The Swiss control programme for *Salmonella* Enteritidis in laying hens: experiences and problems

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Summary

The Swiss control programme for *Salmonella* Enteritidis began at the end of 1993. All efforts are focused on the elimination of infected parent and layer flocks and on the production of *S.* Enteritidis-free eggs. The new Zoonosis Order and more stringent import regulations help to identify *S.* Enteritidis-positive parent layer and layer flocks. Other measures, such as additional voluntary monitoring of parent layer flocks, hatcheries and layer flocks, increased hygiene on poultry farms and the use of heat-treated feed, serve to prevent the spread of *S.* Enteritidis. An important point of concern is the elimination of *S.* Enteritidis from contaminated poultry farms, particularly from free-range farms. In the last two years, the number of reported infections of *S.* Enteritidis in humans has almost fallen to the level of 1988 (the year before the onset of *S.* Enteritidis infection in laying hens in Switzerland).

Keywords

Laying hens - Monitoring - Poultry diseases - *Salmonella* Enteritidis - Switzerland.

Introduction

Since the early 1980s, infections of humans with *Salmonella* Enteritidis have increased significantly in industrialised countries (1, 17). In some of these outbreaks, a strong correlation with the consumption of raw or lightly cooked eggs and egg products was established in trace-back studies (2, 3, 6, 10, 12, 13, 16, 18). Subsequently, *S.* Enteritidis-infected laying flocks were detected in many countries (5, 8, 9, 19). In Switzerland, the first cases of infected parent layer and layer flocks with *S.* Enteritidis, localised in the ovary and/or oviduct, were reported in 1989 (8). Since then, 160 *S.* Enteritidis-infected flocks have been identified and eradicated (Table I).

The detection of infected flocks is difficult because laying hens rarely show signs of clinical disease. Single contaminated eggs with low numbers of bacteria are laid intermittently (7, 11, 14). *S.* Enteritidis is generally isolated mostly from the shell membrane, but also from the egg white and yolk, which means that vertical transmission to the hatching chick may occur and that hygienic measures during egg collection do not stop transmission of the agent (15).

Several infected consignments of day-old parent layer and layer chicks were imported into Switzerland as late as 1993 because the risk of this zoonosis had not been fully recognised. However, the severe increase in human infections...
salmonellosis led to the introduction of improved control measures for this infection in poultry.

Surveillance and control measures in Switzerland

The Swiss control policy aims to stop the two most important routes of infection with S. Enteritidis.

The vertical route is, in the opinion of the author, still the most important route of transmission. This route is controlled by surveillance of imported living poultry and by monitoring the breeding flocks. Such control is facilitated by the fact that all breeding birds and some laying birds are imported as day-old chicks. By intensified monitoring during the 15-week quarantine, the vertical introduction of S. Enteritidis can be identified. Serological surveillance at the port of entry is an important aid for following up suspect flocks.

The horizontal spread of S. Enteritidis is maintained through inadequate cleansing and disinfection measures and insufficient vector control on contaminated farms. One of the main difficulties in reducing the horizontal spread is the increasing number of small laying flocks, particularly with free-range management. The banning of cages for poultry facilitates the spread of S. Enteritidis but enables easier identification of infected flocks.

The material submitted to the laboratory is listed in Tables II-V. Liver, caecal contents and yolk from 10 chicks are pooled and homogenised. Pre-enrichment in buffered peptone water (1:10; 37°C; 18-24 h) is used where sublethally damaged Salmonella are expected (e.g., control after disinfection, dry faeces with litter, chick-box liners). All samples are enriched in tetrathionate broth (1:10; 37°C; 18-24 h), followed by plating on selective media (brilliant green agar; xylose lysine tergitol 4 agar) (37°C; 18-24 h). Suspect colonies (5-15 per plate) are confirmed biochemically and by rapid agglutination with polyvalent salmonella-O-antiserum. Monovalent antiserum against the somatic antigen O:9 and swarm agar with monovalent antisera against flagellar antigen-factor g,m are used for identification of S. Enteritidis. Phagetyping is performed by the Bundesstaatliche Untersuchungsanstalt für Salmonellen (Federal Bacteriological Serological Investigation Office in Graz, Austria), according to the typing scheme of the Central Public Health Laboratory, London, United Kingdom.

### Table III

<table>
<thead>
<tr>
<th>Sample collection</th>
<th>Samples examined</th>
</tr>
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<tbody>
<tr>
<td>During rearing</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>20 live chicks</td>
</tr>
<tr>
<td></td>
<td>10 chick-box liners per 1,000 chicks</td>
</tr>
<tr>
<td>Week 1</td>
<td>All dead or culled chicks, faeces*</td>
</tr>
<tr>
<td>Week 2</td>
<td>All dead or culled chicks, faeces*</td>
</tr>
<tr>
<td>Week 3</td>
<td>10 culled chicks per 1,000 chicks</td>
</tr>
<tr>
<td>Week 5</td>
<td>Faeces*, 20 blood samples**</td>
</tr>
<tr>
<td>Week 8</td>
<td>Faeces*</td>
</tr>
<tr>
<td>Week 12</td>
<td>Faeces*</td>
</tr>
<tr>
<td>Week 15</td>
<td>Faeces*, 30 blood samples</td>
</tr>
<tr>
<td>Week 22</td>
<td>Fresh blood slide agglutination with S. Gallinarum of 10% of the birds on the farm</td>
</tr>
<tr>
<td><strong>During production</strong></td>
<td></td>
</tr>
<tr>
<td>Every 8 weeks</td>
<td>Faeces*, 20 blood samples**</td>
</tr>
<tr>
<td>At the hatchery</td>
<td></td>
</tr>
<tr>
<td>From every hatch</td>
<td>Hatcher fluff, eggshells and meconium from 250 chicks</td>
</tr>
</tbody>
</table>

* 60 pooled faecal samples of 1 g
** Tested using the enzyme-linked immunosorbent assay against flagellar antigen

The control of S. Enteritidis in Swiss laying flocks is based on four different subprogrammes: improved import regulations, the voluntary parent layer control programme, the new Zoonosis Order and the supplementary surveillance of laying flocks.
Voluntary parent layer control programme

Since June 1993, the majority of parent layers in Switzerland (95% of the total) have been integrated into a voluntary monitoring programme for S. Enteritidis. The regular examination of culled and dead birds, faeces, drag swabs (two 10 x 10 cm surgical gauze swabs moistened with buffered peptone water are dragged back and forth for the entire length of the pen) and blood samples is intended to identify infected flocks before they go into production. Furthermore, hatchery fluff, eggshells and meconium from every hatch are tested during the layer stage (Table III).

To date, only one of 51 parent layer flocks in production — into which infection was introduced, probably as a result of horizontal transmission from broilers on the same premises — has been identified by a hatchery sample as being infected and has been eliminated.

The new Zoonosis Order

The new Zoonosis Order introduced in 1994 lists infection with S. Enteritidis in poultry as a notifiable disease. A monitoring procedure is enforced on every parent breeder and layer flock containing more than 50 birds (Table IV).

In cases of suspected infection (i.e., where there is a strong correlation with human cases, positive serology or S. Enteritidis is found in drag swabs or faeces), additional samples must be taken as soon as possible by an authorised veterinarian. Infected parent layer and layer flocks are eradicated. Premises must be controlled after cleansing and disinfection. This procedure must be repeated if S. Enteritidis is found to be present. Furthermore, preventive measures are discussed with the farmer (monitoring of new birds, hygiene on the farm, rodent control and building improvements).

This procedure has shown so far that 1% to 2% of the 2,500 smaller flocks (those containing 50 to 500 birds) are still infected.

Supplementary surveillance of laying flocks

Some major food distributors require egg contractors to conduct regular supplementary testing for S. Enteritidis in layer flocks. A company that produces approximately 30% of eggs in Switzerland prescribes the procedure described in Table V. In larger flocks (500-12,000 birds) the incidence of S. Enteritidis is low (< 0.5%).

Furthermore, specifically concentrated efforts to produce Salmonella-free feedstuffs in mills with a hazard analysis and critical control point (HACCP) concept for Salmonella, as well as the improvement of hygienic standards on farms, aid in the attempt to produce and maintain Salmonella-free layer flocks. Increasing use of acid-treated and heat-treated feed during rearing and egg production reduces the prevalence of Salmonella from this source.

Experiences and problems

Although the more stringent measures for the control of S. Enteritidis in Swiss poultry were introduced after some delay, the results of this programme seem very promising. The
spread of S. Enteritidis is reduced by the interruption of vertical transmission, which keeps infection pressure low, and horizontal transmission of S. Enteritidis is rare. The human S. Enteritidis infection rate has been reduced markedly. This improvement has been achieved not only by strict surveillance of poultry but also through additional measures, such as refrigeration of eggs and the use of pasteurised eggs in catering for groups at risk (e.g., in old-age homes and hospitals).

The success of these measures is undisputed and it is clear that such effectiveness is due to the eradication of infected flocks. Various other measures were discussed and tested before the introduction of the control programme as a means of avoiding or reducing the costs of eradication (which have so far reached more than eight million Swiss francs).

Chemotherapy of infected chicks, pullets and laying hens with enrofloxacin (10 mg/kg body weight) for 10 days did not effectively eliminate S. Enteritidis. Part of this failure was due to the fact that poultry in Switzerland must be kept on the floor (animal welfare regulations introduced a ban on cages on 1 January 1992). Reinfection of treated flocks of different age groups was observed in all cases, even if further measures such as competitive exclusion and dislocation of flocks were applied.

Salmonella Enteritidis cannot be eliminated from contaminated farms by vaccination. Inactivated S. Enteritidis vaccine has been used with relative success. Vaccination with live avirulent S. Typhimurium is only accepted as a supporting measure when pullets are being transferred to farms with previously infected flocks, and for parentbroilers. Parent layers are not vaccinated. There are several arguments against the introduction of routine vaccination, such as the success of the control programme in Switzerland, possible interference with planned serological monitoring, farmers having unrealistic expectations of the efficacy of vaccination, consequently followed by reduced efforts in hygiene, and contradictory experiences with the wide use of vaccination abroad.

For economic reasons, the pasteurisation of eggs from infected flocks is no alternative.

Monitoring at the abattoir was rejected as it is a retrospective surveillance instrument. Furthermore, only a small percentage of spent hens (20% to 30%) are sent to the abattoir; the majority are sold to farmers with small flocks. Thus the spread of S. Enteritidis to smaller layer flocks would be an imminent danger.

Regular testing of various samples, such as birds, pooled faecal samples and drag swabs, provided an effective picture of the S. Enteritidis situation in parent layer and layer flocks. The phagetyping showed that phagetype 4 was most prevalent, but other phagetypes, such as 7, 8, 6a, 6 and 23, were found as well. Colonisation of the ovary, oviduct, liver or spleen was observed with most of the S. Enteritidis strains isolated: an indication of the invasive character of these strains.

From the beginning of 1993 until mid-1996, other serovars of Salmonella were found. S. Typhimurium was found 23 times, with 21 isolations of vaccine strains from recently vaccinated parent broiler flocks; S. Hadar: 21; S. Montevideo: 11; S. Agona: 8; S. Ohio: 4; S. Mbandaka: 3; S. Braenderup: 2; S. Infantis: 2; S. Tennessee: 2; S. Berta: 1; S. Brandenburg: 1; S. Heidelberg: 1; S. Orion: 1; S. Senftenberg: 1; and S. Thompson: 1. These strains have never been isolated from internal organs other than from the intestinal contents. Valuable information on the efficiency of cleansing and disinfecting farms is gained by this kind of screening, although other sources of infection, such as rodents, etc., should be considered.

Experience with disinfection on farms has confirmed observations made in other countries (4). Approximately 60% of sanitised houses were still contaminated with S. Enteritidis at final testing. It is principally old buildings and free-range farms that contribute to this unsatisfactory situation. Thus, hygienic measures should always be supported by building improvements.

Conclusion

The identification of infected flocks is a prerequisite for the control of S. Enteritidis. The official measures specified in the new Zoonosis Order and more stringent import regulations are suitable tools to identify S. Enteritidis-positive flocks. Infected flocks must be eliminated. In the future, a combination of cultural and serological screening will be necessary, due to the decreasing prevalence of S. Enteritidis. Various enzyme-linked immunosorbent assays (ELISAs) based on lipopolysaccharides (LPS) or flagellar antigens are already in use at different stages of production (the importation of day-old chicks, dislocation of replacement pullets to the laying farm) and will complete cultural techniques for S. Enteritidis. The improved sensitivity of ELISAs will further help to identify infected laying hens, particularly in backyard flocks.

In Switzerland, all efforts are focused on the establishment of S. Enteritidis-free parent layer and layer flocks. Consumer confidence has returned. Egg consumption has increased by 12 eggs per person in the last two years. During this time, the number of reported infections due to S. Enteritidis in humans has fallen almost to the level of 1988 (the year before the onset of S. Enteritidis infection in laying hens in Switzerland).
Programme suisse de lutte contre Salmonella Enteritidis chez les poules pondeuses : expérience et problèmes

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Résumé

Mots-clés

El programa suizo para el control de Salmonella Enteritidis en gallinas ponedoras: experiencia y problemas

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Resumen
Desde finales de 1993 viene aplicándose en Suiza un programa de control de Salmonella Enteritidis. Todos los esfuerzos se concentran en lograr la eliminación de bandadas infectadas de reproductoras y ponedoras y la producción de huevos libres de S. Enteritidis. El nuevo Decreto sobre Zoonosis, sumado a normas de importación más estrictas, ayudan a detectar las bandadas de reproductoras y ponedoras infectadas por S. Enteritidis. Existen otras medidas que previenen la propagación de S. Enteritidis, entre ellas una vigilancia más intensa, con carácter voluntario, de bandadas de reproductoras, así como de instalaciones de incubación y de bandadas de ponedoras, un mayor nivel de higiene en las granjas avícolas y el uso de pienso sometidos a tratamiento térmico. Especial importancia se otorga a la eliminación de S. Enteritidis de granjas avícolas contaminadas, especialmente de granjas al aire libre. En los últimos dos años, el número de casos de infección humana por S. Enteritidis ha descendido hasta un nivel cercano al de 1988 (año anterior a la aparición en Suiza de las infecciones de gallinas ponedoras por S. Enteritidis).

Palabras clave
References


