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Serological testing for *Trichinella* infection in animals and man: Current status and opportunities for advancements

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ABSTRACT

Serological tests are widely used for the detection of *Trichinella* spp. infections in animals and humans. Despite some limitations, (such as low sensitivity in the early period after infection) ELISA and western blot testing have demonstrated good performance when excretory/secretory products from muscle larvae are used as antigens in agreement with the International Commission on Trichinellosis. Over recent decades, considerable progress has been made in the characterization of *Trichinella*-derived molecules in the hope of improving diagnosis, mainly during the early days post infection. Despite these efforts, validated tests using characterized antigens for early diagnosis are still not available. However, combining currently available sero-diagnostic tools with clinical and epidemiological data provides valuable information on *Trichinella* infections in humans and animals as shown in this review.

1. Introduction

In terms of immunodiagnosis, trichinellosis is among the most widely studied helminthic infections (reviewed by Gómez-Morales and Ludovisi, 2021). In practice, the enzyme-linked immunosorbent assay (ELISA) is widely used in screening, and western blot (WB) testing is used as a confirmatory test (Bruschi et al., 2019). Both tests have demonstrated good performance when using excretory/secretory products (ESA) from muscle larvae (ML) as antigens (Bruschi et al., 2019; Gamble et al., 2004; Gómez-Morales et al., 2008, 2012; Gómez-Priego et al., 2000; Ilić et al., 2014; Robert et al., 1996; Rodriguez-Osorio et al., 2003; Tinoco-Velazquez et al., 2002; Yera et al., 2003).

In humans, the detection of specific antibody production, in particular IgG, to confirm a suspected clinical diagnosis of trichinellosis is a valuable diagnostic tool (Dupouy-Camet and Bruschi, 2007; Bruschi et al., 2019) and is considered to provide a more sensitive and earlier diagnosis than the muscle biopsy (Dupouy-Camet et al., 2021). Seroconversion in infected humans usually occurs between the third and fifth weeks after infection (Dupouy-Camet et al. (2021)), although specific IgG have been detected earlier (12 days post-infection (p.i.)) or later (60 days p.i.) depending on the level of exposure. The period required for antibody appearance in serum depends on the *Trichinella* species and the infectious dose (the lower the dose, the later the antibodies appear). IgG levels do not normally correlate with either the severity or the clinical course of the infection, but IgG levels can be affected by the number of infecting larvae ingested, the immunogenicity of the *Trichinella* species, the individual host responsiveness to the infection and the

Abbreviations: ELISA, Enzyme linked immunosorbent Assay; Wb, Western blot; ESA, excretory/secretory products; ML, muscle larvae; d.p.i., days post infection; ICT, International Commission on Trichinellosis; LPG, larvae per gram; CWE, crude worm extract; NBL, newborne larvae.

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stage of the infection (Bruschi and Murrell, 2002). There are reports indicating that specific IgG can persist in humans for a long time – up to 40 years p.i. (Fröscher et al., 1988; Pozio et al., 1993). For human disease, an early and specific diagnosis will allow for timely and appropriate treatment. Once the acute illness has abated, and the muscles have become fully invaded by the parasites chemotherapeutic intervention is generally less effective, and this factor could lead to complications and sequelae (Campbell and Denham, 1983).

For animals, the International Commission on Trichinellosis (ICT) does not recommend the use of serological tests for testing individual carcasses of food animals at slaughter for the purpose of assuring food safety (Gamble et al., 2004; Bruschi et al., 2019). This recommendation is consistent with the legislation of many governmental bodies, under which meat inspection programs for *Trichinella* in pork, horse and game meats are performed using a direct method such as the artificial digestion (European Commission, 2015; OIE/World Organisation for Animal Health, 2017), because animal hosts can harbour infective ML before detectable antibodies are present (Gamble, 1996, 1998; Gamble et al., 1983). In these hosts the seroconversion period is correlated to the infectious dose (Nöckler et al., 2000). In pigs experimentally infected with 100, 500 and 2500 *T. spiralis* larvae, seroconversion was observed by ES-ELISA at 5–7, 4–5 or 4 weeks after infection, respectively (Nöckler et al., 2000). With higher infectious doses of 8000, 10,000 and 64,000 larvae per pig, seroconversion occurred earlier, at 2.5–5 weeks after infection (Smith and Snowdon, 1989; Kapel and Gamble, 2000; Nöckler et al., 2005; Pozio et al., 2020). Similar results have been obtained in pigs experimentally infected with *T. spiralis, T. britovi and T. nativa* (Kapel et al., 1998). Pigs inoculated with 10,000 *T. pseudospiralis* larvae seroconverted later, at 5–6 weeks p.i (Pozio et al., 2020). Infection of pigs with low numbers of larvae can result in an extended period of seronegativity before anti-*Trichinella* antibodies are detectable in serum (Nöckler et al., 2005). The IgG response lasts for more than two years in pigs experimentally infected with *T. spiralis* and *T. britovi*, whereas this response lasts around one year in pigs infected with *T. pseudospiralis*, as has been observed in long-term studies (Pozio et al., 2020). However, in these animals there is no correlation between the level of the antibody response and the number of larvae per gram (LPG) of tissue, the infecting species or the time-point p.i when antibodies are detected (Pozio et al., 2020).

In wild boars inoculated with *T. spiralis*, *T. britovi*, and *T. nelsoni*, the antibody level increased rapidly between weeks 3 and 5 and remained stable for up to ten weeks (the experimental period). Similarly, in wild boars inoculated with *T. nativa*, *T. murrelli*, and *Trichinella* T6, a rapid increase in antibodies was detected between weeks 3 and 5 followed by a decrease (Kapel, 2000).

In horses, antibody responses persisted in a dose-dependent manner from 14 to 20 weeks post-infection (p.i) and then declined to undetectable levels, whereas viable ML persisted in horse muscle for longer period (Hill et al., 2007; Nöckler et al., 2000; Pozio et al., 2002).

Because of the lack of predictability of antibody responses in animals, serological assays should not be used to detect *Trichinella* sp. infection in individual food animal carcasses for protecting human health. However, antibody detection is both suitable and appropriate for surveillance of *Trichinella* sp. infection in domestic animals and wildlife and its use can contribute to the knowledge on *Trichinella* circulation (Gajadhar et al., 2009; Gamble et al., 2004).

Over recent decades, significant progress has been made on the molecular and immunological characterization of *Trichinella*-derived proteins in the hope of improving detection and diagnostic and potentially developing vaccines. Inasmuch as proteins from pre-adult, adult and newborn larval *Trichinella* stages are exposed to the host immune system prior to the maturation of ML, it has been proposed that these proteins may contain early diagnostic markers for *Trichinella* infection. Therefore, much attention has been focused on the identification and characterization of antigens from these stages in the hope of finding molecules useful for serodiagnosis of *Trichinella* infection during the early stages, when the use of ESA from ML generates false negative results. Indeed, several promising candidates for early diagnosis have been listed and are waiting for validation in further studies (Review by Wu et al., 2021). Despite this, antigens suitable for improved early diagnosis or detection are not yet available.

Currently available diagnostic tools, when used in combination with clinical and epidemiological data, provide enhanced information on *Trichinella* infections in humans and animals, in particular, in outbreak investigations, epidemiological studies and risk surveillance.

2. Outbreak investigations

The identification of the causative agent during an investigation in the course of a trichinellosis outbreak is extremely important in tracing the source of infection; preventing the spread of the vehicle of infection (meats or meat products) to other individuals; and assessing the risk to domestic animals susceptible to these zoonotic pathogens (Gómez-Morales et al., 2021).

When a medical alert of a possible outbreak of trichinellosis is raised following the detection of a suspected index case, the public health service starts an epidemiological investigation to trace the source of infection and to identify the causative agent. Unfortunately, this is not always possible. Recently, an outbreak of trichinellosis occurred in Genoa in northern Italy (Gómez-Morales et al., 2021). The epidemiological link was traced back to a dinner during which most of the guests had consumed raw meat. Clinical and laboratory data on 30 individuals out of the 52 (57.7%) persons who attended the dinner, were consistent with the case definition of trichinellosis, including serological positivity to ELISA confirmed by ES-WB. However, the source of infection was not traced and for ethical reasons – mainly due to the mild clinical patterns – no muscle tissue was biopsied from the patients. Another test was performed, focusing on the serological positivity and on the local epidemiological data indicating the possible circulation of *T. britovi* and *T. pseudospiralis* in the area (Pozio, 2016). This third test was a WB with *T. spiralis* crude worm extract (CWE-WB) which is able to distinguish *Trichinella* infections caused by encapsulated species (such as *T. spiralis* or *T. britovi*) from those caused by the non-encapsulated species *T. pseudospiralis*. This test provides a pattern of proteins within the same range of molecular weight but with different band distributions (Gómez-Morales et al., 2018). It was thus possible to conclude that the outbreak was caused by *T. pseudospiralis* (Gómez-Morales et al., 2021). This finding made it possible to gather more information regarding the clinical pattern of *T. pseudospiralis* in humans. Previous information was limited due to a low number of cases (Ranque et al., 2000). In this case, eosinophilia was confirmed

as a marker of *T. pseudospiralis* infection since it was present in 100% of the infected people (Ranque et al., 2000; Gómez-Morales et al., 2021).

3. Epidemiological studies

Wild carnivores and omnivorous animals are the main reservoir hosts of nematodes of the genus *Trichinella*. The circulation of *Trichinella* spp. in natural hosts should be monitored so that information can be acquired on the risk of transmission to domestic animals and from these to humans.

Serological assays have been, and are, suitable for surveillance and epidemiological investigations in these animal populations and are considered as an appropriate tool for monitoring programs (Gamble et al., 2004). However, parasitological (i.e. digestion) and serological tests used as epidemiological tools to study the circulation of *Trichinella* spp. in these animals have provided controversial data (Wacker et al., 1999; Chávez-Larrea et al., 2005; Cuttell et al., 2014, Gómez-Morales et al., 2014; reviewed by Pozio, 2021), since the exposure of animals to *Trichinella* parasites (the presence of specific IgG) does not always correspond to detectable larvae in the striated muscles. This discrepancy has been explained by 1) cross-reactivity to antigens from other infections, or a reduction in serum sample quality through haemolysis or bacterial or fungal contamination; 2) differences in analytical sensitivity between the artificial digestion test (1 larvae/g (LPG)) and the ELISA (0.01 LPG, OIE/World Organisation for Animal Health, 2017; Gamble et al., 1983; Nöckler and Kapel, 2007) and 3) biological and immunological differences among *Trichinella* species (Gómez-Morales et al., 2014).

Serological results in pigs may be explained by differences in parasitological and antibody responses to different *Trichinella* species. By way of example, in Duroc × Large White pigs experimentally infected with three (*T. spiralis*, *T. britovi* and *T. pseudospiralis*) of the four species circulating in Europe, *T. spiralis* showed the highest survival, since larvae were found in muscles for up to two years p.i. In *T. britovi*-infected pigs, the number of LPG was about 70 times lower than for *T. spiralis* at two months p.i. Only a few degenerated/calcified larvae or empty cysts were detected at six months p.i. and at 18 and 24 months p.i. no larvae or cysts were detected. The larval burden of *T. pseudospiralis*-infected pigs was similar to that of *T. britovi* at two months p.i., and no larvae were detected from six months p.i. onwards. In *T. spiralis* and *T. britovi* infected pigs, IgG levels persisted until the end of the experiment (two years). However in *T. pseudospiralis*-infected pigs, the IgG level showed a significant drop at six months p.i. and declined to the cut-off value at 12 months p.i. (Pozio et al., 2020). These experimental results may explain the controversial data reported using parasitological and serological tools in the course of epidemiological investigations. These results also enable the authors to hypothesize the circulation of *T. britovi* in a population of free-ranging pigs in Sicily, a Mediterranean island where this *Trichinella* species has never been detected in domesticated or wild animals (Pozio et al., 2021a).

4. Risk surveillance

Pig herds officially recognized as being under controlled management conditions, are considered to have a negligible risk of *Trichinella* spp. infection (Pozio, 2014). To meet controlled housing conditions, most farmed pigs should be kept under conditions where they have no outside access. However, in temperate regions (such as Mediterranean countries), sows have access to open fenced areas during gestation and when they are kept on farms applying controlled housing conditions simply to improve their welfare. Yet, if the farm is located in an agricultural area interspersed with wooded and barren zones where *Trichinella* could be present in wildlife, it is questionable whether the risk for *Trichinella* infection can still be considered negligible.

To address this question, sows from a holding applying controlled housing conditions according with the European Commission requirements (European Commission, 2015) were monitored using serological testing for two years (Pozio et al., 2021b). For annual monitoring, serum samples were collected one month before and around three months after outdoor access and tested for anti-*Trichinella* antibodies (Bruschi et al., 2019), and using a CWE-Wb to identify the etiological species. During the first year of monitoring all sera tested negative. During the second year, seroconversion for anti-*Trichinella* IgG was found in 21.8% of breeding pigs, which had outdoor access for two months, even though no larvae of *Trichinella* sp. were detected by artificial digestion in muscle samples of serologically positive animals when they were slaughtered. This suggested that these animals were exposed to *Trichinella britovi* during this period. This study shows the successful application of serology for the surveillance of the *Trichinella* sp. risk in pigs with outdoor access and suggests that planning for a pig farm under controlled conditions should include an accurate parasitological study of the surrounding habitat in which the farm will be located to reduce the risk of transmission of zoonotic agents.

5. Conclusions and future perspectives

Serological methods for the detection of antibodies to *Trichinella* in hosts have a long history. For the human host *Trichinella* infection can be suspected on the basis of clinical manifestations and epidemiological links and can then be definitively diagnosed using the serological tools currently available or by detecting larvae in a muscle biopsy (Dupouy-Camet et al., 2021). For food animals, much effort has been invested in research with the aim of finding a unique, effective, and practical diagnostic test for *Trichinella* spp. infections. This should be inexpensive and easy to use, preferably applied at the farm or slaughter house and should not have any limitations (it should be, able to differentiate current and past infections and with high diagnostic accuracy in the early period after infection). Unfortunately, such a test has not yet been developed.

Trichinella is a highly complex organism with a life cycle that is completed within a single host. Several developmental stages occur – among them ML, adults and new-born larvae (NBL) – and these differ in various ways (ESA and cuticle composition, and mode of locomotion) and colonize different niches (intestine, bloodstream and musculature) within the host (Takahashi, 2021). To ensure their

own survival, these parasites have developed evasion mechanisms allowing an infection to become established (Bruschi and Chiumiento, 2012).These include antigen-dependent mechanisms of evasion (including anatomical seclusion, antigen stage-specificity, shedding and renewal and molecular mimicry), which make it difficult to identify diagnostic molecules. Regarding the specificity of the antigens, *Trichinella* spp. contain thousands of kinds of proteins, some of which have a stage-specific expression, whereas others have a species-differential expression (reviewed by Wu et al., 2021). For example, early antibodies specific for adult worms do not bind to migrating stages. Furthermore, during the first hours of life, NBLs, undergo modifications of their surface proteins (Jungery et al., 1983). The surface of the cuticle of *T. spiralis* expresses protein molecules that change qualitatively following the moulting process and quantitatively during growth of the worms within one stage (Philipp et al., 1980). Given such an antigenic complexity, the use of more than one stage-specific antigen, namely antigens from each developmental stage (adult, NBL and ML), might allow the development of more accurate diagnostic tests. In the meantime, the use of more than one of the currently available tests may help to improve the diagnostic accuracy in establishing *Trichinella* spp. infections.

Declaration of Competing Interest

The authors report no declarations of interest.

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