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Shepherd dogs as a common source for *Salmonella enterica* serovar Reading in Garmsar, Iran

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Abstract: *Salmonella* infection is one of the most important diseases that affect all animal species and is the leading cause of foodborne infections worldwide. One of the challenges in the control and prevention of salmonellosis is the recognition of potential carriers. To assess the potential role of shepherd dogs in the epidemiology of salmonellosis, fecal samples were taken from 38 shepherd dogs in Garmsar Province. *Salmonella* strains were isolated from 4 dogs (10.5%) related to 3 sheep flocks. All of the strains were confirmed at the genus level using *invA* virulence gene PCR. Serotyping confirmed all of the strains as *Salmonella* Reading. To study the clonal relatedness of the isolates, the strains were subjected to RAPD-PCR, and antimicrobial resistance patterns were also determined using 9 antibacterials. The similar RAPD-PCR profiles and resistance patterns strongly suggested that the shepherd dogs in the present study shed a similar clone of S. Reading; therefore, shepherd dogs can be recognized as a common source for this serotype in Garmsar.

Key words: Salmonellosis, shepherd dogs, Garmsar, *Salmonella* Reading, RAPD-PCR

Salmonellosis is one of the most important zoonotic diseases with global distribution and importance. Infection can occur through the consumption of contaminated foodstuffs or contact with infected animals (1–3). Carrier states in animals are of major concern in the epidemiology of salmonellosis, because animals can become the latent carriers of *Salmonella* serovars and shed the organism into the environment without any apparent clinical signs. Although *Salmonella* can cause enteritis and diarrhea in dogs, most infected animals show no sign of disease in spite of infection and fecal shedding (4,5). *Salmonella* serovars readily colonize in the canine large intestine and mesenteric lymph nodes. Fecal shedding of the pathogen in naturally occurring infections probably continues for a period of at least 6 weeks. Since the lymph nodes harbor the agent, the carrier state may persist for much longer periods (6).

Among different species, dogs can be one of the most important reservoirs of *Salmonella* for the following reasons. Dogs are one of the most popular pets in close contact with their owners, including children, and they can shed the organism for weeks without any clinical signs. They are also the inseparable companion of food animals in the nonintensive sheep raising system, which is still common in many countries.

The aim of the present study was to investigate the role of herd dogs in the epidemiology of salmonellosis. Although *Salmonella* infection has been investigated previously in dogs, this appears to be the first study that primarily assesses the potential role of shepherd dogs in the epidemiology of salmonellosis.

Fecal samples were taken from 38 clinically healthy mixed-breed shepherd dogs related to 19 sheep flocks in Garmsar Province, which is located 90 km to the east of Tehran, the capital city of Iran. Fecal samples were taken using sterile swabs and sent to the laboratory within 5 h. The samples were subsequently cultured in 10 mL of selenite F broth (Merck, Germany) and incubated for 12 to 16 h at 37 °C. Following enrichment, a loopful of selenite broth culture was streaked onto selective media, including xylose lysine deoxycholate agar (Merck) and MacConkey agar (Merck). After incubation, the plates were examined and 3 suspected colonies were picked and subcultured, and the isolates were finally tested by means of conventional biochemical tests (7).

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The biochemically identified strains were subjected to polymerase chain reaction (PCR) for the *invA* gene, using the S139 (5'-GTGAAATTATCGCCACTGTCGGGCAA-3') and S141 (5'-TCATCGCACCGTCAAAGGAACC-3') primers to confirm the isolates at the genus level (3). Reactions with these primers were carried out in a 25-µL amplification mixture consisting of 2.5 µL of 10X PCR buffer (500 mM KCl, 200 mM Tris–HCl), 0.5 µL of dNTP mix (10 mM), 2 mM $MgCl_2$, 0.5 µM of each primer, 1 U of *Taq* DNA polymerase, and ultrapure water.

Serotyping of the isolated bacteria, which were confirmed at the genus level by biochemical tests and the presence of the *inv*A virulence marker, was performed using commercial antisera (Difco, USA) according to the manufacturer's instructions.

Resistance profiles of the isolated *Salmonella* strains were determined on Mueller-Hinton agar (Merck) using the Kirby–Bauer method according to Clinical and Laboratory Standards Institute protocols (formerly the National Committee for Clinical Laboratory Standards). Nine antibacterial disks were used, including ampicillin (AM; 10 µg), penicillin G (P; 10 units), streptomycin (S; 10 µg), erythromycin (E; 15 µg), Linco-Spectin (LP; 150 µg), chloramphenicol (C; 30 µg), trimethoprim-sulfamethoxazole (SXT; 1.25/23.75), ceftriaxone (CRO; 30 µg), and cefixime (CM; 5 µg).

To assess the clonal relatedness of the isolates, random amplification of polymorphic DNA (RAPD)-PCR profiles were investigated using 3 sets of arbitrary primers, including P1254 (5'-CCGCAGCCAA-3'), 23L (5'-CCGAAGCTGC-3'), and OPA-4 (5'-AATCGGGCTG-3') (8,9). In brief, a single colony of each isolate from an agar plate was picked and suspended in 200 µL of distilled water. After vortexing, the suspension was boiled for 5 min, and 50 µL of the supernatant was collected after centrifuging for 10 min at 15,000 × *g*. The DNA concentration of the boiled extracts was determined with a spectrophotometer and adjusted to approximately 40 ng DNA/µL. The PCR was conducted in a 25-µL volume containing 40 ng of template DNA, 2 mM $MgCl_2$, 0.5 µM of primer, 1 U of *Taq* DNA polymerase (Fermentas, Lithuania), and 200 µM of each dNTP in 1X PCR buffer. A *Salmonella* Typhimurium strain (Microbial Collection, Faculty of Veterinary Medicine, Tehran University) was used as the positive control and distilled water was used as the negative control. The PCR products were electrophoresed on 2% agarose gel at 80 V for 2 h and visualized after staining with ethidium bromide.

Salmonella spp. was isolated from 4 shepherd dogs (10.5%) belonging to 3 sheep flocks. In other words, 15.8% of sheep flocks in the area have carrier shepherd dogs. All 4 of the isolates produced the 284-bp specific product of the *invA* gene. Serotyping of the strains using commercially available antisera identified all isolates to be *S. enterica* subsp. *enterica* serovar Reading. Four strains revealed similar resistance patterns; in fact, all of the strains were resistant to streptomycin, trimethoprim-sulfamethoxazole, penicillin, and erythromycin, but sensitive against other applied antimicrobials, including ampicillin, Linco-Spectin, chloramphenicol, ceftriaxone, and cefixime. The molecular typing by the RAPD method using 3 sets of primers could not discriminate the isolate genetically and a similar RAPD-type was observed in 4 isolated *Salmonella* strains (Figures 1–3).

Traditional nonintensive sheep raising systems rely on the presence of shepherd dogs as inseparable members of the flock. This traditional system is still fairly common in Middle Eastern countries, and also in some parts of Europe and Australia. Shepherd dogs are usually born near their flocks and live with ruminants through their working life. Therefore, direct contact of these dogs with food animals is unavoidable. Dogs are among the most famous latent carriers of *Salmonella* serovars because they can shed the organism for 3 to 4 weeks, and in rare cases for up to 100 days (10,11). On the other hand, a single sheep flock can provide meat and dairy products for hundreds of people or consumers, and this is when the importance of a single infected dog in a flock seems to be overlooked. Results of this study indicated that shepherd dogs can be a potential reservoir for *Salmonella* spp. and the transmission of the disease to food animals. Although disease transmission

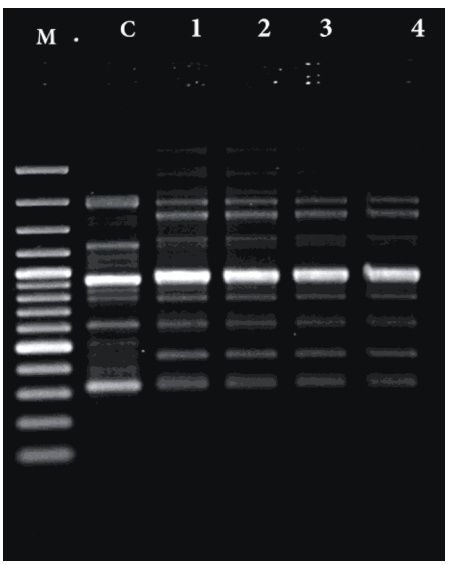

Figure 1. RAPD-PCR (Primer 1254): M, Marker, 100 bp-plus; C, *S.* Typhimurium; Lanes 1–4, *S.* Reading isolated from 4 herd dogs in this study.

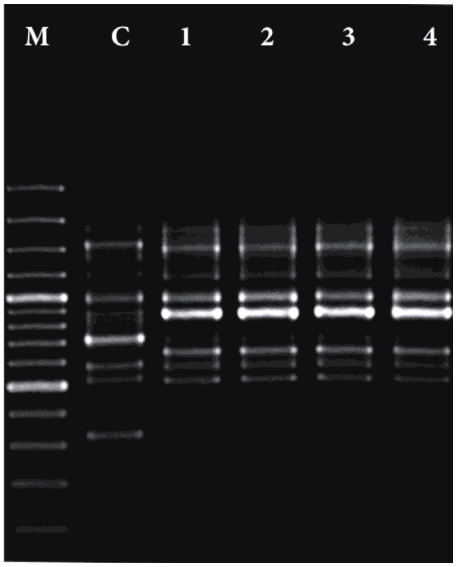

Figure 2. RAPD-PCR (Primer OPA4): M, Marker, 100 bp-plus; C, *S.* Typhimurium; Lanes 1–4, *S.* Reading isolated from 4 herd dogs in this study.

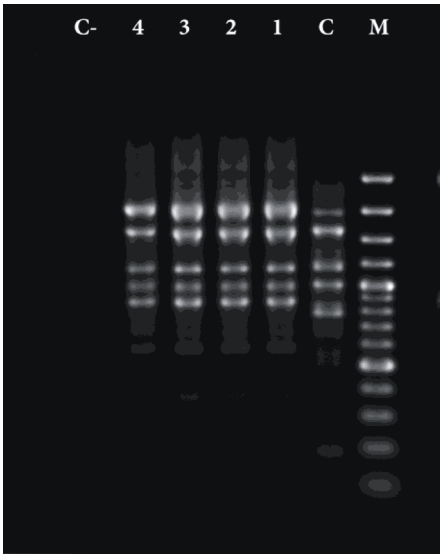

Figure 3. RAPD-PCR (Primer 23L): M, Marker, 100 bp-plus; C, *S.* Typhimurium; Lanes 1–4, *S.* Reading isolated from 4 herd dogs in this study; C–, Negative control.

can occur as a blind circle from sheep and goat to dogs, and from dogs to the animals of the flock, this epidemiological circle may occur several times in a single flock over a long period of time. Furthermore, the feeding of herd dogs with raw meat in urban areas is fairly common; this can lead to higher rates of infection and shedding of *Salmonella* serovars. Finley et al. (4) showed that feeding dogs raw foods that are contaminated by *Salmonella* spp. can be a major public health threat to humans and animals. Poor-quality foods have also been considered as an important source of salmonellosis in dogs (12). Previously, the *Salmonella* spp. shedding of dogs of the same geographical area (Garmsar Province) not related to herds was studied, but *Salmonella* spp. was not isolated (unpublished study). As mentioned earlier, this may be due to the eating of raw foods and also to intimate contact with ruminants.

The present study indicated the presence of *Salmonella* spp. in 10.5% of shepherd dogs in Garmsar Province. The shedding pattern of *Salmonella* serovars in animals is usually intermittent and periodical; therefore, 3 rounds of sampling are recommended to assess the carrier states in animals. In this study, taking only one sample per animal was possible, and as a result of that, the shedding status rate in the herd dogs may be underestimated. To our knowledge, there is no report or study available on the prevalence of salmonellosis in herd dogs, but interestingly, *S.* Reading has been frequently reported in both small ruminants and dogs. In Italy, *S.* Reading was one of the most frequent *Salmonella* serovars isolated from dogs (13). In another study in Ethiopia, *S.* Reading was one of the most prevalent serovars isolated from sheep and goats (14). In 1991, the Centers for Disease Control reported a *S.* Reading-associated outbreak of salmonellosis in humans; the outbreak was reported to be caused by the consumption of improperly cooked turkey meat (15). White et al. (16) presented a study on dog treats derived from samples such as pig ears and other animal parts and followed up on isolated *Salmonella* spp. for their epidemiologic sources using serotyping, pulsed-field gel electrophoresis, and antimicrobial susceptibility testing. Their results indicated that animal-derived dog treats in the United States could be a potential source of animal and human infections with *Salmonella* spp., including multidrug-resistant *Salmonella* strains.

A noteworthy observation in the present study was the shedding of clonally similar *S. enterica* subsp. *enterica* serovar Reading in herd dogs. This finding suggested that the herd dogs act as a common source for a particular serotype of *Salmonella* in the studied area. There is no authentic information available on the epidemiology of salmonellosis in humans and animals in Garmsar Province. Further study on the prevalence and epidemiology of salmonellosis in Garmsar Province can lead to deeper insight into the importance of herd dogs in the transmission and epidemiology of salmonellosis.

References

1. Mirzaie, S., Hassanzadeh, M., Ashrafi, I.: Identification and characterization of *Salmonella* isolates from captured house sparrows. Turk. J. Vet. Anim. Sci., 2010; 34(1): 181–186.
2. Zahraei Salehi, T., Tadjbakhsh, H., Atashparvar, N., Nadalian, M.G., Mahzounieh, M.R.: Detection and identification of *Salmonella* Typhimurium in bovine diarrhoeic fecal samples by immunomagnetic separation and multiplex PCR assay. Zoonoses Public Health, 2007; 54(6–7): 231–6.
3. Zahraei Salehi, T., Mahzounieh, M., Khaksar, E.: Detection of *Salmonella* serovars in zoo and pet reptiles, rabbits, and rodents in Iran by culture and PCR methods. Comp. Clin. Pathol., 2010; 19: 199–202.
4. Finley, R., Ribble, C., Aramini, J., Vandermeer, M., Popa, M., Litman, M., Reid-Smith, R.: The risk of *Salmonella* shedding by dogs fed *Salmonella*-contaminated commercial raw food diets. Can. Vet. J., 2007; 48: 69–75.
5. Fukata, T., Naito, F., Yoshida, N., Yamaguchi, T., Mizumura, Y., Hirai, K.: Incidence of *Salmonella* infection in healthy dogs in Gifu prefecture, Japan. J. Vet. Med. Sci., 2002; 64: 1079–1080.
6. Morse, E.V., Duncan, M.A., Estep, D.A., Riggs, W.A., Blackburn, B.O.: Canine salmonellosis: a review and report of dog to child transmission of *Salmonella* Enteritidis. Am. J. Public Health, 1976; 66(1): 82–84.
7. Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R.: Clinical Veterinary Microbiology. Mosby Ltd., St. Louis, Missouri, USA. 1994; 209–236.
8. Lin, A.W., Usera, M.A., Barrett, T.J., Goldsby, R.A.: Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella* Enteritidis. J. Clin. Microbiol., 1996; 34: 870–876.
9. Madadgar, O., Zahraei Salehi, T., Ghafari, M.M., Ashrafi Tamai, I., Madani, S.A., Yahyareyat, R.: Study of an unusual paratyphoid epornitic in canaries (*Serinus canaria*). Avian Pathol., 2009; 38(6): 437–441.
10. Bagcigil, A.F., Ikiz, S., Dokuzeylul, B., Basaran, B., Or, E., Ozgur, N.Y.: Fecal shedding of *Salmonella* spp. in dogs. J. Vet. Med. Sci., 2007; 69: 775–777.
11. Green, C.E.: Enteric bacterial infections: salmonellosis. In: Green, C.E., Ed. Infectious Diseases of the Dog and Cat. 2nd ed., WB Saunders Co., Philadelphia. 1998; 235–240.
12. Chengappa, M.M., Staats, J., Oberst, R.D., Gabbert, N.H., McVey, S.: Prevalence of *Salmonella* in raw meat used in diets of racing greyhounds. J. Vet. Diag. Invest., 1993; 53: 372–377.
13. Corradini, L., Salami, P., Zavanella, M.: Detection of *Salmonella* carriers among the dogs of the province of Ferrara. Isolations in 1 year and antibiotic-resistance. Ann. Scalvo., 1979; 21: 303–306.
14. Molla, W., Molla, B., Alemayehu, D., Muckle, A., Cole, L., Wilkie, E.: Occurrence and antimicrobial resistance of *Salmonella* serovars in apparently healthy slaughtered sheep and goats of central Ethiopia. Trop. Anim. Health Pro., 2006; 38: 455–462.
15. Centers for Disease Control: Foodborne nosocomial outbreak of *Salmonella* Reading -- Connecticut. MMWR Morb. Mort. Wkly. Rep., 1991; 40: 804–806.
16. White, D.G., Datta, A., McDermott, P., Friedman, S., Qaiyumi, S., Ayers, S., English, L., McDermott, S., Wagner, D.D., Zhao, S.: Antimicrobial susceptibility and genetic relatedness of *Salmonella* serovars isolated from animal-derived dog treats in the USA. J. Antimicrob. Chemother., 2003; 52(5): 860–863.