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Research Note

Evaluation of a Fluid Versus a Powder Pepsin Formulation To Detect *Trichinella spiralis* Larvae in Meat Samples by a Digestion Technique

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ABSTRACT

Pepsin powder constitutes a health risk, potentially causing severe allergic reactions to those handling the chemical. A fluid pepsin formulation was produced and tested, first in a preliminary study and then in a ring trial encompassing four European National Reference Laboratories (NRLs). The purpose of each trial was to ascertain and compare the action of pepsin powder with that of the pepsin fluid for digesting meat and liberating encapsulated *Trichinella spiralis* larvae for subsequent counting. The quality of digestion was furthermore evaluated by assessing the visibility through the digestion fluid and the amount of debris remaining after digestion. For the ring trial, at each laboratory 20 blinded replicate 100-g samples of pork meat containing a known number of encapsulated *T. spiralis* larvae (0 to 30) were digested by the magnetic stirrer method using either the standard pepsin powder (10 samples) or the pepsin fluid (10 samples). With an average recovery rate of 70 to 80%, all NRLs found the pepsin fluid and pepsin powder to be equally effective. The NRLs also found no difference between the two pepsin formulations with regard to debris remnants or visibility through the digestion fluid. The use of pepsin fluid may therefore constitute an improvement of the digestion procedure for the analysts involved.

Several reports have been published regarding the development of occupational asthma, alveolitis, or rhinitis from work with pepsin powder including the allergenic component pepsin A *(1, 2, 7, 8).* Hence, another pepsin formulation is needed, particularly for use with meat digestion when evaluating the presence of *Trichinella* spp. Every year, millions of pig carcasses are inspected for *Trichinella* spp. by employing one of the available digestion techniques *(3, 5, 9),* which involve the use of pepsin powder. Work with pepsin powder should take place under a fume hood to avoid allergic reactions to pepsin A. Use of a fluid would alleviate asthmatic allergic reactions caused by the fine pepsin powder dust, which is aerosolized during weighing and other handling procedures. A fluid pepsin formulation was therefore produced (A/S Orthana, Biofac, Kastrup, Denmark) to have properties similar to those of pepsin powder (1:10,000 N.F. = 2,000 F.I.P.) with regard to chemical activity and stability when measured over a 1-year period *(6).* However, to determine the usefulness of this formulation for detection of *Trichinella* sp. in meat it was necessary to assess its functional properties using the recommended *Trichinella* meat inspection methodology. The objective of this study was to compare the newly developed pepsin fluid formulation with a conventional pepsin powder formulation for their ability to digest meat with encapsulated *Trichinella spiralis* larvae and to liberate the larvae for their detection and enumeration.

MATERIALS AND METHODS

Design of study. The pepsin fluid was tested in two steps. Initially, the performance of the pepsin fluid was assessed in one National Reference Laboratory (NRL) (trial 1). Upon obtaining statistically valid results for the pepsin fluid, three more European Union (EU) NRLs were included in a trial (trial 2). In both trials, testing was done by comparing pepsin fluid and pepsin powder for their ability to digest meat, liberate encapsulated larvae, and render the digestion fluid adequately transparent (visibility) for easy counting of larvae, i.e., without excessive amounts of debris.

Infected tissue. Five mice were inoculated orally with 400 *T. spiralis* muscle larvae at least 6 weeks before the start of the study. Mice were kept according to animal protection laws of Denmark under Permission no. 192000-561-303. At the time of the study, the mice were euthanized by cervical dislocation, skinned, and eviscerated. Minute muscle pieces were removed and examined by trichinoscopy *(9)* for enumeration of encapsulated *T. spiralis* larvae.

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TABLE 1. *Results from a preliminary study in one laboratory (trial 1) for the recovery of* Trichinella spiralis *larvae after digestion using the magnetic stirrer method and either pepsin powder or pepsin fluid*

	Pepsin powder				Pepsin fluid			
	No. of larvae				No. of larvae			
Sample no.	Added	Recovered	% larvae recovered	Undigested tissue (g)	Added	Recovered	% larvae recovered	Undigested tissue (g)
1	25	24	96	0.6	25	21	84	1.8
2	24	22	92	0.3	24	21	88	1.2
3	5	2	40	2.3	5	4	80	0.8
4	6	6	100	0.2	6	6	100	0.7
5	3	3	100	2.2	3	3	100	0.9
6	9	6	67	2.5	9	2	22	1.4
7	25	25	100	0.4	25	24	96	2.3
8	8	8	100	2.1	8	8	100	4.7
9	20	16	80	1.6	20	20	100	2.0
10	10	10	100	1.3	10	7	70	0.3
11	10	9	90	1.9	10	10	100	1.1
12	9	3	33	0.6	8	8	100	1.4
Mean	12.8	11.2	83.1	1.3	12.8	11.2	86.6	1.5
SD			23.9	0.8			22.6	1.1

Sample preparation. In trial 1 and trial 2, 24 and 80 replicate 10-g samples of ground pork, respectively (high grade quality, certified *Trichinella* free), were placed in sealable plastic bags. Known numbers of *T. spiralis* larvae were added by transferring the selected muscle piece(s) from the compressorium into the ground meat samples before sealing the bag. For trial 1, samples were spiked with 3 to 6 (6 samples), 8 to 10 (10 samples), or 20 to 25 (8 samples) encapsulated *T. spiralis* larvae. For trial 2, four samples were spiked with 1 to 3 encapsulated *Trichinella* larvae, six were spiked with 6 to 9 larvae, six were spiked with 11 to 16 larvae, and four were spiked with 20 to 30 larvae. In trial 1, the samples were kept refrigerated at the preparing laboratory until processed. In trial 2, 20 samples were prepared for each of the four NRLs (labs 1 through 4). The samples were kept cool (<10°C) during shipment to three of the NRLs, where they were kept at 4°C until processed. All laboratories had received the pepsin fluid from the producer (A/S Orthana, Biofac) just before the study.

Sample processing. At each laboratory, 90 g of ground pork (high grade quality, certified *Trichinella* spp. free) was added to each 10-g sample to reach a total of 100 g of ground pork for each digestion. At every laboratory, 10 samples were digested with pepsin fluid and 10 samples were digested with pepsin powder. The magnetic stirrer method was employed at all laboratories and was performed according to current EU legislation *(3, 9)*. For the pepsin fluid, 30 ml corresponded to 10 g of pepsin powder at 1:10,000 N.F. (= 2,000 F.I.P.) according to the manufacturer's instructions. The samples in trial 2 were examined at all four labs within 10 days of sample preparation.

Evaluation parameters. For each digestion, the following parameters were evaluated: recovery of *T. spiralis* larvae (quantitative), amount (grams) of undigested muscle tissue left on the 177- or 180-μm-mesh sieve (quantitative), amount of debris in the digestion fluid after 30 min of sedimentation in a separating funnel (qualitative), and visibility through the digestion fluid in the counting chamber (qualitative).

Statistical analyses. An analysis of variance was used to compare the recovery rates in trial 2 after digestion with pepsin powder or pepsin fluid at the four NRLs.

RESULTS

Trial 1. Overall, there was no difference between the two pepsin formulations in their ability to digest ground meat and to liberate *T. spiralis* larvae (Table 1). The mean larval recovery rates were 83 and 86.6% for pepsin powder and pepsin fluid, respectively, and the mean amounts of undigested meat retained on the sieve were 1.3 and 1.5 g for powder and fluid, respectively (Table 1). Visibility was good, and the amount of debris was low in all instances (data not shown).

Trial 2. The average recovery rates for the four NRLs ranged from 63.7% (lab 4, powder) to 90.7% (lab 2, fluid), with overall rates of 76.1% (range, 63.7 to 84.2%) for the pepsin powder and 81.2% (72.6 to 90.7%) for the pepsin fluid (Fig. 1). The recovery rates were evaluated by analysis of variance on untransformed data following removal of the lowest outlier value from all groups. There were no significant differences between the two pepsin formulations, but one of the laboratories (lab 4) had a significantly lower recovery rate compared with the three other laboratories for both the pepsin powder formulation ($P = 0.002$) and the pepsin fluid formulation ($P = 0.04$).

In four instances, the number of larvae recovered was one more than that recorded as added during sample preparation. We cannot exclude the possibility that in these cases an extra larva not observed when examining the meat in the compressorium was added, either because of the presence of more than one larva in a given capsule or the presence of an unrecorded capsule in the selected piece of muscle.

At one NRL (lab 3), excessive numbers of larvae (three, five, seven, or eight) were recorded from four of the samples. These findings could not be explained by the addition of extra larvae to the samples, so the data were excluded from the data set (two from each of the pepsin powder and pepsin fluid experiments, respectively).

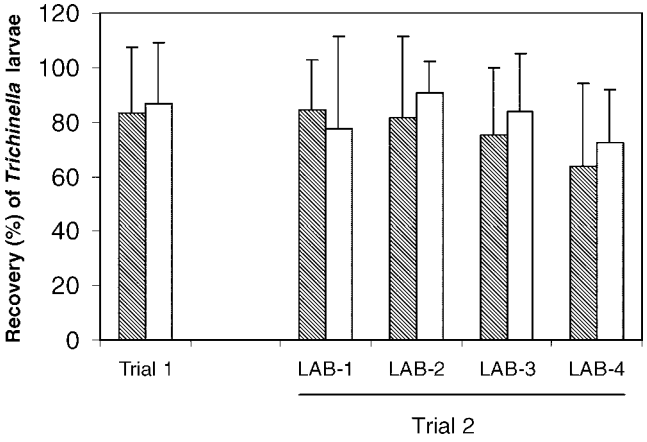

FIGURE 1. *Recovery of* Trichinella spiralis *larvae by digestion with the magnetic stirrer method using either pepsin powder (■) or pepsin fluid (□). (T), standard deviation. In trial 1, 24 samples were processed with either pepsin powder (12 samples) or pepsin fluid (12 samples). In trial 2, each laboratory (labs 1 through 4) processed 10 samples with each of the pepsin formulations.*

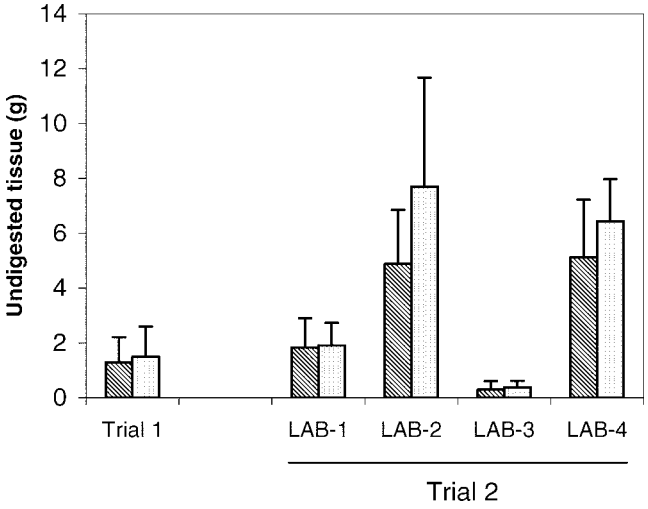

FIGURE 2. *Amount of tissue remaining undigested on the 177- or 180-μm-mesh sieve following digestion of ground pork with the magnetic stirrer method and either pepsin powder (■) or pepsin fluid (□). (T), standard deviation.*

Considerable variation in the amount of tissue remaining on the 177- or 180-μm-mesh sieve was observed after the 30-min digestion period (Fig. 2). Amounts were particularly high at lab 2, where up to 12.9 g of meat (average for 10 samples: 7.7 g) remained after digestion with pepsin fluid.

The amount of debris (particulate or flocculate matter) in the digestion fluid varied among the NRLs. At lab 4, debris was present in all samples, whereas no debris was present at lab 1, and labs 2 and 3 reported intermediate amounts. However, there was no consistent difference between the two pepsin formulations in their tendency to cause precipitation.

Visibility through the fluid was variably judged, ranging from poor in all samples (lab 3) to good in all samples (lab 4) and fair to good (labs 1 and 2). Lab 2 noted that the visibility was fair before washing and good after washing. Only lab 2 performed a wash.

DISCUSSION

The International Commission on Trichinellosis recommends that all slaughter testing methods for *Trichinella* detection in pigs, other livestock, and game animals should be validated by standard procedures and that new methods or modifications are subject to evaluation by at least three reference laboratories *(5)*. The ability to perform validation studies depends on proficiency samples of consistently high quality *(4)*. In the present validation study, there was no significant difference in performance between pepsin powder (1:10,000 N.F.) and pepsin fluid (30 ml equivalent to 10 g of powder) with regard to recovery of *T. spiralis* larvae, digestion of tissue, debris found in the digestion fluid, and visibility through the fluid. The biological properties of the pepsin fluid were not tested towards the end of its 1-year shelf life. However, no difference in performance within its shelf life is anticipated because of its persistent chemical properties.

Low numbers of larvae were used in some of the samples (spiking doses ranged from 1 to 30), putting more pressure on the recovery process to obtain high recovery rates. Thus, in samples with only one or two larvae recovery was 0% in some instances. Unfortunately, this result is expected for this method, which has a recognized average sensitivity of approximately 80% *(9)*; hence, the results of the performed trials adequately reflected the method sensitivity.

The variable amount of tissue retained on the sieve at some NRLs could be due to differences in the amount of tendon or other indigestible matter in the filler meat. Similarly, the variation between NRLs in the amount of debris in the digestion fluid probably was related to the quality of the filler meat used for the digestion or to variations in the degree of meat blending before the digestion process.

The performance of the pepsin fluid formulation, when used according to the manufacturer's instructions of 30 ml corresponding to 10 g of powder, was equal to that of the pepsin powder (1:10,000 N.F.) with regard to digestion of pork and mouse tissue for the liberation of encapsulated *T. spiralis* larvae.

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