and Western blot analyses using three antigens to detect anti-*Trichinella* IgG in horses. *Veterinary Parasitology*, 2002a, *108*, 163-178.

POZIO E., TAMBURRINI A., SACCHI L., GOMEZ MORALES M.A., CORONA S., GOFFREDO E. & LA ROSA G. Detection of *Trichinella spiralis* in a horse during routine examination in Italy. *International Journal for Parasitology*, 1997, *27*, 1613-1621.

POZIO E., VARESE P., GOMEZ MORALES M.A., CROPPO G.P., PELLICCIA D. & BRUSCHI F. Comparison of human trichinellosis caused by *Trichinella spiralis* and *Trichinella britovi*. *American Journal of Tropical Medicine and Hygiene*, 1993, *48*, 568-575.

REASON A.J., ELLIS L.A., APPLETON J.A., WISNEWSKI N., GRIEVE R.B., MCNEIL M., WASSOM D.L., MORRIS H.R. & DELL A. Novel tyvelose-containing tri- and tetra-antennary N-glycans in the immunodominant antigens of the intracellular parasite *Trichinella spiralis*. *Glycobiology*, 1994, *4*, 593-603.

ROBERT F., WELL B., KASSIS N. & DUPOUY-CAMET J. Investigation of immunofluorescence cross-reaction against *Trichinella spiralis* by Western blot (Immunoblot) analysis. *Clinical Diagnosis Laboratory Immunology*, 1996, *3*, 575-577.

RUITENBERG E.J. & VAN KNAPEN F. Enzyme-linked immunosorbent assay (ELISA) as a diagnostic method for *Trichinella spiralis* infections in pigs. *Veterinary Parasitolology*, 1977, *3*, 317-326.

RUITENBERG E.J., STEERENBERG P.A., BROSI B.J. & BUYS J. Serodiagnosis of *Trichinella spiralis* infections in pigs by enzyme-linked immunosorbent assays. *Bulletin of the World Health Organization*, 1974, *51*, 108-109.

SCHELLENBERG R.S., TAN B.J.K., IRVINE J.D., STOCKDALE D.R., GAJADHAR A.A., SERHIR B., BOTHA J., ARMSTRONG C.A., WOODS S.A., BLONDEAU J.M. & MCNAB T.L. An outbreak of trichinellosis due to consumption of bear meat infected with *Trichinella nativa* in two northern Saskatchewan communities. *Journal of Infectious Diseases*, 2003, *188*, 835-843.

SEAWRIGHT G.L., DESPOMMIER D., ZIMMERMANN W. & ISENSTEIN R.S. Enzyme immunoassay for swine trichinellosis using antigens purified by immunoaffinity chromatography. *American Journal of Tropical Medicine and Hygiene*, 1983, *32*, 1275-1284.

SMITH H.J. & SNOWDON K.E. Experimental *Trichinella* infections in Ponies. *Canadian Journal of Veterinary Research*, 1987, *51*, 415-416.

SMITH H.J. & SNOWDON K.E. Comparative assessment of a double antibody enzyme immunoassay test kit and a triple antibody enzyme immunoassay for the diagnosis of *Trichinella spiralis spiralis* and *Trichinella spiralis nativa*. *Canadian Journal of Veterinary Research*, 1989, *53*, 497-499.

SPIEGEL Y. & MCCLURE M.A. The surface-coat of plant-parasitic nematodes-chemical composition, origin and biological role – a review. *Journal of Nematology*, 1995, *27*, 127-134.

SOFRONIC-MILOSAVLJEVIC LJ., POZIO E., PATRASCU I.V., SKEROVIC N., GOMEZ MORALES M.A. & GAMBLE H.R.. Immunodiagnosis of *Trichinella* infection in the horse. *Parasite*, 2001, *8*, 260-262.

SOULÉ C., DUPOUY-CAMET J., GEORGES P., ANCELLE T., GILLET J.P., VAISSAIRE J., DELVIGNE A. & PLATEAU E. Experimental trichinellosis in horses: biological and parasitological evaluation. *Veterinary Parasitology*, 1989, *31*, 19-36.

VAN DER LEEK M.L., DAME J.B., ADAMS C.L., GILLIS K.D. & LITTELL R.C. Evaluation of an enzyme-linked immunosorbent assay for diagnosis of trichinellosis in swine. *American Journal of Veterinary Research*, 1992, *53*, 877-882.

VAN DER GIESSEN J.W.B., ROMBOUT Y., VAN DER VEEN A. & POZIO E. Diagnosis and Epidemiology of *Trichinella* infections in wildlife in the Netherlands. *Parasite*, 2001, *8*, 103-105.

VAN KNAPEN F., FRANCHIMONT J.M., HENDRIKX W.M. & EYSKER M. Experimental *Trichinella spiralis* infection in two horses. *Veterinary Archives*, 1987, *31*, 123-132.

VAN KNAPEN F., FRANCHIMONT J.M., VERDONK A.R., STUMPF J. & UDEUTSCH K. Detection of specific immunoglobulins (IgG, IgM, IgA, IgE) and total IgE levels in human trichinosis by means of the enzyme linked immunosorbent assay (ELISA). *American Journal of Tropical Medicine and Hygiene*, 1982, *31*, 973-976.

VERCAMMEN F., VERVAEKE M., DORNY P., BRANDT J., BROCHIER B., GEERTS S. & VERHAGEN R. Survey for *Trichinella* spp. in red foxes (*Vulpes vulpes*) in Belgium. *Veterinary Parasitology*, 2002, 103, 83-88.

VOIGT W.P., NÖCKLER K., FREISCHEM B., HENRIKSEN S.A., VAN KNAPEN F., MARTINEZ FERNANDEZ A., PFEIFFER G., POZIO E., REUTER G., RING CH., SOULÉ C. & WEISS H. Detection of low levels of *Trichinella spiralis* in experimentally infected horses. Ortega-Pierres G., Gamble H.R., van Knapen F. & Wakelin D. (Eds.), *Trichinellosis*. Germar Press, Nonoalco Tlateloco, Mexico, 1997, 629-634.

WACKER K., RODRIGUEZ E., GARATE T., GEUE L., TACKMANN K., SELHORST T., STAUBACH C. & CONRATHS F.J. Epidemiological analysis of *Trichinella spiralis* infections of foxes in Brandenburg, Germany. *Epidemiology and Infection*, 1999, *123*, 139-147.

WISNEWSKI N., MCNEIL M., GRIEVE R.B. & WASSOM D.L. Characterization of novel fucosyl- and tyvelosyl-containing glycoconjugates from *Trichinella spiralis* muscle stage larvae. *Molecular and Biochemical Parasitology*, 1993, *61*, 25-35.

WU Z., NAGANO I., POZIO E. & TAKAHASHI Y. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for the identification of *Trichinella* isolates. *Parasitology*, 1999, *118*, 211-218.

YERA H., ANDIVA S., PERRET C., LIMONNE D., BOIREAU P. & DUPOUY-CAMET J. Development and evaluation of a western blot kit for the diagnosis of human trichinellosis. *Clinical Diagnosis and Laboratory Immunology*, 2003, *10*, 793-796.

YEPES-MULIA L., ARRIAGA C., VIVEROS N., ADAME A., BENITEZ E. & ORTEGA-PIERRES M.G. Detection of *Trichinella* infection in horses by ELISA and Western blot analysis. *Veterinary Parasitolology*, 1999, *81*, 57-68.

ZARLENGA D.S., CHUTE M.B., MARTIN A. & KAPEL C.M. A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. *International Journal for Parasitology*, 1999, *29*, 1859-1867.

ZARNKE R.L., GAMBLE H.R., HECKERT R.A. & VER HOEF J. Serologic survey for *Trichinella* spp. in grizzly bears (*Ursus arctos*) from Alaska, 1973 to 1987. *Journal of Wildlife Diseases*, 1997, *33*, 474-479.

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