Veterinary Parasitology 194 (2013) 121–124

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Validation of a latex agglutination test for the detection of *Trichinella* infections in pigs

Maria Interisano[a], Gianluca Marucci[a], Maria Angeles Gómez-Morales[a], Walter Glawischnig[b], Marleen Claes[c], Age Kärssin[d], Goran Zakrisson[e], Edoardo Pozio[a,*]

[a] *Department of Infectious Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy*
[b] *Austrian Agency for Health and Food Safety, Innsbruck, Austria*
[c] *Institute of Tropical Medicine, Antwerp, Belgium*
[d] *Veterinary and Food Laboratory, Tartu, Estonia*
[e] *National Veterinary Institute, Uppsala, Sweden*

ARTICLE INFO

Keywords:
Latex agglutination
Trichinella
Detection
Pig
Digestion

ABSTRACT

An antigen detection kit (Trichin-L), based on latex agglutination and developed by the Bio-Rad company was validated at five European laboratories. The validation parameters included specificity, sensitivity, robustness and reproducibility. Specificity was evaluated by testing parasite antigens from five non-*Trichinella* parasites in addition to the *Trichinella* genus. To evaluate sensitivity, 10 pork samples spiked with 1, 3, 6 or 15 *Trichinella* larvae were tested in each laboratory. To evaluate the robustness of the test, the solubilized antigens were maintained at room temperature and tested at different times. Reproducibility was assessed in each laboratory using 40, 100 g minced pork samples, each spiked with *Trichinella spiralis*. The use of larval homogenates obtained from the Trichin-L kit as a template for parasite identification at the species level by a multiplex PCR, was also evaluated. The results showed a high specificity and sensitivity where solubilized antigens maintained their stability and reactivity for up to three days. Reproducibility was high, as similar results were obtained in the five laboratories. The larval homogenates obtained using the Trichin-L kit were successfully used in multiplex PCRs to identify *Trichinella* species.

1. Introduction

Zoonotic nematodes of the genus *Trichinella* show a cosmopolitan distribution and affect livestock (pigs and horses) and wild animals (mainly wild pigs and carnivores) (Pozio and Murrell, 2006). In the European Union, it is mandatory to test for the presence of *Trichinella* sp. larvae by artificial digestion in all susceptible animals prior to marketing their meat (European Commission, 2005). Today in the European Union, laboratories performing the digestion test must follow one of the approved methods (European Commission, 2005). According to the European Commission's Directorate General for Health and Consumer Policy (DG SANCO), validation is required prior to adopting any new method or apparatus for digestion. These guidelines entrust such validation to the European Union Reference Laboratory for parasites (EURLP) and four National Reference Laboratories (NRLs) for Trichinella (www.iss.it/binary/crlp/cont/Guidelines_for_the_validation_of_apparatuses_for_the_detection_of_Trichinella_larvae.pdf). On April, 2010, BIO-RAD (Marnes la Coquette, France) contacted the EURLP to request validation of the Trichin-L kit. The procedure is based on the current digestion protocol, but sedimentation

* Corresponding author. Tel.: +39 06 4990 2304; fax: +39 06 4990 3561.
E-mail address: edoardo.pozio@iss.it (E. Pozio).

0304-4017/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2013.01.035

steps and microscopic diagnosis of the larvae are replaced by antigen detection based on latex agglutination. The aim of the present work was to validate the Trichin-L kit, according to the guidelines approved by the DG SANCO.

2. Materials and methods

2.1. Equipment and consumables

As indicated in the Guidelines, four NRLs should be selected among those which have demonstrated good performance in the most recent proficiency test for the detection of *Trichinella* larvae in muscle samples. The selected NRLs were those of Austria, Belgium, Estonia and Sweden. BIO-RAD provided each laboratory (EURLP and NRLs) the following: a blender, a magnetic stirrer with a thermometer probe, a vacuum pump, a 0.5 l steel funnel, a 10 l plastic tank to collect digestion fluid, a steel sieve, a pestle for 15 ml Falcon tubes, and a rocker. Furthermore, the company provided the Trichin-L kits, containing the following disposable materials and reagents: 300 g of pepsin, a sample diluent, forceps, 20 μm nylon mesh filters, negative and positive controls, latex beads, sticks and latex agglutination cards.

2.2. Tested samples

As prescribed by the Guidelines, a total of 200, 100 g samples of minced pork, prepared according to a previously published protocol (Marucci et al., 2009), were tested (under blind conditions) in the selected laboratories by the Trichin-L kit. Each laboratory tested: (1) 10 negative samples (to evaluate the amount of undigested meat on the sieve and the kit specificity); (2) 10 samples containing 15 *T. spiralis* larvae; (3) 10 samples containing 6 *T. spiralis* larvae; and (4) 10 samples containing 3 *T. spiralis* larvae.

2.3. Specificity

Since worms of the genus *Trichinella* share many antigens with other nematodes, the cross reactivity of the Trichin-L kit was tested against 0.1 and 0.5 mg of crude antigens from the following nematodes: *Ascaris suum*, *Trichuris suis*, *Toxocara cati*, and *Anisakis pegreffii*; as well as 0.1, 0.3 and 0.5 mg of crude antigens from the tissue protozoa *Toxoplasma gondii*. Furthermore, 100 muscle samples of different weights (range 48–102 g; average 72.17 g) from diaphragm pillars from backyard pigs collected at slaughter and that tested negative by the digestion method, were also examined by the Trichin-L kit.

2.4. Sensitivity

To verify that the Trichin-L kit was able to detect larvae belonging to different *Trichinella* species, meat ball samples were spiked with 1, 3 or 5 *Trichinella* larvae (five replicates for each number of larvae), belonging to each of eight species of *Trichinella* (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis*). Furthermore, to check the sensitivity of the Trichin-L kit to detect *Trichinella* sp. larvae in undigested muscle tissues, 10 pieces of mouse muscle (average 177 mg, range 125–232 mg), infected with 1–4 larvae of *T. spiralis*, were tested without prior digestion, directly by the Latex-Agglutination in the Trichin-L kit.

2.5. Robustness

To evaluate the *Trichinella* antigen stability over time, Trichin-L kit filters with the larva homogenate were left in 15 ml Falcon tubes with sample diluent at room temperature for different periods of time (from 4 up to 96 h) and then 50 μl aliquots were tested using the Trichin-L kit.

To determine if the Trichin-L kit can correctly identify the presence of *Trichinella* sp. larvae in the presence of excess antigens, 5000 *T. spiralis* live larvae were spiked on one filter and tested, in triplicate, using the Trichin-L kit protocol.

2.6. Species identification

The ability to determine the *Trichinella* species by PCR using the residual larval homogenate present on the filter of the Trichin-L kit was investigated. Briefly, 1, 3, 5, 10 or 20 larvae were added to the center of filtration membranes using a micropipette under a stereomicroscope. The samples were treated according to the Trichin-L kit protocol and first tested by latex agglutination. Then, 10 μl of the homogenized sample were used for the multiplex PCR reaction (Pozio and La Rosa, 2010). Because the PCR failed, presumably because of the chaotropic nature of the sample diluent in the Trichin-L kit, this experiment was repeated by replacing the buffer with PBS. To remove any trace of the buffer and to concentrate the DNA before PCR, 450 μl of the homogenized sample (i.e., the residual liquid after testing by the latex agglutination) were first subjected to DNA purification (QIAmp DNA MiniKit, Qiagen, MD, USA).

3. Results and discussion

A total of 200 pork samples were tested in the five laboratories. Furthermore, in two NRLs and at the EURLP, eight additional *Trichinella*-negative samples were tested in each of the three labs. Thus, a total of 150 *Trichinella*-positive samples and 74 *Trichinella*-negative samples were tested. The average undigested material on the sieve was 1.7 g (range 0.0–<5.0). All but one of the *Trichinella*-positive samples tested positive; the one which tested negative was due to a technical mistake (Table 1). Out of the 50 negative samples, 37 (74%) tested negative, 10 (20%) tested doubtful in three labs, and three (6%) samples tested positive in one laboratory. Since 26% of negative controls tested doubtful (20%) or positive (6%) and these results were obtained in two out of the five labs, eight additional negative samples were tested in these two NRLs and at the EURLP. In one NRL, the first three negative samples tested positive, the fourth sample tested doubtful, whereas the other four samples tested negative. In the 2nd NRL and in the EURLP, all the eight samples tested negative. The investigation carried out at the NRL with false positive or doubtful results showed that, after digestion, detergent traces present on the apparatus after washing were the cause of the false