Infectivity and Persistence of *Salmonella* Typhimurium for Bengalees, a Variety of *Lonchura striata*, Using an Isolate from a Bengalee

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(Received 1 July 1996/Accepted 17 August 1996)

**ABSTRACT.** Due to its importance in public health, *Salmonella* Typhimurium originating from a naturally infected bengalee (*Lonchura striata*), a common cage bird, was examined for its infectivity and persistence for the same species. Eight birds per group for each experiment were used. When bengalees were inoculated orally with $10^7$, $10^8$ or $10^9$ colony forming units (CFU) of *S.* Typhimurium and observed for 7 days, all the birds receiving $10^9$ CFU were positive for the organism in the liver, spleen or the intestines, and necrotic foci in the liver were observed in 6 birds. When bengalees were inoculated with $10^9$ CFU of *S.* Typhimurium and observed for 22 days, the organism was found in fecal samples throughout the experimental period and the maximum *S.* Typhimurium counts in feces were $3.9 \times 10^6$ CFU per gram. *S.* Typhimurium was recovered from the liver, spleen and intestines in 7 birds and necrotic foci in the liver were also observed in 7 birds. The results indicate that *S.* Typhimurium originating from a naturally infected bengalee is pathogenic to these birds and the persistence of the pathogen lasts at least for 22 days. — Key words: bengalee, cage bird, infectivity, public health, *Salmonella* Typhimurium.

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There have been many reports concerning *Salmonella* infection in cage birds such as canaries [1, 6, 13], pigeons [14, 19, 24, 30], parrots [9, 26] and psittacine birds [16, 17, 25]. The authors recently reported an outbreak of *Salmonella choleraesuis* subspecies *choleraesuis* serovar Typhimurium (*S.* Typhimurium) infection in bengalees, a variety of *Lonchura striata*, which was the first report in this species and was characterized by high mortality rate reached 74% [20]. The bengalee is one of the most popular cage birds and millions of the birds are reared in contact with humans [12]. The authors also isolated *S.* Typhimurium from zebra finches, *Poephila guttata*, which was the first report in this species [21]. Cage birds are known as an important source of human *Salmonella* infection [7, 15, 31] and there have been many reports on human *Salmonella* infection caused by cage birds such as parakeets [8, 11], canaries [18], parrots [10] and Easter chicks [20]. There are also some reports concerning experimental studies for medication of salmonellosis in cage birds [3, 27]. However, there is a few reports concerning experimental studies of *Salmonella* infection for its infectivity and persistence in cage birds. Due to its importance in public health, the infectivity and persistence of *S.* Typhimurium originating from a naturally infected bengalee for the same species were examined.

**MATERIALS AND METHODS**

*Birds and diets:* Adult male bengalees were obtained from a local pet shop and were kept in wire cages in an isolated room. Each cage was partitioned by solid aluminium dividers to avoid cross contamination. Filter paper was placed on tray-typed floor of each cage and replaced daily after fecal samples had been collected. Fecal samples were taken from these birds for 3 consecutive days before the start of the experiment and cultured to confirm the absence of *Salmonella*. They were given a feed for cage birds throughout the study. Tap water was given and renewed daily.

*Inoculation of Salmonella:* An isolate of *S.* Typhimurium recovered from a bengalee in a field outbreak of paralyphoid infection [20] was used. The *Salmonella* was grown in heart infusion broth (Nissui, Tokyo) at 37°C for 24 hr and resuspended in phosphate buffer solution (PBS) and then kept in a refrigerator at 4°C until the following day. The number of *Salmonella* in the suspension was counted and diluted shortly before inoculation with PBS to attain concentrations of $10^7$, $10^8$ or $10^9$ colony forming units (CFU) per ml. A volume of 0.1 ml per bird was inoculated orally into the crop of each bird.

*Experiment 1:* This experiment was designed to confirm the infectivity and pathogenicity of *S.* Typhimurium originating from a naturally infected bengalee to the same species. Thirty-two bengalees were divided into 4 groups of 8 birds per cage. Each bengalee in group 1 received an oral inoculation containing $10^2$ CFU of *S.* Typhimurium, in group 2 containing $10^4$ CFU, in group 3 containing $10^6$ CFU, and in group 4 containing 0.1 ml of PBS as a control. These birds were observed for 7 days for clinical signs and mortality and fecal samples from each cage were taken daily to confirm the excretion of *S.* Typhimurium. They were then killed using chloroform gas and examined for the presence of *S.* Typhimurium in the liver, spleen and intestines. In the present experiment, the intestines include both small and large intestines except the duodenum.

*Experiment 2:* This experiment was designed to confirm the persistence of *S.* Typhimurium originating from a naturally infected bengalee for the same species. Sixteen
bengalees were divided into 2 groups of 8 birds per cage.
Each bengalee in group 1 was inoculated orally with \(10^6\) CFU of \(S\). Typhimurium and in group 2 with 0.1 ml of PBS as a control. These birds were observed for 22 days after inoculation for clinical signs and mortality and fecal samples from each cage were taken daily to confirm the excretion of \(S\). Typhimurium. The number of \(Salmonella\) in the fecal samples taken from two spots on the floor of each cage was also counted twice a week. The birds were killed using chloroform gas 22 days after inoculation and then examined to confirm the presence of \(S\). Typhimurium in the liver, spleen and intestines.

Bacteriological examination: Hajna tetrathionate broth (Nissui, Tokyo) was used for enriching \(S\). Typhimurium in the fecal sample, liver, spleen and intestines. Desoxycholate hydrogen sulphide lactose (DHL) agar (Nissui, Tokyo) was used to count the number of \(S\). Typhimurium in the fecal samples, and to confirm the presence of \(S\). Typhimurium in the liver, spleen and intestines. The DHL agar was supplemented by 20 mg of novobiocin (Sigma Chemical Co., U.S.A.) per liter to inhibit growth of other \(Enterobacteriaceae\) such as \(Proteus\) spp. The initial dilution of the fecal sample was made by adding 9 volumes (V/W) of sterile PBS and shaken thoroughly until a homogeneous mixture was obtained. Further dilution was made with PBS by serial 10-fold steps. From each of the serial dilutions, including the initial dilution, 0.1 ml of suspension was taken and spread on the DHL agar and incubated at 37°C for 24 hr. Suspected \(Salmonella\) colonies on the cultured plates were counted. For identification, representatives from each of the suspected colonies were tested with Diagnostic \(Salmonella\) Antisera for O and H antigens (Denka, Tokyo) and \(Salmonella\) H sera was used for phase induction (Denka, Tokyo) according to the manufacturer’s instructions.

Histopathological examination: At necropsy, pieces of the liver from each bird were fixed in 10% formalin and their paraffin sections were prepared and stained with hematoxylin and eosin (HE).

RESULTS

Experiment 1: Clinical signs were not observed and all the birds remained healthy following inoculation with \(10^3\), \(10^5\) and \(10^6\) CFU of \(S\). Typhimurium. \(S\). Typhimurium was found in the fecal sample from one day after inoculation in groups 2 and 3, and the excretion of the \(Salmonella\) continued throughout the experimental period in both groups. No \(Salmonella\) excretion was observed in group 1. Seven days after inoculation, \(S\). Typhimurium was recovered from the liver, spleen or intestines in half of the birds in group 2 and in all the birds in group 3. However, no \(Salmonella\) was isolated from those organs in group 1 (Table 1).

At necropsy, white foci in the liver together with enlargement of the liver and spleen (Fig. 1) were seen in nearly half of the birds in groups 2 and 3. Also upon histopathological examination, granulomatous necrotic foci in the liver were noted in 4 birds in group 2 and in 6 birds in group 3 (Table 1).

Experiment 2: Two bengalees died at 14 days and 20 days after inoculation in group 1 shortly after the onset of clinical signs such as severe diarrhea, ruffled feathers and general weakness. \(S\). Typhimurium was found in the fecal sample from one day after inoculation in group 1 and the excretion of the \(Salmonella\) continued throughout the experimental period in this group (Table 2). The \(Salmonella\) in the fecal samples were counted twice a week and the maximum \(Salmonella\) counts in group 1 were \(3.9 \times 10^4\) CFU per gram at 22 days after inoculation (Table 3).

Twenty-two days after inoculation, \(S\). Typhimurium was recovered from the liver, spleen and intestines of 7 birds in group 1 including two birds that died day 14 and 20.

At necropsy, white foci in the liver together with enlargement of the liver and spleen were seen in most of the birds in group 1. Also upon histopathological examination, granulomatous necrotic foci in the liver (Fig. 2) were noted in most of the birds in the group (Table 2).

Table 1. Infectivity of \(S\). Typhimurium originating from a bengalee to the birds (Experiment 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Inoculum</th>
<th>Postinoculation day of fecal collection</th>
<th>7 days after inoculation</th>
<th>Focal necrosis in the liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7</td>
<td>Liver</td>
<td>Spleen</td>
</tr>
<tr>
<td>1</td>
<td>(10^2) CFU of (S). Typhimurium</td>
<td>- - - - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>(10^4) CFU of (S). Typhimurium</td>
<td>+ + + + + +</td>
<td>2/8</td>
<td>2/8</td>
</tr>
<tr>
<td>3</td>
<td>(10^6) CFU of (S). Typhimurium</td>
<td>+ + + + + +</td>
<td>5/8</td>
<td>5/8</td>
</tr>
<tr>
<td>4</td>
<td>Phosphate buffer solution</td>
<td>- - - - - -</td>
<td>0/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

(a) Number of birds positive for \(S\). Typhimurium or lesions per number of birds examined.
DISCUSSION

The bengalees inoculated with $10^4$ CFU or $10^5$ CFU of S. Typhimurium derived from a naturally infected bengalee had excreted the organism throughout the experiment. S. Typhimurium was recovered from the liver, spleen and intestines in most of the birds when they were inoculated with $10^5$ CFU of the Salmonella and histopathological examination revealed severe lesions in the liver. The results indicated that S. Typhimurium originating from a naturally infected bengalee is infective and pathogenic to other birds of the same species.

Cage birds are reported to be an important source of human Salmonella infection because these birds are reared in contact with humans [7, 15, 31]. Kaye et al. [8] reported S. Typhimurium infection in a 7-month-old male infant in which a parakeet was apparently the source of the infection. In that case, 2 parakeets had been purchased from a variety store a few weeks before the onset of the illness. Although both birds were active and appeared healthy, S. Typhimurium was isolated from one of those birds and the infected bird excreted the Salmonella into feces from $3.4 \times 10^5$ to $2.7 \times 10^7$ CFU per gram. The parakeets were kept in a cage suspended 150 cm from the floor where the infant crawled and played. The feathers and droppings occasionally fell to the floor and the infant finally had been infected. Madewell and MaChesney [11] reported also S. Typhimurium infection in a 4-month-old male infant in which 2 parakeets were the probable source of the infection. Anderson et al. [2] reported an outbreak of S. Typhimurium infection in human involving at least 29 persons, mainly infants, and demonstrated that Easter chicks were the source.

Table 2. Excretion and persistence of S. Typhimurium originating from a bengalee to the birds inoculated with $10^5$ CFU of the Salmonella (Experiment 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Inoculum</th>
<th>Postinoculation day of fecal collection</th>
<th>22 days after inoculation a</th>
<th>Focal necrosis in the liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day to 22 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>$10^5$ CFU of S. Typhimurium</td>
<td>all positive</td>
<td>7/8 (v)</td>
<td>7/8</td>
</tr>
<tr>
<td>2</td>
<td>Phosphate buffer solution</td>
<td>all negative</td>
<td>0/8 (v)</td>
<td>0/8</td>
</tr>
</tbody>
</table>

a) Two birds which died at 14 days and 20 days were included.
b) Number of birds positive for S. Typhimurium or lesions per number of birds examined.
Table 3. Number of S. Typhimurium (CFU/g) in the fecal samples in bengaleses inoculated with 10^6CFU of the Salmonella (Experiment 2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Inoculum</th>
<th>Postinoculation day of fecal collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>1)</td>
<td>10^6 CFU of S.</td>
<td>4.0 x 10^5</td>
</tr>
<tr>
<td></td>
<td>Typhimurium</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Phosphate buffer</td>
<td>5.0 x 10^5</td>
</tr>
</tbody>
</table>

a) Fecal samples were taken from two spots on the floor of the cage.

The prevalent rate of Salmonella infection among cage birds is also very important from public health point of view. Grimes and Arizmendi [5] examined 2,407 serum samples from various species of psittacine birds and reported that 1.6% were positive for S. Typhimurium agglutinins indicating a certain prevalence of Salmonella infection of carrier state in the psittacine population. Förster and Burrow [4] reported a prevalent rate of Salmonella infection as 8.3% among 495 cage birds such as finches, parrots, parakeets and canaries and S. Typhimurium was the most common serotype identified. Sawa et al. [25] examined imported cage birds which died soon after arrival and reported that 60 out of 327 finches, 6 out of 22 lories and 2 out of 120 parakeets were infected with S. Typhimurium. Apart from cage birds, Tanaka et al. [28, 29] reported that S. Typhimurium was frequently isolated from healthy dogs and the carrier state was developed in dogs which had been given orally canine feces harboring only 39–92 cells of S. Typhimurium. They concluded that carrier animals play an important role for the dissemination and multiplication of Salmonella organisms [28, 29].

In the present study, the carrier state was developed in 7 out of 8 bengaleses given orally 10^6 CFU of S. Typhimurium and the number of the Salmonella being excreted in the feces reached 10^6 CFU per gram. Furthermore, the persistence and excretion of the Salmonella lasted for 22 days. Although S. Typhimurium is generally known to be ubiquitous with a wide host range, it is very important to assess the infectivity or the pathogenicity of the Salmonella when they are isolated initially from popular cage birds such as bengaleses [20] and zebra finches [21].

The authors have reported that S. Typhimurium originating from the bengalese is infective and pathogenic to chickens and the pathogenicity is almost similar to that of Salmonella derived from the chicken [22]. Moreover, S. Typhimurium from the zebra finch was also infective for those finches and the persistence lasted at least for 22 days [23]. The present findings that S. Typhimurium originating from a naturally infected bengalese is severely pathogenic to other birds of the same species and the persistence of the Salmonella lasts for the long duration clearly suggest that Salmonella infection in bengaleses can be a source of human Salmonella infection. Due to its importance in public health, further studies are needed to define the excretion and persistence of Salmonella in other cage birds.

REFERENCES