The Ability of *Salmonella* Enteritidis Isolated from Chicks Imported from England to Cause Transovarian Infection

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**ABSTRACT.** *S.* Enteritidis HY-1 isolated during quarantine from chicks imported from England was used. Laying hens at the age of 34 weeks were inoculated orally with $10^{10}$ organisms (10 birds), intramuscularly with $10^7$ (5 birds), and intravenously with $10^7$ (5 birds). Egg production did not change in hens infected orally, although it was reduced in hens infected intramuscularly for 2–3 weeks post inoculation. For one month, internally infected eggs of which the shells were not contaminated were found: one out of 65 eggs in hens infected orally and three out of 36 eggs in hens infected intramuscularly. This experiment demonstrated the ability of *S.* Enteritidis isolated from chicks imported from England to cause transovarian infection.—**KEY WORDS:** laying-hen, *S.* Enteritidis, transovarian infection.

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A recent increase in *Salmonella* choleraesuis subspecies choleraesuis serovar Enteritidis infection in poultry flocks has been observed in England, the United States and other countries (1, 2, 12, 14). This condition threatens the poultry industry and *S.* Enteritidis is now recognized as an important pathogen in poultry and has been isolated from broiler, breeder and commercial egg-laying flocks [8, 10, 12]. Moreover, it was associated with a dramatic increase in the number of outbreaks of food poisoning in man due to *S.* Enteritidis [5, 6, 13, 14].

It might be inevitable that Japan will be influenced by this situation, because about one million grandparents and parent stocks are imported each year as newly-hatched chicks from England and other countries. *S.* Enteritidis was isolated from chicks of broiler parent stock imported from England in October, 1988 and March, 1989. These flocks were treated with chloramphenicol during quarantine and the chicks were later released after shedding of *S.* Enteritidis ceased. Moreover, it was also demonstrated that *S.* Enteritidis was isolated from flocks of the grown-up chickens and their offspring (The 109th meeting of the Japanese Society of Veterinary Science, Oct., 1990). Moreover, in April, 1989 *S.* Enteritidis was also isolated from chicks imported from England [17].

On the other hand, a dramatic increase in the number of outbreaks of food poisoning in man due to *S.* Enteritidis has been observed in Japan since 1989 [3].

The present study demonstrates the ability of *S.* Enteritidis isolated from chicks imported from England to cause transovarian infection in laying hens.

*S.* Enteritidis HY-1, isolated from chicks imported from England at Himeji Livestock Hygiene Service Center, Hyogo Pref., March, 1989, was provided by Dr. Yuzuru Ichihara. This *S.* Enteritidis HY-1 was of phage type 4 (Dr. Akiko Nakamura, National Institute of Health, Japan). It was also shown to possess 36 Megadalton plasmid. Twenty white leghorn Pathogen Free chickens (Nippon Institute for Biological Science, Tokyo, Japan) were used at the age of 34 weeks. Ten birds (Group J) were inoculated orally with overnight heart infusion broth (HIB) (Eiken Co., Ltd., Tokyo, Japan) culture with shaking, 5 birds intramuscularly (Group K) and 5 birds intravenously (Group I). Inoculum sizes were 1.0 x $10^5$, 5 x $10^5$, and 5 x $10^6$ colony-forming units (CFU), respectively. Clinical signs, egg production and mortality were recorded.

Eggs were taken four times a day. These were placed for 10 min in 10 ml of Haja tetrahionate broth (HTT) (Eiken) in plastic bags. The eggs were then disinfected with a warm solution of 600 ppm chlorine for 5 min [16]. After washing with sterile water 3 times, the eggs were broken onto plastic petri dishes. All of albumen was collected with a pipette and transferred to 90 ml of HIB. The egg yolk was also collected with a plastic syringe without a needle and transferred into 90 ml of HIB. HTT and HIB were incubated at 42°C overnight and then a loopful of these broth cultures was streaked onto desoxycholate-hydrogen sulfide-lactose (Eiken) agar plates. *Salmonella*-like colonies were purified and biochemically tested with triple sugar iron agar, SIM medium and lysin decarboxylase broth (Eiken). They were then checked by plate agglutination test with an antisera prepared in rabbits.

Figure 1 shows the egg production of Group J and K

![Graph showing egg production over time](image-url)
Table 1. Isolation of S. Enteritidis from egg shell, egg yolk and egg white of hens during first month after inoculation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of eggs examined</th>
<th>No. of positive eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shell</td>
<td>White</td>
</tr>
<tr>
<td>J</td>
<td>65</td>
<td>26</td>
</tr>
<tr>
<td>K</td>
<td>36</td>
<td>13</td>
</tr>
</tbody>
</table>

a) Shells of these eggs were not contaminated.

chickens. The egg production of Group J did not change throughout the experimental study. On the other hand, that of Group K was reduced for about 2–3 weeks post inoculation (PI). No abnormal clinical signs were observed in Group J or K chickens, although one chicken in Group J died. All chickens in Group I died within one week PI.

Table 1 shows the isolation of S. Enteritidis from egg shell, egg white and egg yolk. About one-third of the egg shells were positive for one month PI. One egg white out of 65 eggs in Group J, and one egg yolk, and two egg yolks and whites out of 36 eggs in Group K were positive but the egg shells of these eggs were negative. Two and three months PI, only one egg shell was positive for S. Enteritidis and there were no egg yolks or whites that were positive.

Transovarian infection has been demonstrated in hens infected naturally and artificially [4, 7, 8, 14–16]. The present study indicates that S. Enteritidis HY-1 isolated from chicks imported from England can be transmitted in the albumen or yolk of the eggs.

In a previous study [11], no transovarian infection was demonstrated in eggs produced by 12 laying hens inoculated orally with $10^6$ CFU. The difference between two sets of results might be due to the inoculum doses.

The frequency of infection in eggs was one out of 65 (Group J) and 3 out of 36 eggs (Group K) during the first month PI. Although widely differing frequencies of egg yolk and white infection have been reported, the proportion of naturally or artificially infected eggs has been about 1% or less [6, 9]. Our results in Group J chickens inoculated orally showed similar frequency.

Shivaprasad et al. [14] emphasized the role of the oviduct or peritoneal cavity in egg contamination because albumen was contaminated more frequently than yolk. Although our study was limited because of the small scale of the experimental design, S. Enteritidis was isolated from the oviducts of Group 1 chickens inoculated intravenously. Barrow and Lovell [4] reported that oviduct infection appeared to be the result of haematogenous spread resulting from the intravenous route of inoculation.

This experiment demonstrated the ability of S. Enteritidis HY-1 isolated during quarantine from chicks imported from England to cause transovarian infection.

References