Prevalence and serovars of *Salmonella enterica* isolated from ileocolic lymph nodes of market pigs reared in selected Midwest US swine herds

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**Summary**

Objectives: To describe the prevalence and serovars of *Salmonella enterica* in ileocolic lymph nodes of slaughtered swine in a sample of Midwest US herds and to assess as methods of study pooling and freezing of lymph-node samples prior to bacterial culture.

Materials and methods: Ileocolic lymph nodes from 30 pigs from each of 146 herds were sampled at slaughter. Tissue from five pigs was pooled for one bacterial culture. Retained frozen tissues from the same pigs were cultured individually (n = 82 herds) from a subset of those with *Salmonella*-positive pools (n = 100 herds). A mathematical relationship was described to predict approximate individual prevalence based on number of positive pools. Isolates were serotyped. To test for effects of freezing on test sensitivity, lymph nodes from 100 pigs were cultured both fresh and after freezing.

Results: *Salmonellae* were detected in 100 of 146 herds (68.5%). The mean number of positive pools per herd was 1.75, and the mean within-herd, individual-pig prevalence was 6.98% (95% CI, 4.88% - 9.07%). Freezing of samples did not result in decreased detected prevalence. Individual prevalence could be approximately predicted by pool results, although with low precision.

Implications: *Salmonellae* were found in two-thirds of the herds studied. Culture of pooled samples with subsequent culture of retained frozen tissues from positive pools may be an effective way to test a larger number of herds on a given budget through laboratory-cost savings. However, pooling without culture of individuals from positive pools results in imprecise prevalence estimation.

**Keywords:** swine, *Salmonella* prevalence, microbiologic methods, epidemiology

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**Resumen – Prevalencia y serovariedades de *Salmonella enterica* aislada de los nódulos linfáticos ileocólicos de cerdos de mercado criados en piaras seleccionadas del medio oeste de EU**

**Objetivos:** Describir la prevalencia y serovariedades de *Salmonella enterica* en nódulos linfáticos ileocólicos de cerdos de mercado en una muestra de piaras del medio oeste de EU y valorar cómo métodos de estudio el agrupamiento (pooling) y la congelación de muestras de nódulos linfáticos antes del cultivo bacteriano.

**Materiales y métodos:** Al sacrificio se tomaron muestras de los nódulos linfáticos ileocólicos de 30 cerdos de cada una de las 146 piaras. Se agrupó el tejido de cinco cerdos para un cultivo bacteriano. Los tejidos congelados de los mismos cerdos se cultivaron individualmente (n = 82 piaras) para un subgrupo de muestras agrupadas que resultaron positivas a *Salmonella* (n = 100 piaras). Se describió una relación matemática para predecir la prevalencia individual aproximada basada en el número de agrupamientos positivos. Los aislamientos fueron serotipificados. Para probar el efecto de la congelación en la sensibilidad de la prueba, se cultivaron los nódulos linfáticos de 100 cerdos, tanto frescos como después del congelamiento.

**Resultados:** Se detectó la salmonellae en 100 de las 146 piaras (68.5%). El número promedio de agrupamientos positivos por piara fue de 1.75, y el promedio de la prevalencia individual por cerdo dentro de la piara fue de 6.98% (95% CI, 4.88% - 9.07%). El congelamiento de las muestras no disminuyó la prevalencia detectada. La prevalencia individual podría predecirse aproximadamente con los resultados del agrupamiento, aunque con poca precisión.

**Implicaciones:** Se encontró salmonellae en dos tercios de las piaras estudiadas. El cultivo de las muestras agrupadas con el cultivo subsecuente de los tejidos congelados de los agrupamientos positivos puede ser una manera efectiva de probar un número mayor de piaras con un presupuesto pre-establecido a través de un ahorro en los costos de laboratorio. Sin embargo, el agrupamiento sin el cultivo de muestras individuales de los agrupamientos positivos resulta en una estimación imprecisa de la prevalencia.
Salmonella enterica is an important foodborne pathogen of pork. The US Centers for Disease Control and Prevention (CDC) estimated that nontyphi Salmonella was associated with 16.1 cases per 100,000 population in 2002. The CDC also has estimated that there were more than 1.4 million human Salmonella cases per year in the United States, resulting in 15,000 hospitalizations and 400 deaths annually during 1996-1999. A survey of retail US pork products, both whole muscle and ground product, indicated that 9.6% of 384 tested products were positive for Salmonella serovars, while 1.6% of 613 pork chops collected at retail US markets in 2002 were Salmonella-positive.

While the proportion of human cases directly or indirectly attributable to pork has been incompletely quantified in the United States, one report estimates that, on the basis of an assessment of outbreak data, 6% to 9% of foodborne Salmonella infections in the United States may be associated with pork and pork products. Although data is not directly transferable to the United States situation because of differences in consumption patterns and other factors among countries, reports from other countries also suggest a role for pork in human salmonellosis. A study of Danish surveillance data estimated that 9% of human salmonellosis was attributable to pork, with 75% of all cases attributed to a source. Approximately 20% of human salmonellosis in Germany may be attributable to pork sources. Salmonella causes more severe illness than other common foodborne pathogens in pork, with relatively high hospitalization and death rankings for nontyphi Salmonella. Human infections by nontyphi Salmonella has a substantial economic impact in the United States, estimated at $US 3B.

Foodborne outbreaks of all types attributable to pork have declined in the United States during the period 1973 to 1992. Since 1992, US slaughter plants have instituted process control systems based on Hazard Analysis Critical Control Point (HACCP) principles to improve pork food safety. Salmonella serovars are being monitored by the United States Department of Agriculture (USDA) as an indicator of the success of these plans, and a performance standard specifying a maximum of 8.7% Salmonella-positive carcasses has been enforced since 1998. While the USDA has documented progress in Salmonella control, 2.5% of carcasses tested Salmonella-positive in 2003. Consequently, Salmonella in pork continues to represent a threat to food safety.

We designed this study to quantify the prevalence and serovars of Salmonella among slaughtered pigs from US Midwest commercial swine herds. To better understand and interpret our results, we also assessed the effect of freezing ileocolic lymph-node samples prior to culture for Salmonella isolation, and described the relationship between pooled and individual-sample culture results.

Materials and methods

Study herds
Two major US Midwest slaughter plants agreed to participate in the study on condition of anonymity. The client lists of these plants were reviewed to identify herds routinely capable of delivering at least 30 pigs per lot marketed on a single day. In addition, a state pork-producer association, coordinated marketing groups, and swine-dedicated veterinary practices provided names of herds likely to market to these slaughter plants. Participation request forms were distributed to 333 herds, and 225 agreed to participate (67.6%). The selection criteria for participation in the study were that the herds sold market weight pigs to abattoirs participating in the study, were located in the same state as the slaughter plants, were able to market at least 30 animals as a single group, and agreed to complete a survey. Herds delivered pigs to the slaughter plant on the schedule of their choice. Pigs were sampled on the basis of availability of technical personnel at the time of delivery. Samples were collected between July 17, 1997 and October 9, 1998, with a target to sample up to 150 herds. Herds were selected without prior knowledge of Salmonella occurrence.

Collection of ileocolic lymph nodes
Pigs were transported to slaughter using the herd’s normal delivery methods, and were placed in lairage for variable lengths of time, as was the practice of the slaughter plants. Pigs were marked with a tattoo, segregated into a holding pen, and moved through the slaughter line as a group. For
 lots with more than 30 animals, only the first 30 animals on the slaughter line were collected. After evisceration, the intestinal tract was removed from the slaughter line, and the tracts were placed in collection bins. Care was taken not to place tracts from more than one herd in any bin. These were moved to a separate area for removal of the ileocolic lymph nodes (lymph nodes draining the cecum, ileum, and colon, also commonly referred to as ileocecal lymph nodes). The overlying mesentery was wiped dry with sterile gauze, then carefully reflected using sanitized instruments to prevent contamination of lymph nodes from the surface of the mesentery. The nodes were grasped using sterile gauze held by a clean gloved hand, collecting a sample expected to weigh >4 g per individual and placing each in a separate sterile plastic sample bag. Samples were transported to the laboratory on ice.

Identification of Salmonella serovars in fresh samples
Salmonellae were identified using a modification of a published method. All inoculated media were incubated at 37°C. Within 24 hours of collection, lymph-node tissues from individual were split approximately in half using a sterile scalpel or scissors. One half was placed in a sterile plastic bag and frozen at -70°C for 2 years. The remaining tissue was weighed, and each node was cut in half. Each half of each node was immediately processed. The remainder was placed in a sterile plastic bag, frozen at -70°C for 2 weeks, and then thawed overnight at 2°C. Culture methods for both fresh and frozen tissue were as described for individual samples, except that isolates were not serotyped. Detection concordance was compared by the kappa statistic using exact methods (StatXact).

Results
Of the 225 herds that agreed to participate, 146 herds were sampled and surveys were sent to them. Valid surveys were returned by 113 herds. Of these, 20 (17.7%) obtained some or all growing pigs from at least one outside herd, with the remaining herds rearing all pigs from birth to slaughter. The breeding-female inventory, number of pigs marketed during the previous 12 months, growing-pig inventory in the barn at the time of shipment, and proportion of piglets born in an outside herd were described (Table 1). Batch or all in-all out pig flow was practiced in the finisher barn by 39.4% of herds. Pigs were shipped directly from the farm to the slaughter plant by 104 herds (92.0%); four (3.5%) delivered to a facility where pigs from multiple herds were grouped for shipment to slaughter; and five (4.4%) reported other delivery methods.

Salmonellae were detected in lymph nodes from 100 of 146 herds (68.5%). The distribution of pooled-sample results did not differ between slaughter plants (P = .71). The number of pooled positive samples varied from zero to six of six pools tested per herd, with a mean of 1.75 positive (Figure 1). Thirty-three serovars were detected. Ten serovars were represented by more than ten isolates (Table 2): these serovars accounted for 76.5% of all isolates. The
other serovars detected were 4, 5, 12:i-mono-phasic, Bareilly, Bovis-morbificans, Bredeney, Chailey, Cholerasuis (Kunz), Cubana, Give, Hartford, Heidelberg, Infantis, Johannesburg, Litchfield, Livingstone, Montevideo, Muenchen, Newport, Ohio, Pakistan, Saint-Paul, Schwarzengrund, Tennessee, and Thompson.

Of the 100 samples cultured both as fresh and frozen tissues, a total of 24 were culture-positive by at least one method, and 10 were positive by both methods (Table 3). Prevalence estimated by both methods was the same, although seven individuals that were positive by each method were negative by the other. The kappa statistic was 0.50 (95% CI, 0.28 - 0.73).

A total of 1005 frozen individual lymph-node samples were cultured from 201 positive pools distributed among 82 herds. These samples were frozen for 2 to 14 months, depending on availability of lab resources. Among these herds, the median within-herd prevalence of positive individuals was 6.67% (95% CI, 6.73% - 13.20%) and the mean was 9.96% (95% CI, 0% - 80.0%). No salmonellae were detected among individual cultures from 22 of these herds, although salmonellae were isolated from pooled samples. At the herd level, the number of positive pools was positively correlated with individual prevalence (r = 0.65; P < .01). Transformed individual prevalence was described by a quadratic equation (Table 4) derived by regression analysis. The fitted within-herd prevalence varied from 2.1% (95% CI, 0.7% - 4.0%) for herds with one of six positive pools to 36.8% (95% CI, 24.7% - 51.1%) for herds with six of six pools positive (Figure 2). The distribution of number of Salmonella-positive pools among pool-positive herds was not significantly different in herds with or without individual culture results (P = .78). The mean prevalence for all herds was estimated using frozen tissue for 82 herds and the predictive equation for the 22 culture-positive herds without individual results, and was set at zero for herds with no positive pools. The estimated mean prevalence was 6.98% (95% CI, 4.88% - 9.07%) and the 10th, 25th, 50th, 75th, and 90th percentiles were 0%, 0%, 2.5%, 7.0%, and 20%, respectively, with a range of 0% to 80%.

**Discussion**

Approximately two-thirds of the herds investigated in this study had at least one Salmonella culture-positive result, suggesting the importance of procedures to minimize pork contamination throughout the pork chain. These findings are similar to results of a study of Minnesota herds, where Salmonella were detected in 16 of 25 farms and in 3.6% of 3442 pigs. The Minnesota study differed from this study in culture and collection methods, and in addition, a variable number of pigs per herd were sampled in the Minnesota study (n = 14 to 1172). A survey of 317 pigs in Canadian slaughter facilities during 1985-1986 reported 14.2% positive (mesenteric lymph-node samples). Studies of five North Carolina farms and six Iowa farms reported 21% and 9.15% culture-positive pigs, respectively (ileocolic lymph-node samples). In Europe, findings of large-scale, population-based slaughter studies include 3.3% positive (n = 11,942) in Germany and 15.2% positive (n = 7756) in the Netherlands (ileocolic lymph node samples). In Denmark, 6.2% of cecal content samples were culture-positive (n = 13,468). The herds in this study were not selected in a manner that would ensure that they are representative of midwestern US herds. However, participants were chosen without knowledge of prior or current Salmonella status, farm management, or other herd characteristics other than size. Neither of the participating slaughter processors had

![Image](https://example.com/image.png)

**Table 1:** Summary of responses to herd survey questions among 113 Midwest swine herds

<table>
<thead>
<tr>
<th>Survey question</th>
<th>Mean</th>
<th>Minimum</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding-female inventory*</td>
<td>519.3</td>
<td>10</td>
<td>175</td>
<td>280</td>
<td>600</td>
<td>5000</td>
</tr>
<tr>
<td>No. of pigs marketed previous 12 months</td>
<td>9033.5</td>
<td>100</td>
<td>1950</td>
<td>3500</td>
<td>6880</td>
<td>28,000</td>
</tr>
<tr>
<td>No. of pigs in the barn at the time of shipment</td>
<td>787.2</td>
<td>0</td>
<td>247.5</td>
<td>345</td>
<td>800</td>
<td>10,000</td>
</tr>
<tr>
<td>Pigs born in an outside herd (%)</td>
<td>12.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

* For the 94 herds with breeding stock.

![Image](https://example.com/image.png)

**Figure 1:** Frequency distribution of 146 swine herds by the number of Salmonella culture-positive pools of ileocolic lymph nodes among six pools collected (five pigs sampled per pool).
specific programs linked to herd Salmonella history or current status of herds. Although the smallest herds were excluded from the study, since they were not able to deliver 30 pigs per shipment, most Midwest slaughter pigs are produced in herds at least the size of the study herds. Assuming weekly delivery, this minimum delivery-group size would correspond to a herd size of approximately 100 sows, assuming average production efficiency calculated from National Agricultural Statistical Service (NASS) figures.\(^{22}\) Midwest herds with current total inventory of 1000 pigs or larger, the NASS category closest to the inventory expected for 100 sows, accounted for 70.4% of all Midwest inventories in 1998.\(^{22}\) Only 10% of herds had ≥ 20% prevalence, suggesting the possibility that on-farm interventions might be targeted to a subset of farms. However, the success of such a program would depend on development and implementation of tests that are both cost effective and able to accurately predict high risk of Salmonella shedding at slaughter. Further, the current study represents a one-time snapshot of prevalence. Since the prevalence of Salmonella may vary over time,\(^{23-25}\) identification of marketed groups with high prevalence may require ongoing sampling, which would be expensive and cumbersome using existing methods.

Regional differences have been reported for Salmonella prevalence based on fecal culture, with higher proportions of southeastern US herds positive compared with the herds in the Midwest and other areas.\(^{26}\) Consequently, it is likely that the results reported here are lower than would be found nationwide, assuming that fecal and ileocolic lymph-node detection rates are positively associated.\(^{27}\)

The serovar distribution in swine in this study differed from that of isolates reported for human salmonellosis by the CDC's FoodNet surveillance system.\(^{28}\) Most notably, the proportion of serovar Derby isolates was much higher in study samples compared with human-origin isolates. This discrepancy, among others, has been the basis for speculation that either pork may be a minor source of human salmonellosis in the United States, or that certain serovars, especially serovar Derby, have relatively low infectivity for humans.\(^{29}\) Serovar Derby was the most commonly detected serovar both in this study and in carcass swabs collected nationwide.\(^{11}\) On the other hand, there are commonalities among serovars isolated from pigs and human. First, eight of the 10 most common human serovars reported by the FoodNet system in 1998\(^{28}\) were detected in swine lymph nodes in this study. Second, more than half of isolates from both swine and human FoodNet isolates were common serotypes. Whereas these commonalities do not prove that pigs are a source for human infection, the findings are consistent with such linkage and indicate the need for further study.

Invasive Salmonella infections result in more serious health consequences. Four of the eight most common invasive serovars reported in human disease\(^{30}\) were detected in swine samples in this study, although three of these, Heidelberg, Schwarzengrund, and Choleraesuis, were not among the 10 most common swine serovars detected in this study.

Samples collected at slaughter may reflect bacteria derived from the farm of origin,

<table>
<thead>
<tr>
<th>Table 2: Salmonella enterica serovars detected among ileocolic lymph node samples from slaughtered pigs from 113 Midwest herds*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serovar</strong></td>
<td><strong>No. of isolates</strong></td>
</tr>
<tr>
<td>Derby</td>
<td>106</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>55</td>
</tr>
<tr>
<td>Brandenburg</td>
<td>48</td>
</tr>
<tr>
<td>Uganda</td>
<td>26</td>
</tr>
<tr>
<td>Typhimurium (copenhagen)</td>
<td>24</td>
</tr>
<tr>
<td>London</td>
<td>23</td>
</tr>
<tr>
<td>Anatum</td>
<td>22</td>
</tr>
<tr>
<td>Agona</td>
<td>20</td>
</tr>
<tr>
<td>Mbandaka</td>
<td>14</td>
</tr>
<tr>
<td>Worthingston</td>
<td>11</td>
</tr>
<tr>
<td><strong>Serovars with &lt; 10 isolates</strong></td>
<td>107</td>
</tr>
</tbody>
</table>

* Ileocolic lymph nodes were collected from 30 pigs per herd. Half of each individual pig sample was frozen. The other halves of individual fresh samples were pooled and cultured for Salmonella (five pigs per pool, six pools per herd). For Salmonella-positive pools, individual frozen samples were cultured and Salmonella isolates serotyped.

| Table 3: Salmonella culture results from 100 paired ileocolic lymph-node tissues collected at slaughter and cultured fresh (not frozen) and after storing at -70°C for 14 days (frozen) |
|---------------------------------|--------|----------|
| **Fresh tissue result**         | **Positive** | **Negative** | **Totals** |
| Frozen tissue result            |        |          |          |
| Positive                        | 10     | 7        | 17       |
| Negative                        | 7      | 76       | 83       |
| **Totals**                      | 17     | 83       | 100      |

| Table 4: A regression model of Salmonella prevalence\(^{0.5}\) in ileocolic lymph nodes of slaughtered pigs as a function of the number of culture-positive pooled samples* |
|---------------------------------|--------|--------|-------|
| **Coefficient**                 | **SE** | **P**  |       |
| Intercept                      | 0.142  | 0.064  | .03   |
| No. of positive pools          | -0.014 | 0.048  | .77   |
| No. of positive pools\(^2\)    | 0.015  | 0.007  | .04   |
| \(R^2 = 0.47, F(2,79) = 30.7; P < .01\) |

* Samples from five individuals were combined to form a single pool for bacterial culture (six pools per herd) as described in Table 2.
or those acquired during transportation and lairage. Although farms are an important source of strains isolated at slaughter, rapid *Salmonella enterica* infection of intestinal lymph nodes can occur, and there is evidence for uptake of new strains during transport and lairage. Consequently, isolates collected at slaughter should be regarded as a composite of all three sources.

These findings probably underestimate the true prevalence of *Salmonella* for several reasons, including the effect of pooling samples, freezing samples, and limited sensitivity of the culture methods used. Pooling followed by culturing of individual samples in positive pools was an effective way to reduce laboratory resources required. In this study, the total number of bacterial cultures was reduced by approximately two-thirds, compared with culturing all 30 individuals from every herd. Because the cost of culture media needed for *Salmonella* isolation is substantial for large-scale studies, this efficiency and cost savings can dramatically increase the number of herds and pigs studied. For epidemiologic studies, inclusion of a large number of herds provides for a more robust description of a population. However, pooled samples were not a precise indicator of individual-pig prevalence, suggesting that a two-stage protocol would help define prevalence more accurately. Pooling has the potential to introduce systematic bias if the culture methods for pools are less sensitive than culture methods for individual samples. An assessment of prevalence among samples paired with culture-negative pools would be useful to help interpret results. However, we did not evaluate this potential effect, since the objective of pooling in this study was to make more efficient use of available laboratory and financial resources, and culture of negative pools would have required resources that were instead used for study of additional pigs and herds. However, if this methodology resulted in lower sensitivity at the pig level, as would be expected, a bias toward underestimation of prevalence is the likely result. A further bias was noted in failure to detect *Salmonella* in individual samples paired to 22 of the positive pools. If it is assumed that none of the pooled-sample results were false-positives, then it follows that using individual results where they were available, as was done in this study, would also tend to underestimate prevalence. Possible reasons for these incongruities include potentially heterogeneous distribution of salmonellae in lymph-node tissues, relatively insensitive culture methods, death of bacteria during storage, and potential cross-contamination of pooled tissues, among others. These limitations should be kept in mind when pooled samples are used.

Freezing samples may result in diminished viability of salmonellae, with a consequent bias to underestimate prevalence in frozen samples, as has been reported in food samples, 

Figure 2: *Salmonella* prevalence in ileocolic lymph nodes of swine, modeled as a function of the number of culture-positive pooled samples per herd (six pooled samples per herd, 82 herds). Approximate 95% CIs for the fitted model are shown. Lymph nodes were collected at slaughter and stored at -70°C for 2 to 14 months before culturing.

![Image](146x508 to 155x513)

![Image](147x538 to 154x545)

![Image](148x564 to 152x567)

![Image](189x538 to 197x545)

![Image](190x508 to 196x510)

![Image](190x539 to 196x433)

![Image](191x573 to 195x776)

![Image](191x581 to 195x847)

![Image](262x698 to 269x705)

![Image](276x508 to 280x510)

![Image](276x553 to 280x557)

![Image](276x573 to 280x576)

![Image](276x611 to 280x615)

![Image](276x617 to 280x620)

![Image](276x631 to 280x634)

![Image](297x219)

![Image](303x697 to 312x707)

![Image](319x573 to 323x576)

![Image](319x581 to 323x584)

![Image](337x219)

![Image](361x564 to 365x567)

![Image](361x621 to 365x625)

![Image](361x626 to 365x630)

![Image](361x639 to 365x647)

![Image](388x505)

![Image](388x578)

![Image](388x614)

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![Image](440x948)

![Image](440x960)

![Image](440x972)

![Image](440x984)

![Image](440x996)

![Image](456x97)

![Image](518x296)

![Image](562x296)

While salmonellae were found in 68% of herds, the median prevalence of 6.7% suggests that in most herds, the proportion of culture-positive pigs was low. However, positive pigs pose a likely risk to pork food safety, since many of the serovars detected are also detected in human infection, and four were among the common list of invasive human serovars. These findings suggest the need for continued care in development of pork-chain *Salmonella* control programs, and the need for further research to identify cost-effective methods to reduce *Salmonella* shedding on farms.

**Implications**

- Under the conditions of this study, salmonellae were commonly isolated from ileocolic lymph nodes of slaughtered pigs, with positive results in approximately two-thirds of herds and an average of 7% of individual pigs.
- Among herds studied, a minority of market deliveries provided a disproportionately high prevalence of *Salmonella*-positive pigs.
- Freezing of lymph-node samples...
before bacterial culture appears an effective way to simplify logistics without compromising the ability to detect *Salmonella.*

- Pooling of samples for microbial culture then culturing individual retained (frozen) tissue from positive pools can be an effective way to reduce study costs, increase the number of herds examined, or both.

### Acknowledgments

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### References


3. Pooling of samples for microbial culture then culturing individual retained (frozen) tissue from positive pools can be an effective way to simplify logistics without compromising the ability to detect *Salmonella.*


* Non-refereed references.