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Article

Prevalence, Seasonal Fluctuation, and Molecular Profiles of Bacterial Enteropathogens Causing Diarrhea in Cats in Karbala, Iraq

Zainab Haider Abd ALwahhab[1], Ali Hussein Fadhil[2]

[1,2] Department of internal medicine and preventive, College of Veterinary Medicine, University of Karbala
Correspondence: zainab.hayder@s.uokerbala.edu.iq[1], ali.h.fadhil@uokerbala.edu.iq[2]

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Abstract: Background: Information on the presence of intestinal pathogens is very scarce. Consequently, this research work aimed at isolating and characterizing the primary bacteria that cause feline diarrhea in Karbala, studying their monthly prevalence, and registering their molecular information in the GenBank database. **Methodology**: One hundred clinical samples including rectal swabs and fresh feces were collected from symptomatic cats with diarrhea from different ecological zones in Karbala Governorate during an uninterrupted period of six months from October 2025 to March 2026. Preliminary bacterial isolation and identification were done using common bacterial enrichment media and selective differential media. Identification at the species level was done using the extraction of DNA from isolated bacteria followed by amplification through polymerase chain reaction (PCR) using conserved DNA sequences unique to Pseudomonas oryzihabitans, Escherichia coli, Salmonella enterica, Stenotrophomonas maltophilia, Citrobacter braakii, and Enterobacter cloacae. The genetic characterization of the identified bacteria was achieved through the Sanger sequencing of selected PCR-positive clones. The generated sequences were then analyzed against available nucleotide sequences in NCBI using BLAST and submitted to GenBank for the generation of accession numbers. **Results**: The molecular detection revealed that 67 of the 100 samples (67.0%) were positive to one of the enteropathogenic bacteria investigated. Out of the positive samples, Pseudomonas oryzihabitans was the most common bacterial species with the prevalence of 17.0% (n=17), followed by classic zoonotic bacteria Escherichia coli, which had the prevalence of 16.0% (n=16) and Salmonella enterica subsp (14.0% or n=14). Opportunistic and nosocomial pathogens were present less frequently: Stenotrophomonas maltophilia (8.0%, n=8); Citrobacter braakii (7.0%, n=7) and Enterobacter cloacae (5.0%, n=5). Seasonal fluctuations were detected during the time; October 2025 demonstrated the maximum peak of the infection (19 positive cases), whereas secondary peaks were found in December (13 cases) and March 2026 (11 cases), with the lowest rates in November and January, when only 7 positive cases were recorded. **Conclusion**: In this research work, it is clear that the bacterial pathogens are responsible for feline diarrhea in Karbala, with P. oryzihabitans, E. coli, and Salmonella being the primary bacteria causing the infection. The dramatic increase in the incidence of infections in October means that there is the critical danger associated with the change in weather in autumn, probably caused by the climatic factors which result in the physiological stress and weaken animals' immune systems. Also, the

successful deposit of the sequences in the GenBank means that the basic data on the genetics of feline enteric bacteria in Iraq have been created.

Keywords: Molecular Profiles, Felis catus, Bacterial Enteropathogens, Pseudomonas oryzihabitans, Stenotrophomonas maltophilia

Introduction

Gastrointestinal diseases, in particular those associated with diarrhea, are some of the most common clinical signs seen in cats worldwide [1]. Acute and chronic diarrhea in domestic cats (Felis catus) can cause a variety of physiological disorders in cats such as electrolyte imbalance, severe dehydration, malabsorption and life-threatening enteritis if left untreated [2]. Diarrhea in cats can be caused by diet, parasites, or viral infection. But bacterial enteropathogens are the most frequent cause of severe gastroenteritis with high rates of morbidity and mortality, especially in kittens and immunocompromised cats [3]. In addition to the veterinary aspects, these infections in cats represent a serious threat to public health because of the zoonotic potential of these bacterial pathogens [4]. Domestic cats are in close proximity with their human owners, they can act as major carriers of various pathogenic bacteria infecting humans, children and veterinary staff. Zoonotic agents such as Escherichia coli and Salmonella enterica are capable of crossing the species barrier via fecal-oral transmission, and can cause a variety of serious foodborne-like diseases in humans, including hemolytic uremic syndrome. Furthermore, the isolation of opportunistic and environmental bacteria such as Pseudomonas oryzihabitans, Stenotrophomonas maltophilia, Citrobacter braakii and Enterobacter cloacae from animal feces suggests an epidemiological change, as these microorganisms are increasingly recognized as nosocomial and opportunistic pathogens in human and veterinary medicine [5], [6]. Climate and geography are important determinants of infection distribution patterns and seasonality. Environmental stressors such as rapid shifts in temperature levels at season change can hamper the immunity of an animal host thus making it more susceptible to bacterial colonization, proliferation and shedding [7].

The traditional phenotypic methods for detection of these pathogens based on cultivation and biochemical assays are often limited in their accuracy and speed of diagnosis due to the fastidious nature of some bacterial strains or atypical biochemical properties[8]. Thus the advent of molecular techniques including the use of PCR technology has revolutionized the veterinary diagnostics by enabling rapid and precise identification of the specific genotypes of bacteria from clinical samples [9]. It is also important to confirm molecular results by DNA sequencing and to deposit the sequences in international databases such as NCBI GenBank to confirm local findings and to create valuable genetic references for monitoring the molecular evolution and worldwide transmission of pathogens[10].

The prevalence, seasonality and genetics of bacteria causing diarrhea in cats in Iraq, particularly in the Karbala Governorate, are poorly studied and there is a clear lack of molecular data and surveillance. The peculiarity of the socio-environmental conditions of the territory, in particular the growing popularity of keeping pets and the number of stray animals, makes the study of the local bacterial profile relevant.

Therefore, this study was performed with the following objectives: 1.To detect and identify the primary enteric bacterial pathogens of diarrheic cats from Karbala Governorate by using PCR. 2. To describe the temporal and monthly distribution of these infections in a period of six months. 3. To create a permanent genetic bank of isolated bacterial strains with official registration in international GenBank database.

Diarrhea as the major clinical sign is one of the most common presentations in feline veterinary practice worldwide. In domestic cats (Felis catus) acute and chronic diarrhea can result in a spectrum of physiological disorders to the cat including electrolyte imbalance, dehydration, malabsorption and potentially fatal enteritis if untreated. Tho dietary factors, parasitic and viral

infections may be associated with feline diarrhea, bacterial enteropathogens are usually the main cause of gastroenteritis with high morbidity and mortality rates, especially in kittens and immunocompromised animals. The zoonotic potential of these bacterial pathogens makes infections in cats a significant public health threat beyond veterinary concerns. Close contact with their human owners makes domestic cats a major carrier of a variety of pathogenic bacteria infecting humans, children and veterinary staff [11]. For example, fecal-oral transmission of zoonotic agents, including Escherichia coli and Salmonella enterica, can cross species barriers and cause different severe foodborne-like diseases in humans, such as hemolytic uremic syndrome [12]. The isolation of opportunistic and environmental bacteria such as Pseudomonas oryzihabitans, Stenotrophomonas maltophilia, Citrobacter braakii and Enterobacter cloacae from the feces of animals also indicates an epidemiological transition, in which these microorganisms are increasingly being recognized as nosocomial and opportunistic pathogens in human and veterinary medicine. Climatic and geographical factors have a great influence on the distribution and seasonality of infections. Rapid temperature changes during seasonal shifts, for instance, are environmental stressors that can weaken an animal host's immunity, thus making it more prone to bacterial colonization, proliferation and shedding.

The traditional phenotypic methods for the detection of these pathogens based only on cultivation and biochemical tests are often limited in their accuracy and speed of diagnosis due to the fastidiousness of some bacterial strains or unusual biochemical characteristics. Thus, the advent of molecular techniques such as PCR technology has revolutionized the veterinary diagnostic, enabling rapid and precise identification of specific genotypes of bacteria from clinical samples. Molecular results should also be confirmed by DNA sequencing and deposited in the international databases such as NCBI GenBank to validate local findings and create useful genetic references for monitoring the molecular evolution and worldwide transmission of pathogens.

In Iraq, specifically, in the Karbala Governorate, there is an apparent lack of molecular data and surveillance concerning the prevalence, seasonality, and genetics of bacteria causing diarrhea in cats. Given the unique socio-environmental conditions in the area, including rising popularity of keeping pets as well as the high number of stray animals, studying the local bacterial profile becomes critical.

Methodology

1. Ethical Issues and Geographical Limitations.

The current study obtained the official approval of the Institutional Animal Care and Use Committee (IACUC) and Ethics Committee of the College of Veterinary Medicine, University of Karbala, Iraq. The clinical examination and sample collection were performed in the province of Karbala only. Diagnostic and sampling procedures were performed following the internationally accepted standards for the care of veterinary animals with special consideration of avoiding causing any stress and discomfort in the examined animals.

2. Collection and Handling of Samples

During a 6 months period between October and March, 100 samples of feces and rectal swabs were collected from domestic cats (Felis catus) suffering from acute diarrhea with the appearance of watery or mucoid stools. The samples were collected from veterinary hospitals, private clinics, shelters for animals and stray cats in the Karbala Province. Rectal swabs were immediately placed in Amies transport medium (Oxoid, UK), while feces were collected in sterile plastic containers. Each sample was marked according to the month of its collection, stored at 4°C in the insulated ice boxes and sent to the laboratory within two hours [13].

3. Isolation and Phenotypical Characterization of Bacteria

All the samples were initially cultured in appropriate enriching media, including Selenite F broth for isolation of Salmonella spp. and Nutrient broth (Oxoid, UK) for enrichment of enteric bacteria. Further the bacteria were cultured on selective and differential media, which included MacConkey agar, Eosin Methylene Blue (EMB) agar, Xylose Lysine Deoxycholate (XLD) agar, and Cetrimide agar (Oxoid, UK). Media plates were incubated aerobically at 37°C for 24 hours. The suspected bacteria colonies were observed for their morphology, Gram-staining and selected biochemical tests, including catalase, oxidase, Triple Sugar Iron (TSI) and IMViC tests [14],[15].

4. **Isolation of Genomic DNA**

The genomic DNA was isolated from overnight cultures of the suspected bacteria using commercially available DNA isolation kit, such as Qiagen DNeasy Blood & Tissue Kit (Germany) or Bioneer kit (South Korea) according to manufacturer's instructions. The quality and concentration of the DNA were estimated using NanoDrop spectrophotometer based on the ratio of the absorbance at 260/280 nm. The purified DNA was stored at −20°C until the subsequent molecular analyses [16].

5. Molecular Identification with PCR

Polymerase chain reaction (PCR) was used to identify Pseudomonas oryzihabitans, Escherichia coli, Salmonella enterica, Stenotrophomonas maltophilia, Citrobacter braakii and Enterobacter cloacae using species-specific primers targeting conserved genes of bacteria, including 16S rRNA, uidA for E. coli and invA for Salmonella. Each PCR reaction was made in 25 μL reaction volume, containing 12.5 μL Master Mix (Promega, USA), 1 μL of each forward and reverse primers (10 pmol), 2 μL of DNA template and 8.5 μL of nuclease-free water. The PCR was performed in the thermal cycler with the following profile: initial denaturation at 95°C for 5 minutes, 30-35 cycles consisting of denaturation at 94°C for 45 sec, primer-specific annealing for 45 sec, extension at 72°C for 1 min with final extension at 72°C for 7 minutes. The amplified fragments were subjected to separation by electrophoresis in 1.5% agarose gels stained with Ethidium bromide or GelRed and visualized under UV light [17],[18].

6. Sequencing and Deposit into GenBank

In order to verify PCR results and investigate the genetic variability of selected PCR products, the selected positive amplicons were further purified using PCR purification kit [19]. Next, purified amplicons were sequenced in a bidirectional manner using the same primers, used for PCR amplification. The nucleotide sequences were assembled, edited using the BioEdit software and compared with the reference sequences from NCBI database using BLAST search tool. Confirmed sequences were deposited into GenBank for obtaining unique accession numbers [20].

Results

1. Total Prevalence and Composition of Feline Enteric Pathogens

Out of the 100 fecal samples and rectal swabs analyzed from diarrheic cats (Felis catus) in Karbala Governorate, PCR-based molecular testing confirmed that 67 samples (67.0%) tested positive for the targeted enteric bacterial pathogens. The remaining 33 samples (33.0%) did not show any amplification for the targeted species.

Among the positive isolates (n = 67), Pseudomonas oryzihabitans was the most prevalent pathogen detected, with 17 isolates in total, accounting for 17.0% of the entire sampled population.Escherichia coli was the second most frequent pathogen, detected in 16 samples (16.0%), closely followed by Salmonella enterica subsp. with 14 isolates (14.0%). Opportunistic and nosocomial bacterial species were recovered at lower prevalence rates: Stenotrophomonas maltophilia was detected in 8 samples (8.0%), Citrobacter braakii strain in 7 samples (7.0%), and Enterobacter cloacae in 5 samples (5.0%).

All representative isolates were successfully sequenced and deposited into the NCBI GenBank

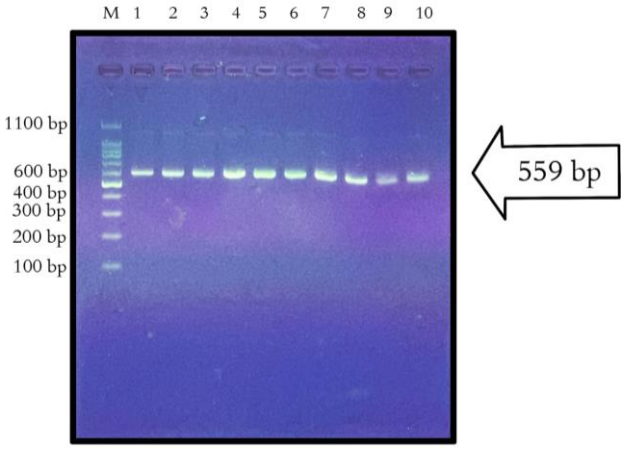

Figure 1: Agarose gel electrophoresis of PCR products for the detection of the ompT gene in Escherichia coli isolates. Lane M represents the DNA molecular size marker, while lanes 1-10 represent the examined E. coli isolates.Specific amplification products corresponding to the ompT gene were detected as distinct DNA bands at the expected size of 559 bp.

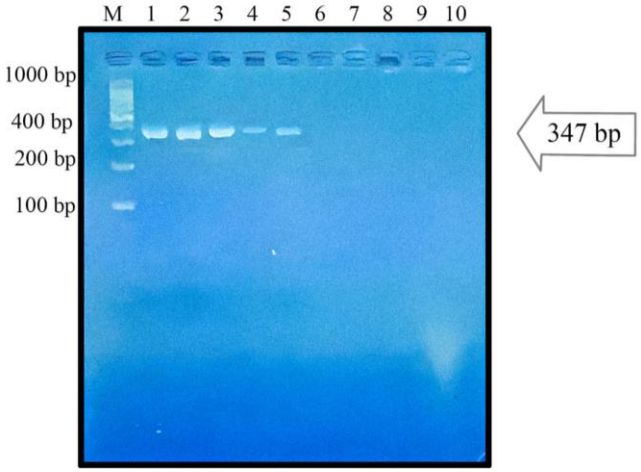

Figure 2. Agarose gel electrophoresis of PCR- amplified products targeting the exo gene in Pseudomonas oryzihabitans isolates. Lane M represents the DNA molecular size marker, while lanes 1-10 represent the examined bacterial isolates. Specific amplification bands corresponding to the target exo gene were detected at the expected amplicon size of 347 bp in lanes 1-5, whereas no specific amplification bands were observed in lanes 6-10.

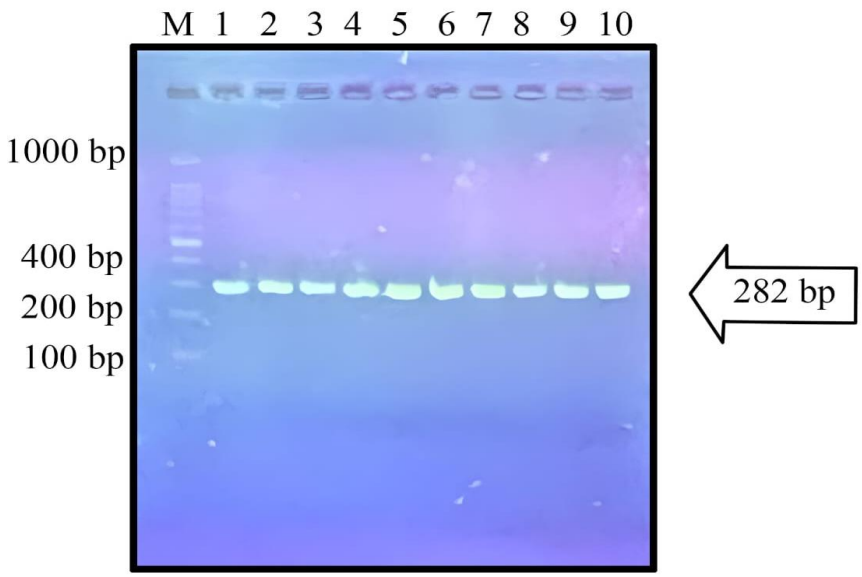

Figure 3. Agarose gel electrophoresis of PCR- amplified products targeting the inv gene of Salmonella enterica subsp. Lane M represents the DNA molecular size marker, while lanes 1-10 represent the examined Salmonella enterica subsp. isolates. Specific amplification products corresponding to the target inv gene were detected as distinct DNA bands at the expected amplicon size of 282 bp in all examined isolates.

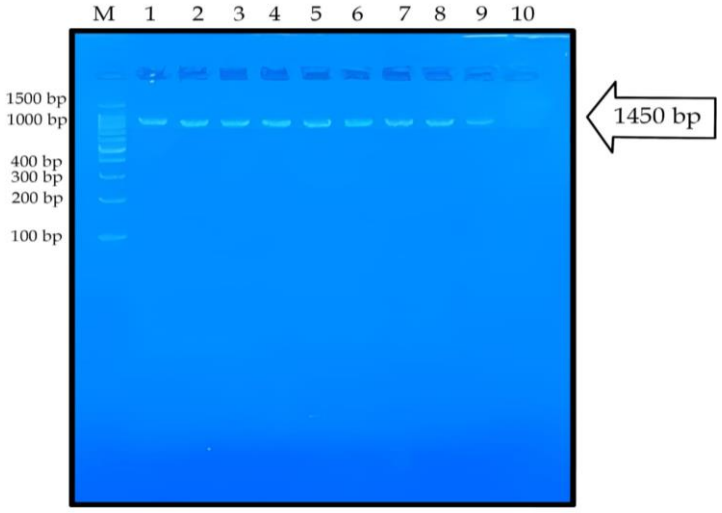

Figure 4. PCR amplification of the bacterial 16S rRNA gene analyzed by agarose gel electrophoresis. Lane M indicates the DNA molecular size marker, and lanes 1-10 represent the examined bacterial isolates. All isolates exhibited distinct amplification products at approximately 1450 bp, corresponding to the expected size of the targeted 16S rRNA gene fragment.

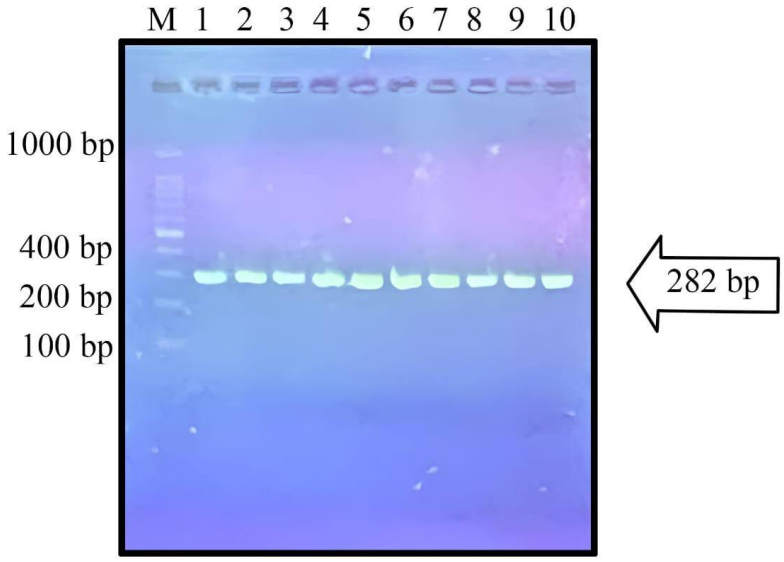

Figure 5. Agarose gel electrophoresis of PCR- amplified products targeting the bacterial 16S rRNA gene. Lane M represents the DNA molecular size marker, whereas lanes 1-9 represent the examined bacterial isolates. Specific amplification bands of approximately 1500 bp were observed in lanes 1-8, corresponding to the expected size of the targeted 16S rRNA gene fragment, while no distinct amplification band was detected in lane 9.

2. Temporal and Monthly Distribution of Isolates

Bacterial shedding and infection rates were found to fluctuate during the six month-period of monitoring. The monthly distribution of positive cases is shown below in the order of descending: Total Monthly Positive Cases: The maximum positive isolates were detected in October with 19 positive isolates obtained from 31 samples. This was followed by December, which recorded 13 cases from 20 samples; March, with 11 cases from 15 samples; and February, with 10 cases from 14 samples. The lowest counts occurred in both November and January, each with only 7 positive cases from 10 samples per month (Table 4-1).

Table 1. Monthly Frequency and Distribution of Isolated Bacterial Species (October to March)

Bacterial Species / Strain	October	November	December	January	February	March	Total
Pseudomonas oryzihabitans	4	2	3	3	2	3	**17**
Escherichia coli	4	2	3	2	2	3	**16**
Salmonella enterica **subsp.**	4	2	2	2	2	2	**14**
Stenotrophomonas maltophilia	2	1	2	0	1	2	**8**
Citrobacter braakii **strain**	3	0	2	0	1	1	**7**
Enterobacter cloacae	2	0	1	0	2	0	**5**
Monthly Total	**19**	**7**	**13**	**7**	**10**	**11**	**67**

Discussion

One of the most interesting results of this study is the very high prevalence of bacterial pathogens (67.0%) in cats with diarrhea in Karbala Governorate, which reveals the important role that bacteria play in the etiology of gastroenteritis in cats[21]. The high prevalence indicates the susceptibility of both domestic and wild cats to bacterial colonization, which may be due to poor hygiene, the scavenging behavior of stray cats or environmental contamination.

It should be noted that Pseudomonas oryzihabitans (17.0%) is one of the prominent pathogens. Previously thought of as rare environmental saprophytes, the microorganisms are currently increasingly regarded as emerging opportunistic pathogens in veterinary medicine. Their high isolation rate reflects the high degree of environmental contamination from either stagnant water sources or poor hygiene in feline environment. P. oryzihabitans will further proliferate in the guts of cats with suppressed gut immunity caused by stress or infection.

The significant detection of two zoonotic pathogens Escherichia coli (16.0%) and Salmonella enterica (14.0%) raises serious concerns regarding human public health. The latter does not normally reside in the gut of cats but is associated with clinical salmonellosis, which can lead to severe enteritis and septicemia. The existence of these pathogens in the gastrointestinal tract of companion animals in Karbala is indicative of the danger of possible transmission of the bacteria to humans, including children, via the fecal-oral route when petting or cleaning litter boxes [4]. Furthermore, the detection of Stenotrophomonas maltophilia (8.0%), Citrobacter braakii (7.0%) and Enterobacter cloacae (5.0%) indicates that there is an increasing variety of opportunistic enteric pathogens in felines. S. maltophilia and E. cloacae are known for their natural resistance to multiple antimicrobial drugs and ability to create biofilm, therefore posing a significant nosocomial threat in veterinary practice [22].

As regards temporal aspect of this issue, the significant rise in the number of positive cases registered in October (19 isolates) correlates with the autumn transitional climate in Iraq. During the transition between summer and autumn, the variations in temperature and humidity levels pose significant thermal and environmental stresses on the animals [23]. This type of stress is known to weaken mucosal immunity of the gut and make cats susceptible to the development of opportunistic infections and increased fecal shedding. On the other hand, the decrease in the number of cases in November and January shows that there was some kind of stability of the gut microflora or decreased bacterial loads in the environment during the winter.

Finally, the molecular identification of the pathogens using PCR, followed by submission to NCBI GenBank database with the designated accession numbers [See section 3.1 for specific accession keys] is a scientific breakthrough[24]. The global database becomes a significant source of information on the particular strain of bacteria existing in the Middle East region, specifically in Iraq. It provides the researchers with opportunities for conducting future phylogenetic research in order to trace the changes in virulence factors and identify cross-border routes of zoonoses' transmission.

Conclusion

The present study confirms that the pathogens associated with causing feline diarrhea in Karbala Governorate include Pseudomonas oryzihabitans, E. coli, and Salmonella as the main causative agents. The peak prevalence during October indicates that seasonal changes in weather conditions act as significant risky periods. Moreover, the deposition of the pathogens to GenBank acts as a genetic benchmark for the region, indicating the need for proper hygiene precautions among pet owners to prevent zoonotic infection.

The present study confirms that bacterial enteric pathogens are important contributors to diarrhea among cats in Karbala Governorate, with an overall detection rate of 67.0% among the examined diarrheic cats. The findings demonstrated that Pseudomonas oryzihabitans, Escherichia coli, and Salmonella enterica were the most frequently detected bacterial pathogens, while Stenotrophomonas maltophilia, Citrobacter braakii, and Enterobacter cloacae were also identified at

notable frequencies. These findings demonstrate the diversity of bacterial agents associated with feline gastrointestinal disease in the study area.

The relatively high prevalence of bacterial pathogens highlights the importance of routine bacteriological and molecular investigation of cats presenting with diarrhea, particularly in environments where domestic cats, stray animals, and humans have frequent contact. The detection of potentially zoonotic organisms such as E. coli and Salmonella enterica is of particular public-health importance because infected or asymptomatic animals may contribute to environmental contamination and transmission through fecal-oral contact. Therefore, appropriate hygiene practices, regular veterinary examination, safe handling of feces, and responsible pet ownership should be encouraged to minimize the potential risk of zoonotic transmission.

The observed monthly variation in bacterial detection, with the highest number of positive cases recorded in October, suggests that seasonal and environmental conditions may influence bacterial occurrence and shedding in diarrheic cats. However, the present study was conducted over a six-month period, and therefore longer-term surveillance covering all seasons would be valuable for determining whether the observed pattern represents a consistent seasonal trend.

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