

DEVELOPMENT OF A QUALITATIVE EXPOSURE ASSESSMENT FOR SALMONELLA IN GRADE A SHELL EGGS ON THE ISLAND OF IRELAND

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Introduction

In the 1980s the incidence of salmonellosis in the human population in Europe rose markedly due to the emergence of *Salmonella* Enteritidis PT4. This organism caused a systemic infection in laying hens with the result that grade A shell eggs were laid with the organism present in the egg contents. Subsequently foods prepared from eggs which were not fully cooked could act as vehicles for human infection. Consequently measures were introduced to obviate this threat to human health. In the UK, the Lion Quality code of practice was re-introduced in 1998 and now covers approximately 85% of eggs produced. This scheme requires the vaccination of commercial layer flocks against *Salmonella* Enteritidis, in addition to controls for welfare, hygiene and bio-security. This scheme is widely adopted in Northern Ireland (NI). In contrast, in the Republic of Ireland (RoI) vaccination of flocks is not permitted. Egg production is regulated by law requiring routine monitoring of feeds and flocks for *Salmonella*. Any flocks found to be infected with *S. Enteritidis* or *Salmonella* Typhimurium must be slaughtered under this legislation. Also, since 1999, eggs produced under the voluntary Bord Bia (Irish Food Marketing Board) Egg Quality Assurance scheme have been subject to further controls governing aspects of hygiene, flock welfare, packaging of eggs and environmental protection.

To determine the current prevalence of salmonellas in eggs produced under NI and RoI control regimes a major survey was conducted by Queen's University Belfast and University College Dublin. This data was required for the development of a qualitative exposure assessment for salmonellas in eggs produced on the island of Ireland.

Microbiological risk assessment (MRA) as outlined by Codex Alimentarius Commission (CAC, 1999) can be divided into 4 stages: hazard identification, exposure assessment, hazard characterisation and risk characterisation. The exposure assessment stage aims to track a pathogen in a food from production to consumption, accounting for changes in the prevalence and pathogen numbers due to growth, death or cross-contamination occurring at each step in the pathway. In qualitative MRA the levels of risk are classified using defined descriptive terms, such as low, moderate and high, based on evaluation of available data and expert opinion.

Materials and Methods

Egg survey. Between March 2005 and April 2006, samples of 12 grade A eggs, from a specific flock, were collected at the pre-grading stage. Eggs were placed in new cardboard cartons and transported to the laboratories within 24 h. On delivery samples were kept cool (< 20°C) until required for analysis, which was initiated within 6 d of receipt at the laboratory. Salmonellae were isolated using methodology based on BS EN 12824: 1998 (Anon. 2001), as used in a major UK survey (Anon. 2001), biotyped and serotyped. For each sample, six eggs were aseptically broken open and the shell separated from contents, taking care to avoid contaminating the contents with pieces of shell. A small amount of buffered peptone water (BPW) (Lab M, Bury Lancashire, UK) was added to the contents and homogenized (1min) in a stomacher blender at normal power (Model 400, Seward, London, UK). Further BPW was added in order to create a 50:50 dilution and the mixture again blended (1min). Incubation was at 37°C for 24 h in the stomacher bags. Shells were transferred to a sterile plastic jar (300 ml), crushed down with a sterile gloved hand and sufficient BPW added to cover the shells. The jar was shaken gently then incubated (37°C, 24 h). Incubated BPW (0.1ml) was inoculated into 10 ml soya peptone Rappaport-Vassiliadis broth (Oxoid, Basingstoke, UK) and incubated at 41.5°C for 24 h. BPW (10 ml) was also inoculated into 100 ml selenite cystine broth (E&O Labs, Bonnybridge, UK) and incubated (37°C, 24 h). After incubation both media were streaked onto modified brilliant green agar (Oxoid) and xylose lysine desoxycholate agar (Oxoid) and incubated, 24 h at 37°C. Up to 5 suspect colonies were streaked to purity on nutrient agar plates (24 h, 37°C) and identified using standard biotyping and serotyping methods (Anon. 1998).

Exposure assessment. The exposure pathway was divided into modules covering production and packing, distribution and storage and preparation and consumption. Each module was further divided into steps representing defined stages in the chain. At each step both the probability of *Salmonella* being present and the level of contamination can be classified as negligible, low, moderate or high. These two assessments are then combined using the matrix of Moutou et al. (2001) to give an overall evaluation of risk.

Results and Discussion

Egg survey. In total 5,018 samples, comprising a total of 30,108 eggs, were analysed with 2503 samples produced in Northern Ireland and 2515 in the Republic of Ireland. Only two samples were positive for *Salmonella*, and in both cases only the shells were contaminated. One serovar was isolated from each positive sample and these were identified as *Salmonella* Infantis and *Salmonella* Montevideo. No significance difference ($p>0.05$) between the frequency of isolation of salmonellae in samples from Northern Ireland and the Republic of Ireland was found; hence the data were used to estimate of the prevalence on the island of Ireland as a whole. This data was used as a starting point in the development of the exposure assessment for eggs produced on the island of Ireland.

Exposure assessment. The results of the above survey indicated a negligible incidence of *Salmonella* contamination of eggs produced on the island of Ireland, at time of sampling. Most available evidence suggests that internal egg contamination typically involves small initial numbers of *S. Enteritidis*. Studies of eggs produced by naturally infected birds have demonstrated contamination levels of less than 10 CFU per egg after 7 days (Humphrey et al., 1989). Cogan et al., (2001) suggested numbers introduced at time of lay could be as low as only 1 or 2 cells, and the former data may represent numbers occurring after an initial growth phase see in eggs inoculated less than 24 h after lay (Gast & Holt, 2000). Based on this data the level of *Salmonella* in eggs, when present, was judged to be low. The combination these two parameters resulted in a low risk from *Salmonella* spp. in eggs produced on the island of Ireland, at time of sampling.

Conclusions

The survey results represent a reduction in the frequency of *Salmonella* isolation from eggs compared with the 2003 survey of UK-produced retail eggs (Anon, 2004) and also indicate the two control strategies used on the island of Ireland are equally effective in reducing *Salmonella* spp. in eggs. Based on this data, the prevalence, in conjunction with the likely low numbers of *Salmonella* in eggs at time of lay gives a low estimation of risk associated with eggs at their time of lay. However, changes in both prevalence and level of contamination of *Salmonella* may occur at subsequent steps in the production chain. Work to develop a full farm-to-fork exposure assessment to address these issues is ongoing.

References

1. Anonymous. 2004. Report of the survey of *Salmonella* contamination of UK produced shell eggs on retail sale. Food Standards Agency, HMSO, London, UK. Available at: <http://www.food.gov.uk/science/surveillance/fsis2004branch/fsis5004eggs>. Accessed 11 October 2006.
2. Anonymous. 2001. Second report on *Salmonella* in eggs. Advisory Committee on the Microbiological Safety of Food, HMSO, London, UK.
3. Anonymous. 1998. Microbiology of food and animal feedstuffs-Horizontal method for the detection of *Salmonella*. BS EN 12824: 1998. British Standards Institute, London.
4. Codex Alimentarius Commission. 1999. Principles and guidelines for the conduct of microbiological risk assessment. CAC/GL-30.
5. Cogan, T. A., Domingue, G., Lappin-Scott, H. M., Benson, C. E., Woodward, and M. J., Humphrey, T. J. (2001). Growth of *Salmonella enteritidis* in artificially contaminated eggs: the effects of inoculum size and suspending media. *International Journal of Food Microbiology*, 70, 131-141
6. Gast, R. K., and Holt, P. S. (2000). Influence of the level and location of contamination on the multiplication of *Salmonella enteritidis* at different storage temperatures in experimentally inoculated eggs. *Poultry Science*, 79, 559-563
7. Humphrey, T. J., Baskerville, A., Mawer, S., Rowe, B., and Hopper, S. (1989). *Salmonella enteritidis* phage type 4 from the contents of intact eggs: a study involving naturally infected hens. *Epidemiology and Infection*, 103, 415-423
8. Moutou, F., Dufour, B., and Ivanov, Y. (2001). A qualitative assessment of the risk of introducing foot and mouth disease into Russia and Europe from Georgia, Armenia and Azerbaijan. *Revue scientifique et technique (International Office of Epizootics)*, 20, 723-730.

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