

PREVALENCE AND MOLECULAR CHARACTERIZATION OF YERSINIA SPECIES ISOLATED FROM DOGS AND CATS IN BAGHDAD CITY, IRAQ

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ABSTRACT

Domestic animals, such as cats and dogs, can spread diseases affecting humans, particularly young children. They possess zoonotic pathogens in their gastrointestinal tracts and can infect their owners. *Yersinia* bacteria are regarded as prevalent pathogens responsible for illness in youngsters. This study aims to determine the infection prevalence rate in clinically sick dogs and cats in Baghdad, Iraq. Seventy-five rectal specimens from canines and felines were examined using a conventional PCR approach with primers for the detection of *Yersinia* species. Eleven specimens (seven felines and four canines) tested positive for *Yersinia*. The prevalence rate of *Yersinia* was 17% in felines and 11% in canines. The findings indicate that *Yersinia* was present in 11–17% of symptomatic companion animals exhibiting diarrhoea. Contaminated products from animals could be the principal root of disease. These findings may aid in the formulation of control and preventative strategies.

KEYWORDS: *Yersinia*, Dog, Cat, PCR, Iraq.

INTRODUCTION

Pet animals may be the main source of zoonotic infections because they are regarded as members of the family and share the same surroundings as people.^[1] The genus *Yersinia* comprises 11 species, three of which have been definitively established as causative agents of the human disease yersiniosis. The enteropathogenic species *Yersinia enterocolitica* (*Y. enterocolitica*) affects both humans and animals and is prevalent in nature.^[2,3] The genus *Yersinia* belongs to the Enterobacteriaceae family and includes three human and animal pathogens: *Y. enterocolitica*, *Y. pestis*, and *Y. pseudotuberculosis*. You get yersiniosis from the pathogenic strains of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*.^[4] Yersiniosis was the third most reported gastrointestinal disease in 2022 inside the EU, and the greatest detection rate was observed in under-five-year-old children.^[5,28] Little is known about the epidemiology of enteric yersiniosis. There are multiple ways that *Y. enterocolitica* and *Y. pseudotuberculosis* might spread. Pork eating and occupational contact with pigs are strongly linked to sporadic *Y. enterocolitica* infections.^[6,7] Common symptoms of *Yersinia* species in children and pet animals are fever, abdominal pain, and diarrhea, which is often bloody.^[8] As previously mentioned, traditional culture-dependent techniques have a number of drawbacks, including lengthy incubation

periods of up to four weeks, a lack of species identification, and a lack of discrimination between strains that are harmful and those that are not.^[9] The first isolation and description of *Yersinia pseudotuberculosis* as the cause of tuberculosis-like lesions in guinea pigs occurred in 1884, marking the beginning of the *Yersinia* species' history.^[10] After being described, the bacterium was given the name *Bacillus pseudotuberculosis*.^[11] Soon later, in 1894, the French bacteriologist Alexandre Yersin isolated *Y. pestis* for the first time in Hong Kong, where the infamous bubonic plague agent had spread from mainland China.^[12,28] Humans can contract *Yersinia pseudotuberculosis* intermittently or in large quantities from domestic pets.^[13] Dogs and cats are examples of pets that can become a source of *Y. pseudotuberculosis* infection because they contract the pathogen throughout the winter.^[14]

MATERIALS AND METHODS

Fecal samples isolation: Isolation of faecal specimens: a maximum of 75 faecal specimens were collected from canines and felines. with referrals to small animal veterinary clinics in Baghdad, Iraq (40 cats and 35 dogs). The animals from which these samples were taken showed brown-greenish diarrhea.

DNA extraction: Following the manufacturer's instructions, 200 microliters of sample was used to extract total DNA using a genomic CTB DNA extraction kit (Applied biosystems, USA). 200 microliters of sample suspensions were incubated with 200 microliters of lysis buffer and 10 microliters of proteinase K. Following incubation, the lysate was mixed with 250 microliters of binding buffer and 250 microliters of 80% ethanol. After that, the samples were cleaned in accordance with the manufacturer's instructions. 50 microliters of the elution buffer included in the kit was used to elute the nucleic acid. Without cultivating the material, DNA was retrieved.

Identification using a PCR assay targeting the 16S rRNA gene: A designed conventional PCR method that amplifies a 1485 bp for *Yersinia* PCR amplicons primers

was used to detect the nucleotide sequences of the 16S rRNA gene in *Yersinia*, as indicated in Table 1. All amplification processes utilised the master mix red Taq DNA polymerase 2X (Thermo Fisher Scientific, USA). The amplification of PCR was conducted in 25 microliters reaction quantities, consisting of 9.5 microliters of double-distilled water, 2 microliters of template DNA, 0.5 microliters of each primer (10 mM), and 12.5 microliters of master mix. All amplified processes of PCR were conducted in a PCR Express thermal cycler. (Applied Biosystems, USA) as detailed in Table 2. Subsequently, 10 microliters of PCR amplicon were subjected to electrophoresis on a 2.5% agarose gel. Gels were visualised using a UV illuminator and captured via a gel documentation method. A 100-bp DNA ladder (Bioneer, Korea) served as a size marker for DNA molecules.

Table 1: Primers used in this study to detect the 16S rRNA.

Primer	Size of BP	Sequence (5'-3')	Reference
16S rRNA	1485	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-GGTTACCTTGTTACGACTT-3'	Hao et al., 2016

Table 2: Amplification of 16S rRNA gene was achieved using the following condition.

Phase	Temperature	Time	No. of cycle
Initial Denaturation	94°C	5 min	1 cycle
Denaturation	94°C	30 sec	32 cycles
Annealing	62°C	30 sec	
Extension	72°C	6 min	1 cycle

RESULTS

In this article this investigation assessed the presence of *Yersinia* spp in faecal samples from ostensibly diarrhoeic dogs and cats. Eleven samples of 7 cats and 4 dogs showed 1485 bp PCR product in 16S rRNA gene that were positive for *Yersinia* spp as shown in (figure 1) and (figure 2). The average percentage of *Yersinia* spp was 17% in felines and 11% in canines respectively. Regarding cat samples, the data depicted in (figure 1)

highlighted that 7 of 40 cats' samples were positive for *Yersinia enterocolitica* with 17% ratio and the dogs' samples resulted in (figure 2) 4 of 35 fecal samples were positive for 16S rRNA gene of *Yersinia enterocolitica* with 11% ratio. Conventional PCR was utilised to identify the existence of pathogenic genes in *Y. enterocolitica* obtained from faecal samples. of cats and dogs. On the other hand, the genome 16S rRNA of *Y. enterocolitica* was identified in all examined samples.

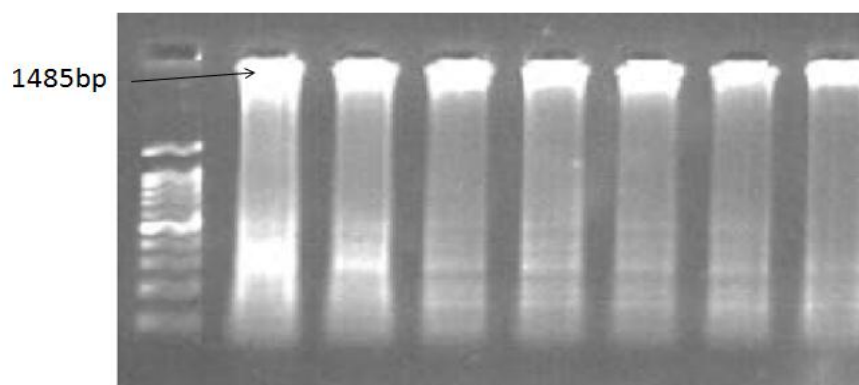


Figure 1: Agarose gel electrophoresis of *Yersinia enterocolitica* gene 16S rRNA Lane 1, lane 2, lane 3, lane 4, lane 5, lane 6 and lane 7 at 1485 bp in cats.

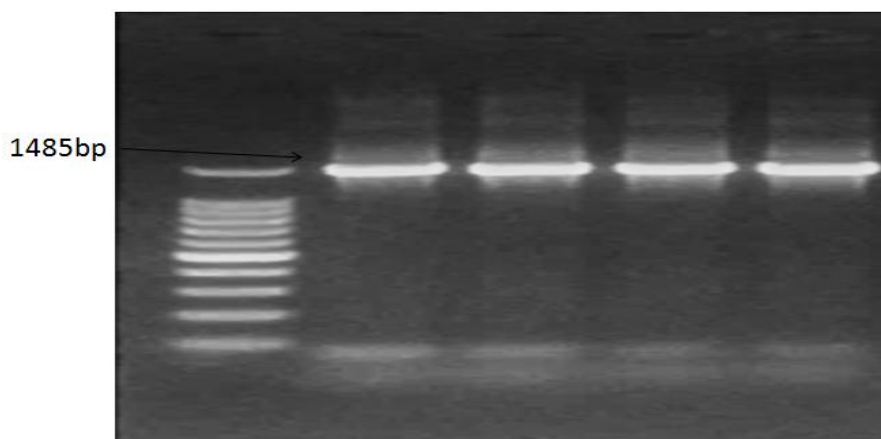


Figure 2: Agarose gel electrophoresis of *Yersinia enterocolitica* gene 16S rRNA Lane 1, lane 2, lane 3 and lane 4 at 1485 bp in dogs.

DISCUSSION AND CONCLUSION

Maintaining pets inside the house entails significant responsibilities. Animal rights and welfare, healthcare, routine veterinarian examinations, and preventive measures are all significant ethical considerations. If a homeowner abandons their duties, it may face severe repercussions in social relationships and public health.^[15] Yersiniosis is a severe disease of public health hazard.^[16] Among the gastrointestinal disease in Europe, yersiniosis takes the third level of foodborne gastrointestinal disease.^[17] Because some animal-transmitted diseases are common and, regrettably, never recognized, they are extremely essential. Thus, they might release harmful substances through their secretions and spread them to people.^[18] The leading cause of death in poor nations is diarrhea. According to WHO records, 5 million cases of diarrheal illnesses and 12 million child deaths are documented annually.^[19, 30, 31] In Germany and other EU nations, yersiniosis is the third zoonotic bacterial disease. It is among the five primary bacterial gastrointestinal disorders that affect people. Alongside pigs, pathogenic *Y. enterocolitica* was commonly found in companion animals, particularly dogs and cats.^[20] According to the results, in the present study *Yersinia* was found in 11-17% of domestic animals with symptoms of diarrhea. Contaminated animal food may be the primary source of infection. In 1986, Nastasi *et al.* recovered one *Yersinia enterocolitica* from 212 canine faeces in Italy.^[21, 27] In a 2013 study, Stamm *et al.* conducted a study in 2013 in which they analysed 4,325 faecal samples from dogs and 2,624 faecal samples from cats. They found that *Y. enterocolitica* strains were present in 198 (4.6%) of the dog samples and 8 (0.3%) of the cat samples.^[13] Regardless of species, households with pets were often associated with individuals at higher risk of illness. Households contain people who are at a higher risk of obtaining infectious diseases, and those who remember being taught about the hazards of diseases that are associated with pets, adopted comparable behaviors as families without these people or instruction. Households with individuals at greater risk of infection and those with risky species should put first their educational

initiatives.^[22,32] Disease transmission to humans is typically complicated, requires close interaction with pets or their waste, and frequently entails a violation of good hygiene practices. Additionally, animals should not be fed uncooked meat. This article presents A general agreement regarding the diagnostic criteria and epidemiology, therapy, and management of the primary enteropathogenic bacteria in dogs and cats, emphasising *Yersinia* spp. However, according to Sulakvelidze 2000, some of these organisms may represent potential emerging pathogens with putative virulence factors that differ from those found in typical "pathogenic" *Yersinia* strains and may be undetected by traditional virulence assays.^[23] The most common symptoms of this study were diarrhea and abdominal pain which has come in harmony with Saebo *et al* 2005 who reported *Yersinia enterocolitica* normally causes self-limiting gastroenteritis. The most common symptoms are abdominal pain and diarrhea, like gastroenteritis caused by other enteric infections. Other symptoms, including fever, nausea, and vomiting, may also develop.^[24, 33, 34] On the other hand, this study concluded and confirms the presence of infection with yersinia species bacteria in some cats and dogs in Baghdad city, Iraq and may be transmitted to humans through direct contact with them. *Yersinia* species demonstrated in vitro susceptibility to antibiotics typically effective against gram-negative rods, with the exception of earlier β -lactam drugs, such as aminopenicillins and first-generation cephalosporins. No multiresistant strains were identified. Aminopenicillins, first-generation cephalosporins, and amoxicillin/clavulanate, when administered independently, proved ineffective. Third-generation cephalosporins, frequently utilised in conjunction with other antibiotics, achieved success in 85% of instances. Fluoroquinolones—either singularly or in conjunction—effectively resolved all 15 infections, with patients demonstrating rapid improvement and achieving apyrexia within 1 to 4 days. Consequently, these drugs appear to represent the optimal treatment.^[25, 26, 29]

REFERENCES

- Overgaauw, P. A., Vinke, C. M., van Hagen, M. A., & Lipman, L. J. (2020). A one health perspective on the human-companion animal relationship with emphasis on zoonotic aspects. *International journal of environmental research and public health*, 17(11): 3789.
- Aziz, M., and Yelamanchili, V.S. *Yersinia enterocolitica*: 'StatPearls [Internet]' (StatPearls Publishing, 2021).
- Sabina, Y., Rahman, A., Ray, R.C. and Montet, D. *Yersinia enterocolitica*: mode of transmission, molecular insights of virulence, and pathogenesis of infection. *Journal of Pathogens*, 2011; 1: 429069.
- Abdelwahab, A.M., El-Tawab, A., Awad, A., Abdallah, F. and Maarouf, A.A. Phenotypic and genotypic studies on antibiotic resistant *Yersinia enterocolitica* isolated from milk and milk products in Kaliobia, Egypt. *Benha Veterinary Medical Journal*, 2021; 40(2): 149-153.
- European Food Safety Authority (EFSA), & European Centre for Disease Prevention and Control (ECDC). (2023). The European Union One Health 2022 Zoonoses Report. *EFSA Journal*, 21(12): e8442.
- Guillier, L., Fravallo, P., Leclercq, A., Thebault, A., Kooh, P., Cadavez, V., & Gonzales-Barron, U. (2021). Risk factors for sporadic *Yersinia enterocolitica* infections: a systematic review and meta-analysis. *Microbial Risk Analysis*, 17: 100141.
- Rosner, B. M., Stark, K., Höhle, M., & Werber, D. (2012). Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009–2010. *Epidemiology & Infection*, 140(10): 1738-1747.
- Reust, C. E., & Williams, A. (2016). Acute abdominal pain in children. *American family physician*, 93(10): 830-837.
- Cocolin, L., & Comi, G. (2005). Use of a culture-independent molecular method to study the ecology of *Yersinia* spp. in food. *International Journal of Food Microbiology*, 105(1): 71-82.
- Malassez, L., & Vignal, W. (1884). Sur le micro-organisme de la tuberculose zoologique. *Arch Physiol Norm Pathol*, 3: 81-105.
- Pfeiffer, A. (1889). *Über die bacilläre Pseudotuberkulose bei Nagethieren*. G. Thieme.
- Yersin, A. (1894). La peste bubonique a Hong-Kong. *Ann. Inst. Pasteur*, 8: 662-667.
- Stamm, I., Hailer, M., Depner, B., Kopp, P. A., & Rau, J. (2013). *Yersinia enterocolitica* in diagnostic fecal samples from European dogs and cats: identification by Fourier transform infrared spectroscopy and matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Journal of Clinical Microbiology*, 51(3): 887-893.
- Stenkova, A. M., Isaeva, M. P., & Rasskazov, V. A. (2008). Development of a multiplex PCR procedure for detection of *Yersinia* genus with identification of pathogenic species (*Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*). *Molecular Genetics, Microbiology and Virology*, 23(3): 119-125.
- Uddin, M. M., Talukder, H., Islam, O., Asaduzzaman, M., Das, M., Ahsan, M. I., & Islam, S. (2021). Magnitudes of diseases in dogs vary among different levels of age, gender, breed, and season: A hospital-based, retrospective cross-sectional study. *Heliyon*, 7(11).
- Aziz, M., & Yelamanchili, V. S. (2021). *Yersinia Enterocolitica*: StatPearls.
- Bucher, M., Meyer, C., Grötzbach, B., Wacheck, S., Stolle, A., & Fredriksson-Ahomaa, M. (2008). Epidemiological data on pathogenic *Yersinia enterocolitica* in Southern Germany during 2000–2006. *Foodborne pathogens and disease*, 5(3): 273-280.
- Hadinejad, F., Morad, H., Jahanshahi, M., Zarrabi, A., Pazoki-Toroudi, H., & Mostafavi, E. (2023). A novel vision of reinforcing nanofibrous masks with metal nanoparticles: antiviral mechanisms investigation. *Advanced Fiber Materials*, 5(4): 1273-1317.
- Keusch, G. T., Walker, C. F., Das, J. K., Horton, S., & Habte, D. (2016). Diarrheal diseases.
- Le Guern, A. S., & Pizarro-Cerdá, J. (2022). *Yersinia*. *Pathogenesis of Bacterial Infections in Animals*, 200-220.
- Nastasi, A., Massenti, M. F., Scarlata, G., Mammina, C., Calco, C., & Villafrate, M. R. (1986). Salmonella and *Yersinia enterocolitica* in soil and dog faeces. *Bollettino dell'Istituto sieroterapico Milanese*, 65(2): 150-152.
- Kucirka, L. M., Sarathy, H., Govindan, P., Wolf, J. H., Ellison, T. A., Hart, L. J., ... & Segev, D. L. (2011). Risk of window period hepatitis-C infection in high infectious risk donors: systematic review and meta-analysis. *American Journal of Transplantation*, 11(6): 1188-1200.
- Sulakvelidze, A. (2000). *Yersiniae* other than *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*: the ignored species. *Microbes and infection*, 2(5): 497-513.
- Saebo, A., Vik, E., Lange, O. J., and Matuszkiewicz, L. (2005) Inflammatory bowel disease associated with *Yersinia enterocolitica* O:3 infection, *Eur J Intern Med*, 16: 176-182.
- Tavassoli, M., Afshari, A., Drăgănescu, D. O. I. N. A., Arsene, A. L., Burykina, T. I., & Rezaee, R. (2018). Antimicrobial resistance of *Yersinia enterocolitica* in different foods. A review. *Farmacia*, 66(3): 399-407.
- Dawah, Z. A. (2022). LD50 and affective dose of *Eruca sativa* mill (gergeer) ethanolic extract. *Thi-Qar University Journal for Agricultural Researches*, 11(2).
- Qasim, D. A., Lafta, I. J., & Iyiola, O. A. (2023). Antibacterial Activity of *Lactiplantibacillus*

- plantarum from Dairy Products Against Some Foodborne Bacteria. *The Iraqi Journal of Veterinary Medicine*, 47(1): 44-51.
28. AL-Ta'an, S. A., AL-Jobori, A. H., & AL-Bana, A. S. (2004). Study about pathogenic bacteria associated with bovine mastitis. *The Iraqi Journal of Veterinary Medicine*, 28(1): 227-234.
29. Abdulridha, R. N., & Saliem, A. H. (2023). Effect of Ultrasonic Extract of Capparis spinosa Fruits Against E. coli O157: H7. *The Iraqi Journal of Veterinary Medicine*, 47(1): 86-92.
30. Saher, M. H. (2009). Study on bacterial isolation from dogs affected with malignant tumor: Saher. MH, Mohamed. A. Abdulrazak, Ali. S. Aboud, Al-khaban. JM, and Iesa. AM. *The Iraqi Journal of Veterinary Medicine*, 33(1): 120-131.
31. AL-Nassry, B. S. (2011). Isolation and Identification of bacterial isolates from ear infection and their sensitivity to usual antibiotics in human and dogs. *The Iraqi Journal of Veterinary Medicine*, 35(1): 159-166.
32. Al-abbas, A. A. A. (2010). Inhibitory effect of local Honey on Bacteria in culture media and in laboratory animals: Ali, A. Abed Al-abbas Saffaa, AA Abbas, HN Al-saeed. *The Iraqi Journal of Veterinary Medicine*, 34(1): 1-8.
33. Abdulrasool, M. I. (2011). The rapid detection of E. coli 0157 Antigen in meat products by using ELISA Test Kit. *The Iraqi Journal of Veterinary Medicine*, 35(2): 61-65.
34. Dakheel, M. M., Al-Mnaser, S. A., Quijada, S., Woodward, M. J., & Rymer, S. (2021). Use of tannin-containing plants as antimicrobials influencing the animal health.