Incidence of Salmonella Infection in Healthy Dogs in Gifu Prefecture, Japan

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ABSTRACT. A total of 1,013 feces samples and 8 mesenteric lymphonodus samples obtained from apparently healthy dogs were examined for the incidence of salmonella infection. One strain of S. typhimurium (ST) was isolated from feces of one dog, and S. enteritidis (SE) was isolated from the mesenteric lymphonodus of one dog. Sera obtained from 330 apparently healthy dogs were examined for Salmonella antibodies using an ELISA with heated whole cells of SE and ST. Fifty-one of the 330 serum samples were considered to be positive for salmonella antibodies, including 12 which were SE-positive and 39 which were ST-positive. These results indicate that dogs cause possible environmental problems as Salmonella carriers.

KEY WORDS: canine, ELISA, Salmonella.

Although there have been many reports on the isolation of salmonellae from apparently healthy dogs [4, 10, 11, 13], clinical salmonellosis is uncommon in dogs. Information on the incidence of salmonellae infections in dogs, including those in the carrier state is very important for public health, because dogs are usually kept in close contact with humans [4, 7]. There are some reports on transmission of salmonellae from dogs to humans [4–8], but there have been no serological studies on the incidence of salmonella infection in dogs.

The objects of this study were to determine the incidences of salmonellae infections in healthy dogs using bacteriologic and serologic techniques.

A total of 1,013 feces samples were obtained from healthy dogs at Gifu University Veterinary Medical Teaching Hospital, and 8 samples of the mesenteric lymphonodi were obtained from healthy dogs at the Laboratory Animal Center of the same university.

Each feces and lymphonodus sample was placed in a tube containing approximately 10 times of Hajna tetrathionate broth (Eiken, Tokyo, Japan) as volume. The tube was shaken thoroughly and kept at 37°C for 1 or 2 days. Then, one loop of cultured broth was spread on DHL agar (Nissui, Tokyo, Japan) or mannitol lysine crystal violet brilliant green agar (MLCB; Nissui), and these agar plates were incubated at 37°C for 24 hr. Suspicious colonies were confirmed by agglutination tests using serum against Salmonella antigens (Denka Seiken, Tokyo, Japan).

Salmonella typhimurium (ST) was isolated from the feces of one dog, and Salmonella enteritidis (SE) was isolated from the mesenteric lymphonodus of one dog (Table 1).

A total of 330 serum samples were obtained from healthy dogs at Gifu University Veterinary Medical Teaching Hospital.

SE [2] and ST [1] which are the main pathogens of human salmonellosis were used as whole antigens for ELISA. Each bacterium was grown statically in Tryptoseoy broth (Nissui) at 37°C for 20 hr. The concentration of each bacterium suspension was about 10⁷ colony forming units/ml. Whole Salmonella antigen was prepared by heating (65°C, 1 hr) each of the bacterium suspensions and purification by centrifugation of each of the suspensions at 500 × g for 30 min. Flat-bottomed 96-well ELISA plates (Nalge Nunc International, NY, U.S.A.) were coated with 100 µl of 1:200 diluted Salmonella whole antigen. After washing four times with 0.05% Tween 20/PBS, non-specific binding was blocked by incubating the plate with 1% skim milk in PBS at 37°C for 1 hr. After washing one time with 0.05% Tween 20/PBS, 5 µl of a test serum sample was added to each well, and the plates were incubated at 37°C for 30 min with 50 µl goat anti-dog IgG-heavy and light chain: HIPO conjugate (Bethyl Laboratories, TX, U.S.A.). The plates were washed again four times and incubated with 50 µl of O-phenylenediamine for 30 min at room temperature. The single wavelength absorbance was measured at 495 nm by an ELISA plate reader (Bio-RAD Model 550, Bio-Rad Laboratories, Hercules, CA, U.S.A.). When the OD level of the serum sample divided by the OD level of the SE-positive control was over 2.5, the serum sample was considered to contain SE antibodies, and in the test for ST antigen the serum sample was considered to contain ST antibodies if

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<tr>
<th>Table 1. Numbers of Salmonella species isolated from feces and mesenteric lymphonodus samples in healthy dogs</th>
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<tbody>
<tr>
<td>Salmonella enteritidis</td>
</tr>
<tr>
<td>positive</td>
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<tr>
<td>Feces</td>
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<tr>
<td>Mesenteric lymphonodus</td>
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this value was over 2.0.

It was considered that of the 330 serum samples, ten had SE antibodies only, 25 had ST antibodies only, and 16 had both antibodies.

Salmonella enteritidis-antibody test kit (IDEXX, Me, U.S.A.) [9], a kit for identifying SE using gm-flagellum by ELISA, was used to confirm the presence of both antibodies in those 16 samples.

Two of the 16 samples were confirmed to have SE antibodies. Based on these results, 51 of the 330 serum samples were considered to have Salmonella antibodies, 12 samples were considered to have SE antibodies, and 39 samples were considered to have ST antibodies (Table 2).

The incidence of salmonella infection in the feces samples in this study was lower than that reported previously [3, 10, 12]. However, the high incidence of Salmonella antibodies in the serum detected by ELISA in the present study is similar to previously reported high incidences in fecal samples. Although bacteriologic and serologic techniques for detection of salmonella have different sensitivities, the high incidence of salmonella antibodies detected by ELISA indicates that many dogs are infected with salmonellae and that large numbers of salmonellae are present in areas in which dogs move around. Since dogs are usually in close contact with humans, knowledge of the incidence of salmonella infection including the carrier state in dogs is very important for public health.

The results of the ELISA assays showed that serum samples from dogs positive for Salmonella in feces or lymphonodus samples did not have Salmonella antibodies. It was considered that the salmonellae passed only transiently through the digestive tract of the dogs, and that the organisms did not stimulate or did not indicate the immunological system.

These results indicate that many dogs, even those negative for salmonellae antibodies, can in fact be infected with Salmonella and that these dogs spread salmonellae to other dogs when they are being taken for walks.

REFERENCES

Table 2. Numbers of serum samples positive for Salmonella-specific antibodies

<table>
<thead>
<tr>
<th>Salmonella species</th>
<th>positive serum/total serum (%)</th>
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<tbody>
<tr>
<td>Salmonella enteritidis</td>
<td>12/330 (3.6)</td>
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<tr>
<td>Salmonella typhimurium</td>
<td>39/330 (11.8)</td>
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<tr>
<td>Total</td>
<td>51/330 (15.5)</td>
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