Veterinary Parasitology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

# Validation studies of the latex agglutination test for the detection of *Trichinella* larvae in meat products

Jennifer Gayda*, Sabine Reckinger, Nora Thaben, Karsten Nöckler, Anne Mayer-Scholl

*Federal Institute for Risk Assessment, Diedersdorfer Weg 1, 12277 Berlin, Germany*

ARTICLE INFO

*Article history:*
Received 1 February 2016
Received in revised form 4 July 2016
Accepted 9 July 2016

*Keywords:*
*Trichinella spiralis*
Detection
Cured meat products
Sausage
Latex agglutination test
Validation

ABSTRACT

Human trichinellosis is a foodborne disease caused by ingestion of meat infected with *Trichinella* muscle larvae. To control *Trichinella* spp. infection in the European Union, all slaughtered pigs from holdings that are not officially recognized as applying controlled housing conditions and other animals susceptible to *Trichinella* infection and intended for human consumption should be examined by one of the approved digestion methods described in Regulation (EU) No. 2015/1375.

In the past, *Trichinella* outbreaks due to the consumption of cured wild boar or pork products have been described in several European countries, making the identification of the larvae from these products relevant for *Trichinella* control.

Therefore, this study aimed to validate the newly approved latex agglutination test (Trichin-L) for routine testing of cured meat products. The test was validated based on the OIE Guidelines using pork products spiked with *Trichinella* larvae. The sensitivity of the method varied greatly depending on the investigated meat product and was usually lower than for the gold standard, the magnetic stirrer method. The detection rate reached 80% for three larvae and 60% for one larva in cured pork sausages. A detection rate of 100% for three larvae and 50% for one larva was found in bacon. For frozen samples (−20 °C) the Trichin-L kit is similarly sensitive as for cured samples. Further, to determine the performance of the test under field conditions, pork products from regions with known high *Trichinella* prevalences confiscated by customs authorities at two German international airports were analyzed. Problems associated with the Trichin-L test were incomplete digestion due to fatty ingredients, spices and very dry meat products, resulting in data which could not be evaluated. Therefore, the test is currently not suitable for the detection of *Trichinella* larvae in cured meat products and needs further adaptation steps to increase both usability and sensitivity.



## 1. Introduction

Nematodes of the genus *Trichinella* are one of the ten foodborne parasites with the greatest global impact (FAO, 2014). Trichinellosis occurs worldwide through the consumption of raw or inadequately processed meat or meat products containing *Trichinella* larvae (Gottstein et al., 2009). This zoonosis is characterized by a large diversity of symptoms in humans such as high fever, headache, periorbital or facial edema and myalgia (Dupouy-Camet et al., 2002). Depending on the infectious dose, human trichinellosis can be a debilitating and occasionally fatal disease.

In addition to some wild animals such as wild boars, bears, and badgers, pork is the most important source of human infection (Murrell and Pozio, 2011). Depending on the *Trichinella* infection status of European Union countries or livestock holdings, it is mandatory to examine certain animal populations susceptible to *Trichinella* infection and intended for human consumption (European Commission, 2015). To date, the magnetic stirrer method is considered the gold standard for the detection of *Trichinella* larvae in fresh meat. However, this method is subjective and highly dependent on the examiners knowledge on parasite morphology, which often results in low test sensitivity (Riehn et al., 2013). Also, the final step of the magnetic stirrer method, the visualization, is technically the most uncontrollable and thus considered error-prone (Riehn et al., 2013).

Recently, a new non-microscopic method was developed for the detection of *Trichinella* in fresh meat. The *Trichinella* Antigen Test Kit® (Trichin-L) detects *Trichinella* antigen using monoclonal

* Corresponding author at: Federal Institute for Risk Assessment, Department of Biological Safety, Diedersdorfer Weg 1, 12277 Berlin, Germany.
*E-mail address:* jennifer.gayda@bfr.bund.de (J. Gayda).

http://dx.doi.org/10.1016/j.vetpar.2016.07.012
0304-4017/

antibodies and enables an objective assessment of the results by agglutination. The test has been validated and included in Regulation (EU) No. 2015/1375 (European Commission, 2015; European Union Reference Laboratory for Parasites, 2011), but is only considered suitable for the testing of meat of domestic swine.

Cured meat products are popular in many parts of the world. Even though cured meat products are a known source of food borne diseases they remain a traditional and popular food item in many European countries. In the past, *Trichinella* outbreaks due to the consumption of cured wild boar or pork products have been described in countries such as Germany, Italy and Romania (Dobrescu et al., 2014; Faber et al., 2015; Fichi et al., 2015).

Therefore, reliable and sensitive methods for the identification of *Trichinella* larvae in meat products are relevant for *Trichinella* control. The objective of this study was to validate the Trichin-L test for routine testing of cured meat products.

## 2. Materials and methods

### 2.1. Trichin-L test kit validation

The Trichin-L test was validated for meat products based on the OIE Guidelines, Principles and methods of validation of diagnostic assays for infectious diseases, section B "Assay validation pathway" (OIE World Organisation for Animal Health, 2013).

A total of 120 samples of cured pork sausages (German 'Grobe Mettwurst', Mago) and 33 samples of bacon (German 'Gelderländer Bauchspeck', Chantos) were used for validation. These two pork products are representative of the two main curing methods used for popular German food products. Each sample group was tested at least ten times.

All examinations were performed with *T. spiralis*, the most common species implicated in human *Trichinella* infection (Pozio and Darwin Murrell, 2006). *T. spiralis* larvae (ISS 003) were isolated from pork in our own laboratory by the magnetic stirrer method and were utilized for spiking meat products.

#### 2.1.1. Analytical sensitivity

To determine the limit of detection (LOD) a total of 25 g of cured pork sausage or bacon were spiked with 10, 5 or 1 larvae. All cured pork sausages were tested in both cured and frozen (−20 °C) state. Additionally, duplicate samples were tested by the magnetic stirrer method and a fresh meat control was carried out for each sample group.

#### 2.1.2. Analytical specificity

To verify that the Trichin-L test was able to distinguish *Trichinella* antigen from antigens of other nematodes, the analytical specificity of the test kit was assessed. The antigenic cross reactivity was examined by spiking 25 g of cured pork sausage with 1 worm of *Metastrongylus* sp., *Oesophagostomum dentatum, Toxocara cati* and 40 mg of *Ascaris suum,* respectively and analyzing the samples with the Trichin-L test.

#### 2.1.3. Repeatability

To test for the repeatability of the magnetic stirrer method and the Trichin-L test, ten samples of cured pork sausage and bacon were spiked with 10 larvae and tested in replicates on two different days by two different technicians.

### 2.2. Field study

Within the scope of the ZooGloW project ("Zoonoses and Food Safety Along Global Supply Chains") 29 different pork products from regions with known high *Trichinella* prevalence was collected at the German international airports Berlin Schönefeld and Frankfurt (Beutlich et al., 2015). Further, illegally imported Serbian sausages, which resulted in a trichinellosis outbreak in Germany (Willen, 2015) was tested. To determine the performance of the test under field conditions 25 g of the raw sausages or bacon was analyzed by the Trichin-L kit. All samples were also tested with the gold standard magnetic stirrer method. Further, the fitness of use was assessed. Sausages and bacon were stored at −20 °C prior to testing.

### 2.3. Laboratory examinations

The Trichin-L test and the magnetic stirrer method were carried out according to the EU regulation (EU) No. 2015/1375 (European Commission, 2015). Samples are digested with pepsin and transferred into a filtration funnel through a nylon mesh filter membrane. *Trichinella* larvae are disrupted by pestle to allow for antigen solubilization, followed by immune-detection of larval antigens. Here, latex beads forming blue aggregates in the presence of *Trichinella* antigens provide direct detection of *Trichinella* antigens without microscopic observation. For both methods, 25 g of meat product was digested and the amount of undigested meat from the sieve weighed. Both tests were performed in 1 l digestive fluid. For the magnetic stirrer method, the number of larvae per gram (lpg) was determined. The agglutination reaction of the Trichin-L test can only be classified as positive or negative.

### 2.4. Ethics statement

All animal work to obtain *T. spiralis* larvae (ISS 003) were conducted according to the guidelines of the German Protection of Animals Act Tierschutzgesetz in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206, 1313). The State Office of Health and Social Affairs Berlin approved the described animal work (approval No. H 0078/00).

### 2.5. Data management and statistics

To assess the analytical sensitivity, the proportion of correctly identified *Trichinella* positive samples was calculated for any number of larvae. 95% confidence intervals were determined from the Binomial distribution as described by Clopper and Pearson (1934). Non-overlapping error bars indicate statistical significance at $p \leq 0.05$.

## 3. Results and discussion

As listed in Table 1, the Trichin-L test achieved a lower analytical sensitivity for cured pork sausages compared to the gold standard. All samples spiked with ten native larvae were identified correctly with both methods. In eight of ten repeats, larvae were detected in the samples spiked with three larvae by the Trichin-L test, resulting in a sensitivity of 0.8 (95% CI = 0.444–0.975). In contrast, larvae were identified in all samples by the magnetic stirrer method. With one larva only 60% (95% CI = 0.262–0.878) of the cured samples were identified by the Trichin-L test, whereas the magnetic stirrer method was still able to identify all positive samples correctly. Recovery rates achieved by the magnetic stirrer method in this study were comparable with results of proficiency samples examined with the same method (Gajadhar and Forbes, 2002). At a significance level of 95% the differences between the Trichin-L test and the gold standard method were not significant.

For frozen samples the sensitivity of the magnetic stirrer method and the Trichin-L test was slightly less in comparison to the cured samples. While the results for samples spiked with ten and three larvae were comparable to the results of the cured samples, a sensitivity of 0.4 (95% CI = 0.122–0.738), was achieved for one

Please cite this article in press as: Gayda, J., et al., Validation studies of the latex agglutination test for the detection of *Trichinella* larvae in meat products. Vet. Parasitol. (2016), http://dx.doi.org/10.1016/j.vetpar.2016.07.012