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# Serological tools for detection of *Trichinella* infection in animals and humans

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ABSTRACT

Trichinellosis is a serious foodborne zoonotic disease. It is an important threat to public health in both developing and developed countries. Human infections are strongly associated with consuming undercooked meat containing infective *Trichinella* larvae. The development of serological tools has enabled seroepidemiological studies and contributed to our knowledge on the importance of this parasite. Serological tests can also help the diagnosis of parasite infections in humans and the surveillance of animals. Generally speaking, serological techniques include detection methods for specific antibodies and for circulating parasite antigens in the serum or tissue fluids. Here, we present a comprehensive review of various methods used in the detection of antibodies against *Trichinella* and circulating parasite antigens in animals and humans.



**Contents**



## 1. Introduction

Trichinellosis is one of the most important food-borne parasitic zoonoses throughout the world. Humans acquire trichinellosis by ingesting

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raw or undercooked meats containing the infective *Trichinella* larvae. In the past several decades, human outbreaks have been reported in many parts of the word [24,33]. Trichinellosis is regarded as an emerging or re-emerging disease in some parts of the world. The global importance of Trichinellosis has prompted the development of a number of serological tools for the detection of *Trichinella* infection in humans and animals. Serological techniques include detection methods for specific antibodies and for circulating parasite antigens in the serum or tissue fluids. For detection of *Trichinella* infection in humans, serological tests for detecting *Trichinella*-specific antibodies are valuable methods for human trichinellosis diagnosis. For detection of *Trichinella* infection in animals, according to the International Commission on Trichinellosis, serological methods are not recommended as substitutes for meat inspection of individual carcasses. However, serological methods for antibody detection are suitable for the surveillance of domestic animals and wildlife and contribute to the knowledge on *Trichinella* circulation [19]. This paper summarizes the progress on serological test tools for the detection of *Trichinella* infections, as well as their advantages and shortcomings.

## 2. Antigens used in serological tests

*Trichinella* antigens are divided into a fast-responding group (group I) and a slow-responding group (group II). The group I antigens are mainly composed of somatic antigens and are detected after two weeks of infection. The group II antigens are mainly composed of cuticular and excretory/secretory (ES) antigens of the muscle larvae (ML) and are detected after 4–5 weeks of infection.

### 2.1. Cuticular antigens

The cuticle is the most obvious point of contact between a parasite and its host. Thus, it is very useful in the indirect fluorescent-antibody test. Investigations on surface antigens indicated that cuticular antigens are stage-specific. Four major antigens are present on the new born larvae (NBL) cuticle, with molecular masses of 20, 30, 58, and 64 kDa. First-stage larvae were shown to contain four major antigens with molecular masses of 47, 55, 90, and 105 kDa. The adult cuticle contains three major antigens with molecular masses of 20, 33 and 40 kDa [8].

### 2.2. ES antigens

ES antigens are synthesized by *Trichinella* from different developmental stages, and the source of ES antigens is the stichosome. The antigenicity and composition of ES antigens of *Trichinella* vary according to the developmental stage. The ES antigens of the ML consist of a group of structurally related glycoproteins with molecular weights of 45–53 kDa [39]. *Trichinella* ML antigens have been classified into eight groups (TSL-1 to TSL-8) based on their recognition by different monoclonal and polyclonal antibodies. TSL-1 (45–100 kDa in the non-reduced form), TSL-2 (45 kDa in the non-reduced form), TSL-3 (45 kDa in the non-reduced form), and TSL-5 (35 kDa in the non-reduced form) are present in ES antigens of the ML. TSL-1 is the most abundant ES antigen [31]. A immunocytolocalization study showed that the antibodies are distributed in the hypertrophic nuclei and cytoplasm of parasitized nurse cells and in the lumen of the larvae oesophagus and intestine tissues. On the contrary, ES antigens of adult parasites are sometimes poorly immunogenic and lack the specific 45–53 kDa antigens [31]. The NBL cannot excrete/secrete any antigen; however, the NBL starts to form a stichosome after invading muscle cells.

### 2.3. Somatic antigens

Somatic antigens are less specific and can cross with antibodies against other parasites. This cross-reaction is due to the presence of phosphorylcholine within somatic antigens. They are distributed in many internal structures in both the ML and adult worms. Phosphorycholine has been found in many parasites, including *Ascaris suum*, *Nippostrongylus brasiliensis*, *Toxocara canis*, and *Trichuris suis*. Additionally, phosphorycholine is an immunodominant bacterial and fungal cell wall component. Moreover, Boireau et al. classified eleven groups of antigens with monoclonal antibodies (mAbs) against somatic *Trichinella* ML extracts. An indirect fluorescent assay indicated that most groups belong to the surface and ES antigens, and only those of group 11 are restricted to the gut [5].

### 2.4. Antigen purification and cloning

TSL-1 antigens share an immunodominant carbohydrate epitope (tyvelose), which is unique for *Trichinella* and elicits the major antibody response in the late stage of *Trichinella* infection. They can be purified by affinity chromatography with mAbs. TSL-1 antigens are specific to the ML stage and are lost during the accelerated larval moulting [6]. Immunocytolocalization studies showed that TSL-1 antigens are primarily at the cuticle and in alpha- and beta stichocytes. mAbs against TSL-1 recognized the 40–70 kDa antigens from ML homogenates under reducing conditions, and they recognized the 45–55 kDa antigens in ES products under non-reducing conditions [31]. TSL-1 antigen epitopes are highly conserved and can been recognized by antibodies that are induced by different *Trichinella* species. Thus, enzyme-linked immunosorbent assays (ELISAs) that are based on TSL-1 antigens can detect any *Trichinella* species infection [19]. The 45-, 49-, and 53 kDa glycoproteins are the major ES antigens and they were purified by affinity chromatography with mAbs [13].

A TsA-12 clone encoding a 53 kDa glycoprotein was identified by immunoscreening of a *Trichinella spiralis* ML cDNA expression library. The 53 kDa glycoprotein of *T. spiralis* is expressed in the postcapsule larvae (15-day-old ML) and adult worms but not in the precaspule (35-day-old ML) or NBL. The glycoprotein showed high sequence similarities (90.7% and 89.5%, respectively) to the *Trichinella britovi* and *Trichinella native* 53 kDa proteins (encapsulated species); however, they showed low sequence similarities (66.6% and 68.8%, respectively) to the *Trichinella pseudospiralis* and *Trichinella papuae* 53 kDa proteins (non-encapsulated species) [27]. The 53-KDa glycoprotein of *T. spiralis* contains species-specific epitopes. The antibody response induced by the 53 kDa glycoprotein is mainly due to protein epitopes, and the antibody response against glycan epitopes is less important [34]. Western blot analysis with the different *Trichinella* species 53 kDa recombinant proteins indicated that the 53-kDa antigens induced an early and species-specific antibody response in mice that were infected with *Trichinella* [27].

In recent years, a number of antigens have been identified by immunoscreening of cDNA expression libraries from different *T. spiralis* developmental stages, including serine protease inhibitors, serine proteases and some early antigens [44,47]. Some of these antigens showed promising potential in early detection of *Trichinella* infection in pigs. Furthermore, epitope mapping was performed on specific immunodominant antigens using various approaches, including overlapping synthetic peptides or cDNA fragments expressed in *Escherichia coli* [4,32] and a phage display strategy combined with a monoclonal antibody [42]. However, the immunodominance of linear epitopes that were identified by screening overlapping synthetic peptides could not replace the ES antigens in the indirect ELISA. Recently, proteomic or transcriptomic (subtractive cDNA libraries) approaches were successfully used to select immunodominant targets or to identify new antigenic components [41,25].

Longitudinal studies in various host species underline the possibility of variation in the antigenic stimulation. After several weeks post infection, encapsulated *Trichinella* does not stimulate the immune system in several hosts and most ML antigens seem hidden. Table 1 illustrates the antigenic variation of the expression of some antigens that differ before and after cyst formation [43].