Archives of Razi Institute, Vol. 75, No. 1 (2020) 93-99

DOI: 10.22092/ARI.2018.123213.1242

Original Article

A case control study of *Salmonella* SPP. infection in stray dogs in Tehran shelters and the correlation between paraclinical tests results and clinical findings

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Received 17 September 2018; Accepted 18 December 2018
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ABSTRACT

Salmonellosis as a zoonotic disease in dogs is not fully understood, and various reports have pointed to the transmission of antibiotic-resistant *salmonella* from dogs to humans. The current study aimed to evaluate the serologic and bacteriologic prevalence of *Salmonella* spp. in stray dogs placed in animal shelters around Tehran, compare the results to those of asymptomatic dogs, and determine the serotype of isolated species, as well as their antibiotic susceptibility pattern. A total of 100 fecal swab and blood samples were obtained from symptomatic and apparently healthy dogs (clinically) placed in four animal shelters around Tehran, Iran. Fecal and blood culture, as well as dog food culture, tube agglutination test, serotyping, and antibiotic susceptibility testing were performed on the samples. Fever, lethargy, diarrhea, and abdominal pain were observed in all the dogs in the case group, and bloody diarrhea was the least commonly detected symptom in clinical examination. A number of 11 and 4 collected fecal swabs from the case and control groups were positive for *Salmonella* spp., respectively. The polymerase chain reaction (PCR) also confirmed the laboratory tests results. Blood culture on the selective medium was negative for all the cases. Moreover, 60% and 100% of dogs in the case and control groups showed inflammatory markers in their blood test. The tube agglutination test was positive for 12% of the samples from the case group, while it was positive only for 5% of cases in the control group. The highest and lowest antibiotic resistance was observed against gentamicin and ciprofloxacin from the case group, respectively. *Salmonella* spp. infection in stray dogs placed in animal shelters is a great public health concern. In this regard, it is recommended that these animals be regularly monitored since they serve as *Salmonella* carriers.

Keywords: *Salmonella* spp., Stray dogs, Shelters, Zoonotic diseases

La Salmonellose chez les Chiens Errants dans les Refuges de Téhéran et la Corrélation entre les Résultats des Tests Paracliniques et les Résultats Cliniques: Une Étude Cas-Témoins

Résumé: La salmonellose en tant que maladie zoonotique chez le chien n'est pas entièrement comprise. Divers rapports ont montré la transmission de salmonelles résistantes aux antibiotiques du chien à l'homme. Cette étude visait à évaluer la prévalence sérologique et bactériologique des espèces de salmonelle chez les chiens errants dans les refuges pour animaux de la banlieue de Téhéran, en Iran. Les résultats obtenus ont été comparés à ceux des chiens asymptomatiques afin de déterminer le sérotype des espèces isolées, ainsi que leur profil de sensibilité aux antibiotiques. Au total, 100 écouvillons fécaux et échantillons sanguins ont été prélevés sur des chiens symptomatiques et apparemment sains (cliniquement) placés dans quatre refuges pour animaux autour de Téhéran. Les échantillons ont été étudiés en termes de culture fécale et sanguine, de culture d'aliments pour chiens, de test d'agglutination en tube, de sérotypage et de test de sensibilité aux antibiotiques.

Selon nos résultats, des symptômes diverses incluant fièvre, léthargie, diarrhée et douleurs abdominales ont été observés chez tous les chiens du groupe infecté symptomatique, tandis que la diarrhée sanglante était le symptôme le moins fréquemment détecté à l'examen clinique. Au total, 11 et 4 prélèvements fécaux étaient respectivement positifs pour la salmonelle dans les groupes symptomatiques et contrôle. La réaction en chaîne de la polymérase a également confirmé ces résultats. L'hémoculture sur milieu sélectif était négative pour tous les cas. De plus, les résultats des tests sanguins ont montré la présence de marqueurs inflammatoires dans respectivement 60% et 100% des chiens des groupes symptomatiques et témoins. Le test d'agglutination en tube était positif pour 12% des chiens symptomatiques; alors qu'il était positif pour 5% des cas dans le groupe témoin. La résistance aux antibiotiques la plus élevée et la plus faible a été respectivement observée contre la gentamicine et la ciprofloxacine chez les ciens infectés. La salmonellose est une grande préoccupation de santé publique chez les chiens errants placés dans des refuges pour animaux. Il est recommandé de surveiller régulièrement ces animaux car ils sont considérés comme porteurs potentiels de la salmonelle.

Mots-clés: Salmonella spp., Chiens errants, Refuges, Maladies zoonotiques

INTRODUCTION

Zoonotic diseases caused by *Salmonella* spp. are widespread and observed in most animals. The incidence rate of such diseases has recently increased in humans and animals, especially dogs, as compared to last four decades (Zahraei Salehi, 1999; Schmidt and Tirado, 2001; Tsai et al., 2007). *Salmonella* is the etiologic agent of salmonellosis which is a very common intestinal infection in humans and animals. The bacteria are among the most common causes of acute and chronic diarrhea, as well as mortality in human and different livestock (Thompson et al., 2001).

The prevalence of *Salmonella* spp. in dogs is not fully understood since dogs can serve as asymptomatic carriers and excrete the bacteria with no clinical demonstrations. The dogs carrying *Salmonella* spp. can excrete the bacteria for more than 6 weeks (Greene, 1998; Marks and Kather, 2003). The clinical symptoms of salmonellosis in dogs include fever, loss of appetite, diarrhea, bloody diarrhea, abdominal pain, and abortion (Greene, 1998; Marks and Kather, 2003). A part of these symptoms usually occurs 3-5 days after infection with the bacteria; nonetheless, the clinical symptoms may appear even 12 hours after exposure to bacteria (Marks et al., 2011). Nevertheless, the clinical salmonellosis infection in dogs is rare and the subclinical form of the disease is the dominant type in dogs (Sanchez et al., 2002). Dogs kept in animal shelters can be a good source for the distribution of different serovars of *Salmonella* in the environment (Jackson et al., 2013). Animals, especially carnivores, as the asymptomatic carriers of *Salmonella* spp. play a significant role in this cycle. However, most of the studies in Iran and many other countries have focused on salmonellosis in poultry populations. Different reports have highlighted the transmission of antibiotic-resistant *Salmonella* spp. from animals to humans living in contact (Sanchez et al., 2002). Although *Salmonella* serovars are severely host-limited, they can contaminate a wide range of hosts (Ojo and Adetosoye, 2009). Therefore, determining the antibiotic susceptibility pattern of *Salmonella* species isolated from such animals can provide valuable information for the identification of ineffective medicines and replacing them with useful ones in order to promote and maintain public health. With this background in mind, the current study aimed to evaluate the serologic and bacteriologic prevalence of salmonellosis among stray dogs placed in the animal shelters around Tehran, Iran,

compare the results with those of asymptomatic dogs, and determine the serotypes, as well as antibiotic susceptibility patterns of the isolated species.

MATERIAL AND METHODS

A 10-mL blood sample was taken from each dog, transferred to blood culture bottle, and subcultured on MacConkey and Salmonella-Shigella agar plates after 24, 48 and 72 h. The current study was conducted on 100 fecal swab samples obtained from dogs with clinical symptoms of diarrhea, loss of appetite, and fever (as the case group) and another 100 fecal swab samples collected from apparently (clinically) healthy dogs or those recovered from the abovementioned symptoms (as the control group) placed in four animal shelters around Tehran from spring 2016 to winter 2017. The fecal specimens were collected in the feces sampling containers. The rectal swab technique was also employed if the fecal specimen was not available.

Culture test. The specimens were transferred on ice to laboratory, and the samples were cultured on selective medium on the day of sampling. For this purpose, the specimens were initially cultured directly in Rappaport Vassiliadis enrichment broth and were inoculated on the selective media as MacConkey and Salmonella-Shigella agar plates after 24 hours. After performing the biochemical differentiation tests, three colonies from each medium were stored at -20°C. Moreover, to determine the genotype of the microbiologically identified species, DNAs of the stored species were extracted. Thereafter, the polymerase chain reaction (PCR) was used to confirm the identifications with four pairs of primers (Fratamico, 2003). In addition, the food of the dogs was cultured every 20 days in order to monitor contamination with *Salmonella* spp. To trace bacteremia, blood samples taken from the animals were cultured. For this purpose, after shaving, the area was cleaned with alcohol-soaked cotton balls and the blood samples were then taken based on the standard guidelines (Quinn et al., 1994). A 1mL blood sample was obtained from each dog, cultured on MacConkey and Salmonella-Shigella agar plates, and biochemically assessed based on the fecal culturing technique in order to evaluate bacteremia.

Serological evaluation and serotyping. The tube agglutination test was performed using the Widal antigens (Iran Pasteur Institute, Karaj, Iran) (Waltman et al., 1998), based on manufacturers instruction. Serotyping was performed to confirm the results of microbiological tests, and serum grouping was conducted based on manufacturer's instructions (Difco Co., Maryland, USA) and specific antisera (Agasan et al., 2002).

Antibiotic susceptibility pattern. After the isolation and identification of *Salmonella* species using the Clinical and Laboratory Standards Institute (CLSI) guidelines and standards, the Kirby-Bauer disk diffusion method was used to determine the susceptibility pattern of the species. To this end, after providing a bacterial suspension adjusted to 0.5 McFarland turbidity standard, the sterile swabs were used to inoculate the suspension onto Mueller-Hinton agar plates. Subsequently, four disks of gentamicin, amikacin, streptomycin, and ciprofloxacin (Mast Co., Merseyside, United Kingdom) were planted on the plates (Salehi et al., 2005).

Statistical analysis. The obtained results were analyzed and compared using Chi-squared test at the probability level of %5.

RESULTS

In general, as evidenced by the results of the current study, a total number of 100 samples were obtained from clinically symptomatic dogs. No significant difference was detected between the case and control groups in terms of gender (60 female and 61 male dogs) (P=0.89). In addition, there were no significant differences between the groups regarding length of shelter stay (P=0.81). Some symptoms,

including fever, lethargy, diarrhea, and abdominal pain, were observed in all symptomatic dogs (case group). Moreover, the most and least common clinical symptoms were reported as loss of appetite and bloody diarrhea with 90% and 22% prevalence. In addition, among the evaluated symptoms, bloody diarrhea and leanness were the most commonly observed symptoms in the Salmonella-positive cases, compared to the negative subjects. Moreover, a significant correlation was observed between tube agglutination positivity and bloody diarrhea/ leanness in the evaluated dogs.Based on the culture results of 100 fecal swab samples obtained from the case group with suspected salmonellosis, 11 samples (11%) were positive for *Salmonella* spp. The results of the repeated cultures were the same as what was obtained in the first one. Similar results were also reported from the same number of fecal swabs (n=100) obtained from the control group dogs with 4 positive cases (4%). In addition, the results of food culturing were negative, similar to those of blood culture test. The tube agglutination test was positive for 12(12%) and 5 (5%) serum samples in the case and control groups, respectively. The number of positive samples obtained from culture and tube agglutination tests in the case group was significantly higher, as compared to that of the control group (P=0.03 and P=0.003, respectively). Out of 11 samples with positive culture, 7 cases were identified as *S. typhimurium* and 4 as *S. enteritidis*. No significant differences were observed between the groups in terms of the type of serovars (P=0.49). Polymerase chain reaction (PCR) was amplified on the positive samples of both control and case groups grew on selective media and the results of PCR were the same as those achieved in the culture test (Figure 1). The antibiotic susceptibility testing was performed on the species grown on the selective media, the results are displayed in Table 1. The highest resistance was observed in *S. enteritidis* against gentamicin, as well as *S. typhimurium* against amikacin (Table 1). No significant difference was reported between the serovars in terms of antibiotic resistance (P>0.05).

DISCUSSION

While animals do not contribute to the epidemiology of typhoid fever, they play a significant role in the epidemiology of salmonellosis caused by the other serotypes of *Salmonella* (Zahraei Salehi, 1999). There exists a massive population of stray dogs in cities with no certain monitoring and control systems over their nutritional habits. Consequently, these animals can pose a serious challenge to public management in urban communities since they can play a significant role in the transmission of zoonotic serovars to humans (Kaufmann, 1966; Sato et al., 2000). As a matter of fact, stray dogs are considered the major causes of global failure of programs which were developed to control or eradicate *Salmonella* in animal (Sato et al., 2000). Therefore, the provision of information regarding salmonellosis in dogs, particularly the carrier status, can be of great help in promoting public health.

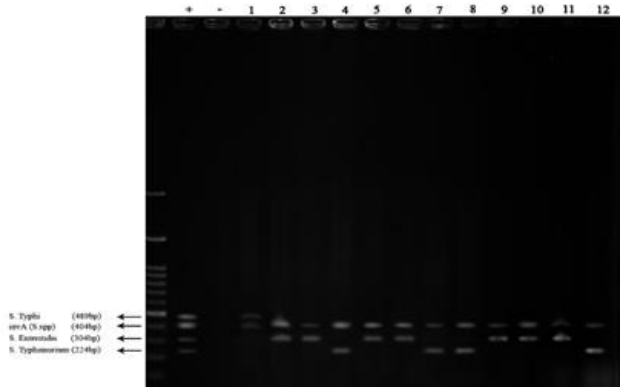

Figure 1. Gel electrophoresis image of polymerase chain reaction products.

Table 1. Results of Antibiotic Susceptibility Testing

Antibiotic	Gentamicin	Amikacin	Streptomycin	Ciprofloxacin
Number of Antibiotic susceptible (%)	7 (43.75)	6 (37.5)	2 (12.5)	1 (6.25)

Since salmonellosis in dogs is usually subclinical and asymptomatic, the mysterious role of dogs in the transmission of the infection should be investigated in

further studies (Zahraei Salehi, 1999). It is rather difficult to diagnose salmonellosis based on clinical symptoms since none of the symptoms is disease-specific and most of them are common among different diseases. Therefore, it is necessary to consider symptoms of salmonellosis along with laboratory results (Zahraei Salehi, 1999). The traditional conventional diagnosis of salmonellosis in dogs relies on the isolation of microorganisms corresponded to the targeted clinical symptoms, as well as the evaluation of risk factors, such as hospital stay, age, environmental factors, and antibiotic administration (Jajere et al., 2014). Several culture tests should be performed in the case of diagnosis merely based on microbiological findings, (Marks and Kather, 2003) since the sensitivity and specificity of culture test is significantly lower than PCR (Schuurman et al., 2007). The isolation of *Salmonella* spp. was also reported from apparently healthy dogs (Kaufmann, 1966; Tanaka et al., 1976; Sugiyama et al., 1993; Weber et al., 1995). In general, the prevalence of *Salmonella* species in asymptomatic dogs is estimated at 0-43% (Jajere et al., 2014). In their study, Ojo and Adetosoye (2009) failed to detect *salmonella* spp.in the intestinal contents of 100 stray dogs. However, Sanchez et al. (2002) pointed to the intestinal carriage of *Salmonella* spp. by 36% of healthy dogs. The results of the current study were in agreement with those obtained by Seepersadsingh et al. (2004) who reported 3.6% prevalence for the non-diarrheal Salmonella-carrier dogs. On the other hand, the results of the present study were inconsistent with a study that reported the prevalence of 20% for the Salmonella- carrier dogs (Jajere et al., 2014). Nonetheless, the results of the later study was in agreement with those of aforementioned studies since the non-diarrheal asymptomatic stray dogs can be the carriers for the bacteria and play the role of reservoir by excreting the bacteria to the environment. The isolation of *Salmonella* species from the fecal specimens of healthy dogs may lead to misleading interpretations, particularly in the case of diarrheal animals (Cantor et al., 1997). However, the clinical salmonellosis has also been reported in dogs, and in case of the exacerbation of this disease, nausea, vomiting, fever, depression, abortion, and even death may occur (Jajere et al., 2014). The prevalence of *Salmonella* spp. among the stray dogs dropped from 23.5% in 1970 to 5.5% in 1975 (Jajere et al., 2014). Hackett and Lappin (2003) performed a study on the prevalence of intestinal diseases among dogs based on the findings from fecal specimens. They indicated that *Salmonella* species account for 3.2% of factors which cause diarrhea in these animals. Based on the results of a study carried out by Tsai et al. (2007), the prevalence of *Salmonella* spp. among pet and stray dogs was 2.1% and 6.3%, respectively with Salmonella enterica var. Düsseldorf dominance in the both groups. Hackett and Lappin reported that the prevalence of *Salmonella* spp. among the stray dogs was 12% which was higher than the rate reported in the studies conducted by Tsai et al. (2007), lower than that obtained by Khan (Hackett and Lappin, 2003). Nonetheless, it was very close to the rate indicated by Salehi et al. (2013) who reported that about 10.5% of stray dogs living around Garmsar, Iran, carried *Salmonella* species. Based on the annual report of the United States Department of Agriculture (USDA), the National Veterinary Services Laboratories (NVSL) 2011, *S. typhimurium* and *S. enterica* serotype Senftenberg, followed by *S. enterica* serovar Muenchen, Newport, and Javiana were the most common *Salmonella* species and serotypes in dogs (Jajere et al., 2014). However, the dogs even can carry a wider range of serovars. Morse and Duncan isolated 35 different *Salmonella* serovars from dogs, while Seepersadsingh isolated 28 different serovars. Nevertheless, *S. typhimurium* was the most common species isolated from these animals (Seepersadsingh et al., 2004; Jajere et al., 2014). The antibiotic susceptibility pattern of the species isolated in the current study was rather alarming. Therefore, it can be

concluded that indiscriminate use of antibiotics in animals may result in the emergence of new antibiotic-resistant species. Consequently, the management of antibiotics should be monitored properly and only be prescribed after the confirmation of their efficacy (Ojo and Adetosoye, 2009). In the current study, the lowest resistance was against ciprofloxacin which was in line with the results of the study conducted by Tsai et al. (2007). The reported susceptibility to ciprofloxacin and ceftriaxone in all the isolated *Salmonella* species. On the other hand, they indicated the highest resistance against tetracycline (77.5%), followed by chloramphenicol (52.5%) and ampicillin (50%) (Tsai et al., 2007). Zoonotic diseases are regarded as public health concerns, and dogs are the active carriers of Salmonella species which is one of the most important human pathogens. In light of the abovementioned facts, the present study pointed to the great importance of regular monitoring of stray dogs, particularly the ones placed in shelters. It is due to the fact that high microbial continuous contamination in such places is a potential risk for public health and can cause outbreaks in dogs and consequently in humans, if being neglected.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Study concept and design: Siamak Mashhady Rafiee
Acquisition of data:Nader Askari
Analysis and interpretation of data: Nader Askari
Drafting of the manuscript: Nader Askari
Critical revision of the manuscript for important intellectual content: Siamak Mashhady Rafiee
Statistical analysis: Kumarss Amini
Administrative, technical, and material support: Siamak Mashhady Rafiee

Acknowledgment

Our special appreciation and thanks go to Faculty of Veterinary Medicine, Islamic Azad University, Tehran, for funding this research project.

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