J. Vet. Med. B **53,** 194–196 (2006)

ISSN 0931–1793

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Detection of *Salmonella* spp. in Stray Dogs in Bursa Province, Turkey: First Isolation of *Salmonella* Corvallis from Dogs

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Received for publication January 6, 2006

Summary

The prevalence and antimicrobial sensitivity of *Salmonella* spp. in stray dogs in Bursa province, Turkey were determined from a total of 82 faecal samples by bacteriological methods. Of the dogs, nine (11%) were positive for salmonellae-carrying. All *Salmonella* isolates were serotyped as *S*. Corvallis, and were sensitive to amoxycillin/clavulanic acid, ampicillin, cephalothin, chloramphenicol, enrofloxacin, gentamicin, kanamycin, nalidixic acid, neomycin, oxytetracycline and trimethoprim/sulphamethoxazole, while 66.7% of them were resistant to streptomycin. It was concluded that stray dogs could be an important carrier of salmonellae as well as a source of human salmonellosis in Turkey. Additional studies are needed to clarify the epidemiological relationship between *S*. Corvallis isolated from dogs and humans with regard to public health. This is the first report on the isolation of *S*. Corvallis from dogs in the country.

Introduction

Salmonellae, worldwide distributed bacteria, are responsible for large numbers of infections in both humans and animals (Timoney et al., 1988). A number of animal species including ruminants, carnivores, birds and reptiles can play a major role as a carrier in the spread of salmonellae and transmit them to other healthy animals and humans (Sanchez et al., 2002).

Because of the close contact with human beings, the incidence of *Salmonella* infections in dogs or the intestinal carriage of salmonellae by dogs is very important to public health (Morse and Duncan, 1975; Morse et al., 1976). There have been several reports on transmission of salmonellae from dogs to humans (Morse et al., 1976; Birnbaum et al., 1980; Sato et al., 2000).

Stray dogs, which are closely related with human living in the environment of man, have been documented to pose public health problems throughout the world (Butcher, 1999). The prevalence of the isolation of *Salmonella* spp. from the faeces of stray dogs was reported to be between 0.0% and 23.5% (Khan, 1970; Timbs et al., 1975; Fukushima et al., 1985; Ojo, 1994).

Materials and Methods

Samples

Rectal swabs from 82 stray dogs kept in six dog shelters of Nilufer Municipality in Bursa province, Turkey were aseptically placed in tubes containing Carry-Blair transport medium (Oxoid Ltd., Basingstoke, UK) and transported ice-cooled to the laboratory within 8 h of collection. The dogs were from various quarters of Nilufer district, which has an area of 425 km^2. Every shelter had approximately 20 animals and approximately 15 of them were sampled. It was reported that the dogs had been kept for up to 4–8 weeks in their shelters. Forty-nine (60%) of the animals were apparently healthy and the others (40%) were diarrhoeic at the time of sampling. The dogs were crossbreeds except for four (two terriers, one kangal, one boxer) and were between the ages of 1 month and 5 years. Of the dogs, 45 (55%) were males and 37 (45%) were females.

Isolation

Salmonella isolation procedure previously described by Seepersadsingh and Adesiyun (2003) was used. Each rectal swab was placed in a tube containing 9 ml of tetrathionate (TT) broth (Oxoid) for enrichment. The tube was shaken thoroughly and kept at 37°C for 18–24 h. Then one loop of cultured TT broth was spread on xylose-lysine-desoxycholate (XLD) agar (Oxoid) and the agar plates were incubated at 37°C for 24 h. Presumptive *Salmonella* colonies on XLD agar were picked and subjected to biochemical tests.

Identification

Species and subspecies differentiations of the isolates were carried out according to the scheme of Popoff (2001) by standard methods using 14 biochemical reactions.

Serotyping

Salmonella isolates were serotyped according to the Kauffman–White scheme (Rowe and Hall, 1989). The determination of cell wall (O) and flagellar (H) antigens was performed using slide agglutination tests by means of O and H factor sera supplied by Bio-Rad Laboratories, Inc. (Richmond, CA, USA), Denka Seiken Co., Ltd. (Tokyo, Japan) and Statens Serum Institute (Copenhagen, Denmark).

Antimicrobial Sensitivity Testing

The sensitivity of *Salmonella* isolates to amoxycillin/clavulanic acid (30 μg), ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), enrofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), oxytetracycline (30 μg), streptomycin (10 μg) and trimethoprim/sulphamethoxazole (25 μg) (Oxoid) was examined by the Kirby–Bauer disc diffusion method (Bauer et al., 1966) on Mueller–Hinton agar (Oxoid).

Results

Of a total of 82 dogs examined, nine (11%) were positive for salmonellae-carrying. Of the dogs, from which salmonellae were isolated, six (66.7%) were from shelter 1, two (22.2%) were from shelter 2 and one (11.1%) was from shelter 5; six (66.7%) were apparently healthy and three (33.3%) were diarrhoeic; five (55.6%) were male and four (44.4%) were female. All positive animals were crossbreeds and were aged between 1 month and 5 years at the time of sampling.

In serotyping, the strains had the following antigenic formula: O8, 20: z_4, z_{23}: [z_6] corresponding to *S*. Corvallis according to the Kauffman–White scheme. In antimicrobial sensitivity testing, all *S*. Corvallis strains were sensitive to all antibiotics except streptomycin, to which six (66.7%) strains were resistant to.

Discussion

Dogs have been reported to be the carrier of *Salmonella* spp. worldwide which have the potential to serve as sources of exposure or infection for humans (Carter and Quinn, 2000). It was reported that the intestinal carriage of salmonellae by dogs is more common than the prevalence of clinical disease. The frequency of faecal isolation of *Salmonella* spp. from clinically healthy dogs was reported to be between 0.0% and 43.0% (Carter and Quinn, 2000; Sanchez et al., 2002).

The prevalence of *Salmonella* spp. in stray dogs in New Zealand (Timbs et al., 1975) and Sudan (Khan, 1970) has been reported to be 5.5% and 23.5%, respectively. However, Ojo (1994) failed to detect salmonellae in the intestinal contents of stray dogs in Trinidad, West Indies. In Japan, 5.9% of stray or unwanted apparently healthy dogs were positive for the presence of salmonellae in their intestinal contents and various serovars except *S*. Corvallis were identified (Fukushima et al., 1985). In the present study, salmonellae were isolated from 11% of stray dogs examined. This isolation rate is between the lowest (0.0%; Ojo, 1994) and the highest (23.5%; Khan, 1970) prevalence values of salmonellae in stray dogs mentioned above. The differences in the sample sizes of dogs, year of sampling, type of faecal sample, geographical properties, and sampling strategies and isolation methods performed in the various countries may all affect the prevalence (Seepersadsingh et al., 2004).

Salmonella Corvallis was first described in 1949 in USA and was isolated from the pooled caecal contents of young chickens affected with enteritis (Edwards and Hermann, 1949). In Turkey, the first isolation of *S*. Corvallis was performed from the faeces of two persons with gastroenteritis in 1993 (Erdem et al., 1995). Up to date, there is no new documentation regarding the isolation of *S*. Corvallis from a human or animal origin in Turkey. In 2001, *S*. Corvallis was involved in a food poisoning outbreak in a hospital in Japan and was isolated from both the stool specimens of patients and the suspected meal served in the hospital (Hamada and Tsuji, 2001). An other food poisoning outbreak caused by *S*. Corvallis occurred in Italy in 1985 (Nastasi et al., 1987). In a comprehensive study performed in Tunisia between 1989 and 1993, the red meats and poultry meats sampled from public stores and slaughterhouses were examined for the salmonellae contamination and *S*. Corvallis was reported as one of the most frequently isolated *Salmonella* serovars (Guellouz and Ben Aissa, 1995).

We describe the first isolation of *S*. Corvallis in prevalence of 11% from dogs in Turkey. However, in a study in which a total of 1391 dogs were examined in Trinidad (Seepersadsingh et al., 2004), 28 different *Salmonella* serovars, the predominant serovars of which were Javiana, Newport, Arechavaleta and Heidelberg, but not Corvallis, were identified. In Sweden, 12 different *Salmonella* serovars with the exception of *S*. Corvallis, the predominant serovar of which was *S*. Typhimurium, were identified in dogs during a 5-year period (1993–1997; Boqvist et al., 2003). The number and occurrence of *Salmonella* serovars isolated from dogs can change from country to country and is related to the animal's diet or the general environment (Carter and Quinn, 2000).

Salmonella Corvallis strains were isolated from three of the shelters sampled in the study. A great proportion (66.7%) of the strains were isolated from the animals kept in the same shelter (shelter 1). Thus, the source of the infection might be the one or more of the dogs, which acquired *S*. Corvallis from the environment or eating contaminated food prior to sheltering. Consequently, cross-infection might have taken place by direct contact within 4–8 weeks period in which the dogs were kept in the shelters. Sanchez et al. (2002) reported that the environment, contaminated foodstuffs, fomites, and animal handlers can be sources of *Salmonella* spp. for dogs.

Salmonella Corvallis strains, identified in our study and a food poisoning outbreak in a hospital in Japan (Hamada and Tsuji, 2001), were similar with regard to their sensitivity to various antibiotics. All *S*. Corvallis strains belonging to two groups were sensitive to ampicillin, chloramphenicol, gentamicin, kanamycin, nalidixic acid and trimethoprim/sulphamethoxazole with the exception of streptomycin; none of the Japanese strains, but 66.7% of our strains were resistant to streptomycin. It was reported that a great proportion (80.6%) of *Salmonella* strains belonging to various serovars except *S*. Corvallis, isolated from dogs in Japan, were resistant to streptomycin (Seepersadsingh et al., 2004).

In conclusion, it was thought that stray dogs could be an important carrier of salmonellae and a source of human salmonellosis in Turkey. Additional studies are needed to clarify the epidemiological relationship between *S*. Corvallis isolated from dogs and humans in Turkey with regard to public health.

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