

## Estimation of the vertical and horizontal bacterial infection of hen's table eggs

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### Introduction

There are two possible routes of bacterial infection of shell eggs: either vertically or horizontally. In the transovarian route (vertical transmission), the yolk (very infrequently the yolk itself), the albumen and/or the membranes are directly contaminated as a result of bacterial infection of the reproductive organs, *i.e.* ovaries or oviduct tissue, before the eggs are covered by the shell (7). Vertical transmission can originate from infection of the ovaries of a laying hen via systemic infection, or from an ascending infection from the contaminated cloaca to the vagina and lower regions of the oviduct (5; 8). In the horizontal transmission the micro-organisms penetrate through the eggshell. The egg passes through the highly contaminated cloaca area at the moment of lay; this is often illustrated by visible faecal contamination on the shell. Following oviposition, the shell acquires contamination from all surfaces with which it makes contact (1). While being wet and entering an environment with a temperature of approximately 20°C below the hen's body temperature, the egg will cool immediately. The egg content will contract and a negative pressure establishes inside the egg, thereby moving contaminants through the shell (9). The aim of this study was to estimate the incidence of vertically and horizontally contaminated whole eggs and to identify the contaminants to species level.

### Materials and methods

#### *Farms*

14 flocks of laying hens from 11 different commercial farms were included in this study. Cage as well as non-cage systems were included. To study the influence of the housing system six of those flocks housed in non-cage systems and 4 of those flocks housed in furnished cages were sampled on a fixed moment; when the birds were about 60 weeks of age. On each farm eggs were sampled at the egg belts (no ground eggs).

#### *Sampling, collection and transport of eggs*

The eggs were sampled by hand, placed in open carton filler-flats and brought by car, in ambient conditions, to the laboratory (2). From the 14 flocks, in each case circa 80 intact eggs (no cracks) were sampled at the hen house the morning of lay (day 0). Half of the eggs (circa 40) were used to determine the egg content contamination the same day of sampling; the other half (circa 40 eggs) was stored at room conditions for 21 days, followed by the determination of the egg content contamination.

#### *Eggshell contamination*

For the recuperation or recovery of bacteria from the eggshell, the intact egg was placed in a plastic bag with 10 ml quarter-strength Ringer's solution (Oxoid, Hampshire, UK) and the egg was rubbed through the bag for 1 minute (2). The diluent was plated on Nutrient Agar (Oxoid). Plates were incubated for 3 days at 30°C.

#### *Egg content contamination*

To remove the egg content aseptically the eggshell was first disinfected with hydrogen peroxide (30%) by dipping the eggs for 10 sec in the solution, followed by sprinkling the egg with 75% ethanol and burning off the alcohol during circa 5 sec. The disinfected egg was broken by hand using a sterile blade and sanitized plastic gloves. The egg content was enriched in BPW (Oxoid) at 30°C for 24h and plated out on Nutrient Agar. Plates were incubated at 30°C for 72h. (3)

#### *Species identification*

Shell and egg content contaminants were identified to species level using Api (bioMérieux, Marcy l'Etoile, France) and 16S rRNA partial sequencing (10).

## **Results and discussion**

### *Eggshell decontamination*

Dipping the eggs in 30% hydrogen peroxide solution for 10 sec, followed by sprinkling the egg with 75% ethanol and burning off the alcohol during circa 5 sec seemed to be the most suitable method. Only burning off the eggshell, as described in previous studies (4; 3), was not suitable to eliminate all and especially spore-forming bacteria from the eggshell.

### *Incidence of vertically and horizontally contaminated whole eggs*

Contamination % was respectively 2,7% (15/554 eggs) immediately after lay (day 0) and 3,4% (18/532 eggs) after 21 days storage. Comparing these contamination percentages the importance of horizontal transmission would probably be low for intact (no broken or hair-cracked) whole eggs.

Due to eventual contamination problems in our experimental procedure we do not dare to conclude on the prevalence for vertical contamination (day 0). Although the contamination we observed can be due to vertical transmission, the probability of an eventual contamination during our experimental procedure can not be excluded. In the case the observed egg content contamination would be due to an experimental bias, we consider its probability equal for eggs tested directly after lay and after 21 days storage so that our conclusion about the relative low importance of horizontal transmission stays relevant.

### *Egg content contamination in relation to the housing system*

Egg content contamination was 1,9% (5/269 eggs) for furnished cages compared to 2,3% (10/432 eggs) for non-cage systems. Although only a limited number of eggs was tested, these preliminary results indicate no significant difference in egg content contamination between both housing systems.

### *Species identification*

The natural eggshell contamination we found in our study was dominated by Gram-positive *Staphylococcus* spp. (*S. linens*, *S. equorum*, *S. lentus* and *S. xylosus*). Board and Tranter (1995) reported that because of their tolerance for dry conditions, the microflora of the eggshell is dominated by Gram-positive bacteria which may originate from dust, soil or faeces (1). We found that *Staphylococcus* was also the most dominating microflora in the air of the poultry houses.

As major egg content contaminants of our study; Gram-negative bacteria as *Escherichia coli*, *Salmonella* and *Alcaligenes* sp. were found and Gram-positive bacteria like *S. lentus*, *S. xylosus* and *Bacillus* sp.. Mayes and Takeballi (1983) and Board and Tranter (1995) found rotten eggs normally contain a mixed infection of Gram-negative and a few Gram-positive organisms. Some of the most common spoilage types in their studies were members of the genera *Alcaligenes*, *Pseudomonas*, *Escherichia*, *Proteus* and *Aeromonas* (6; 1).

## **Conclusions**

Comparing the contamination percentages the importance of horizontal transmission would probably be low for intact (no broken or hair-cracked) whole eggs. We do not dare to conclude on the prevalence for vertical contamination because of the eventual contamination

during our experimental procedure. Working on a method of aseptically opening eggs without risk of contaminating the content from the shell of membrane with all and especially spore-forming bacteria is a challenge. The little information available on the influence of the housing systems on the egg content contamination indicate no large differences in egg content contamination between cage eggs and non-cage eggs (ignoring floor eggs). Further research is recommended.

## References

1. Board, R. G. and Tranter, H. S. (1995). The microbiology of eggs. Egg science and technology. W. J. Stadelman, Cotterill, O.J. (Eds.). New York, Food Products Press - The Haworth Press, Inc.: 81-104.
2. De Reu, K., Grijspeerdt, K., Heyndrickx, M., Uyttendaele, M. and Herman, L. (2005). The use of total aerobic and Gram-negative flora for quality assurance in the production chain of consumption eggs. *Food Control* 16: 147-155.
3. De Reu, K., Grijspeerdt, K., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J. and Herman, L. (2006). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella* Enteritidis. *International Journal of Food Microbiology* 112: 253-260.
4. Himathongkham, S., Riemann, H. and Ernst, A. (1999). Efficacy of disinfection of shell eggs externally contaminated with *Salmonella enteritidis*. Implications for egg testing. *International Journal of Food Microbiology* 49: 161-167.
5. Keller, L. H., Benson, C. E., Krotec, K. and Eckroade, R. J. (1995). *Salmonella* enteritidis colonization of the reproductive tract and forming and freshly laid eggs of chickens. *Infection and Immunity* 63: 2443-2449.
6. Mayes, F. J. and Takeballi, M. A. (1983). Microbial contamination of the hen's egg: A review. *Journal of Food Protection* 46: 1092-1098.
7. Messens, W., Grijspeerdt, K. and Herman, L. (2005). Eggshell penetration by *Salmonella*: a review. *World's Poultry Science Journal* 61: 71-85.
8. Miyamoto, T., Baba, E., Tanaka, T., Sasai, K., Fukata, T. and Arakawa, A. (1997). *Salmonella* Enteritidis contamination of eggs from hens inoculated by vaginal, cloacal and intravenous routes. *Avian Diseases* 41: 296-303.
9. Padron, M. (1990). *Salmonella typhimurium* penetration through the eggshell of hatching eggs. *Avian Diseases* 34: 463-465.
10. Scheldeman, P., Goossens, K., Rodriguez-Diaz, M., Pil, A., Goris, J., Herman, L., De Vos, P., Logan, N. and Heyndrickx, M. (2004). *Paenibacillus lactis* sp. nov., isolated from raw and heat-treated milk. *International Journal of Systematic and Evolutionary Microbiology* 54: 885-891.

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