

Taking steps towards a salmonella free hatchery

by Dr Olivier Leon, Hubbard,
part of Groupe Grimaud, France.

Salmonella infection in poultry is a recurrent worldwide issue which threatens both animal and public health. Efficiently fighting against salmonella requires knowing its biology: the genus can be divided into two groups:

- Non-motile salmonellae, which comprise *Salmonella gallinarum* and *pullorum* (SGP). Some countries do not separate the two serotypes. This group belongs to serogroup D, and is host-specific to avian species. While chicken is the natural host, infections have been described in other species, with variable sensitivities to infections.

SGP is responsible for fowl typhoid and Pullorum disease,

respectively a chronic disease of mature birds and an acute infection of chicks and poults. This group has virtually been eradicated through proper management, testing and culling measures in many countries like USA, Canada or in Western Europe. Other parts of the world are however still facing economic losses related to it.

- Motile salmonellae, sometimes called Paratyphoid Salmonellae (PTS), which are non host-specific and responsible for foodborne illness in humans through consumption of contaminated poultry products.

The increasing incidence of salmonellosis in humans has been the result of the coincidental high poultry meat consumption and high salmonella prevalence in poultry flocks.

Step 1: Identifying potential sources

This step is obviously critical. The sources of infection are multiple and their relative importance can greatly vary depending on the local epidemiological context: farm density, presence of backyard poultry, presence of wild birds, salmonella prevalence in the country, existence of a national control plan, etc.

The first source to consider is the hatching eggs. Salmonella can be transmitted vertically through the eggs, but can also contaminate the shell membranes at time of lay and the following egg cooling process. The main consequence is that a few infected eggs can easily spread the contamination to the whole hatchery and the whole hatching room during hatching. Therefore, guarantees regarding the salmonella status of the donor flocks have to be provided by the egg supplier.

The water used at the hatchery is a frequent source of infection. Whether using a well or public network, drainage of contaminated soil into the water source is the main way of contamination. Contaminated equipment (egg trays, shoes, wheels, etc) can also disseminate the bacteria easily.

Salmonella can be healthily carried in the digestive tract or mechanically transported by numerous vertebrates, among which rodents and wild birds. Similarly, invertebrates like flies, beetles or red mites can be a potential risk.

Humans can serve as a source of infection as well. Members of staff working at the hatchery are at risk, especially those who own poultry or pigs at home, or have recently suffered from food poisoning without proper medical care. The US Center for Diseases Control estimates that for one reported human case of salmonella infection, 29.3 cases are not!

The hatchery itself can be the origin of the infection, when a former contamination has not been properly managed. Even if the eggs are coming from salmonella-free flocks, contamination arises during the process.

Step 2 Specific measures to tackle potential sources

In most cases, a salmonella-free status can be maintained by proper management techniques. Let us review the main ways of dealing with each aforementioned potential source.

- Hatching eggs: most measures have obviously to be taken at the donor flock level, and a few can be implemented in the hatchery. A summary of keys point are as follows:

At farm level:

- Ensuring day-old chicks are coming from salmonella-free flocks.
- All-in/all-out, and avoiding mixing ages within the building.
- Managing strict pest control (wild birds, rodents, flies and mites).
- Using decontaminated feed, either by heat treatment or by the use of acids. Even if pelletisation cannot be considered as a decontamination process, pelleted feed is safer than mash or crumbles. The status of raw materials is of utmost importance. Proteins of animal origin are a well described risk factor.
- Implementing strict staff entry and clothing procedures: dedicated shoes and outfit per building, head covers.
- Litter management: deteriorated litters favour salmonella survival.
- In countries allowing it: vaccination of the birds, which will decrease birds' sensitivity to infection. Both live and inactivated vaccines have been developed, with different targets. Salmonella immunity is both humoral and cell-mediated. Literature shows that the most effective immune response is obtained by combining a live vaccine primo-vaccination with the injection of an inactivated one a few weeks later. While cell-mediated immune response decreases salmonella colonisation at the gut level, high antibody levels strongly reduce organ colonisation, and egg transmission of salmonella.
- Using adequate egg management on farm: frequent collections, efficient disinfection protocols: the most widespread method is fumigation with a formaldehyde-based product at the right conditions: above 24°C/85% relative humidity, 20 minutes of contact time.

At the hatchery:

- Minimising multiple hatches within a hatch room to avoid cross contamination.
- Implementing strict staff entry and clothing procedures: dedicated shoes and outfit per building, head covers.
- Incubating eggs of known salmonella-free status.
- Using competitive exclusion by using complete or specific flora in order to inhibit salmonella colonisation in immature newly hatched chicks.
- Water: disinfection at hatchery entry point. Salmonella is sensitive to chlorine, but chlorine disinfection is only effective in specific conditions: pH below 7, low iron and organic matter. Chlorine dioxide is better suited when chemical conditions of the water supply are unworthy. Acidification of water can also help since salmonella rather develops in more basic environments.
- Pest control: traps and rodenticides should be placed on several critical passage points, flies develop more easily with heat and liquid manure where larvae usually dwell. Beetles and mealworms should be dealt with at each cleaning and disinfection stage by spraying insecticide on the lower part of walls to target adults, and the litter to target larvae.
- Humans: the specific measures mentioned at farm level can be applied at the hatchery too. Ensuring staff do not own poultry or other farm animals reduces the risk, as well as regularly checking carrier status.

Step 3

Monitoring the efficacy of the implemented measures

Any risk reduction strategy is useless if no monitoring and recording of its efficacy is in place. The monitoring strategy is the key to success for salmonella freedom: the proper samples taken at the right time analysed with the proper method. Three methods are commonly used for salmonella detection: bacteria culture, serology and PCR.

Sample types and methods:

Salmonella detection can be performed on bird material (liver, caeca) or environmental samples (swabs, bootswab). For SGP detection, it is preferable to use bird material only, as environmental samples will lead to false negative results. Caeca and caecal tonsils are the most reliable sample for that purpose. At farm level, along with footswabs on the litter, swabs of nests, egg belts, feeding trough, walls or any other dusty zone within the bird living area are also highly sensitive for PTS detection.

At the hatchery level, box liners along with swabs of the hatcher or chick belts are recommended material. Salmonella can also be isolated from all other potential sources, such as feed, water, raw material and pests.

Bacterial culture methods have been extensively described in numerous official documents (OIE, US NPIP, ISO 6579 v.2007 for instance) and the reader should refer to it for further technical information.

The major antibody response associated with salmonella infections in poultry allows for the use of serology. Rapid agglutination techniques are widespread, along with ELISA techniques. Antibodies in egg yolks are easily detected as well. However there are limitations to the use of serology only. A positive result is not necessarily predictive of an ongoing infection. Besides, there is a delay between the time of infection and the onset of detectable antibodies. Subclinical or low-level infections can sometimes appear undetectable by serology, whereas bacterial cultures are positive. Finally, the use of serology is not relevant in vaccinated birds as it becomes impossible to differentiate vaccinated from infected birds.

Sampling frequency:

A sampling schedule should be elaborated taking into account legal regulations if any and local epidemiological constraints. An example of a thorough sampling protocol at farm level is shown in Table 1. In the presented schedule, testing every hatcher at every hatch by swabbing is an easy and efficient method to control and keep track of salmonella infections occurring at the hatchery due to donor flocks. Water is checked twice a year. SGP serology is performed on 60 birds, which allows for a detection of 5% prevalence with a 95% confidence interval.

Pressure has been placed by official bodies upon poultry producers to control and monitor for salmonellae. Consequently, control plans have been elaborated on national levels to coordinate the actions aiming at reducing the prevalence of infections in both poultry flocks (especially chicken and turkey) and human populations.

Two examples of such policies can be found in Europe (200-2010 EU Regulation for broiler breeders) or North America (US National Poultry Improvement Plan). Moreover, the globalisation of modern poultry trade has created a new epidemiological dimension to salmonella dissemination, and exporting countries have to abide by importers' specific regulations regarding salmonella status.

Given its ubiquitous nature and the easiness of both horizontal and vertical transmission, any strategy aim-

ing at reducing its prevalence has to encompass all the stages of the production process.

The objective of this article is to focus on measures to undertake at the hatchery level to ensure salmonella-free status.

Conclusion

Salmonella freedom status at hatchery level requires the involvement of all. Elaborating the strategy to obtain, and more importantly keep a salmonella free status grounds itself on a set of rules which remain identical wherever the hatchery, whatever the species.

The 360° approach, as shown in Fig. 1, can be envisioned as the equilibrium between the five pillars balancing the strategy. Whatever the angle of attack, the hatchery will remain undefeated. ■

When?	Sample	No. of samples	Test
Once or twice a year	Water	5 litres	Bacteriology, PTS
Every hatch	Hatcher swab	Every hatcher	Bacteriology, PTS
Every hatch	Chicks	10 per flock	Freezer storage, 40-60 days
When breeders are 34 and 50 weeks old	Pipped unhatched eggs	20 per flock	Bacteriology, SGP
Every 14 days	Hatching room swabs	Post disinfection monitoring: as many as required	Bacteriology PTS
Depending on local epidemiology	Chick blood	60	SGP Serology
Every month	Incubation room swabs	Post disinfection monitoring: as many as required	Bacteriology PTS

Table 1. Example of a hatchery salmonella monitoring schedule. SGP: *Salmonella gallinarum pullorum*. PTS: *Para-typhoid salmonella* (i.e. non SGP: *typhimurium*, *enteritidis*, etc.)

Step 4

Catering for the worst case scenario

A contingency plan has to be ready in case a salmonella detection occurs at the hatchery. The bacteria are usually sensitive to most chemical agents, but care should be taken to use the recommended dose as exposition to sub-optimal concentrations may elicit resistance. A common strategy combines soap detergents, spraying an accredited disinfectant on all surfaces, and finishing by fumigation of an airborne disinfectant: ammonium/glutaraldehyde and formaldehyde are efficient and widely used, orthophenylphenol is an alternative.

Antibiotic treatment is a hot debate and the authors advise against use as it entails a potential selection of resistance which can then be easily transmitted. Moreover, the efficacy of antibiotic treatments is inconsistent: from actual protection to potentiation of salmonella infection due to eradication of natural microflora in the first days of age.

The plan should include a full investigation of the potential causes of infection, in order to prevent recurrent detection.

Fig. 1. The 360° approach to salmonella freedom at the hatchery level.

