Sampling Methods and Frozen Storage of Samples for Detection of Campylobacter jejuni on Freshly Processed Broiler Carcasses

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ABSTRACT

Swab, rinse and excision sampling methods are commonly used for detection of microorganisms on poultry carcasses. Swabbing has been the most frequently reported sampling method for Campylobacter jejuni on poultry. We evaluated the three methods for C. jejuni detection on freshly processed poultry in the following ways: (a) the interior and exterior surfaces of half of a carcass were each thoroughly rubbed with separate swabs which were combined in a test tube containing 2 ml of appropriate medium; (b) 25 g of skin and tissue samples from neck and abdominal opening cut areas were deposited in a stomacher bag with 5 ml of brucella broth (BB) and stomached for 2 min; and (c) half carcasses were shaken for 1 min with 100 ml BB in plastic bags. One drop of each sample was streaked for isolation on brucella agar containing 10% defibrinated sheep blood and Skirrow antibiotics. Isolates were identified by microscopy and appropriate cultural tests. All three sampling techniques were essentially equivalent for detection of C. jejuni. Inclusion of protective agents was an effective method to preserve swab samples during frozen storage.

Since the recognition of Campylobacter jejuni as an important human pathogen and its association with food animals, numerous reports cited in a recent article by Blaser (1) have shown that commercially processed poultry is frequently contaminated by this organism. Investigators have employed conventional sampling methods in these studies, including swabbing carcass surfaces (10), cloacal areas and fecal material (4,7), and rinsing (9,12). Methods for transporting samples intended for C. jejuni detection have mainly been concerned with fecal material (7,8).

If an in-plant survey was done to determine the incidence of C. jejuni contamination of freshly processed poultry within a large geographical area, adequate sampling and transport methods would be needed in the event samples were to be analyzed at a distant laboratory. We report a comparison of sampling methods for freshly processed poultry and methods for frozen transport.

MATERIALS AND METHODS

Sampling methods

Freshly processed broiler carcasses were obtained (immediately after chilling) from a commercial slaughter plant, placed in individual plastic bags and transported in ice to the laboratory within 1 h. Each carcass was split and half was used for fresh sampling. The remaining half was placed in a plastic bag and stored frozen in a -30°C room for 10 d before sampling. Sampling methods were selected that we felt could be conveniently used for an in-plant survey by inspection personnel. Groups of 10 half carcasses each were sampled in one of three ways as follows: (a) 25 g of skin plus tissue were excised, placed in a plastic bag, mixed with 5 ml of brucella broth (Difco) and macerated 2 min in a Colworth Stomacher (model 80); (b) the exterior surface of each half was swabbed with one nylon swab and the interior surface with another; both swabs were deposited in a test tube with 2 ml of brucella broth and vortex mixed for 1 min; and (c) half carcasses were shaken with 100 ml of brucella broth for 2 min. Two groups of ten half carcasses each were sampled by each method. One drop of broth from each sample was used to streak isolation plates. In addition, after a drop of rinse sample (method c) was taken for streaking, the remaining broth from each sample was centrifuged for 10 min at 13,200 × g, the supernatant fluid removed, the pellet resuspended in 2 ml of brucella broth, and a drop was used to streak for isolation. Frozen halves were thawed overnight at 2 to 4°C and sampled in the same manner as described above. Also, after sampling of thawed carcasses and streaking for isolation, the same broth samples were incubated 4 h at 37°C to allow time for recovery of cells from possible freeze-injury. Samples were then streaked again for isolation.

Isolation procedures

Brucella agar (Difco) containing Skirrow antibiotics (Oxoid, No. SR-69) and 10% sheep blood was used for all isolations. Plates were incubated at 43°C in plastic desiccators which were evacuated to 0.25 atmosphere followed by replacement with a gas mixture containing 5% O2, 10% CO2, 85% N2. After 48 h of incubation, typical colonies were picked and streaked for purification on brucella agar supplemented with ferrous sulfate, sodium pyruvate and sodium bisulfite according to George et al. (5). Isolates were confirmed to be C. jejuni according to previously described characteristics (11).

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Cryoprotective agents for frozen sample storage

Survival (after frozen storage) of a laboratory strain of C. jejuni was tested by inoculating tubes of brucella broth supplemented with various cryoprotective agents. Media tested included brucella broth, double strength brucella broth and brucella broth supplemented (10\% vol/vol or vol/vol) with one of the following: polyvinyl pyrrolidine (PVP; Eastman), sucrose (Difco), dimethylsulfoxide (DMSO; Mallinckrodt), casamino acids (Difco), bile salts (Difco) and glycerol (Mallinckrodt). All media were adjusted to pH 7.2, filter sterilized and aseptically dispensed in 2-ml volumes into sterile screw-cap test tubes. Sets of 10 tubes of each medium were inoculated with cells from a 24-h culture of C. jejuni to achieve one of four approximate final cell concentrations: 60, 300, 800 and 4600 cells/ml. The cell concentrations were achieved by diluting the 24-h culture to a predetermined absorbance (A600nm) of known cell density and then making further dilutions so that the desired inoculum was delivered in 0.1 ml to each tube. Actual counts were confirmed by plating samples of appropriate dilutions used to inoculate tubes. Immediately after inoculation and mixing, one drop from each tube was used to streak isolation plates. Tubes were then frozen at -30°C for 7 d, thawed and again sampled. In addition, three groups of 30 freshly processed broiler carcasses were sampled by swabbing one-half of both the outside and inside surfaces each with one swab. The two swabs were then deposited in a test tube containing 2 ml of either brucella broth, brucella broth + 10\% PVP or brucella broth + 10\% sucrose. Tubes were mixed for 1 min on a Vortex mixer, a drop taken for plate inoculation, and the tubes were stored frozen for 7 d at -30°C. Frozen tubes were subsequently thawed, mixed and again a drop from each was used to inoculate an isolation plate.

**TABLE 1. Comparison of sampling methods for detection of C. jejuni on fresh and frozen broiler carcasses.**

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>No. positive&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Frozen</th>
<th>Frozen + 4 h recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excised skin</td>
<td>19</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Rinse</td>
<td>19</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Conc. rinse</td>
<td>18</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Swab</td>
<td>20</td>
<td>7</td>
<td>NT&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>See text for details of procedures.

<sup>b</sup>Number positive among 20 samples.

<sup>c</sup>NT, not tested.

**TABLE 2. Effect of cryoprotective agents on survival of C. jejuni during frozen storage.**

<table>
<thead>
<tr>
<th>Approximate No. cells/ml</th>
<th>BB</th>
<th>2 × BB</th>
<th>BB × 10% Glycerol</th>
<th>BB × 10% Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 d</td>
<td>7 d</td>
<td>0 d</td>
<td>7 d</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>300</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>800</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>4600</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>BB + 10% CA</td>
<td>0 d</td>
<td>7 d</td>
<td>0 d</td>
<td>7 d</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
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<td></td>
<td>6</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>See text for details of procedure.

<sup>b</sup>BB, brucella broth; Glycerol, glycerol; Suc, sucrose; CA, casamino acids; BS, bile salts; DMSO, dimethyl sulfoxide; PVP, polyvinyl pyrrolidone.

<sup>c</sup>0-d samples were taken before freezing.

**RESULTS AND DISCUSSION**

The level of C. jejuni contamination on any carcass would be expected to influence the success of a given sampling method to qualitatively detect the organism. If a low number of C. jejuni occurred on most carcasses, differences in detection rates among sampling methods would likely be observed. However, if higher numbers occurred, it might be expected that similar detection rates would be seen among methods. The results given in Table 1 suggest that the contamination level on carcasses sampled shortly after processing was of such magnitude that all methods tested appeared to be about equally effective for qualitative detection of C. jejuni.

The incidence of detection on carcasses after frozen storage declined substantially suggesting that the numbers of C. jejuni surviving were below levels detectable by the sampling methods employed. Similar results were observed by Simmons and Gibbs (10) with frozen broilers, and Luechtfeld et al. (8) reported that frozen storage of turkey cecal samples greatly reduced detection of C. jejuni. The beneficial concentrating effect of centrifuging rinse samples was shown by the greater detection of C. jejuni after frozen storage of carcasses compared to the other sampling methods. Park et al. (9) concentrated by centrifuging rinse samples of carcasses from retail stores but did not evaluate its effectiveness. No detection advantage was observed as a result of incubating samples 4 h at 37°C. This procedure seemed to increase the proportion of non-Campylobacter colonies appearing on isolation plates. Since no selective antibiotics were included in broth used for sampling, it might be expected that there would be more faster growing non-Campylobacter bacteria than C. jejuni.

Transporting microbiological field samples to central laboratories in the frozen state has the advantages of arrest all microbial growth and also preventing sample deterioration. However, freezing was shown to be clearly detrimental to survival of C. jejuni (Table 1). Stern and
Kotula (13) recently reported substantial declines in viability during frozen storage of C. jejuni inoculated into ground beef mixed with Cary-Blair diluent or 0.1% peptone. Consequently, we next tested a series of cryoprotective agents added to brucella broth for their protective effect during frozen storage of C. jejuni. The results of these experiments are shown in Table 2. Detection of C. jejuni in tubes containing the lowest number of cells/ml was quite variable before freezing. Brucella broth (BB) alone yielded the highest incidence (10 of 10 positive samples), whereas all other media showed reduced incidence ranging from 1 of 10 positive for BB + 10% casamino acids to 8 of 10 positive for BB + 10% sucrose. These results suggest that various supplements added to brucella broth had an inhibitory effect at the lowest cell level tested. However, at higher cell concentrations, C. jejuni was detected in nearly all tubes except in those to which glycerol or casamino acids was added. These supplements resulted in lower initial recovery. A protective effect from freeze-damage was observed at the lowest level of cells only in the cases of DMSO-, PVP- and sucrose-supplemented broth. At higher cell concentrations (300/ml and above), little or no reduction in detection incidence was observed between non-frozen and frozen samples when DMSO-, PVP-, or sucrose-supplemented broth and double strength brucella broth were employed. Single strength brucella broth and broth supplemented with glycerol, casamino acids or bile salts showed little or no protective effect. Hence, some cryoprotective agents were useful in protecting C. jejuni from freeze-damage, but at cell numbers below about 300 cells/ml there was a reduced probability of detecting all positive samples when a drop of sample was used to streak isolation plates.

A simulated field trial was done to evaluate the effectiveness of cryoprotective agents for natural contaminants. Thirty freshly processed broiler carcasses were sampled on each of 3 d by the swab method. The 30 carcasses were divided into groups of 10 each and the swabs from individual carcasses of each group were placed in either brucella broth alone or supplemented with PVP or sucrose. Detection was performed by direct plating before and after 7 d frozen storage at -30°C. The swab method was chosen because of general familiarity with the technique, the practicality of transporting frozen samples in test tubes, and the finding that swab sampling was equivalent to other methods for detection of C. jejuni on freshly processed broiler carcasses. Results are shown in Table 3. The incidence of initial C. jejuni detection among the three groups of carcasses indicated a high degree of contamination. Other investigators have obtained similar results with poultry products (7,8,12). Results of laboratory experiments, wherein samples frozen in brucella broth alone showed a reduced incidence, were confirmed in the simulated trial (unfrozen 100% vs. frozen 43%) with freshly processed carcasses. Incidence of detection among samples frozen in broth supplemented with PVP declined only 10%, whereas samples frozen in sucrose supplemented broth declined 23%. Although not tested, similar results might be expected with the use of DMSO-supplemented broth. Because only naturally occurring C. jejuni contaminants were detected in the field trial, it is difficult to compare the incidence among the three groups of carcasses. Also, no attempt was made to determine the number of C. jejuni per carcass. However, considering the results presented in Table 2, the lower incidence observed among the two groups of carcasses in which PVP- and sucrose-supplemented broths were used could be attributed to (a) very low levels or lack of contaminants on some carcasses or (b) a possible inhibitory effect of the cryoprotective agents in cases where carcasses were contaminated with low levels of C. jejuni. The use of various cryoprotective chemicals has been used successfully to protect bacteria from injury or destruction during frozen storage in other media and products (5,6). Stern and Kotula (13) recently reported improved recovery of C. jejuni from ground beef stored frozen in 10% DMSO or glycerol. Although selective enrichment procedures were not tested in this study, it would be expected that the use of these procedures, such as reported by other researchers (2,9,12), would improve detection of C. jejuni in frozen samples.

The results of this study suggest that for purposes of a broad geographical survey for C. jejuni incidence among freshly processed poultry carcasses samples in-plant, the swab sampling method would be useful. Further, the use of brucella broth supplemented with 10% PVP would be a suitable medium for transport of swab samples in the frozen state to a central laboratory for analyses.

REFERENCES


TABLE 3. Effect of cryoprotective agents on detection of C. jejuni during frozen storage of swab samples of fresh poultry.a

<table>
<thead>
<tr>
<th>Results</th>
<th>BBb</th>
<th>BB + 10% PVP</th>
<th>BB + 10% Suc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positived</td>
<td>0 d</td>
<td>7 d</td>
<td>0 d</td>
</tr>
<tr>
<td>Incidence (%)</td>
<td>100</td>
<td>43</td>
<td>87</td>
</tr>
</tbody>
</table>

aSee text for details of procedures.
bBB, brucella broth; PVP, polyvinyl pyrrolidone; Suc, sucrose.
c0-d samples were taken before freezing.
dNumber positive among 30 samples.

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Speroni et al., con't. from p. 509


