Research Note

Analysis of Cured Meat Products for *Cryptosporidium* Oocysts following Possible Contamination during an Extensive Waterborne Outbreak of Cryptosporidiosis

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ABSTRACT

An outbreak of waterborne cryptosporidiosis in a town in northern Sweden during winter 2010 resulted in the potential exposure of cured meat products to *Cryptosporidium* oocysts during their manufacture. The purpose of this work was to develop a method for analyzing cured meat products for contamination with *Cryptosporidium* oocysts and use this method to analyze potentially contaminated product samples. A simple method of elution, concentration, separation, and detection was used, based on work with other food matrices but adapted for the relatively high fat content of cured meat surfaces. Using spiking experiments, the recovery efficiency of this method was found to be over 60%. In the analysis of the potentially contaminated products, only one putative *Cryptosporidium* oocyst was detected, and this was sufficiently deformed so that it could not be confirmed as an oocyst; if it was an oocyst, it was considered to have been probably deformed and inactivated prior to analysis. Based on the results of the analyses, together with data on the probable extent of contamination of the products and on our knowledge of factors, such as water activity, which affect oocyst survival, the products were safely released to the market.

In November 2010, an outbreak of waterborne cryptosporidiosis occurred in the town of Östersund in northern Sweden. An estimated 12,700 people were infected, and it was suspected that sewage containing *Cryptosporidium hominis* oocysts had been discharged to a stream that fed the local water supply (6). *Cryptosporidium* oocysts of subgenotype IbA10G2 (the same subgenotype that was associated with the Milwaukee outbreak of 1993) were identified both in fecal samples from patients and in the implicated water supply. A boil-water notice was implemented as soon as the outbreak was identified, and this was not withdrawn until 18 February 2011; other prevention and control measures applied included cleaning of the water distribution system, enhanced protection of the water supply area, and incorporation of a UV treatment at the water treatment works.

Among the various businesses affected by the outbreak was a supplier of cured meat products. In the preparation of the cured meats, a prolonged process including salting, smoking, and drying stages, the products are exposed to tap water on all surfaces for several hours. As the cured products are then consumed without further preparation (cooking) in the home, it was considered that, if the products had become contaminated with *Cryptosporidium* oocysts during their water contact, then they could potentially act as vehicles for further transmission of *Cryptosporidium* to the consumer. Various foodborne outbreaks of cryptosporidiosis have been documented in the literature; although the outbreaks are apparently from diverse areas of the world (the Far East, the United States, Australia, and Europe), it is interesting to note that Scandinavia is strikingly overrepresented (14). It is speculated that this may reflect consumer habits or elevated diagnostic proficiency or that *Cryptosporidium* oocysts thrive best in the moist, cool conditions that predominate in Scandinavian countries (14).

The development of a robust, reproducible, and efficient method for detection of *Cryptosporidium* oocysts on food has been considered a challenge for many years (14). Although various methods have been developed for fruit and vegetables (2, 3, 15, 16) and also for shellfish (12, 17), a validated standard method has yet to be described (14). In this article, we describe the development and use of a method for analyzing the surfaces of cured meat products for *Cryptosporidium* oocysts and the rationale for subsequent release onto the market of the products that had been exposed to the potentially contaminated water.

MATERIALS AND METHODS

Cured meat products for method development and analysis. Two different cured meat products were used in the method development, pæreskinke (PS) and lufttørket skinke (LS), both of which are cured ham products. In the preparation of PS (approximately 19% fat), the ham is salted for a total of 21 days before being rinsed by hand for approximately 30 s in fresh water. The ham then stands in a freshwater bath overnight (approximately...
14 h) before being smoked in a smoking chamber (20 to 25°C dry temperature) for 24 h and then dried for 6 to 10 weeks, depending on water activity (such that the final product has a water activity of less than 0.910 aₜ).

In the preparation of LS, the ham is salted for between 30 min and 4 h before being submerged in a brine solution bath (20% salt solution) for 10 days. This is followed by a freshwater rinse, using a continuous freshwater flow, for 3 to 4 h. The ham is then smoked in a smoking chamber (20 to 25°C dry temperature) for 4 days and then air dried for 4 to 10 weeks, depending on water activity (such that the final product has a water activity of less than 0.910 aₜ).

Both ham products are netted before immersion in water, and potential contamination is surface only.

Cryptosporidium oocysts for seeding studies. Viable Cryptosporidium parvum oocysts (Iowa isolate, mouse source) were purchased from Waterborne, Inc. (New Orleans, LA) and kept refrigerated until use. Dilutions for seeding studies were made in phosphate-buffered saline, and control counts were made on at least three replicates for each seeding study.

Contamination of unexposed cured meat products with Cryptosporidium oocysts. Cured meat products (both PS and LS) that were considered not to have been exposed to the contaminated water were obtained from the supplier. As only surface contamination was considered to have potentially occurred, the surfaces of the hams were removed with a sharp knife and cut into approximately equal squares, which were measured and weighed. The outside surfaces of these sample squares were contaminated with Cryptosporidium oocysts by distributing 100 µl over the surfaces with a pipette as evenly as possible and ensuring no runoff. The ham surface squares were left to dry overnight before analysis.

Analysis of artificially contaminated cured meat products: method development. Although a reasonable quantity of literature has been published on elution of Cryptosporidium oocysts from the surfaces of fruits and vegetables (2, 3, 14–16) and also from shellfish (12, 14, 17), very little has been published on the elution of Cryptosporidium oocysts from meat products, and we were unable to identify any previous publications on the detection of Cryptosporidium oocysts on cured meat products. Therefore, based on our extensive experience with the development of methods for the isolation of protozoan parasites from fruits, vegetables, and shellfish, an analytical protocol outline was described (Fig. 1). Immunomagnetic separation (IMS) and detection by immunofluorescent antibody test (IFAT) followed instructions from the manufacturers and standard procedures for analysis for Cryptosporidium and Giardia as extensively described previously. However, due to the nature of the food matrix, with PS being particularly fatty, different variables within the method were compared in order to improve elution and minimize losses in centrifugation and IMS. In addition, modifications of the detection procedure (step 4) were also investigated. The variables investigated are listed in further detail below. Due to the requirement for producing an adequate method within a limited time frame, the variables investigated were limited in scope, and relatively few replicates were included per trial. Thus, although the method developed for use was satisfactory (it should be noted that published methods for analyzing fruit and vegetable produce for Cryptosporidium oocysts report recovery efficiencies of between 40 and 60%), no attempt at validation by interlaboratory trials was attempted. Indeed, such validation, which is costly in terms of both time and resources, would have been inappropriate in the circumstances. The selection of variables was based on previous experience in method development and on the ongoing results obtained during the method development.

For step 1, elution, two different elution buffers were used (1 M glycine and a detergent-based buffer) and different periods of stomaching compared (Table 1).

For step 2, concentration, it was considered that fat from the meat surfaces could present a potential problem that might affect recovery efficiency. Thus, the effects on recovery efficiencies of two fat dispersants (diethyl acetate and 1% deoxycholate [DOC]) were investigated. In trials C1 and C2 (Table 2), oocysts were placed directly on the meat samples, which were first stomached (parameters as in trial E3) before parameters were compared. For trials C3 to C6 (Table 2), negative (nonspiked, unexposed) samples noted to be particularly fatty were first stomached (parameters as in trial E4), and the resultant concentrated eluant divided into tubes into which the oocysts were added before comparisons of the effect of DOC on recovery efficiency were made. Details of the parameters investigated are described in Table 2. For all trials, the eluate was concentrated by centrifugation at 3,000 rpm (1,100 × g) for 10 min using a swing-out rotor before fat dispersal was investigated.

In step 3, detection by IFAT, the final 50-µl sample of concentrate was air dried and methanol fixed to a welled slide, incubated with monoclonal antibody (Aqua-Glo, Waterborne, Inc., New Orleans, LA) for 30 min, and subsequently incubated with
TABLE 1. Elution parameters tested (step 1)

<table>
<thead>
<tr>
<th>Trial (elution)</th>
<th>Pretreatment</th>
<th>Stomaching conditions (1)</th>
<th>Stomaching conditions (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Soak in 80 ml of ice-cold 1 M glycine, 5 min</td>
<td>40 ml of ice-cold 1 M glycine, 15 min</td>
<td>40 ml of ice-cold 1 M glycine, 15 min</td>
</tr>
<tr>
<td>E2</td>
<td>Soak 30 min in 50 ml of ice-cold detergent-based buffer a</td>
<td>Use soak glycine, 15 min</td>
<td>Use soak glycine, 15 min</td>
</tr>
<tr>
<td>E3</td>
<td>Soak 30 min in 50 ml of ice-cold detergent-based buffer a</td>
<td>50 ml of ice-cold detergent-based buffer, 30 s a</td>
<td>40 ml of ice-cold 1 M glycine, 15 min</td>
</tr>
<tr>
<td>E4</td>
<td>Soak 30 min in 50 ml of ice-cold detergent-based buffer a</td>
<td>Use soak buffer, 1 min</td>
<td>40 ml of ice-cold 1 M glycine, 1 min</td>
</tr>
</tbody>
</table>

a Detergent-based buffer consisted of 0.1% sodium dodecyl sulfate, 0.1% Tween 80, 0.01% antifoam A, and various salts (15, 16).

4',6-diamidino-2-phenylindole (DAPI) by standard procedures before visualization by fluorescence microscopy. Using paired samples, the effect of adding 50 μl of 0.1% Evans Blue, a well-known quenching agent, to the staining with the monoclonal antibody step was investigated.

**Analysis of cured meat products exposed to contaminated water during production.** Based on the results of the method development trials described above, a protocol with satisfactory recovery efficiency was developed and used for the analysis of products that had been exposed to the potentially contaminated water.

Two products of each type (PS and LS) were obtained from the manufacturer. Although salami is also prepared by the supplier and could have been exposed to the contaminated water, the volume of freshwater used in the production of salami is considerably less (1 liter/110 kg, compared with 4,400 liters/1,020 kg), and therefore, salami was excluded from the analyses. From each product, at least three sections of surface were removed with a sharp knife, measured, and weighed. The sections were soaked for 30 min in 50 ml of ice-cold elution buffer (15, 16) containing 0.1% sodium dodecyl sulfate, 1% Tween 80, and 0.01% antifoam A with the outer surface downward. The product sections and the buffer in which they had been soaked were then transferred to individual stomacher bags and stomached for 1 minute. The fluid was collected into 50-ml centrifuge tubes, and 40 ml of ice-cold 1 M glycine was added to the stomacher bag containing the sample and stomached for 1 min. The fluid was then collected into 50-ml centrifuge tubes, and the bag and sample discarded. The buffers were centrifuged together by centrifugation for 10 min at 3,000 rpm (1,100 × g), using a standard benchtop centrifuge. If the final concentrate was particularly fatty or the sample itself had been noted as being particularly fatty (some PS samples), then 5 ml of DOC was added to 10 ml of the final concentrate and the mixture shaken for 5 min on a vortex mixer and centrifuged again, and the pellet washed twice in water before proceeding to IMS. IMS (Dynabeads anti-Cryptosporidium kit, Invitrogen) was conducted according to the manufacturer’s instructions for water concentrates, using buffers SL-A and SL-B and rotating beads, buffers, and samples for 1 h in an L10 tube. The final concentrate (50 μl) was then dried to welled slides, methanol fixed, and incubated with 50 μl of Aqua-Glo monoclonal antibody for 30 min at 37 °C in a humid chamber. For PS samples, 50 μl of 0.1% Evans Blue was added with the monoclonal antibody and incubated with the sample. After removing the stains, the sample was incubated with DAPI for staining of nuclei before examination by fluorescence microscopy using appropriate filter blocks and examination of any suspect objects by both fluorescence microscopy and use of Nomarski optics. The recovery efficiency of the entire method was over 60%, based on the results for spiked samples.

**Estimation of potential for contamination of cured meat products exposed to Östersund’s drinking water supply during the outbreak period.** Estimates of the concentration of Cryptosporidium oocysts in the water at peak contamination, the water usage per production batch of cured meat products (LS), and the finished product weight (batch size) were used to estimate the potential extent of contamination of the products. These data, in combination with the results of the analyses, were used to decide whether the products should be released to the market for sale to consumers.

**RESULTS**

**Weights and areas of cured meat product surface samples used for method development.** The mean weight of the 45 different product sample surface squares seeded with Cryptosporidium oocysts in the method development was 18 g (standard deviation [SD], 8.3 g), and the mean area was 31 cm² (SD, 8.7 cm²; area data missing for 14 samples).

**Selection of elution parameters (step 1) in method development.** Data from previous studies on extraction of

TABLE 2. Fat dispersal methods investigated following concentration of eluate (step 2)

<table>
<thead>
<tr>
<th>Trial (concn)</th>
<th>Fat dispersant used</th>
<th>Volume of dispersant added to 10-ml pellet</th>
<th>Wash step prior to IMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 a</td>
<td>Ethyl acetate</td>
<td>10 ml, vortex vigorously 5 min</td>
<td>Proceed directly to IMS</td>
</tr>
<tr>
<td>C2 a</td>
<td>1% DOC</td>
<td>1 ml, vortex vigorously 5 min</td>
<td>Centrifuge, then wash pellet twice in water before IMS</td>
</tr>
<tr>
<td>C3 a</td>
<td>1% DOC</td>
<td>2 ml, vortex vigorously 5 min</td>
<td>Centrifuge, then wash pellet twice in water before IMS</td>
</tr>
<tr>
<td>C4 b</td>
<td>1% DOC</td>
<td>5 ml, vortex vigorously 5 min</td>
<td>Centrifuge, then wash pellet twice in water before IMS</td>
</tr>
<tr>
<td>C5 b</td>
<td>1% DOC</td>
<td>10 ml, vortex vigorously 5 min</td>
<td>Centrifuge, then wash pellet twice in water before IMS</td>
</tr>
</tbody>
</table>

a Oocysts were added directly to cured meat product, which was first stomached (parameters as in trial E3) before concentration by centrifugation.

b Oocysts were added to stomached eluate of fatty samples rather than directly to cured meat product.
**TABLE 3. Comparison of elution and fat dispersal trial results during method development**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Mean no. of oocysts in inoculum</th>
<th>Mean no. of oocysts recovered</th>
<th>95% CI</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 (elution)</td>
<td>102.7 (n = 3)</td>
<td>29.5</td>
<td>18.3–40.7</td>
<td>28.7</td>
</tr>
<tr>
<td>E2</td>
<td>102.7 (n = 3)</td>
<td>20.5</td>
<td>16.3–24.7</td>
<td>20.0</td>
</tr>
<tr>
<td>E3</td>
<td>162.3 (n = 3)</td>
<td>94.3</td>
<td>73.8–114.7</td>
<td>58.1</td>
</tr>
<tr>
<td>E4</td>
<td>98.3 (n = 3)</td>
<td>62.4</td>
<td>54.6–70.2</td>
<td>63.5</td>
</tr>
</tbody>
</table>

Step 2 (fat dispersal after concn)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Mean no. of oocysts in inoculum</th>
<th>Mean no. of oocysts recovered</th>
<th>95% CI</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>162.3 (n = 3)</td>
<td>94.3</td>
<td>73.8–114.7</td>
<td>58.1</td>
</tr>
<tr>
<td>C2</td>
<td>162.3 (n = 3)</td>
<td>60.3</td>
<td>28.2–92.3</td>
<td>37.1</td>
</tr>
<tr>
<td>C3a</td>
<td>104 (n = 4)</td>
<td>57.0</td>
<td>43.2–70.8</td>
<td>54.8</td>
</tr>
<tr>
<td>C4a</td>
<td>104 (n = 4)</td>
<td>53.0</td>
<td>23.7–82.3</td>
<td>51.0</td>
</tr>
<tr>
<td>C5a</td>
<td>104 (n = 4)</td>
<td>70.7</td>
<td>62.7–78.7</td>
<td>68.0</td>
</tr>
<tr>
<td>C6a</td>
<td>104 (n = 4)</td>
<td>68.7</td>
<td>56.4–80.9</td>
<td>66.1</td>
</tr>
</tbody>
</table>

*Oocysts were added to stomached eluate of fatty samples rather than directly to cured meat product.

**Use of Evans Blue in detection of Cryptosporidium oocysts by IFAT (step 4) in method development.** Samples that were particularly fatty, particularly those of PS, were difficult to analyze by IFAT due to background fluorescence from small globules, presumably of fat (Fig. 2a). Although the globules themselves were smaller than Cryptosporidium oocysts (between 2 and 3 μm in diameter), they were distracting for the microscopist, and clusters of globules, in particular, somewhat resembled oocysts. The addition of 0.1% Evans Blue to the sample at the same time as the monoclonal antibody reduced this effect, although as the antibody fluorescence was also somewhat quenched (Fig. 2b), it was only used for samples that were clearly fatty. Evans Blue is a well-known quenching agent that works by shifting the autofluorescent spectrum to longer wavelengths.

**Analysis of cured meat products exposed to contaminated water during production.** Five samples of LS (from two LS products) and six samples of PS (from two PS products) that had been exposed to the contaminated drinking water were analyzed using the method described. The mean weight and surface area of the LS samples were 37.3 g (SD, 9.8 g) and 56.2 cm$^2$ (SD, 7.7 cm$^2$), and those of the PS samples were 45.4 g (SD, 2.1 g; data from four samples missing) and 50.5 cm$^2$ (SD, 8.8 cm$^2$). The approximate total surface areas analyzed were 280 cm$^2$ (LS) and 303 cm$^2$ (PS).

Objects resembling Cryptosporidium oocysts were not detected in 10 of the samples analyzed. In one sample of PS, an object was detected that reacted with the monoclonal antibody and was of the correct size for a Cryptosporidium oocyst (ca. 5 μm in diameter). However, morphological criteria regarding sporozoite number and form for confirming the detection of a Cryptosporidium oocyst were not met, as DAPI staining and Nomarksi microscopy demonstrated that the object lacked internal contents. In addition, the "wall" structure of the object, to which the monoclonal antibody had bound, was deformed and the fluorescence was patchy. Thus, the object was recorded as a putative

Cryptosporidium oocysts from vegetables and fruit in our laboratory (data not presented here) have demonstrated that, provided the food matrix is not macerated, stomaching is an effective method to remove oocysts from food surfaces. The duration of stomaching is a balance between maximizing the removal of oocysts and minimizing other debris from the matrix also becoming suspended in the buffer. As the material was fatty, ice-cold buffers were used to minimize fat becoming dissolved in the buffer during stomaching. Of the elution methods used, E4 was considered to provide the best recovery (over 60% recovery efficiency) (Table 3). As the cut edges of the meat sample were rather hard, the stomacher bag was pierced by the sample edges on two occasions, resulting in loss of the sample. Soaking the samples for a period up to 30 min was found to soften the samples and thereby reduce the potential for loss of the sample through the bag being pierced.

**Exploration of fat dispersal following eluate concentration (step 2) in method development.** The surface layer of some parts of the samples was particularly fatty, especially for PS samples, and despite the use of ice-cold elution buffer, the fluids collected after stomaching were also fatty. As fat can coat the paramagnetic beads and limit the efficiency of IMS and may also negatively affect the IFAT detection step, efforts were made to remove the fat from the concentrated sample washings. Ethyl acetate was not found to improve recovery efficiency (Table 3), perhaps due to some entrapment of the oocysts in the fat. However, DOC treatment was not found to have an adverse effect (Table 3), and as most fatty samples were visually cleaner after DOC treatment, this step was included for PS samples and/or for samples that seemed particularly fatty after stomaching and concentration by centrifugation. It should be noted that the results from trials C1 and C2 can be compared with each other but not with the results from trials C3 to C6 (although these can be compared with each other), as in the latter trials, the oocysts were added to the eluate following stomaching of negative, unexposed fatty samples rather than to the samples themselves.
ocyst with the additional note that, if it was an oocyst, then it had been sufficiently deformed and inactivated and that this was unlikely to have been due to the analytical procedure and could be a result of the smoking or drying treatments to which the cured meats had been subjected.

Estimation of potential for contamination of cured meat products exposed to Östersund’s drinking water supply during the outbreak period. In the preparation of LS, water is used (i) for cleaning of production equipment, (ii) in the salt brine in which the meat is immersed for 10 days, and (iii) in the water used to rinse the product before smoking and drying. As all water used for cleaning is heat treated and all equipment dried before use, no risk of potential contamination with infectious oocysts was assumed from cleaning of production equipment.

The maximum number of oocysts in the water supply was assumed to be 50 oocysts per 1,000 liters (actual numbers detected were lower than this [Östersund kommune, personal communication]) distributed evenly throughout the water. As a worst-case scenario, all oocysts were assumed to be viable and infective, and the infectious dose assumed to be 1 oocyst.

For the salt brine, the data used were water usage per production batch (1,100 liters), batch size (finished product weight of 1,020 kg), and consumer portion size, estimated as 25 g, which is considered a large but not excessive portion size per meal. For the water for rinsing prior to smoking and drying, the data used were water usage per production batch (3,300 liters), the batch size (finished product weight of 1,020 kg), and the portion size, estimated again as 25 g.

Thus, for the water for the salt brine, it could be estimated that the theoretical maximum number of oocysts per kilogram of product was 0.054 oocyst and that the infection probability (that is, the probability of ingesting a single oocyst with the product) was 0.00135. For the rinsing water used prior to smoking and drying, the theoretical maximum number of oocysts per kilogram of product was estimated to be 0.162 oocyst and the infection probability (that is, the probability of ingesting an oocyst with the product) to be 0.0040. Thus, as the products were exposed to both the brine and the rinse water, the combined infection probability (ingestion of a single oocyst with the product) was estimated to be 0.00535.

DISCUSSION

Cryptosporidiosis is recognized to have the potential for foodborne transmission, and indeed, there have been various outbreaks of cryptosporidiosis associated with food (14). These have mostly been associated with raw vegetables (5, 9) or beverages made with contaminated fruit (1, 7) or it has not been possible to pinpoint the food item involved (10). Indeed, the potential for contamination of vegetables and fruit is probably higher than for meat products, due not only to agricultural practices but also to handling during the farm-to-fork chain (14), and in addition, most meat products are cooked almost immediately prior to consumption, thus inactivating any contaminating oocysts. Nevertheless, one small meatborne outbreak of cryptosporidiosis has been reported in the literature (18), in which four people were infected with Cryptosporidium parvum, probably via consumption of either “yukke” (Korean-style beef tartare) or raw liver. Thus, there has been relatively little focus on developing methods for analyzing meat products for Cryptosporidium oocysts, although one method has been published for isolating and detecting Cryptosporidium oocysts from beef carcass surfaces (8). This method, which considers fat beef tissue and lean beef tissue separately, reported recovery efficiencies of over 85% (fat tissue) and of over 128% (lean tissue). Thus, this research group also found lower recovery efficiencies from fatty tissue, although the proportion of fat in the samples is not reported.

In the studies on lean beef tissue, it is slightly worrying that the number of recovered oocysts exceeded the size of the initial inoculum, which suggests problems either in the
initial preparation of the inocula (as suggested by the authors) or in subsequent identification of the oocysts (misidentification of other objects reacting specifically or nonspecifically with the monoclonal antibody); as neither DAPI nor Nomarski or differential interference contrast microscopy was used as an identification aid in this study, the latter possibility seems relatively likely, particularly as we experienced considerable nonspecific cross-reactivity with the monoclonal antibody in our study. It should be noted that methods for analyzing fruit and vegetable produce for Cryptosporidium oocysts (food matrices that are potentially easier to work with as they do not contain fat) generally report recovery efficiencies of between 40 and 60%, although these recoveries are often lower in less experienced laboratories (2, 3, 14–16).

Other reasons for the surprisingly high recovery efficiencies previously described for meat analysis (8) could be that the initial oocyst inocula were given relatively little time to bind to the food matrix (allowed to stand for only 15 min between inoculation and recovery) and the use of a Pulsifier rather than a stomacher for removing the oocysts. However, a Pulsifier was not available for our analyses. These authors (8) also report that they found that using a swing-out rotor during centrifugation improved recovery efficiencies. We also used a centrifuge with a swing-out rotor but at a slower centrifugation speed, as we have found that a high speed compresses the pellet and makes it more difficult to resuspend. It was reported (8) that the centrifugation speed did not affect recovery efficiency (lowest speed used, 1,200 × g).

Attempting detection using molecular methods (PCR and real-time PCR) was considered in our work but not explored in the laboratory, largely due to IFAT being the “gold standard” in International Organization for Standardization and U.S. Environmental Protection Agency methods for analyzing water samples, and because published studies comparing detection of Cryptosporidium oocysts on fruits and vegetables have found that molecular methods performed more poorly (lower detection levels) than microscopy-based methods (11).

Although the recovery efficiency of the method used in our study was over 60%, indicating approximately 40% losses, we felt that it was satisfactory for analyzing the potentially contaminated products as it compares very well with efficiencies for recovery of Cryptosporidium oocysts from other matrices, such as water and fresh produce. Given the relatively low concentration of oocysts estimated per gram of product, it is unsurprising that most samples analyzed were negative. However, it should be noted that the surfaces of the samples were selected for analysis, thus increasing the probability of detection. The majority of the weight of product is protected from external contamination beneath the surface layer, and thus, in any serving of the cured meats, the vast majority of the serving will not have been exposed directly to water. Due to the anticipated low numbers of oocysts on the potentially contaminated products, somewhat larger samples were used for the actual analyses than for the seeding trials. The volumes of reagents used were still sufficient to cover the samples adequately, but if yet larger samples were to be used, we would suggest that larger volumes of buffers might also be necessary. In addition, greater quantities of fat and particles would be eluted into the buffer and might affect recovery.

The single putative oocyst detected in the analyses of the products exposed to potentially contaminated water had uncharacteristic morphology, suggesting deformation, and also had no internal features. Should the products have been contaminated, then the salting, smoking, and drying procedures are likely to have had a deleterious effect on viability and morphology, and desiccation has been previously demonstrated to inactivate oocysts (13). However, as the surfaces of the cured meat products are not entirely desiccated, inactivation could not be assumed without analysis. Additionally, it has been previously noted that reduced water activity may have a dramatic effect on oocyst infectivity, with pancake syrup (aw value, 0.85) and salt-dextrose solution (aw value, 0.95) resulting in significantly reduced sporozoite die-off (4). As the cured meat products investigated here had a final water activity of less than 0.910 aw, this would suggest reduced oocyst survival, although again, this should not be assumed without sufficient supportive analytical data.

Although the data available for estimating the likelihood of ingesting a single oocyst were not perfect (data were not easily available, analysis of the water probably was not conducted at the point of peak contamination, and also, oocysts may not be distributed evenly in the water but may aggregate in clumps), the low likelihood estimated using a worst-case scenario, together with the results of the laboratory analyses and our knowledge of factors affecting oocyst survival, were used to justify releasing the products to the market, with no apparent ill effect to consumers.

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REFERENCES


