Journal of Food Protection, Vol. 62, No. 11, 1999, Pages 1308–1313

A Validated *Trichinella* Digestion Assay and an Associated Sampling and Quality Assurance System for Use in Testing Pork and Horse Meat

LORRY B. FORBES* AND ALVIN A. GAJADHAR

Centre for Animal Parasitology, Canadian Food Inspection Agency, 116 Veterinary Road, Saskatoon, Saskatchewan, Canada S7N 2R3

MS 99-95: Received 9 April 1999/Accepted 23 July 1999

ABSTRACT

A revised digestion method, developed for efficiency and quality assurance, was validated for the detection of *Trichinella* larvae in pork and horse meat to meet requirements for food safety testing and facilitate access to international markets. The method consisted of a tissue homogenization step and a spin bar digestion procedure conducted at 45°C to free larvae from muscle tissue, followed by two sequential separatory funnel steps to concentrate the larvae for detection using a stereomicroscope. Critical control points were determined for the method and monitored during testing. Under conditions of a defined protocol, test capacity was suitable for industrial applications, since multiples of up to 100 g of tissue could be analyzed at one time. The overall sensitivity of the test system depended on the size and origin of the sample taken from individual infected carcasses. Data from swine indicated that the currently accepted sample size of 1 g from individual carcasses consistently detected larval loads of ≥3 larvae per gram. Larval loads of 1.0 to 1.9 larvae per gram required 3- to 5-g samples of muscle tissue for reliable detection. Five-gram samples were considered optimal, because they consistently detected more tissues than 3-g samples, although the difference was not statistically significant. Tissue localization studies in experimental pigs indicated that the tongue and diaphragm were the tissues of choice for the most sensitive larval recovery. A system of analyst training, laboratory certification based on ISO guide 25, and on-site proficiency panel testing was used to ensure that external laboratories would consistently produce reliable test results. The system developed for pork was successfully modified for the testing of horse meat.

Requirements designed to ensure that meat products are safe for human consumption must be met to qualify meat for international trade. Increasingly, consumers are demanding safe food, and multinational agreements are requiring adequate food safety standards and stipulating that tests used to qualify meat for export are scientifically valid and conducted under recognized quality assurance systems such as ISO/IEC guide 25 *(9)*. A number of tests for *Trichinella* in pork have been approved by the European Union for use *(3)*. In these tests, the parasites in meat are detected following tissue compression (trichinoscopy) or artificial digestion in a pepsin-HCl solution. Unfortunately, validation data are not available for these tests, and quality control systems are not specified or required. Because of the continuing outbreaks of human trichinellosis, particularly in Europe and Asia, the Canadian Food Inspection Agency developed a testing system for *Trichinella* in pork and horse meat to ensure safe meat and accessibility to international markets. The official Canadian digestion protocol *(5)* is based on the principles of traditional digestion methods, with modifications to increase the efficiency of large volume testing at abattoirs and to provide reliable results through the use of an appropriate quality control system. The quality system includes test-specific critical control points (CCPs), laboratory accreditation, analyst training programs, and proficiency panel systems *(4)*. Prototype test development was done using pork muscle spiked with *Trichinella spiralis* cysts obtained from experimentally infected rats. The study described herein was conducted to validate the official prototype protocol for pork, including determination of the optimal tissue for sampling and sample size. In addition, a modification of this procedure was evaluated for use with horse meat *(6)*.

* Author for correspondence. Tel: 306-975-5345; Fax: 306-975-5711; E-mail: lforbes@em.agr.ca.

MATERIALS AND METHODS

Digestion test. The digestion test used in this study was developed for pork and horse meat in part by modifying the digestion and larval concentration phases of the assay. Essentially, the test was conducted as follows. Fascia and fat were trimmed from meat samples. Three liters of 0.12 N HCl, hereafter referred to as 1% HCl, was prepared by adding 30 ml of concentrated HCl (37 ± 1% wt/wt) to 2970 ml of water. The 1% HCl solution was preheated to 45 ± 2°C, and approximately 50 to 100 ml was placed in a bladed blender (Waring blender base model no. 31BL41, jar and blade stock no. CAC32, Waring Products Division, Dynamics Corporation of America, New Hartford, Conn.) with a 100-g sample and pureed (several 1- to 3-s blender pulses). An additional 100 to 200 ml was added, and the mixture was blended for 5 to 10 s until uniformly liquid. Finally, 30 g of pepsin (pepsin 1:10,000 NF XII, granular, American Laboratories Inc., Omaha, Neb.) and 200 ml of 1% HCl were added to the liquid, and the mixture was blended for 5 s (CCP 1). The homogenate was then poured into a 4-liter beaker, and the blender jar was rinsed using the remainder of the 3 liters of the 1% HCl. Any

tissue debris still adhering to the sides or top of the blender was rinsed into the digest solution using 10 to 20 ml of tap water in a squirt bottle. The beaker containing the digestion mixture was placed on a magnetic stirrer in a 45 ± 2°C incubation chamber. The temperature of the digest and the incubation chamber was recorded. If not already at 45 ± 2°C, the digestion mixture was brought to 45 ± 2°C before digestion timing was started. The beaker was covered with aluminum foil or a plastic top, and the stirrer speed was adjusted to create a deep whorl in the liquid. After 30 min (pork) or 45 min (horse meat), the temperature of the incubator and the digest fluid was recorded (CCP 2). Within 5 min of removal from the incubation chamber, the digestion fluid was poured through a 180-μm sieve and into a 4-liter separatory funnel (Pyrex, 4 liters, Squibb, pear-shaped, Corning #6402–4 L, Science Products Division, Corning Inc., Corning, New York, N.Y.). The beaker was rinsed onto the sieve with 10 to 20 ml of room temperature tap water from a squirt bottle, and the sieve was rinsed into the 4-liter separatory funnel using the same procedure (CCP 3). The fluid in the separatory funnel was allowed to settle undisturbed for 30 min (CCP 4). The stopcock of the 4-liter separatory funnel was opened to drain 125 ml of fluid into a 500-ml separatory funnel (Kimax, 500 ml, Squibb, pear-shaped with drip tip and 24/40 joint on delivery stem, Kimble 29055F–500, Kimble Glass Inc., Vineland, N.J.) (CCP 5). Three hundred seventy-five milliliters of room temperature tap water was added to the 500-ml separatory funnel, and the fluid was allowed to settle undisturbed for at least 10 min. The stopcock on the 500-ml separatory funnel was opened to release 22 to 27 ml of fluid directly into a gridded petri dish (CCPs 6, 7, and 8). The petri dish was allowed to sit undisturbed for 1 min to allow larvae to settle to the bottom; then the drawn fluid was screened using a stereomicroscope at 10 to 16 power by systematically examining each grid for the presence of *Trichinella* larvae (CCPs 9, 10, 11, and 12). Rarely, suspensions in the petri dish were not clear enough for accurate assessment and were clarified as follows. The contents were transferred into a 50-ml conical tube using a pipette. The plates were rinsed using tap water, and the rinse was added to the tube. The volume in the tube was increased to 45 ml using tap water and allowed to sit undisturbed for 10 min. The supernatant was withdrawn with a pipette leaving the bottom 7.5 ml, which was resuspended and transferred back into a petri dish. The tube was then rinsed into the petri dish twice using 5 ml of tap water each time. The petri dish was allowed to stand undisturbed for at least 1 min then examined. The clarification process was repeated if necessary (CCPs 13 and 14).

The initial sample of muscle must be large enough to allow follow-up testing to identify infected animals in a positive pool or retesting in case of technical error. To identify the infected pig(s) in a positive pool of 100 pigs, initial retesting is done using 10 pools of 10 pigs each. Each of the 10 pigs in the resultant positive pool(s) is then individually tested. The sensitivity of the retest can be increased by using >1 g of tissue for each pig.

CCPs. CCP 1 affirms that HCl must be mixed with water before it is added to the pepsin-sample mixture to avoid inactivation of the pepsin by concentrated acid.

CCP 2 affirms that the digest solution must remain at 45 ± 2°C for the digest to be complete in 30 min for pork (45 min for horse meat) to avoid killing any *Trichinella* larvae present and to avoid inactivating the pepsin.

CCP 3 affirms that the presence of undigested muscle on the sieve indicates incomplete digestion. When present, the muscle tissue is rinsed off the sieve into a beaker using 800 ml of 1% HCl. Eight grams of pepsin is added, and the digestion procedure is conducted as previously described until all muscle is digested or for 60 min.

CCP 4 affirms that the 4-liter separatory funnel must remain undisturbed for 30 min to ensure larvae do not remain suspended in the solution.

CCP 5 affirms that the stopcock on the 4-liter separatory funnel must be opened quickly and completely to ensure a rapid flow of liquid and prevent larvae from becoming trapped on the edge of the drain opening

CCP 6 affirms that the stopcock on the 500-ml separatory funnel must be opened quickly and completely to ensure a rapid flow of liquid and prevent larvae from becoming trapped on the edge of the drain opening.

CCP 7 affirms that if less than 22 ml is drained into the petri dish, an additional aliquot of 22 to 27 ml must be drained into a second petri dish and both plates read to ensure larvae were not lost.

CCP 8 affirms that if more than 27 ml is drained into the petri dish, resulting in excess debris that compromises reading of the plate, the sample must be clarified (as described below).

CCP 9 affirms that the suspension must be allowed to settle for at least 1 min before examination to avoid missing suspended larvae.

CCP 10 affirms that the sample in the petri dish must be clear to avoid missing larvae.

CCP 11 affirms that the digests must be examined on the day of the digestion to avoid deterioration of the sample and compromised trace back to the carcass of origin.

CCP 12 affirms that the microscope must have at least ×10 magnification, be properly maintained, and have sufficient resolution to clearly see a single human hair to detect larvae in the petri dish.

CCP 13 is a clarification step. The supernatant must be withdrawn slowly from the top of the 50-ml conical tube to prevent resuspension of the sediment. If the sediment is disturbed, the supernatant must be returned to the tube and allowed to settle for an additional 10 min.

CCP 14 is a clarification step. To ensure complete transfer of the sediment from the 50-ml tube to the petri plate, the sediment must be resuspended before transfer and residual sediment in the tube must be rinsed into the plate as per instructions in the protocol.

Source of infected tissue. Three groups of five weanling pigs from a *Trichinella*-free swine herd were infected orally with *T. spiralis* in doses ranging from 40 to 1,000 larvae per pig (Table 1). The strain of parasite used (CAPTS1) was maintained in rats but was of porcine origin and was confirmed by DNA analysis as T1 *(2).* Pigs were killed 61 to 147 days after infection. Samples were collected for digestion testing based on meat cuts to approximate consumer exposure and to determine the reliability of samples from various sites to harbor parasites. Fourteen samples were selected and consisted of tongue, diaphragm, and right and left portions of the masseter muscle, shoulder, abdomen, ham, back loin, and tenderloin. One hundred grams from each cut was initially tested to determine larval loads. One to five additional digestion tests of 100 g each were conducted on larger cuts, i.e., loin and ham, to verify the reliability of 100-g samples. Large cuts of meat were divided into quadrants for sampling, and 25 samples of 1 g each were taken randomly from each quadrant to address any uneven distribution of cysts within a cut. Small cuts, such as the diaphragm, tongue, and masseter muscles were chopped into approximately 1-g pieces and mixed, and 100 g was removed for testing.

TABLE 1. *Larvae per gram of tissue for each muscle group based on the digestion of 100-g samples*

	Pig identification and number of larvae in oral infective dose														
Tissues sampled	P1 40[a]	P2 40	P3 50	P4 50	P5 80	P6 120	P7 120	P8 150	P9 150	P10 200	P11 300	P12 300	P13 500	P14 500	P15 1,000
Tongue	3.3	4.0	0.0	0.01	2.4	3.0	12.8	0.3	5.2	57.0	19.0	65.0	174.0	66.0	267.0
Diaphragm	3.3	5.6	0.2	0.0	2.8	3.4	12.2	1.9	5.8	29.0	13.4	79.0	46.0	58.0	142.0
Masseter	1.6	1.1	0.0	0.0	1.4	1.5	5.9	0.1	2.5	35.5	8.4	87.0	76.0	38.0	109.0
Left shoulder	1.8	1.7	0.0	0.0	1.1	1.1	5.2	0.1	2.0	17.0	5.8	31.0	78.0	24.0	60.0
Right shoulder	1.2	1.7	0.0	0.0	1.2	1.2	5.2	0.1	2.4	29.0	3.2	28.0	34.0	31.0	89.0
Left tenderloin	1.5	1.6	0.0	0.0	0.5	1.0	4.7	0.2	1.2	11.0	6.2	35.0	59.0	23.0	71.0
Right tenderloin	1.6	1.4	0.0	0.0	0.9	0.8	4.5	0.2	1.4	15.0	5.7	24.0	53.0	35.0	53.0
Left abdomen	1.8	1.7	0.0	0.0	0.8	0.9	5.0	0.2	1.6	9.0	5.4	14.3	34.0	15.0	77.0
Right abdomen	1.5	1.6	0.0	0.0	1.0	1.2	4.2	0.2	1.9	20.0	4.3	22.3	36.0	14.3	77.0
Left ham	1.3	1.4	0.0	0.0	0.7	0.8	3.2	0.1	1.6	5.0	3.7	17.5	31.0	28.0	62.0
Right ham	1.2	1.0	0.1	0.0	0.7	0.9	3.4	0.0	1.4	9.0	5.0	24.0	27.0	17.5	41.0
Left back loin	0.7	1.0	0.0	0.0	0.6	0.5	2.5	0.1	0.7	4.5	3.5	22.0	16.0	22.3	21.3
Right back loin	0.8	0.9	0.0	0.0	0.6	0.5	2.6	0.1	0.8	4.8	3.2	23.0	17.0	22.0	27.0

[a] Number of larvae in oral infective dose.

Tissue localization. The number of larvae per gram (lpg) of tissue was determined for each cut of meat using the digestion assay and sampling method described above. For each pig, individual tissues were ranked according to their larval concentration. Tissues with the highest lpg were ranked as 1, and rank value increased as the lpg decreased. Ties were accorded the same rank, and the next lowest tissue was ranked as it would have been if the ties were separate. For example, in pig 1 the rankings were assigned in the following manner: tongue and diaphragm (3.3 lpg) were both ranked as 1, left shoulder and left abdomen (1.8 lpg) were ranked as 3, and masseter and right tenderloin (1.6 lpg) were ranked as 5. Preferred localization sites and hence preferred sampling sites were determined by observing the data from all 15 pigs and determining which tissues consistently ranked highest. In addition, the lpg among tissue types was compared statistically. Since the data for larval concentrations within each tissue type were not normally distributed, the data were transformed by taking the logarithm of one plus the larval concentration, which is appropriate for data where the standard deviation is proportional to the mean, with some values close to zero *(11)*. A two-way analysis of variance was performed on the transformed data using a randomized complete block design, where individual pigs were the blocking factor and the muscle group was the treatment factor. Since the muscle group had a significant effect on the concentration of larva in the tissue, pairwise comparisons of means using the least significant difference test were performed *(10)*. The results of the comparisons are presented in Table 2, and mean lpg for each tissue presented is the antilog of the means calculated from the transformed data (geometric means) *(11)*. Statistical analysis was performed using the computer program Statistix (Statistix version 1.0, Analytical Software, Tallahassee, Fla.).

TABLE 2. *The distribution of* Trichinella *larvae among the various muscle groups of 15 experimentally infected pigs*

Muscle group	Mean[a] larvae per g
Tongue	10.47 A[b]
Diaphragm	9.48 A
Masseter	6.29 B
Left shoulder	4.85 C
Right shoulder	4.75 C
Right tenderloin	4.39 CD
Left tenderloin	4.39 CD
Right abdomen	4.23 CD
Left abdomen	3.87 CD
Left ham	3.50 DE
Right ham	3.45 DEF
Right loin	2.74 EF
Left loin	2.65 F

[a] Geometric mean; $n = 15$.

[b] Means within the column having different letters are significantly different at $P < 0.05$.

Detection limits. Tissues from infected pigs with larval loads ranging from 0.01 to 17.7 lpg as determined in the 100-g assays were divided into eight groups (Table 3). Ten to 67 samples each of 1-, 3-, or 5-g amounts were taken from each of the eight groups for a total of 640 samples. These samples were combined with 99, 97, or 95 g of uninfected pork diaphragm to make up 100 g and tested as described above. The frequency of identification of an infected sample (at least one larva detected) was compared between sample sizes for each group using Pearson's chi-square test of homogeneity in a contingency table or Fisher's exact test if an expected frequency was less than 5 *(1)*. The results were used to determine the detection limits (sensitivity) for each sample size and compare their relative effectiveness for use in testing for consumer protection.

Assessment of the digestion method for horse meat. Fifty proficiency samples, each weighing 20 g, were prepared as previously described *(4)*. Each sample contained between 6 and 34 *Trichinella* cysts and was combined with 80 g of uninfected horse tongue to perform a 100-g digestion. Each sample was tested using the procedure described above as for horse meat.

RESULTS

Tissue localization. Larvae were recovered from the tissues of all experimentally infected pigs and the lpg of tissue detected are listed in Table 1. In general, larval densities increased with dose. The tissues from each pig are ranked by lpg in Table 4. The tongue and diaphragm most frequently contained the highest number of lpg in each pig

TABLE 3. *Percentage of* Trichinella*-infected tissues detected by a modified digestion assay using 1-, 3-, or 5-g samples*

Sample size (g)	*Trichinella*-infected tissues grouped by larvae per g of tissue							
	0.01–0.9	1.0–1.4	1.5–1.9	2.8	3.0–3.4	4.2	5.8	8.0–17.7
1	40% (8/20) A[a]	73% (49/67) A	67% (16/24) A	80% (8/10)	100% (37/37)	95% (19/20)	100% (15/15)	100% (31/31)
3	75% (15/20) B	96% (64/67) B	100% (23/23) B	100% (10/10)	100% (37/37)	95% (19/20)	ND[b]	100% (31/31)
5	75% (15/20) B	100% (68/68) B	95% (21/22) B	100% (10/10)	100% (37/37)	100% (20/20)	ND	100% (31/31)

[a] Percentages within the column having different letters are significantly different at $P \leq 0.0252$.
[b] ND, not done.

and were ranked 1 or 2 in 13 of 15 pigs and 12 of 15 pigs, respectively. The diaphragm ranked first in 7 of 9 pigs who received an oral dose of ≤150 larvae, whereas the tongue ranked first in 5 of 6 pigs who received ≥200 larvae orally. Masseter muscles were third (ranked 1 to 3 in 10 of 15 pigs). There were smaller ranking differences among the remaining tissues, but the trend was as follows: shoulder (4), tenderloin and abdomen (5), ham (6) and back loin (7). There was a significant difference in the larval concentration in different muscle groups ($P = 0.0000$) when the effect of individual pigs was controlled for. Comparisons for individual muscle group mean lpg are presented in Table 2. The tongue and diaphragm contained significantly more larvae than the other groups but did not differ significantly from each other. Masseter muscle had significantly fewer larvae than the tongue and diaphragm but more larvae than the other muscle groups. There was considerable overlap among the remaining muscle groups, although, in general, ham and loin ranked lowest.

Detection limits. Detection results for the 1-, 3-, and 5-g sample sizes are summarized in Table 3. Tissues containing 3 lpg or more were effectively detected using all three sample sizes. Below this level, 1-g samples were inadequate for the detection of infection in any given tissue. The highest level of detection per site was achieved using 5-g samples, which identified all 68 tissues containing 1 to 1.4 lpg. At this same larval load, 3-g samples gave positive results with 64 of 67 tissues. When categories of larval load were combined to create a category of tissues containing 1 to 1.9 lpg, 5-g samples detected 89 (98.9%) of 90 tissues, 3-g samples detected 87 (96.7%) of 90, and 1-g samples detected 65 (71.4%) of 91 tissues. Chi-square test results indicated a significant reduction in detection with 1-g samples compared with 3-g samples for the 0.01- to 0.9-lpg group ($P = 0.0252$), the 1.0- to 1.4-lpg group ($P = 0.0004$), the 1.5- to 1.9-lpg group ($P = 0.0039$), and the combined 1.0- to 1.9-lpg group ($P = 0.0000$). A similar significant reduction in detection was observed with 1-g samples compared with 5-g samples. There were no other significant differences ($P > 0.05$) in detection between sample sizes for any of the groups.

Assessment of the digestion method for horse meat. The mean recovery of larvae from the 50 proficiency samples was 98% (SD = 5%). Larval recovery was ≥81% in 50/50 samples, ≥95% in 42/50 samples, and 100% in 38/50 samples.

DISCUSSION

The double separatory funnel digestion method and its associated quality control system were designed to reliably

TABLE 4. *Relative ranking*[a] *of tissues from each pig based on larvae per gram in each tissue*

Tissues sampled	Pig identification and number of larvae in oral infective dose														
	P1 40[b]	P2 40	P3 50	P4 50	P5 80	P6 120	P7 120	P8 150	P9 150	P10 200	P11 300	P12 300	P13 500	P14 500	P15 1,000
Tongue	1	2	0	1	2	2	1	2	2	1	1	3	1	1	1
Diaphragm	1	1	1	0	1	1	2	1	1	3	2	2	6	2	2
Masseter	5	10	0	0	3	3	3	7	3	2	3	1	3	3	3
Left shoulder	3	3	0	0	5	6	4	7	5	6	5	5	2	7	9
Right shoulder	10	3	0	0	4	4	4	7	4	3	12	6	8	5	4
Left tenderloin	7	6	0	0	13	7	7	3	11	8	4	4	4	8	7
Right tenderloin	5	8	0	0	7	10	8	3	9	7	6	7	5	4	10
Left abdomen	3	3	0	0	8	8	6	3	7	9	7	13	8	12	5
Right abdomen	7	6	0	0	6	4	9	3	6	5	9	9	7	13	5
Left ham	9	8	0	0	9	10	11	7	7	11	10	12	10	6	8
Right ham	10	11	2	0	9	8	10	0	9	9	8	7	11	11	11
Left back loin	13	11	0	0	11	12	13	7	13	13	11	11	13	9	13
Right back loin	12	13	0	0	11	12	12	7	12	12	12	10	12	10	12

[a] The tissue with the highest number of larvae per gram ranked 1; ties were ranked equally; and no larvae detected ranked 0.
[b] Number of larvae in oral infective dose.

detect a single *Trichinella* larva in a 100-g sample. This level of sensitivity was indicated in an earlier study *(4)* and was supported in this study, which showed that 20 of 29 tissues containing approximately 1 lpg were detected when 1-g samples taken from these tissues were combined with 99 g of uninfected muscle and tested. Failure to detect all 29 tissues was attributed to uneven distribution of larvae within the tissues sampled. A previous study using this method showed that ≥75% of the larvae in spiked samples were detected in 94.3% of the samples tested *(4).* Since the use of spiked samples eliminated sampling error, failure to detect all larvae in these samples was attributed to minor technical, mechanical, and biological factors that were beyond the control of the quality system. Based on these data, approximately 1.3 larvae would have to be present in a 100-g sample to ensure detection at least 94% of the time. Therefore, if larval distribution was not a factor, it could reasonably be expected that all tissues in this study containing at least 2 lpg would be detected by testing a 1-g sample of each tissue in a pool with 99 g of uninfected tissue. Regrouping of the experimental data resulted in a set of 24 tissues that contained 2.4 to 2.8 lpg each, and only 20 (83%) of these were detected. Thus, uneven distribution of larvae within tissues appears to be the greatest limiting factor for detection using this test system.

The data indicate that detection failures caused by uneven distribution of the larvae within tissues can be overcome by increasing the size of the sample taken from each tissue. Samples of 1 g reliably detected tissues containing ≥3 lpg. However, muscle tissue containing 1 lpg is considered a food safety hazard; therefore, 1-g samples are inadequate for detection. Our data show that a sample size of 3 to 5 g is required for reliable detection of tissues containing 1 lpg. This agrees with previous studies in horses and swine that show that 5-g samples consistently detected tissues containing approximately 1 lpg, whereas 1-g samples did not *(7, 8).* These earlier studies did not assess intermediate sample sizes between 1 and 5 g. In the present study, combined data from tissues containing 1.0 to 1.9 lpg showed no significant difference in detection between 3- and 5-g samples, which detected 87 of 90 and 89 of 90 tissues, respectively ($P = 0.62$). Similarly, data from tissues containing 1.0 to 1.4 lpg showed no significant difference in detection between 3- and 5-g samples, which detected 64 of 67 and 68 of 68 tissues, respectively ($P = 0.12$). If only samples containing 1.0 lpg were selected, there was still no significant difference in detection between 3- and 5-g samples, which facilitated detection of 28 of 30 and 30 of 30 tissues, respectively ($P = 0.49$). The 3 infected tissues that were missed using 3-g samples contained 1 to 1.1 lpg and were detected with 5-g samples. This suggests that 3-g samples may not be dependable when tissue larval densities approach 1 lpg. However, this statement is not proven by the data, which indicate that these numbers could occur by chance alone. Test data from a larger population of tissues containing 1 lpg would be useful to demonstrate that 3-g samples have adequate sensitivity for food safety purposes.

Determination of an optimal sample size has implications for industrial testing and food safety. In industrial applications, the current 1-g sample requirement per pig allows 100 carcasses to be tested as a pool at one time. Increasing the sample size to 5 g, as is required for horse meat, ensures detection at the 1-lpg level but reduces test capacity to 20 carcasses per test. Additional research using 3- and 4-g samples would be useful to determine if a sample size of less than 5 g is adequate, since even a 1-g reduction in sample size would result in a resource saving for industry and simplify modification of the existing method to maintain high carcass throughput.

Clinical trichinellosis in humans is associated with the consumption of tissues containing more than 1 lpg of tissue, and this level of infection has become the benchmark for the sensitivity of diagnostic test systems. The digestion test described herein, in conjunction with a sample size of 5 g and appropriate quality control, can reliably detect tissues containing 1 lpg and thereby minimize the risk of clinical disease. The use of larger samples sizes to increase the sensitivity to less than 1 lpg of tissue is not necessary, since exposure to less than 1 lpg of *Trichinella* usually results in asymptomatic infections that are not life threatening and rarely detected in their active phase.

Selecting the optimal tissue for sampling can further reduce the risk of human exposure. The data in Table 1 show that tongue, diaphragm, and, to a lesser extent, masseter muscle consistently contained more larvae than the other tissues. This agrees with previous studies using pigs *(7).* In heavy infections, this is inconsequential, since all tissues are easily detected by existing tests. In low-level infections, however, sample selection is important. For example, in Table 1, five pigs had several tissues containing 0.5 to 1.4 lpg. All five pigs had at least 2.4 lpg in the tongue or diaphragm, and four of these had 3 to 5.8 lpg. If tongue or diaphragm were selected for testing, our data indicate that even 1-g samples would have a good chance of detecting these carcasses. Therefore, sampling tongue or diaphragm in pigs will not only enhance the detection of lightly infected carcasses, but will also create a margin of safety, since low-larval densities in the tongue and diaphragm are associated with subclinical doses for humans in other tissues. Our data suggest that the diaphragm was the best tissue for sampling, because it contained the highest number of lpg in seven of the nine pigs with the lowest level of infection in the study.

The digestion test method used in this study was developed to minimize operator error, improve efficiency, incorporate quality control steps, and process large numbers of samples without compromising sensitivity. Modifications over existing procedures included a sample/digest volume ratio of 1:30, an increased digestion temperature (45 ± 2°C), an incubation chamber to maintain digestion temperature, a double separatory funnel system for clarification of the digested sample, and determination of a number of CCPs for the method. The method was integrated with standardized reagent preparation, sampling, recording, reporting, and record maintenance to create a fully documented test system. Following test validation, procedures were developed to ensure that test results from all laboratories per-

forming the test were reliable. This included a requirement for all technicians performing the test to successfully complete training at the reference laboratory and then pass on-site proficiency sample panels at their home laboratory. In addition, laboratory sites performing this test were required to have a quality system in place based on ISO/IEC guide 25 before they were allowed to test. Finally, a system of external proficiency sample panels was developed and implemented to provide ongoing monitoring and assessment of competence *(4)*.

With the exception of minor modifications to the digestion protocol as listed in the ''Materials and Methods'' section, an identical test system was put in place to test horse meat. Validation of the digestion test for horse meat using proficiency samples compared favorably to the proficiency sample data previously reported for pork *(4)*. This study indicated that ≥81% of larvae were recovered from 100% ($n = 50$) of the horse meat samples compared with ≥75% of the larvae from 94% ($n = 404$) of the pork samples. Validation studies using infected horses were not done, since previous work by others demonstrated that horse tongue was the tissue of choice for sampling and that 5-g samples were required to reliably detect tissues containing 1 lpg using digestion tests *(8)*. However, future studies using infected horses and a test of adequate quality for use under industrial conditions would be required to determine whether test sensitivity can be maintained with horse meat sample sizes of less than 5 g.

The primary function of digestion tests is to detect larval densities of *Trichinella* that constitute a food safety hazard. This study and the work of others have shown that digestion tests will not consistently detect low levels of infection in individual animals without an impractical increase in the amount of tissue required for testing *(7, 8)*. Conversely, serological tests for trichinellosis cannot be used to reliably detect disease in individual carcasses, since large numbers of infective larvae may be present in some animals before an immune response is generated. Serological tests, however, can provide both economical and reliable disease prevalence data with well-defined confidence intervals when conducted on a population basis. It is, therefore, necessary to maintain both serological and digestion test capabilities for trichinellosis to meet requirements for surveillance and food safety.

ACKNOWLEDGMENTS

We acknowledge Dr. Sarah Parker for advice on statistical analysis; Dr. Andrijana Rajic for contributions to initial test development; Dr. Brad Scandrett for assistance with postmortem sample collection; Shawna Peace, Garth Mathie, and other staff at the Centre for Animal Parasitology, Saskatoon, Saskatchewan, for technical assistance; and Dr. Bill Bulmer, Director (retired), Health of Animals Laboratory, Saskatoon, Saskatchewan, for administrative support. Financial support for this study was provided in part by the Canadian Meat Council, Canadian Meat Research Institute, Canadian Pork Council, Saskatchewan Pork International, and the Market and Industry Services Branch of Agriculture and Agri-Food Canada. Intercontinental Packers Limited, Saskatoon, provided the negative pork tissue used in this study.

REFERENCES

1. Altman, D. G. 1991. Comparing groups—categorical data, p. 229–276. *In* Practical statistics for medical research. Chapman & Hall, New York.
2. Appleyard, G. D., D. Zarlenga, E. Pozio, and A. A. Gajadhar. 1999. Differentiation of *Trichinella* genotypes by PCR using sequence specific primers. J. Parasitol. 85:556–559.
3. European Economic Community. 1984. Commission directive 84/319/EEC. Off. J. Eur. Comm. 167:34–43.
4. Forbes, L. B., A. Rajic, and A. A. Gajadhar. 1998. Proficiency samples for quality assurance in *Trichinella* digestion tests. J. Food Prot. 61:1396–1399.
5. Gajadhar, A. A., L. B. Forbes, and A. Rajic. 1996. The double separatory funnel technique for the detection of *Trichinella* larvae in pork, version 1.0, official protocol. Canadian Food Inspection Agency, Ottawa, Canada.
6. Gajadhar, A. A., L. B. Forbes, and A. Rajic. 1997. The double separatory funnel procedure for the detection of *Trichinella* larvae in horse meat, version 1.0, official protocol. Canadian Food Inspection Agency, Ottawa, Canada.
7. Gamble, H. R. 1996. Detection of trichinellosis in pigs by artificial digestion and enzyme immunoassay. J. Food Prot. 59:295–298.
8. Gamble, H. R., A. A. Gajadhar, and M. B. Solomon. 1996. Methods for the detection of trichinellosis in horses. J. Food Prot. 59:420–425.
9. International Organization for Standardization. 1990. ISO/IEC guide 25, p. 1–7. *In* General requirements for the competence and calibration of testing laboratories. International Organization for Standardization, Geneva, Switzerland.
10. Snedecor, G. W., and W. G. Cochrane. 1980. Statistical methods. Iowa State University Press, Ames.
11. Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics; a biometrical approach. McGraw-Hill Book Co., Toronto, Ontario.