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Efficacy of an aerosol-resistant pepsin powder used in artificial digestion for the detection of *Trichinella* larvae in meat

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Abstract

Trichinellosis is an important worldwide foodborne zoonosis. The gold standard test to detect *Trichinella* spp. larvae in muscle samples of animals intended for human consumption is the artificial digestion method. Handling and dispensing of conventional pepsin powder present significant safety risks for analysts. The use of pepsin powder that is resistant to aerosolization should alleviate these safety concerns. The aim of this study was to compare the efficacy of an aerosol-resistant pepsin powder to conventional pepsin powder in the artificial digestion method. Proficiency samples of pork diaphragm containing specific numbers of viable *Trichinella spiralis* larvae were tested in two laboratories. The results revealed that aerosol-resistant pepsin was simple, effective and convenient to use, and showed good solubility and larval recovery that met the requirements of the European Union regulation EU 2015/1375. Overall, the efficacy of the aerosol-resistant pepsin was comparable to the conventional pepsin and safer for analysts.

Introduction

Trichinellosis is an important parasitic zoonosis that can be prevented with currently available tools (Pozio, 2020). Artificial digestion of muscle samples is used for the detection of infective and non-infective *Trichinella* spp. larvae. In the traditional magnetic stirrer digestion method, hydrochloric acid (HCl) activated pepsin enzyme is incubated with homogenized muscle samples to release *Trichinella* spp. larvae which are isolated and detected by microscopy (European Union, 2015; ISO Standard 18743:2015). It has been estimated that the annual cost for *Trichinella* meat inspection in the European Union is about 570 million USD (Caccio *et al.*, 2018). A critical control issue for the satisfactory performance of the magnetic stirrer method is quality of the pepsin (Noeckler *et al.*, 2019). The liquid and powder forms of pepsin are important considerations for accuracy, convenience, safety and health of laboratory analysts. It is well recognized that handling the powdered pepsin represents a health risk because of potential allergic skin reaction (Cartier *et al.*, 1984; Maddox-Hyttel *et al.*, 2007), occupational allergic asthma (Cartier *et al.*, 1984) and work-related rhino-conjunctivitis due to inhalation of aerosolized pepsin by analysts performing *Trichinella* testing at slaughterhouses (Marquès *et al.*, 2006). To mitigate these risks other formulations of digestion enzymes have been adopted for the artificial digestion method, including liquid pepsin (Maddox-Hyttel *et al.*, 2007), and serine protease as part of the digestion kit – PrioCHECK™ *Trichinella* AAD (Gajadhar *et al.*, 2018).

Despite the availability of alternative enzymes for the artificial digestion method, the conventional pepsin powder is still widely used because of its proven reliability for digestion, and the long-standing habit of its use at laboratories worldwide. As accurate weighing of pepsin powder represents a critical control point in the artificial digestion procedure (ISO standard 18743:2015) and dust formation is an operator's safety issue (Cartier *et al.*, 1984; Marquès *et al.*, 2006), an aerosol-resistant pepsin powder could present a suitable solution for such problems. However, whether this modification affects the pepsin activity and digestion efficiency remains to be examined. The aim of this study was to compare the efficacy of an aerosol-resistant pepsin powder to conventional pepsin powder in the artificial digestion method.

Materials and methods

The efficacy of the new aerosol-resistant pepsin powder (Parasitix, Canada) was investigated independently in two separate laboratories at the University of Belgrade: Laboratory A – the Institute for the Application of Nuclear Energy (INEP); and Laboratory B – the Faculty of Veterinary Medicine. The aerosol-resistant pepsin powder (10,000 NF) for artificial digestion

was provided as a gift by Parasitix Lab Services Inc, Canada. Samples of fresh pork diaphragm muscles used in this study were obtained from a local slaughterhouse near Belgrade, Serbia. The artificial digestion method was performed according to the regulation EU 2015/1375 (European Union, 2015) and ISO Standard 18743:2015. We used 10 g of new aerosol-resistant pepsin for digestion of muscle samples (100 g).

The study was conducted in three phases. The first phase compared the solubility and efficacy in digesting tissue samples of the aerosol-resistant pepsin powder vs. the conventional pepsin powder (10,000 NF) (Biognost®S, Croatia). We measured the time required for both types of pepsin to dissolve completely and efficacy in artificial digestion of three pig muscles samples (100 g each) in both laboratories according to EU regulation 2015/1375 and ISO Standard 18743:2015.

The second phase of the study was conducted in the form of proficiency testing (PT) according to quality assurance requirements for the production and use of proficiency samples in *Trichinella* digestion testing (Gajadhar *et al.*, 2018). *Trichinella spiralis* muscle larvae were obtained by artificial digestion of the muscles of an experimentally infected laboratory rat. Details of the preparation of test samples have been previously described (Marucci *et al.*, 2016; Vasilev *et al.*, 2019). Larvae were obtained by very short digestion of *T. spiralis* infected rat carcasses. Larvae were counted under a stereo microscope and transferred individually to each 100 g meat ball. The glass was examined twice under the stereo microscope to ensure that all larvae were transferred. Every single meat ball was enclosed in a bag and sealed under vacuum. A total of 30 PT samples were prepared in a manner that blinded all analysts of the two testing laboratories to the spiking identification. A total of 30 PT samples were spiked with viable *T. spiralis* muscle larvae (L1). Each of the two laboratories were provided with 15 samples comprising three groups, each containing 10, 5 or 3 L1 in compliance with the guidelines of ISO 17043:2010. Laboratories A and B tested separately their assigned test panels over a period of three days after preparation of the samples, using the magnetic stirrer method for pooled sample digestion (European Union, 2015; Noeckler *et al.*, 2019).

The third phase of the study was performed in Laboratory B by using pork proficiency samples spiked with encapsulated *T. spiralis* first stage larvae (L1) and tested for parasite recovery using aerosol-resistant pepsin powder and pepsin powder. PT samples were prepared with pork diaphragm obtained from a local abattoir, negative for *Trichinella* spp. Minced pork samples, free of fat and fascia and weighing 100 g, were spiked with five *T. spiralis* larvae that were taken with muscle of rats that were experimentally infected. Small pieces of muscle were squeezed in a compressorium and under a stereomicroscope the number of encapsulated larvae were counted by two experienced technicians. Muscle pieces that contained five larvae were transferred into the meat samples. Every single meat ball was enclosed in a bag and sealed under vacuum.

To compare differences between numbers of larvae recovered and remaining tissue on the sieve the Student's *t*-test was performed. The formula (detected number of larvae/expected number of larvae) × 100 was used for calculating percentage of recovered larvae.

Table 1. Results of the proficiency testing organized to test the larvae recovery after use of the aerosol-resistant pepsin powder.

Laboratory A				Laboratory B			
Larvae			Undigested debris (g)	Larvae			Undigested debris (g)
Spiked	Recovered			Spiked	Recovered		
(*n*)	(*n*)	%		(*n*)	(*n*)	%	
3	3	100	1.63	3	3	100	2.9
3	3	100	2.24	3	3	100	3.72
3	3	100	2.64	3	3	100	3.84
3	3	100	2.14	3	3	100	2.94
3	3	100	2.63	3	3	100	2.84
5	5	100	1.93	5	4	80	4.14
5	4	80	3.71	5	5	100	3.3
5	5	100	1.73	5	5	100	2.83
5	5	100	2.7	5	4	80	4.04
5	5	100	2.47	5	5	100	3.85
10	10	100	2.18	10	8	80	4.41
10	9	90	3.66	10	9	90	4.28
10	10	100	2.64	10	10	100	4.12
10	10	100	1.88	10	10	100	3.64
10	10	100	1.44	10	9	90	3.74
Average		98.0	2.38	Average		94.7	3.64
Standard deviation		5.6	0.69	Standard deviation		8.3	0.55

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Table 2. Results of the effectiveness of the aerosol-resistant pepsin powder to release encapsulated *Trichinella spiralis* larvae in proficiency testing samples compared with classical powder pepsin.

Aerosol-resistant pepsin powder				Pepsin powder			
Larvae			Undigested debris (g)	Larvae			Undigested debris (g)
Spiked encapsulated	Recovered			Spiked encapsulated	Recovered		
(*n*)	(*n*)	%		(*n*)	(*n*)	%	
5	4	80	3.23	5	5	100	3.94
5	4	80	3.51	5	5	100	3.85
5	5	100	2.63	5	4	80	3.52
5	5	100	3.67	5	4	80	4.21
Average		90.0	3.26	Average		90.0	3.88
Standard deviation		11.5	0.46	Standard deviation		11.5	0.28

Results and discussion

The results of the first phase of the investigation showed that new aerosol-resistant pepsin powder pepsin have a good solubility. After placing the aerosol-resistant pepsin in a glass beaker (with preheated water, HCl and a stirring rod) on magnet stirrer for performing artificial digestion, a short period of time is needed to completely resolve it, similar to control powder pepsin. We found that pepsin dissolves in 3.0–3.5 min, while aerosol-resistant pepsin powder dissolves in 3.0–3.5 min. There was no statistical difference between dissolving time for aerosol-resistant pepsin powder and classical pepsin powder. The pepsin solubility and sample digestion time (less than 60 min) met criteria as prescribed in the EU regulation 2015/1375 (European Union, 2015) and the standard procedure (ISO, 2015) to achieve the acceptable level of ≤5% residue of undigested debris in both laboratories.

The aerosol-resistant pepsin powder makes less dust when weighing than conventional powdered pepsin in the preparation of digestive fluid (analysts' observations). Handling of the weighed aerosol-resistant pepsin powder ensures the accuracy of test method because the loss of pepsin from aerosolization is negligible. Measurement errors could affect one of the critical control points for artificial digestion (Djordjevic *et al.*, 2013; Riehn *et al.*, 2013; Mayer-Scholl *et al.*, 2017; International Commission on Trichinellosis guidelines, 2022). The pepsin weighing process also represents a safety issue for analysts (Cartier *et al.*, 1984; Marquès *et al.*, 2006; Maddox-Hyttel *et al.*, 2007). The use of the aerosol-resistant pepsin powder minimizes the chance of dust from pepsin powder being inhaled by analysts or direct skin contact. Thus, aerosol-resistant pepsin powder has advantages for the health of analysts as well as the environment.

The results of the second (table 1) and third (table 2) phase of investigation showed that the aerosol-resistant pepsin powder has a good efficacy in the release of *Trichinella* larvae from meat. Specific formulation of the aerosol-resistant pepsin did not affected viability of the larvae (all recovered larvae were viable).

In the second phase the undigested debris percentage (table 1), obtained performing the test according digestion time indicated in the ISO and EU regulations (min 30 min maximum 60 min) ranged from 1.44 g to 3.66 g (average 3.28 g) for Laboratory A and from 2.84 g to 4.41 g (average 3.64 g) for Laboratory B. Visibility through the digestion fluid was good in the initial sedimentation step, and only in six tests (20%) a repeat of the final step was necessary to obtain a sufficiently clear sediment for microscopic detection of *Trichinella* spp. larvae. Recovery of the larvae in the two participating laboratories ranged from 94.7 ± 8.3 in Laboratory B to 98.0 ± 5.6 in Laboratory A (table 1).

In the third phase the undigested debris percentage, according to the ISO and EU regulations (min 30 min maximum 60 min) ranged from 2.63 g to 3.67 (average 3.26 g) after digestion using aerosol-resistant pepsin (10 g for 100 g meat sample) and from 3.52 g to 4.21 g (average 3.88 g) for classical pepsin powder (table 2). Recovery of the larvae was 90 ± 11.5 for both types of pepsin (table 2). There were no statistical differences between aerosol-resistant pepsin and pepsin powder in recovery of the encapsulated larvae and undigested debris percentage (table 2).

These results were much better than those obtained by participants in two PT trials in Serbia in 2017 (Vasilev *et al.*, 2019) and 2021 (Vasilev *et al.*, 2021). Our results were similar to recoveries obtained in a validation study of the PrioCHECK *Trichinella* AAD assay and comparable with those of conventional pepsin powder (Gajadhar *et al.*, 2018). The acceptable larval recoveries in all of the above studies were attributable, in part, to certified and experienced analysts, the use of validated protocols and quality controls, and strict adherence to laboratory quality assurance standards.

Conclusion

These results show good solubility and effectiveness of aerosol-resistant pepsin powder. The digestion time, amount of undigested tissue, and level of larval recovery are consistently within the acceptable limits as prescribed by the EU regulation. These conclusions suggest that an aerosol-resistant pepsin powder could be used in all laboratories performing *Trichinella* examination as a safer option for analysts' health and environmental protection than the conventional powder pepsin.

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Conflicts of interest. None.

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Ethical standards. The care and use of animals complied with institutional policies and all national regulations, and received required approval.

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