*Eimeria tenella* Infection Induces Recrudescence of Previous *Salmonella enteritidis* Infection in Chickens


Department of Veterinary Medicine, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 593, Japan

**ABSTRACT**

Four experiments were conducted to examine the effect of *Eimeria tenella* infection on the recrudescence of *Salmonella enteritidis* in previously infected chickens. Significant recrudescence of cecal colonization in the birds challenged with *E. tenella* was observed at 3 wk after *S. enteritidis* infection in Experiment 1, at 3 and 4 wk in Experiment 2, and at 3, 4, and 5 wk in Experiments 3 and 4. The recrudescence was indicated by .81 to 6.31 logs increase in cecal *S. enteritidis* counts and by higher percentages of ceca that were culture-positive. The possible prolonged cecal *S. enteritidis* shedding from chickens infected with coccidia into the environment might be important for the perpetuation of *S. enteritidis* infectious cycle. Except for Experiment 1, in which a significant higher culture-positive rate of the liver was detected in the coccidia-infected group, no significant difference of culture-positive rate of liver and spleen between the treatments was observed. The recrudescence of previous *S. enteritidis* infection caused by *E. tenella* infection was obviously related to the initial *S. enteritidis* dose size and time of exposure to coccidia.

*(Key words: Salmonella enteritidis, recrudescence, Eimeria tenella, coccidia)*

INTRODUCTION

*Salmonella enteritidis* has become the predominant *Salmonella* serotype isolated from both human and poultry in an increasing number of countries in recent years, including Japan (Nakamura, 1994), Italy (Binkin et al., 1993), Spain (Lujan et al., 1990), Poland (Glosnicka and Kunikowska, 1994), Portugal (Machado and Bernardo, 1990), Germany (Kist, 1991), and the Netherlands (Noordhuizen and Franken, 1994). As chicken eggs were implicated as the main source of human infection (St. Louis et al., 1988), the control of *S. enteritidis* infection in chickens may be a key to the prevention of the infection in humans.

Extensive field investigations have shown that infection of *S. enteritidis* within contaminated flocks was usually at relatively low levels (Ebel et al., 1992; Poppe et al., 1991a,b, 1992). When chickens were infected with *S. enteritidis*, some of the *S. enteritidis* may exist in the ceca and other organs and be shed into the environment intermittently for a long time (Barrow, 1991; Humphrey et al., 1989; Nakamura et al., 1993a).

Experimental work has indicated that *S. enteritidis* infection and shedding into environment could be exacerbated by various stresses, including induced molting (Holt, 1993), heat, introduction of young chickens, temporal removal of feed and water (Nakamura et al., 1993b,c), and some underlying diseases (Opitz et al., 1990; Qin et al., 1995). The stresses and diseases, therefore, may act as inductives and amplifiers in the epidemic of *S. enteritidis* infection.

Coccidiosis has been one of the most common diseases in chickens all over the world, even though anticoccidial agents
EIMERIA TENELLA AND SALMONELLA ENTERITIDIS

have been extensively administered in the feed. Prior work in authors' laboratory has indicated that E. tenella infection enhanced the severity of S. enteritidis infection in chickens and might prolong the shedding of S. enteritidis into the environment (Qin et al., 1995). The present study was conducted to evaluate the effects of E. tenella infection on chickens that were infected with S. enteritidis 3, 4, and 5 wk prior to coccidia exposure.

MATERIALS AND METHODS

Birds and Diets

White Leghorn Hy-Line® cockerels were purchased from a local commercial hatchery. They were caged in battery brooders in an air-conditioned room with continuous artificial illumination and given basal feed (Arakawa and Ohe, 1975). Chickens were euthanatized by cervical dislocation for bacteriological examination.

Salmonella and Coccidia

A strain of S. enteritidis was isolated from eggs that had been implicated in an egg-borne outbreak of S. enteritidis infection in humans. It was confirmed to be phage type 4 by standard laboratory procedures.

Oocysts of E. tenella were prepared from donor chickens 1 wk after oral inoculation. A single oral inoculum of 4 × 10^4 sporulated oocysts suspended in 1 mL tap water was given to the crop of each bird through a long blunt-end injection needle. The caecal lesions at necropsy were scored 3 to 4 according to the thickness of cecal wall, cecal contents, and amounts of blood and caseous core as described by Johnson and Reid (1970).

Media

Trypto soya broth¹ was used to prepare S. enteritidis inoculum. Mannitol lysine crystal violet brilliant green (MLCB)² agar plate was utilized to count S. enteritidis in the inoculum and cecal contents, and to confirm the presence of S. enteritidis in liver and spleen. Hajna tetraphionate broth² was employed to selectively enrich S. enteritidis in liver and spleen samples.

Experimental Designs

Experiment 1. There were three treatment groups of 30 birds each in a trial: an uninfected control, a group infected with 10^2 cfu of S. enteritidis per day consecutively at 2 and 3 d of age, and a group infected with S. enteritidis in the same way as the former group, in which 10 birds were infected with E. tenella 1 wk prior to necropsy. Ten birds from each treatment group were killed at 3, 4, and 5 wk after S. enteritidis infection for bacteriological examination. This experiment consisted of two identical trials.

Experiment 2. The design was identical to that of Experiment 1, except that birds were infected with 10^4 cfu of S. enteritidis.

Experiment 3. This experiment was similar to that of Experiment 1, except that there were 15 birds in each group and the S. enteritidis dose was 10^6 cfu/d. Five chickens were exposed to E. tenella 1 wk before each necropsy, and five birds from each treatment were necropsied at a time. The three trials were replicated.

Experiment 4. Chickens were infected with 10^8 cfu of S. enteritidis. The other procedures were the same as those of Experiment 3.

Bacteriological Examination

At necropsy, disposable gloves and scissors, sterilized over flame, were used for each sampling. Samples of liver, spleen, and ceca were enriched in 10 mL of Hajna tetraphionate broth at 37 C for 24 to 28 h, and one loopful of the contents was transferred to a MLCB plate to confirm the presence of S. enteritidis. At the same time, .1-g sample of cecal contents was diluted by serial 10-fold procedures. Then, a .1-mL suspension from 10^1, 10^3, 10^5, and 10^7 diluted contents were each spread on a MLCB agar plate. Colonies with a diameter of 3 to 5 mm, a convex surface, and a black center were counted after 24 h incubation.

¹Nissui, 2-11-1, Sugamo, Toyoshima-ku, Tokyo, Japan.
²Eiken Chemical Co., Ltd., 33-8 Hongo 1-Chome, Bunkyo, Tokyo, Japan.

Downloaded from https://academic.oup.com/ps/article-abstract/74/11/1786/1574523 by guest on 14 November 2018
presumptive *S. enteritidis* colonies were further confirmed biochemically and serologically.

Cecal samples, without growth in either Hajna tetrathionate broth enrichment or the MLCB plate, were considered to be negative for recoverable *S. enteritidis* and assigned a value of 1 cfu/g of sample. Samples that were negative in the MLCB plates but were culture-positive following tetrathionate enrichment were considered to be positive for recoverable *S. enteritidis* and assigned a value of 99 cfu/g when the results were logarithmically transferred for statistical analysis.

**Statistical Analysis**

Data on the number of *S. enteritidis* in the cecal contents after logarithmic transformation were subjected to Student's *t* test, and the difference between treatments in the frequency of recovery of *S. enteritidis* in organs was determined by chi-square analysis (Steel and Torrie, 1960). As no significant variation was observed among the replicate trials within an experiment, the results were combined for analysis.

**RESULTS**

**Experiment 1**

The data of cecal *S. enteritidis* and number of organs positive for *S. enteritidis* are shown in Table 1. At 3 wk postinoculation, exposure to *E. tenella* significantly induced the recrudescence of *S. enteritidis* infection in the ceca (*P < .01*) and liver (*P < .05*). By 4 and 5 wk, no *S. enteritidis* was found in any of the groups.

**Experiment 2**

Results of Experiment 2 are shown in Table 2. *Eimeria tenella* infection resulted in a significant recurrence of cecal *S. enteritidis* colonization, as indicated by the difference of cecal counts at 3 and 4 wk and number of culture-positive samples at 3 wk between the two treatments were significant (*P < .01*). However, no remarkable recurrence of *S. enteritidis* infection was observed by 5 wk.

A higher culture-positive percentage of liver and spleen was observed in the group infected with coccidia and *Salmonella* group at Table 3 than in the *Salmonella*-infected group, but the differences were not significant.

**Experiment 3**

Results of this experiment are summarized in Table 3. *E. tenella* infection led to a significant exacerbation of *S. enteritidis* infection (*P < .01*). The percentages of chickens that were culture-positive (ceca) in coccidia-infected chickens were significantly (*P < .05*) greater than those in the uninfected birds. *E. tenella* infection did not cause a significant exacerbation of *S. enteritidis* colonization in liver and spleen in this experiment.

**Experiment 4**

Results are presented Table 4. Cecal counts of *S. enteritidis* was significantly increased by *E. tenella* infection (*P < .01*). The difference in the isolation frequency from liver and spleen between the two groups was not significant.

In all the experiments, control groups remained negative throughout the observation period.

**DISCUSSION**

When chickens were infected with *S. enteritidis*, most of the organisms were cleared within several weeks (Gast and Beard, 1990), but a small number of *S. enteritidis* could survive in the ceca and other organs for extended period of time (Barrow, 1991; Nakamura et al., 1993a). The present study examined whether *E. tenella* infection could induce a recrudescence or exacerbation of *S. enteritidis* infection. The results indicated that *E. tenella* infection caused a .8 to 6.3 log increase of cecal *S. enteritidis* population within 5 wk after *S. enteritidis* infection.

The cecal shedding of an increased number of *S. enteritidis* would result in greatly amplified environmental contamination, which may play an important role in the perpetuation of *S. enteritidis* infections among chickens. However, extensive field epidemiological investigations showed that the infection rate within a
TABLE 1. Effect of *Eimeria tenella* infection on the recrudescence of *Salmonella enteritidis* infection in chickens previously infected with *S. enteritidis* for 2 consecutive d, Experiment 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Ceca1</th>
<th>Logs2</th>
<th>Liver</th>
<th>Spleen</th>
<th>Ceca</th>
<th>Logs</th>
<th>Liver</th>
<th>Spleen</th>
<th>Ceca</th>
<th>Logs</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected with <em>S. enteritidis</em></td>
<td>1/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>.11 ± .45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**No *S. enteritidis* was found in the control group during the observation.**

**A,B**Values within a column with no common superscript differ significantly (*P < .01*).

1Number of samples positive for *S. enteritidis* per number of samples examined.

2Means and standard deviations after logarithmic transformation.

TABLE 2. Effect of *Eimeria tenella* infection on the recrudescence of *Salmonella enteritidis* infection in chickens previously infected with *10^4* cfu of *S. enteritidis/d* for 2 consecutive d, Experiment 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Ceca1</th>
<th>Logs2</th>
<th>Liver</th>
<th>Spleen</th>
<th>Ceca</th>
<th>Logs</th>
<th>Liver</th>
<th>Spleen</th>
<th>Ceca</th>
<th>Logs</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected with <em>S. enteritidis</em></td>
<td>5/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>.81 ± 1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 ± .7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**A,B**Values within a column with no common superscript differ significantly (*P < .05*).

1Number of samples positive for *S. enteritidis* per number of samples examined.

2Means and standard deviations after logarithmic transformation.
TABLE 3. Effect of *Eimeria tenella* infection on the recrudescence of *Salmonella enteritidis* in chickens previously infected with $10^8$ cfu of *S. enteritidis*/d for 2 consecutive d, Experiment 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Results at week postinoculation with <em>S. enteritidis</em></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 wk</td>
<td>4 wk</td>
<td>5 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceca¹</td>
<td>Log⁵</td>
<td>Liver</td>
<td>Spleen</td>
<td>Ceca</td>
<td>Log⁵</td>
<td>Liver</td>
</tr>
<tr>
<td>Uninfected control</td>
<td>No <em>S. enteritidis</em> was found in the control group during the observation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected with <em>S. enteritidis</em></td>
<td>5/15B (33%)</td>
<td>2.12 ± 1.8B</td>
<td>4/15</td>
<td>3/15B</td>
<td>4.28 ± 1.9A</td>
<td>2/15</td>
<td>14/15A (100%)</td>
</tr>
<tr>
<td></td>
<td>Infected with <em>S. enteritidis</em> and <em>E. tenella</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15/15A (100%)</td>
<td>7.5 ± 1.6A</td>
<td>9/15</td>
<td>14/15A</td>
<td>4.28 ± 1.9A</td>
<td>2/15</td>
<td>11/15A (100%)</td>
</tr>
</tbody>
</table>

A, B Values within a column with no common superscript differ significantly ($P < .01$).

¹Number of samples positive for *S. enteritidis* per number of samples examined.

²Means and standard deviations after logarithmic transformation.

TABLE 4. Effect of *Eimeria tenella* infection on the recrudescence of *Salmonella enteritidis* infection in chickens previously infected with $10^8$ cfu of *S. enteritidis*/d for 2 consecutive d, Experiment 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Results at week postinoculation with <em>S. enteritidis</em></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 wk</td>
<td>4 wk</td>
<td>5 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceca¹</td>
<td>Log⁵</td>
<td>Liver</td>
<td>Spleen</td>
<td>Ceca</td>
<td>Log⁵</td>
<td>Liver</td>
</tr>
<tr>
<td>Uninfected control</td>
<td>No <em>S. enteritidis</em> was found in the control group during the observation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected with <em>S. enteritidis</em></td>
<td>5/15B (33%)</td>
<td>2.18 ± 2.9B</td>
<td>6/15</td>
<td>3/15B</td>
<td>0.60 ± 1.3B</td>
<td>2/15</td>
<td>14/15A (100%)</td>
</tr>
<tr>
<td></td>
<td>Infected with <em>S. enteritidis</em> and <em>E. tenella</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15/15A (100%)</td>
<td>6.83 ± 1.5A</td>
<td>4/15</td>
<td>14/15A</td>
<td>4.9 ± 2.1A</td>
<td>0/15</td>
<td>8/15A (100%)</td>
</tr>
</tbody>
</table>

A, B Values within a column with no common superscript differ significantly ($P < .01$).

¹Number of samples positive for *S. enteritidis* per number of samples examined.

²Means and standard deviations after logarithmic transformation.
infected flock were relatively low. A reasonable explanation might be that the amounts of *S. enteritidis* in the environment was usually not enough to initiate an infection in the normal birds, but birds that are immunodepressed or that lack normal protective cecal flora, may get infected. This may be partially supported by the fact that certain amounts of *S. enteritidis* were required to establish experimental infections in both laying hens (Holt, 1993) and chicks (Qin et al., 1995). In the large-scale and intensified poultry industry, birds are always exposed to their own excreta. The increased susceptibility to *S. enteritidis* infection and excretion of a large number of *S. enteritidis* into the environment caused by coccidial infection might amplify the menace of horizontal transmission for the flock. *Salmonella enteritidis* could survive in the dry conditions for a relatively long time; therefore, the increased *S. enteritidis* population might also be a hazard for the future flocks that would occupy the house.

The heavily contaminated sewage may also contaminate the environment and surface water outside of the chicken house. A big outbreak of *S. enteritidis* infection in Nagano of Japan caused by supply water (Muramatsu and Nishizawa, 1992) focused attention on the possibility of *S. enteritidis* contamination from chicken houses.

The recurrence of cecal *S. enteritidis* colonization by *E. tenella* infection in the present study may reflect an alteration of local intestinal conditions. As the resistance to *S. enteritidis* was directly associated with the concentrations of propionic acid and other volatile fatty acids (VFA) in the cecal contents (Corrier et al., 1994), *E. tenella* infection might affect the population of some bacteria in the normal cecal flora (Kimura et al., 1976) that are related to the production of VFA and other organic acids (Corrier et al., 1991).

The present study also indicated that dose size was closely related to persistence of *S. enteritidis* infection in ceca. This was similar to what Humphrey et al. (1991) observed in laying hens.

*Eimeria tenella* infection increased the number of liver positive for *S. enteritidis* in the poultry infected with $10^2$ cfu but not in those infected with higher doses, regardless of the dramatic increase of cecal *S. enteritidis* counts. The lack of correlation between cecal *S. enteritidis* population and systemic *S. enteritidis* infection suggested a likelihood of “saturated cecal number” for systemic invasion. Identical phenomena were reported. Qin et al. (1995) noticed that the concurrent infection of *E. tenella* and *S. enteritidis* increased cecal counts of *S. enteritidis*, but did not increase the number of liver and spleen samples positive for *S. enteritidis*. Relatively high doses (Humphrey et al., 1991) caused a prolonged shedding, but did not cause a significant increase in systemic infection.

**ACKNOWLEDGMENT**

The authors wish to thank T. Tsukamoto of the Osaka Prefectural Institute of Public Health for sharing a strain of *S. enteritidis*.

**REFERENCES**


