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A study on the suitability of inactivated *Trichinella spiralis* larvae for proficiency samples

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ABSTRACT

The consumption of raw or undercooked *Trichinella* infected meat, especially pork and horse meat, can have important implications for public health. Therefore each animal carcass from a *Trichinella* susceptible species intended for human consumption must be examined for *Trichinella*. Laboratories carrying out testing of official control samples must undergo a quality assurance program and should regularly participate in proficiency testing schemes. To date, *Trichinella* proficiency samples are prepared with live larvae, which, as a level 2 pathogen, require specific shipping and disinfection procedures. Therefore, the suitability of using inactivated *Trichinella* larvae as proficiency samples was tested. We found that *Trichinella* larvae treated with 2% formaldehyde for 24 h had lost their infectivity and showed a comparable recovery rate to naïve larvae after artificial digestion, albeit with a prolonged sedimentation time.

1. Introduction

Trichinellosis is a food-borne disease caused by nematode worms of the genus *Trichinella* which constitute one of the most widespread zoonotic pathogens in the world. Human trichinellosis has been documented in 55 countries around the globe and the reported average yearly incidence of the disease in humans worldwide is approximately ten thousand cases with a mortality rate of 0.2% (Gottstein et al., 2009). The occurrence of trichinellosis in humans is strictly related to the consumption of raw or undercooked infected meat of different animal origins; however, *Trichinella* derived especially from pork, horse meat and wild boar have an important impact on public health (Gamble et al., 2000).

Worldwide there are various approaches for controlling trichinellosis. Regulations of the European Union (EU) require that each carcass of domestic swine, wild boar, horses and other animal species intended for human consumption be examined for *Trichinella* (European Community, 2005). This results in the testing of more than 250 million pig carcasses per year in the EU.

To ensure a high level of consumer protection, the effective diagnosis of *Trichinella* positive meat is essential. Uniform testing and reduced diagnostic error are desirable and make implementation of quality assurance programs necessary (Gajadhar et al., 2009). Official meat inspection should be carried out using appropriate techniques and experienced staff. Further, adequate facilities and equipment should be available and laboratories carrying out analyses of samples taken during official meat inspection must be accredited according Regulation (EC) No. 882/2004 (European Community, 2004). Laboratory accreditation is defined by the ISO/IEC17025 as the formal recognition that the testing laboratory is competent to carry out specific tests or specific types of tests. As such, proficiency testing is an integral part of quality assurance schemes and accredited laboratories should regularly participate in proficiency testing provided by a reference laboratory.

Usually, positive proficiency samples are prepared using live *Trichinella* larvae. *Trichinella*, as a level 2 infectious pathogen, must be sent under UN3373 packaging, which

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increases the overall costs. Due to the use of infectious material in the laboratory, glassware and laboratory waste must be treated with hot water to efficiently kill the larvae and prevent re-introduction of the larvae into the environment, which is particularly important in non-endemic regions (Office International des Epizooties, 2008). Therefore, this study aimed to evaluate the suitability of formaldehyde-inactivated *Trichinella* larvae for proficiency samples.

2. Materials and methods

2.1. Recovery of Trichinella spiralis larvae and formaldehyde treatment

Artificial digestion of pig muscle tissue infected with *T. spiralis* (*T. spiralis* ISS 003 cultivated in guinea pigs) was used to obtain individual *T. spiralis* larvae. All samples were recovered by the magnetic stirrer method for pooled sample digestion according to Regulation (EC) No. 2075/2005 (European Community, 2005). After isolation from pig muscle tissue, *T. spiralis* larvae were washed in water and treated with 5%, 2% and 1% formaldehyde for 24 h and with 1% formaldehyde for 48 h. The negative control larvae were treated with 0.9% sodium chloride (NaCl) for identical lengths of time. The larvae were washed once in 0.9% NaCl and rehydrated in 0.9% NaCl for 24 h.

2.2. Assessment of larval survival and infectivity

Following formaldehyde treatment and rehydration in 0.9% NaCl, the larvae were heated at 37 °C for 30 min and directly examined under the stereo-microscope at a 15–20-fold magnification. In vitro assessment of larval viability was based on larval motility (active vs. non-active). Non-motile larvae were defined as alive when coiled or dead when C-shaped.

Infectivity of larvae treated with 2% formaldehyde for 24 h was subsequently bio-assayed in a guinea pig. For this purpose, 500 formaldehyde-treated larvae were administered orally and the guinea pig euthanized 8 weeks post inoculation. Muscle tissue (3 × 100 g samples) was digested and the number of *Trichinella* larvae determined after a 60 min sedimentation period.

2.3. Spiking of meat samples with T. spiralis larvae

For each experiment, 10 hand-formed "meatballs" with a weight of 10 g were prepared from ground, fat-free *Trichinella*-negative pork. *T. spiralis* larvae were poured into a Petri dish and observed with a stereomicroscope. For all experiments, 10 larvae were collected with a Pasteur pipette and transferred into a cavity in the meatballs. The cavity was closed with minced meat, and each meatball containing *T. spiralis* was individually packaged in a plastic bag, labelled, and stored at 4 °C for 7 days. Only tightly coiled larvae were selected for the spiking experiments.

2.4. Recovery of T. spiralis larvae from proficiency samples

The proficiency samples spiked with dead or live larvae were digested as described above; only the chopping of the meatballs in a blender was omitted. The meatballs were placed into the digestion fluid and crushed against the glass beaker with a fork. Fat-free, *Trichinella*-negative pork (90 g) was chopped in a blender and transferred to a glass beaker.

The meatballs were digested as described above and the number of larvae determined after 30 min and 60 min sedimentations.

2.5. Statistics

For each treatment experiment, the recovery rate for 10 meatballs spiked with 10 live or dead larvae was evaluated. The recovery rate was calculated from the means of each treatment panel. In order to compare the results of formaldehyde treated vs. untreated samples the unpaired *t*-test was applied and box plots produced.

3. Results

The digestion of the meatballs spiked with untreated (live) larvae showed a 98% recovery rate after a 30 min sedimentation period. Additional larvae could not be recovered after extending the sedimentation time to 60 min. The majority of untreated larvae displayed a coiled morphology (Fig. 1A) and were mostly active.

The recovery rate of larvae treated with 1% formaldehyde for 24 h was comparable (96%) to the untreated control. However, only 10% of the recovered larvae were motile when heated to 37 °C.

The recovery rates of larvae after 30 min sedimentation periods and treated with either 1% formaldehyde for 48 h or 2% formaldehyde for 24 h were both 87%, and for larvae treated with 5% formaldehyde for 24 h the recovery was 86%. All recovery rates for dead larvae were significantly reduced in comparison to the untreated live larvae ($p = 0.01$, 0.03 and 0.007 respectively).

When the sedimentation time was prolonged to 60 min, the larval recovery rate increased. The recovery rate was 92% for larvae treated with 1% formaldehyde for 48 h, 93% for larvae treated with 2% formaldehyde, and 91% for larvae treated with 5% formaldehyde. Only the larvae treated with 1% or 2% formaldehyde did not show a significant reduction in recovery relative to the untreated live larvae ($p = 0.077$ and 0.145 respectively). All larvae treated with 1% (48 h), 2% or 5% formaldehyde were non-motile. The morphologies of all treated larvae did not vary from the untreated larvae (Fig. 1A–D).

Treatment experiments (1% for 48 h and 2% for 24 h, 60 min sedimentation time) beginning with 10 larvae per sample both showed the highest recoveries with a median of 9.5 worms (Fig. 2). The number of recovered larvae treated with 1% formaldehyde ranged between 8 and 10. The upper three quartiles of this treatment panel ranged between 8.25 and 10 larvae. The range of the 2% formaldehyde treated samples was between 7 and 10 worms, but 75% of all samples of the treatment panel ranged between