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# Detection of *Yersinia spp* and *Salmonella spp*. in apparently healthy cats and dogs in Tehran, Iran

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## Abstract

**Introduction**: Companion animals, such as cat and dog, are potential sources of transmissible diseases to humans, especially children. They harbor zoonotic agents in gastrointestinal tracts as carriers which are capable of infecting their owners. *Salmonella* and *Yersinia* bacteria are considered as frequent causes of illness in children. This study was aimed at finding out the prevalence rate of infection in apparently healthy dogs and cats in Tehran, Iran.

**Materials and method**s: A total of 100 rectal swabs from dogs and cats were analyzed by a multiplex PCR method with specific primers for detection of *Yersinia* and *Salmonella* species.

**Results**: Fifteen samples (4 cats and 11 dogs) were positive for *Yersinia* and 20 samples (9 cats and 11 dogs) were positive for *Salmonella*. So the prevalence rate of *Yersinia* was 8% in cats and 22% in dogs and the prevalence rates of *Salmonella* were 18 and 22% in cats and dogs respectively.

**Discussion and conclusion:** According to the results, *Yersinia* and *Salmonella* were detected in 8- 22% of pet animals without any clinical signs. The contaminated animal foods may be the main source of infection. These results may be useful in planning control and preventive programs.

**Key words:** *Salmonella*, *Yersinia*, Dog, Cat, Iran

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## Introduction

Since companion animals are treated as members of the family and live in common environment with human, they may be an important reservoir of zoonotic diseases (1). In many parts of the World, household pets have been found to play a direct role in transmitting zoonosis (1). Although the precise proportion of human diseases for which pets are specifically responsible is largely unknown, more than 70 pathogens of companion animals are known to be transmissible to people (2). Contact with animals has been clearly confirmed as a risk factor to transmit pathogens. For some pet species, their role in human disease has been more clearly identified. Pet animals such as cats and dogs, are potential sources of transmissible agents for humans specially children, such as *Yersinia* and *Salmonella* spp. bacteria. Two species include *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are important agents for humans. *Yersinia enterocolitica* can cause yersiniosis in humans and animals as well. The predominant symptom in humans, particularly in young children, is diarrhea (3). Secondary immunologically induced sequelae, such as reactive arthritis, are not uncommon, especially in HLA-B27- positive individuals. *Yersinia pseudotuberculosis* causes sporadic and epidemic infections in humans and is widely distributed among domestic pets (4-8), farm animals (7), and wild animals (7, 9 & 10). Pets such as dogs and cats may become a source of infection with *Y. pseudotuberculosis,* since they are infected with this organism during the cold months and excrete up to $10^4$ cells per mass of feces expressed in gram (4- 5). Although *Salmonella* may be found in environment, common species of *Salmonella* (except *S. Typhi*) usually have an animal reservoir that shed bacteria without any clinical sign and symptoms. Animal contact transmission of microorganisms by pets accounts for 15-20% of total cases of *Salmonella* infections (11). The agent can be transmitted by direct or indirect contacts. Patients at high risk for pet- transmitted salmonellosis are infants and young children, and immunocompromised persons such as HIV positive individuals. Patients suffered from malignancy or hemoglobinopathies such as sickle cell anemia disease are also at risk of salmonellosis. People can get infected when they eat food or drink water or milk, but they can be transmitted by infected pets such as rodents, cats, dogs and rabbits in contacts (12- 13). In this study, we try to find out the prevalence rate of infection among apparently healthy dogs and cats in Tehran, Iran.

## Materials and methods

**Fecal samples isolation:** A total of 100 fecal samples collected from dogs and cats (50 cats and 50 doges) that referred to small animal veterinary clinics in Tehran, Iran. These samples were collected from animals without clinical signs.

**DNA extraction:** Total DNA was extracted from 200 µl of sample with i-genomic CTB DNA extraction mini kit (Intron, South Korea) according to the manufacturer's instructions. Briefly, 200 µl of samples suspensions was incubated with

200 µl lysis buffer and 10 µl proteinase K at 65°C for 30 min. After incubation, 250 µl of binding buffer and 250 µl of ethanol 80% were added to the lysate. The samples were then washed following the manufacturer's recommendations. Nucleic acid was eluted with 50 µl of elution buffer provided in the kit. DNA was extracted from sample without cultivation.

**PCR**: The nucleotide sequences of *invA* gene in *Salmonella* and *ompF* gene in *Yersinia* were detected by a developed Multiplex PCR method, that amplify a 428 bp for *Yersinia* and 263 bp for *Salmonella* PCR amplicons (Table 1 (14- 15)). The Taq DNA polymerase 2X master mix red (Ampliqon, Copenhagen, Denmark) was used in all amplification reactions. PCR amplification was carried out in 25 µl reaction volumes as follows: 12.5 µl master mix, 0.5 µl of each primer (10 mM), 9.5 µl dobble distilled water and 2 µl of template DNA. All amplification reactions were performed in a PCR Express thermal cycler (BioRad) as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 61°C for 1 min and 72°C for 30 Sec, followed by a final extension step at 72°C for 5 min. The PCR products were visualized by electrophoresis in 2% agarose gels containing Rima sight DNA stain. The electrophoresis was run for 20 min at 100 V, and the gels were photographed under ultraviolet illumination.

Table 1: Primer sequences used for identifying *Yersinia spp* and *Salmonella spp*.

| Primers Name | Nucleotide sequence 5'- 3' | Product size | Annealing tepmrature |
|---|---|---|---|
| 227Fmod<br>*Yersinia spp*<br>669R | GTCTGGGCTTTGCTGGTC<br>GCGTCGTATTTAGCACCAACG | 428 bp | 61°C |
| Sal spp- R<br>*Salmonella spp*<br>Sal spp- F | GTGAAATTATCGCCACGTTCGGGCAA<br>TCATCGCACCGTCAAAGGAACC | 263 bp | |

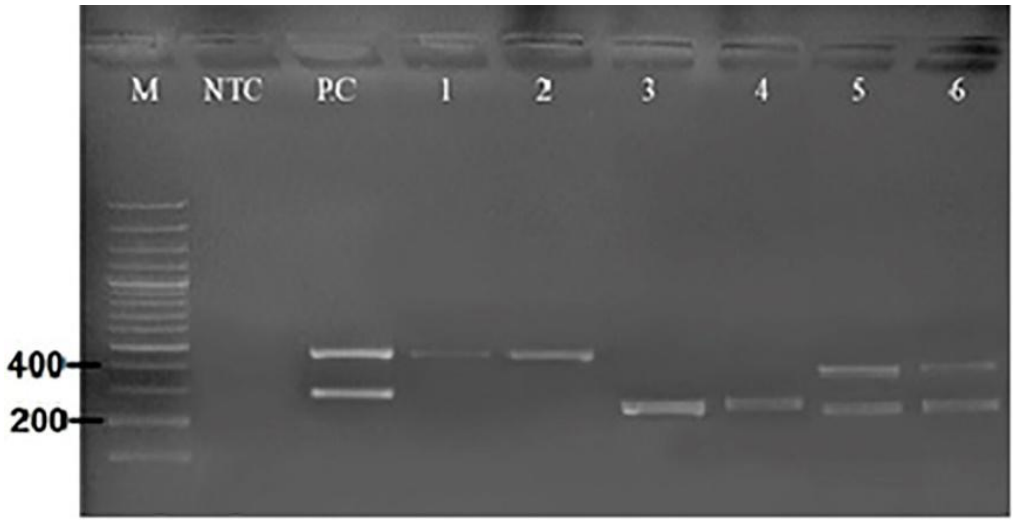


Fig 1- Agarose gel electrophoresis of *Yersinia ompF* gene and *Salmonella invA* gene.
1, 2 *Yersinia* positive, 3, 4 *Salmonella* positive, 5, 6 both *Yersinia* and *Salmonella* positives.

**Results**

Fifteen samples (4 cats and 11 dogs) showed 428 bp PCR product that were positive for *Yersinia* and 20 samples (9 cats and 11 dogs) showed 263 bp PCR product that were positive for *Salmonella*. Some samples were positive for both *Salmonella* and Yesinia (Fig. 1). So the prevalence rate of *Yersinia spp*. was 8% in cats and 22% in dogs and the prevalence rates of *Salmonella* spp. were 18 and 22% in cats and dogs respectively.

**Discussion and conclusion**

Keeping a pet at home is a huge responsibility. There are several factors regarding ethics including animal rights and welfare, health care, regular check- ups by the veterinarian and precautionary measures. If the owner neglects his/ her responsibilities, it may encounter dangerous consequences in contact with persons and public health (16).

In many parts of the world, companion pets have played a direct role in transmitting zoonoses (1). Animal-transmitted diseases are very important because some of them are frequent and unfortunately they are always unrecognized. So they may shed the pathogenic agents via secretions and transmit them to humans.

Infectious gastrointestinal diseases affect man and animals throughout the world. Certain etiologic agents (e.g. *Salmonella spp.*, *Campylobacter jejuni*, *Yersinia enterocolitica*, Cryptosporidia, *Strongyloides stercoralis*, *Echinococcus granulosa*) seem to have the potentiality to be transmitted from pets to humans, causing severe disease in the latter.

Diarrhea is the most common cause of deaths in developing countries. Every year 12 million child death and 5 million related cases to diarrheal diseases are reported according to WHO documents (17). Infectious diarrhea may be caused by many pathogens, but *E. coli*, *Salmonella*, *Shigella*, and *Y. enterocolitica* are the most important bacterial agents (18).

*Yersinios* is the third zoonotic bacterial disease in Germany and other countries of the Union of Europe**.** It is one of the five main bacterial gastrointestinal diseases of humans. Beside pigs, companion animals, especially dogs and cats, were frequently source of pathogenic *Y. enterocolitica* (19).

Salmonellosis was the second most often reported zoonotic disease in humans in 2008.

According to the results, *Yersinia* and *Salmonella* were found in 8- 22% of pet animals without any clinical signs. The contaminated animal foods may be the main source of their infection.

Previously in Italy in 1986, Nastasi *et al* isolated five *Salmonella* and one *Y.enterocolitica* from 212 dog feces (20). In another study in 2013, Stamm *et al* tested a total of 4,325 fecal samples from dogs and 2,624 samples from cats, they isolated *Y. enterocolitica* strains from 198 (4.6%) of the dog and 8 (0.3%) of the cat fecal samples (21).

Individuals at higher risk of infections were frequently present among pet- owning house- holds, regardless of species. With few exceptions, house- holds with members

at higher risk for infectious disease and those who recalled having received education on pet- associated disease risks followed similar practices to households without these individuals or education. Targeted educational efforts are indicated for households with individuals at higher risk of infections and those with high- risk species (22).

Transmission of diseases to man is usually complex, requiring close contact with pets or their excretions and frequently involves a breach of sound hygienic practice. It is also necessary to prevent feeding raw meat to animals.

This report offers a consensus opinion on the diagnosis, epidemiology, treatment and control of the primary enteropathogenic bacteria in dogs and cats, with an emphasis on *Yersinia* and *Salmonella*.

Further research is needed to determine the reasoning behind household infection control and husbandry practices and the respective roles of education and perceptions in shaping these practices.

These findings are useful to optimize the identification and management of enteropathogenic bacteria in dogs and cats and prevalent to be illness in men.

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# جداسازی *یرسینیا* و *سالمونلا* از سگ و گربه‌های به ظاهر سالم در تهران، ایران

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## چکیده

**مقدمه:** حیوانات خانگی مانند گربه و سگ منبع بالقوه انتقال آلودگی به انسان، به ویژه کودکان هستند. آن‌ها حامل عوامل بیماری‌های مشترک دستگاه گوارش هستند که می‌توانند به صاحبانشان منتقل شوند. باکتری‌های *یرسینیا* و *سالمونلا* همواره به عنوان عامل بیماری‌زای کودکان در نظر گرفته شده‌اند. این مطالعه به منظور بررسی میزان شیوع عفونت در سگ و گربه‌های به ظاهر سالم در شهر تهران در ایران انجام شده است.

**مواد و روش‌ها:** در مجموع ۱۰۰ نمونه سواب مدفوع سگ و گربه با روش Multiplex PCR با پرایمرهای اختصاصی برای تشخیص گونه‌های *یرسینیا* و *سالمونلا* بررسی شد.

**نتایج:** ۱۵ نمونه (۴ گربه و ۱۱ سگ) از نظر *یرسینیا* و ۲۰ نمونه (۹ گربه و ۱۱ سگ) از نظر *سالمونلا* مثبت بودند. در نتیجه میزان شیوع *یرسینیا* ۸ درصد در گربه و ۲۲ درصد در سگ و میزان شیوع *سالمونلا* ۱۸ درصد در گربه‌ها و ۲۲ درصد در سگ تخمین زده شد.

**بحث و نتیجه‌گیری:** با توجه به نتایج حاصل میزان شیوع *یرسینیا* و *سالمونلا* در ۸ تا ۲۲ درصد از حیوانات خانگی بدون هیچ گونه علایم بالینی تشخیص داده شد. مواد غذایی آلوده حیوانات ممکن است منبع اصلی عفونت باشد. نتایج حاصل در برنامه‌ریزی روش‌های کنترل و پیشگیرانه می‌تواند مفید باشد.

**واژه‌های کلیدی:** *سالمونلا*، *یرسینیا*، سگ، گربه، ایران

* نویسنده مسؤول مکاتبات
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