A Research Note

Evaluation of a Centrifugation Method for the Detection of Salmonella enteritidis in Experimentally Contaminated Chicken Eggs

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ABSTRACT

Current recommendations for identification of Salmonella enteritidis (SE) contaminated eggs, as outlined by the USDA SE Task Force, require the combination of yolk and albumen from several eggs for room-temperature enrichment for 3 days prior to culture on solid medium. We describe the use of enzymatic digestion and chemical reduction to reduce viscosity and allow for concentration of Salmonella cells in egg albumen by centrifugation prior to enrichment and plating. In these studies, sanitized, intact, fresh shell eggs were inoculated with low numbers of viable SE. Albumen and yolk from six eggs in each group were separated; the albumen was pooled, treated with the proteolytic enzyme papain and the reducing agent N-acetyl-l-cysteine, and centrifuged, and the resulting pellet was incubated in tetrathionate broth (5 ml) at 37°C for 24 h prior to plating on brilliant green agar (BGA). For comparison, yolk and albumen from six eggs in each group were pooled, thoroughly mixed, and incubated at room temperature for 3 days prior to direct plating on BGA. Results from three trials indicated that the two culture techniques were equally sensitive for detection of Salmonella (range: 72 to 0.6 colony-forming units (CFU) of SE per pool). While as sensitive as the technique recommended by the SE Task Force, the centrifugation technique allowed more rapid (48 h) presumptive identification of Salmonella.

Key words: Salmonella, eggs, centrifugation

The frequency of Salmonella enteritidis (SE) isolations from fresh shell eggs has increased in recent years, along with an increasing frequency of human SE-related illness (2, 10, 11, 13). Data from the SE Traceback program indicates that in the 1990s, 32% of all food-borne illness caused by SE were traced back to eggs (1). It has been demonstrated in numerous studies with both naturally infected and experimentally infected hens that intact shell eggs may be contaminated through vertical transmission from the hen (3, 4, 6–8). SE-contaminated eggs produced by naturally and experimentally infected flocks are produced intermittently at low frequencies and generally contain small numbers of SE (6, 8–10). Also, the albumen, as opposed to the yolk, has been demonstrated to be the primary site of SE contamination within the egg (5, 9, 12).

Mandatory testing and required consignment of eggs to breaker plants have cost producers billions of dollars annually (10). Additionally, SE phage type 4, a more virulent strain of SE, has caused extensive flock losses and human illness in Great Britain and has recently been reported in the United States in a commercial flock (15). Considering the increased number of SE isolates from eggs, the human illness concerns, and the recent emergence of SE phage type 4 in the United States, the need for a rapid and sensitive detection method for SE in eggs is further increased. It is clear that mandatory screening of eggs from table-egg breeder flocks, and perhaps table-egg production flocks, will continue and possibly expand (9, 10).

Currently, the SE Task Force recommends screening eggs for SE, utilizing pools of whole egg (yolk and albumen) contents with a minimum of 3 days of enrichment prior to plating (14, 16). The objective of the present study was to develop and preliminarily evaluate a method for the concentration of low numbers of SE cells in pools of experimentally contaminated egg albumen, which would allow sensitive and rapid culture detection of SE-contaminated eggs.

MATERIALS AND METHODS

Preliminary studies involving partial digestion of albumen and concentration of SE cells

Prior to these studies, several methods of reducing the viscosity of egg albumen with NaOH, mechanical blending, proteolytic digestion with papain (ICN Biomedicals, Aurora, OH) and chemical reduction with N-acetyl-l-cysteine (NALC: Sigma Chemical Co., St. Louis, MO), and combined treatment with papain and NALC were evaluated in our laboratory (data not shown). In these preliminary studies, a combination of
papain digestion and NALC reduction was determined to provide sufficient liquification of the albumen for concentration of SE by centrifugation as described below.

Salmonella source

A primary poultry isolate of SE (phage type 13A) was obtained from the National Veterinary Services Laboratory (Ames, IA) and was selected for resistance to novobiocin (NO) and nalidixic acid (NA). Media used to culture the resistant isolate contained 25 μg NO per ml and 20 μg of NA per ml to inhibit the growth of other bacteria. Inocula of SE cells for injection into the eggs were prepared in double-distilled H₂O (ddH₂O). The approximate viable cell concentration of the inoculum was determined spectrophotometrically, serially diluted, and confirmed by colony counts of four replicate samples (100 μl) of the spectrophotometrically determined dilutions spread plated on brilliant green agar (BGA) plates (Difco Laboratories, Detroit, MI) containing NO and NA. In each trial, the colony-forming units (CFU) determined by spread plating were reported as the actual number of SE cells contained in each pool (CFU inoculated per egg × number of eggs per pool).

Inoculation of eggs

Intact whole eggs were obtained from the Texas A&M Poultry Science Research Farm, dipped in an aqueous solution containing 76% ethanol (vol/vol) and 2.5 wt% iodine, and allowed to air dry under a laminar-flow hood. The small end of each sanitized egg was gently perforated using an 18-gauge needle. The appropriate CFU of SE (100 μl) was then injected 0.5 cm into the albumen through the perforation using a 25-gauge needle. Following SE injection, the holes were sealed with paraffin, and the eggs were incubated at room temperature (22°C) in a laminar flow hood for approximately 24 h prior to pooling.

SE inoculation levels

In trial 1, the CFU of SE injected averaged 1.5, 3, 6, and 12 CFU per egg, based on spread plating of higher dilutions, resulting in 9, 18, 36, and 72 CFU of SE per pool; in trial 2, the CFU of SE injected averaged .0008, .004, .02, .10, and .50 CFU per egg, resulting in .0048, .024, .12, .6, and 3 CFU of SE per pool; the CFU of SE injected for trial 3 averaged .002, .01, .07, .34, and 1.7 CFU per egg, resulting in .012, .06, .42, 2.04, and 10.2 CFU of SE per pool.

Comparison of culture methods

For each level of inoculation in each trial, six eggs were randomly selected for partial digestion of albumen and centrifugation, and six eggs were randomly selected for the room-temperature whole-egg culture as recommended by the USDA SE Task Force (16). Briefly, for the currently recommended room-temperature culture method (14, 16), the contents (albumen and yolk) of six eggs per inoculation level were aseptically combined into individual Ziploc® quart-size freezer bags (DowBrands, Indianapolis, IN), thoroughly hand mixed to ensure complete blending of the yolk and albumen, and allowed to incubate (enrichment) for 3 days at room temperature. Following enrichment, 4 replicate 100-μl samples from each group were spread plated onto BGA plates. The plates were incubated at 37°C for 24 h and examined for the presence of lactose-negative, NO- and NA-resistant colonies of SE.

Six eggs from each inoculation level also were selected randomly and aseptically cracked under a laminar flow hood, and the albumen was separated from the yolk using sterile plastic egg separators (EKCO Housewares Inc., Franklin Park, IL). The albumen from each group of six eggs was placed in sterile, polypropylene 250-ml centrifugation bottles and 6 ml (100 mg/ml) of papain was added to each bottle to achieve a final concentration of 2.4 mg/ml. The bottles were placed in a shaking water bath (Lab-Line Instruments, Inc., Melrose Park, IL) at 37°C for 60 min followed by the addition of 6 ml (200 mg/ml) of NALC to each bottle to achieve a final concentration of 4.8 mg/ml. Bottles were returned to the shaking water bath for 30 min and then centrifuged for 40 min at 3,650 × g. Following centrifugation the supernatant was decanted and the pellet was resuspended in 5 ml of tetrationionate broth (Difco Laboratories, Detroit, MI) and incubated (enriched) at 37°C for 18 h. Following enrichment, 4 replicate 100-μl samples of each suspension were spread plated onto BGA plates, incubated at 37°C for 24 h, and examined for the presence of lactose-negative, NO- and NA-resistant colonies of SE.

RESULTS AND DISCUSSION

In trial 1 (Table 1), concentration of the SE cells by liquefaction of the albumen and centrifugation and the whole-egg room-temperature culture technique were equally sensitive for detection of SE, ranging in concentration from 72 to 9 CFU per pool, from pools of albumen and whole-egg contents. Similarly, in trial 2 (Table 1), both culture techniques allowed detection of SE in pools containing averages of 3 and 0.6 CFU per pool, while the pools containing fewer CFU of SE were culture-negative. In trial 3, pools containing an average of 10.2 CFU were SE culture-positive by both culture techniques. However, the albumen pool containing an average of 2 CFU was SE culture-positive by the centrifuge technique, and the whole-egg pool at this level of inoculation was culture-negative by the whole-egg culture technique. In contrast, the whole-egg pool containing an average of 0.41 CFU was determined to be SE culture-positive by the whole-egg culture technique.

<table>
<thead>
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<th>Trial 1</th>
<th>Avg. # SE/pool</th>
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<tr>
<td>72</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>36</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td>18</td>
<td>(+)</td>
<td>(+)</td>
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</tr>
<tr>
<td>9</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td>0</td>
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<td>3</td>
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<td>(+)</td>
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</tr>
<tr>
<td>0.6</td>
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<td>(+)</td>
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</tr>
<tr>
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<tr>
<td>0.02</td>
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</tr>
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<td>10.2</td>
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<td>2</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<td>(+)</td>
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<tr>
<td>0.02</td>
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* Protocol of the SE Task Force: room temperature incubation; culture of pools of homogenized yolk and albumen.

b Concentration technique: enzymatic digestion and chemical reduction of pools of albumen, concentration of SE by centrifugation, incubation at 37°C, and culture.

c Detection of Salmonella enteritidis on one or more plates, +; absence of Salmonella enteritidis on all plates, -.
nique while the matched albumen pool cultured follow-
ing liquification and centrifugation was SE culture-
negative. The noninoculated control eggs were deter-
mined SE culture-negative in each of the three trials.

Mandatory testing of flocks implicated as potential
carriers of SE substantiates the need for sensitive,
expedient culture procedures enabling large volumes
of eggs to be evaluated. Previous studies have indi-
cated that the albumen is the primary site of internal con-
tamination with SE (5, 8, 12). The three trials in this
experiment indicated that partial digestion and subse-
quent centrifugation of pools of egg albumen may
provide concentration of low numbers of SE cells,
allowing detection by culturing. Furthermore, while
utilization of this digestion and centrifugation tech-
nique was equal in sensitivity to the currently recom-
manded whole-egg culture technique, these studies sug-
gest that preliminary results can be obtained at least 48
h sooner using the concentration and centrifugation
technique than the procedure that is currently followed
(14, 16). Whether this technique is applicable to and
cost-effective for testing eggs from commercial flocks
remains to be determined.

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