Research Note

Prevalence of *Salmonella* in Grade A Whole Shell Eggs in the Island of Ireland

LAURA MURCHIE,1 PAUL WHYTE,2 BIN XIA,2 SARAH HORRIGAN,2 LOUISE KELLY,3,5 AND ROBERT H. MADDEN1,4*

1Food Science Department, Queen’s University of Belfast, Newforge Lane, Belfast BT9 5PX, UK; 2Centre for Food Safety, School of Agriculture, Food Science and Veterinary Medicine, Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland; 3Department of Statistics and Modelling Science, University of Strathclyde, Richmond Street, Glasgow G1 1XH, UK; 4Food Microbiology Branch, Agri-Food and Biosciences Institute, Newforge Lane, Belfast BT9 5PX, UK; and 5Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, UK

MS 06-540: Received 18 October 2006/Accepted 22 January 2007

ABSTRACT

Following the emergence of *Salmonella* Enteritidis as a widespread contaminant of eggs and the role of eggs in the transmission of human salmonellosis, control measures were introduced to curb the spread of infection. Two approaches to *Salmonella* control are currently used by egg producers in Ireland, because Northern Ireland producers, like those in the rest of the United Kingdom, widely adopted a vaccination regime, whereas the Republic of Ireland does not permit vaccination but introduced controls based on routine monitoring for specific *Salmonella* serovars and subsequent culling of infected flocks. To compare the efficacy of these two approaches and determine the prevalence of salmonellae in eggs produced for retail sale in the island of Ireland, a major survey of approximately 30,000 grade A eggs was undertaken. Egg shells and contents were analyzed separately for salmonellae by procedures based on International Organization for Standardization methodology. The survey yielded only two positive samples, with *Salmonella* Infantis and *Salmonella* Montevideo isolated from shells; no egg contents yielded salmonellae. There was no statistically significant difference in the prevalence of salmonellae between eggs produced in Northern Ireland and those from the Republic of Ireland; hence, both regimes appeared to be equally effective in controlling salmonellae. The prevalence was also significantly lower than that found in a recent major United Kingdom survey. Hence, shell eggs produced in the island of Ireland are unlikely to be a source of human salmonellosis.

The dramatic rise during the 1980s in the number of human cases of *Salmonella* Enteritidis phage type 4 in the United Kingdom (9, 10) and much of western Europe (20, 24) and the role of eggs in the epidemic have been well documented (1, 13–17, 19, 22, 23). However, in recent years, there has been a decline in the incidence of human salmonellosis in the Republic of Ireland (18) and in cases of *Salmonella* Enteritidis phage type 4 in England and Wales (10), Scotland (8), and Northern Ireland (9). These reductions coincide with the introduction of legislation, industry codes of practice, and quality assurance schemes intended to control *Salmonella* in laying flocks (12).

In the United Kingdom, the Lion Quality code of practice was reintroduced in 1998 and now covers approximately 85% of the eggs produced (7). This scheme requires the vaccination of commercial layer flocks against *Salmonella* Enteritidis, in addition to controls for welfare, hygiene, and biosecurity (4, 7). In contrast, in the Republic of Ireland, the vaccination of flocks is not permitted. Egg production is regulated by laws that require the routine monitoring of feeds and flocks for *Salmonella*. Any flocks found to be infected with *Salmonella* Enteritidis or *Salmonella* Typhimurium must be slaughtered under this legislation (2). Also, since 1999, eggs produced under the voluntary Bord Bia (Irish Food Marketing Board) Egg Quality Assurance scheme have been subject to further controls that govern aspects of hygiene, flock welfare, packaging of eggs, and environmental protection (5).

Eggs imported into Southern Ireland must come from unvaccinated flocks. Hence, some producers in Northern Ireland base the vaccination of flocks on the intended market for their eggs. The efficacy of these different approaches for the control of *Salmonella* in eggs has not been determined. Therefore, a survey of eggs produced in Northern Ireland, under United Kingdom regulations, and in the Republic of Ireland, under Irish regulations, was undertaken. The results also allow the current incidence of salmonellae in eggs produced in the island of Ireland to be compared with previous United Kingdom and Irish surveys and provide information for use in a comparative risk assessment model for the presence of *Salmonella* in shell eggs and the subsequent effects on human health.

MATERIALS AND METHODS

Eggs sampled. Staff members who had been trained in egg grading selected eggs of grade A standard at packing stations. The eggs were taken from incoming material prior to normal grading to ensure that the samples came from a single flock and to allow information from the supplying farms to be collected.
included the type of flock sampled (i.e., intensive, barn, free range, or organic) as well as the flock age and *Salmonella* control measures taken (e.g., birds vaccinated or not, quality assurance scheme).

**Sample collection.** In the Republic of Ireland, the employees of egg packing stations collected eggs at the pregrading stage and then delivered them to the laboratory at the Center for Food Safety, Dublin, for analysis. In Northern Ireland, staff members of the Department of Agriculture and Rural Development collected samples at the same stage of production and delivered them to the Food Microbiology Laboratory at Queen’s University of Belfast. Each sample consisted of 12 eggs, from a specific flock, which were judged as being grade A. Eggs were placed in new cardboard cartons and transported to the laboratories within 24 h. On delivery, the samples were kept cool (<15°C) until required for analysis. All analyses were initiated on a Monday or Tuesday; hence, the analysis of samples was normally initiated within 6 days. Sampling took place from March 2005 until April 2006, inclusively.

**Salmonella isolation procedure.** *Salmonella* were isolated by methodology based on BS EN 12824: 1998 (3), as used in a major United Kingdom survey (4), and biotyped and serotyped. Immediately prior to analysis, eggs were inspected to confirm grade A status and rejected if any flaws, such as cracks or adhering material, were found. Staff members wore a new pair of disposable gloves for each sample, and great care was taken to minimize the potential for cross-contamination. For each sample, six eggs were aseptically broken open, and the shell was separated from the contents, taking care to avoid contaminating the contents with pieces of the shell. A small amount of buffered peptone water (BPW; Lab M, Bury, Lancashire, UK) was added to the contents and homogenized (1 min) in a stomacher blender at normal power (model 400, Seward, London, UK). Further BPW was added to create a 50:50 dilution, and the mixture was again blended (1 min). Incubation was at 37°C for 24 h in the stomacher bags. Shells were transferred to a sterile plastic jar (300 ml) and crushed with a sterile gloved hand, and sufficient BPW was added to cover the shells. The jar was shaken gently and then incubated (37°C, 24 h).

Incubated BPW (0.1 ml) was inoculated into 10 ml of soya peptone Rappaport-Vassiliadis broth (Oxoid, Basingstoke, UK) and incubated at 41.5°C for 24 h. The BPW (10 ml) was also inoculated into 100 ml of selenite cystine broth (E&O Labs, Bonnybridge, Stirlingshire, UK) and incubated (37°C, 24 h). After selective enrichment, both media were streaked onto modified brilliant green agar (Oxoid) and xylose lysine desoxycholate agar (Oxoid), and the plates were incubated for 24 h at 37°C. Up to five suspect colonies were then streaked to purity on nutrient agar plates (24 h, 37°C) and identified by standard biotyping and serotyping methods (3).

Results were statistically analyzed by GenStat Release 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, UK). A binomial regression model was fitted to configure the percentage of positive samples over the various locations. Pairwise comparisons were then used to test for differences between the locations.

**External quality assurance samples.** Four batches of 10 external quality assurance samples inoculated with three different levels of *Salmonella* Poona were prepared by Hannah Research Institute (Ayr, UK). At regular intervals during the survey, external quality assurance samples were transported overnight to the testing laboratories, arriving before noon. External quality assurance samples were analyzed blind on the day they were received. *Salmonella* Poona was used, as it is a rare serovar; hence, any cross-contamination within the laboratory would readily be identified.

<table>
<thead>
<tr>
<th>Survey area</th>
<th>Survey period</th>
<th>No. of samples tested</th>
<th>No. (%) of samples positive for <em>Salmonella</em></th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Island of Ireland</td>
<td>2005–2006</td>
<td>5,031</td>
<td>2 (0.04)</td>
<td>This study</td>
</tr>
<tr>
<td>Republic of Ireland</td>
<td>2003</td>
<td>1,169</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>UK</td>
<td>2003</td>
<td>4,753</td>
<td>9 (0.34)</td>
<td>6</td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>1996–1997</td>
<td>2,090</td>
<td>9 (0.43)</td>
<td>25</td>
</tr>
<tr>
<td>England</td>
<td>1995–1996</td>
<td>13,970</td>
<td>138 (0.99)</td>
<td>4</td>
</tr>
<tr>
<td>England and Wales</td>
<td>1991</td>
<td>7,045</td>
<td>65 (0.92)</td>
<td>16, 17</td>
</tr>
</tbody>
</table>

* Prevalence statistically adjusted for discrepancies in proportion of type tested, actual market share, and geographical area sampled.

RESULTS AND DISCUSSION

In total, 5,018 samples, from 30,108 eggs, were analyzed, with 2,503 samples produced in Northern Ireland and 2,515 in the Republic of Ireland. Overall, 51% of the samples were from intensive production, 24% were free range, 14% were from barn production, and 11% were organically produced. Flocks in the Republic of Ireland are not vaccinated against *Salmonella* Enteritidis, but 87.6% of the Northern Ireland samples came from vaccinated flocks. Two shell samples were found to be positive for *Salmonella*, and both of these samples were from vaccinated flocks in Northern Ireland. Only the shells yielded salmonellae, with no egg contents found to be positive. A single serovar was isolated from each positive sample, and these were identified as *Salmonella* Infantis and *Salmonella* Montevideo.

Both laboratories analyzed four batches of external quality assurance samples during the study and correctly identified all positive samples submitted. The serovar was in all cases confirmed as *Salmonella* Poona, the organism inoculated into the test samples.

Statistical analysis showed no significance difference (*P* > 0.05) between the prevalence of salmonellae in the samples from Northern Ireland and the Republic of Ireland; hence, the prevalence in the island of Ireland as a whole will be discussed below. The low levels of salmonellae recovered also meant that no statistically significant relationship between, or within, production systems could be identified.

A comparison of the results obtained with other United Kingdom and Irish surveys for the presence of *Salmonella* in retail eggs (Table 1) shows a clear trend toward fewer contaminated samples over the past decade. The *Salmonella* detection methodology of this study was the same as that used by the Food Standards Agency survey in 2003 of United Kingdom–produced eggs (on retail sale), which found 0.34% of the samples positive for *Salmonella*. That prevalence was statistically adjusted by the Food Standards
Agency to allow disparity in the proportion of production type tested, actual market share, and geographical area sampled, and it represented an almost threefold fall in prevalence compared with the earlier surveys in England from 1995 to 1996 and in England and Wales in 1991 (Table 1). This was attributed to the success of intervention methods introduced by the government (6). The survey reported here found significantly fewer positives than the Food Standards Agency survey ($P < 0.05$), indicating a continuing fall in the prevalence of salmonellae in shell eggs.

However, a survey of eggs from Northern Ireland from 1996 to 1997 reported a lower incidence of *Salmonella* (0.43%) than a contemporary English survey (Table 1), suggesting that the prevalence of salmonellae differs regionally in the United Kingdom (5). No significant difference was found between the present survey and that in the Republic of Ireland in 2003 (Table 1).

The continued decrease in the prevalence of *Salmonella* Enteritidis in laying flocks in the island of Ireland is encouraging, especially with its concomitant reduction in human infections. The very low levels found suggest that further surveys are not economically feasible, since to determine a lower prevalence, at the 95% confidence level, would require 7,500 samples to be analyzed with no positive samples found. However, the continued requirement for surveillance programs for salmonellae in eggs has been stressed in light of the potential of other serovars to capitalize on the ecological gap caused by the removal of *Salmonella* Enteritidis (4, 12, 19). For example, *Salmonella* Heidelberg is also able to infect the reproductive tissues of birds (11), and the consumption of eggs prepared outside the home has recently been highlighted as the principal risk factor in sporadic cases of *Salmonella* Heidelberg in the United States (21).

Overall, the results reported indicate that the two control strategies employed in the island of Ireland are equally effective in reducing the prevalence of *Salmonella* in eggs and that salmonellosis in the human population is therefore unlikely to result from the consumption of eggs produced in the island of Ireland.

**ACKNOWLEDGMENT**

This study was funded by safefood, the Food Safety Promotion Board, as project 03-RESR-005.

**REFERENCES**