



Prevalence of Zoonotic Pathogens in Domestic and Feral Cats in Shanghai, with Special Reference to *Salmonella*

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Abstract

Interacting with domestic or feral cats comes with both pleasure and harm. This research has attempted to study the role of cats in Shanghai, China, being vectors of disease and the risk they pose to public health. Polymerase Chain Reaction (PCR), gel electrophoresis, molecular cloning, and DNA sequencing were methods used to compare the prevalence of zoonotic bacteria and parasites in domestic and feral cats. Phylogenetic trees were created for further analysis. From this study involving 30 cats and 12 zoonotic pathogens, four pathogens were identified in our stool samples using PCR: *Escherichia coli*, *Campylobacter spp.*, *Salmonella spp.*, and *Toxocara cati*. In total, 60% of the kittens had enteric zoonoses in their stools while 30% of the adult cats' stools contained enteric zoonoses. *Salmonella enterica* subspecies *enterica*, which is the leading cause of *Salmonellosis* in developed countries, was present in two samples inferred from sequencing and phylogenetic tree analysis.

Keywords

Zoonotic Pathogen, Zoonosis, Cat Stool, Pet, Salmonella, Public Health, Infectious Disease, PCR, Primer, Phylogenetic Tree

Introduction

Zoonotic diseases are transmitted by bacteria, parasites, and viruses between vertebrate animals and humans; at least 61% of all human pathogens are zoonoses [1]. The prevalence of zoonotic diseases in urban settings is far more common than we could imagine. Feral cats, dogs, and house pets are potential reservoirs for a number of zoonoses. Close interactions with these animals significantly increase the risk of humans unknowingly being infected with zoonotic

pathogens [2]. Over the past years, the Chinese population rearing cats and dogs as pets has increased due to improvements in overall lifestyle. By 2017, there were at least 53 million pet cats (<https://www.mapsofworld.com/world-top-ten/countries-with-most-pet-cat-population.html>) and 27 million pet dogs in China. (<https://www.mapsofworld.com/world-top-ten/countries-with-most-pet-dog-population.html>)

In Shanghai, an urban city in China, we often observed students closely interacting with feral cats in university campuses. There were also reports of students rearing feral cats in their dormitory rooms. A lack of awareness that animals could be potential reservoirs of zoonotic diseases is a great risk that increases one's exposure to infection, which poses a zoonotic hazard to both human health and the community [3]. Prominent feline zoonoses include: *Toxoplasma gondii*, *Bartonella henselae*, *Toxocara cati*, *Rickettsia felis*, enteropathogenic *Escherichia coli* and the emerging feline-related *Corynebacterium ulcerans* [4]. *Cryptosporidium parvum* and *Toxoplasma gondii* are high-risk zoonotic pathogens, while *Echinococcus spp.* are low-risk pathogens [5]. In a study involving 9 cats, 67.5% were found to have *Giardia spp.* (57.1%), *Cryptosporidium spp.* (1.4%), *Ancylostoma tubaeforme* (1.1%), and *T. cati* (1.1%) [6].

According to a study conducted in Central China, 360 cat stools were microscopically examined for intestinal parasites: 64 (17.78%) were infected with *T. cati*, 61 (16.94%) with *Isospora felis*, 41 (11.39%) with *Isospora rivolta*, 33 (9.17%) with *Paragonimus*, 23 (6.39%) with hookworms, 11 (3.06%) with *Toxoplasma*-like oocysts, 10 (2.78%) with *Trichuris*, 4 (1.11%) with lungworm, 2 (0.56%) with *Sarcocystis*, and 1 (0.28%) with trematode [7]. Statistical analysis suggested that cats living outdoors are an essential factor contributing to the presence of zoonotic pathogens found in cat stools [7]. Another study in Guangzhou, China, investigated the prevalence of *T. gondii*, where 30.77% of feral cats and 17.98% of domestic cats were positive for *T. gondii*; similarly in Lanzhou, a *T. gondii* prevalence of 21.3% in pet cats was reported, indicating a high prevalence of *T. gondii* in cats from China [3].

We hypothesize that dietary factors may also play an important role in the prevalence of enteric zoonoses in cats. For instance, feral cats hunt rodents and birds, which harbor a number of pathogens, making them more likely to acquire and transmit diseases to humans [8,9]. Additionally, if domestic cats are fed with raw meat, the likelihood of contracting *Salmonella spp.* increases significantly [10]. It is worth mentioning

that some zoonotic pathogens are more likely to be present in kittens than in adult cats, such as *T. cati* and *Giardia spp.* Parasites [11,12].

The present study aims to determine the prevalence of 12 different zoonotic pathogens in cats and compare them according to two risk factors: age (adult or kitten) and living conditions (feral or domestic) in Shanghai, China, with an emphasis on the bacterial zoonosis: *Salmonella*.

Materials and Methods

Stool samples:

This cross-sectional study was carried out throughout June-September 2017 in Shanghai, China. 30 cat stool samples were randomly collected across Shanghai, which was accomplished by: collaborating with volunteers from various shelters in different districts, involving volunteers from different universities to send stool samples of their campus' feral cats, implementing trapping and releasing of feral cats in our university campus, and involving domestic cat owners and their friends and families to send us their cats' stools. In total, 15 stool samples were obtained from domestic cats while the other half were obtained from stray cats. Out of the 15 stool samples, 10 belonged to adult cats while 5 belonged to kittens.

Stool Examination and Culture:

All stool samples collected were stored at -20°C within 24 hours of collection.

PCR Analysis:

Genomic DNA was extracted from 30 stool samples using the QIAamp DNA Stool Mini Kit and stored at -20°C when unused; they were thawed prior to performing PCR. The genomic DNA was subjected to PCR for the amplification of 12 different DNA genes based on their respective primers as shown in **Table-1**. A total of 15 primer pairs were used, of which 4 pairs of primers for *Toxoplasma gondii*, *Toxocara cati*, *Trematoda spp.* and *Entamoeba histolytica* were designed in this study using BioEdit and BLAST (www.ncbi.nlm.nih.gov/BLAST/).

Briefly, standardized PCR protocol was carried out throughout our study in a 20ul reaction mixture using

TaKaRa's PCR Amplification Kit. A total of 30 to 35 min polymerization step at 72°C. Initially, 30 PCR cycles of PCR were performed as follows: initial 3-min denaturation at 94°C, followed by denaturation at 94°C for 15s, annealing at 50°C to 60°C for 30s, polymerization at 72°C for 30s, and a final 7- min polymerization step at 72°C. Initially, 30 PCR cycles were used during the experiment; 35 cycles were implemented for repeats. Optimal annealing temperatures (50°C, 55°C and 60°C) were selected according to the primers' GC content.

Table-1: The primers used in this study to amplify target genes in DNA samples by PCR

Pathogen	Primer	Primer Sequence (5' → 3')	PCR size / bp	Reference
Bacteria				
<i>Escherichia coli (EHEC / EPEC)</i>	Eae-R	GTAAGTCCGTTACCCCAACCTG	482	[13]
	Eae-F	TCAATGCAGTTCCGTTATCAGTT		
<i>Shigella spp.</i>	ipaH-R	CCAGTCCGTAAATTCATTCT	423	[14]
	ipaH-F	TGGAAAACTCAGTGCCTCT		
<i>Salmonella spp.</i>	ipaB-R	GCCTCTCCCAGAGCCGTCTGG	314	[15]
	ipaB-F	GGACTTTTTAAAAGCGGCGG		
<i>Campylobacter spp.</i>	CadF-R	TCAAGTCTTAAAGCCAAAGAATC	119	[16]
	CadF-F	AGCAGGTGGAGGATATGAGG		
Parasites				
<i>Toxoplasma gondii</i>	Tg-SAG-R	GCTTCCTTCTTGATCGTTAG	276	[17]
	Tg-SAG-F	ATCGGTCGTCAATAATGTCG		
	Tg-366-R	AAAGGGGTTTGGATTCTATCATTCCA	366	This study
	Tg-366-F	ATGCGCACATGCCTCTTCCCCTG		
<i>Toxocara cati</i>	Tc-ITS-R	TAGTTTCTTTTCCTCCGCT	375	[18]
	Tc-ITS-F	GGAGAAGTAAACTC		
	Tc-209-R	AAGTCAGAGGTTCTGAAGGCG	209	This study
	Tc-209-F	CAAATTAAGCCGAGGCTCC		
<i>Trematoda spp.</i>	Trematode-R	ACTAAAGAAAACAGCAGT	200-300	This study
	Trematode-F	TTTCAGCATTTGTTTTGG		
<i>Entamoeba histolytica</i>	ITS2-R	TCATTCGCCATTACTTAAGAAATCATTGTT	400-500	[19]
	ITS1-F	AGGTGAACCTGCGGAAGGATC ATTA		
	Entam2-R	CAGATCTAGAAACAATGCTTCTCT	400-500	This study
	Entam1-F	GTTTTATACATTTTGAAGACTTTATG		
<i>Entamoeba coli</i>	Ecoli-R	GATTTCTACAATTCTCTTGGCATA	100	[20]
	Ecoli-F	GAATGTCAA AGCTAATACTTGACG		
<i>Blastocystis hominis</i>	Blast-1017	TGCTTTCGCACTTGTTTCATC	500	[21]
	Blast-505	GGAGGTAGTGACAATAAATC		
<i>Cryptosporidium parvum</i>	Crypto-nest-1R	GCAGCTGCTAATCTTCTTAGTGC	500	[22]
	Crypto-nest-1F	AAAGAAGCACCTCTGTTTCAG		
<i>Giardia spp.</i>	Giar-nest-1R	CTGCTGCCGTCCTTGGATGT	500	[23]
	Giar-nest-1F	AAGTGTGGTGCAGACGGACTC		

Gel Electrophoresis and DNA Sequencing:

The PCR products were subjected to gel electrophoresis using 2% ethidium-stained agarose gel. Genomic DNA were mixed with TaKaRa 6x purple DNA loading dye and pipetted into the wells, which were ran for 20 minutes at 100V. The agarose gel was visualized under 312nm wavelength of ultraviolet light using SmartView.

Positive PCR products were extracted using AxyPrep DNA Gel Extraction Kit and purified with QIAquick PCR Purification Kit prior to sequencing. Weakly positive bands and PCR products that failed to sequence by Thermo Fisher Scientific Company were proceeded with ligation according to TaKaRa's DNA Ligation Kit Protocol, followed by transformation into viable *Escherichia coli* cells, selective bacterial culture, inoculation, harvesting of plasmid DNA post-digestion using HindIII, and finally, performing gel electrophoresis to verify successful DNA ligation before sending for sequencing.

DNA Sequence Analysis:

DNA sequences received from Thermo Fisher

Scientific Company were analyzed by comparing them with nucleotide sequences from the GenBank database (<https://www.ncbi.nlm.nih.gov/>) and using BLAST (www.ncbi.nlm.nih.gov/BLAST/) in order to determine the genomic DNA's identity.

Phylogenetic Analysis:

Analysis of multiple DNA sequences was performed using Molecular Evolutionary Genetics Analysis (MEGA), where phylogenetic trees were constructed using the neighbor-joining method.

Results

PCR Analysis:

From our positive samples seen in **Fig-1**, three out of four zoonotic pathogens are bacterial species. However, the most prevalent zoonotic pathogen is the parasite *T. cati* (42.9%), followed by EPEC / EHEC (35.7%), *Salmonella spp.* (14.3%) and eventually *Campylobacter spp.* (7.1%). In total, more pathogens were present in feral cats (71.4%) than domestic cats (28.4%). Additionally, an equal number of pathogens are found in both feral kittens and domestic kittens (21.4%).

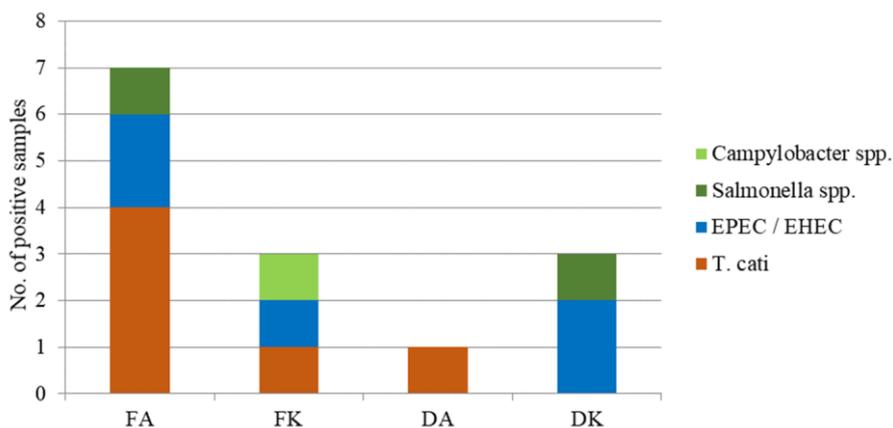


Fig-1: Proportion of pathogens detected in stools of feral adult cats, feral kittens, domestic adult cats and domestic kittens using PCR analysis.

D = Domestic; K = Kitten; F = Feral; A = Adult cat

DNA Sequence Analysis:

Positive stool samples detected using PCR and gel electrophoresis were selected and sent to Thermo Fisher Scientific Company for sequencing. Subsequently, the DNA sequences were analyzed by comparing sequences retrieved from GenBank database and via BLAST to identify sequence similarity.

Table-2 showed samples DK3, DK5, FA3, FA4 and FK1 positive for pathogenic *E. coli* with 99% similarity to the eae nucleotide sequence. Samples DK4 and FA6 were also positive for *S. enterica* with 99% and 96% similarities to the ipaB nucleotide sequence according to the GenBank database.

Table-2: Selected PCR-positive samples sent for sequencing and analyzed using BLAST

Sample	Primer	Amplicon size / bp	Potential Pathogen	BLAST result	
				Similarity	Pathogen identity
DK3	eae	482	<i>E. coli</i> (EPEC/EHEC)	99%	<i>E. coli</i> (EPEC / EHEC)
DK5	eae	482	<i>E. coli</i> (EPEC/EHEC)	99%	<i>E. coli</i> (EPEC / EHEC)
FA3	eae	482	<i>E. coli</i> (EPEC/EHEC)	99%	<i>E. coli</i> (EPEC / EHEC)
FA4	eae	482	<i>E. coli</i> (EPEC/EHEC)	99%	<i>E. coli</i> (EPEC / EHEC)
FK1	eae	482	<i>E. coli</i> (EPEC/EHEC)	99%	<i>E. coli</i> (EPEC / EHEC)
DA1	Entam	600	Unknown	95%	<i>Euphorbiaceae</i>
DA2	Entam	600	Unknown	99%	<i>Gregarina blattarum</i>
DK4	ipaB	314	<i>Salmonella spp.</i>	99%	<i>Salmonella enterica</i>
FA6	ipaB	314	<i>Salmonella spp.</i>	96%	<i>Salmonella enterica</i>
FA9	Tc-ITS	375	<i>T. cati</i>	Unknown	Unknown
FA10	Tc-ITS	375	<i>T. cati</i>	Unknown	Unknown

Samples DA1 and DA2 were positive for our self-designed Entam primer at 600bp for *E. histolytica*, however DNA analysis revealed 99% similarity to a *Euphorbiaceae* plant and a *Gregarina* parasite respectively. No organisms were identified for *T. cati* samples FA9 and FA10 despite showing strong

evidence for ITS gene on gel electrophoresis **Fig-2** and **Fig-3**.

Phylogenetic Analysis:

Construction of Phylogenetic trees using the neighbour-joining method (**Fig-4** and **Fig-5**).

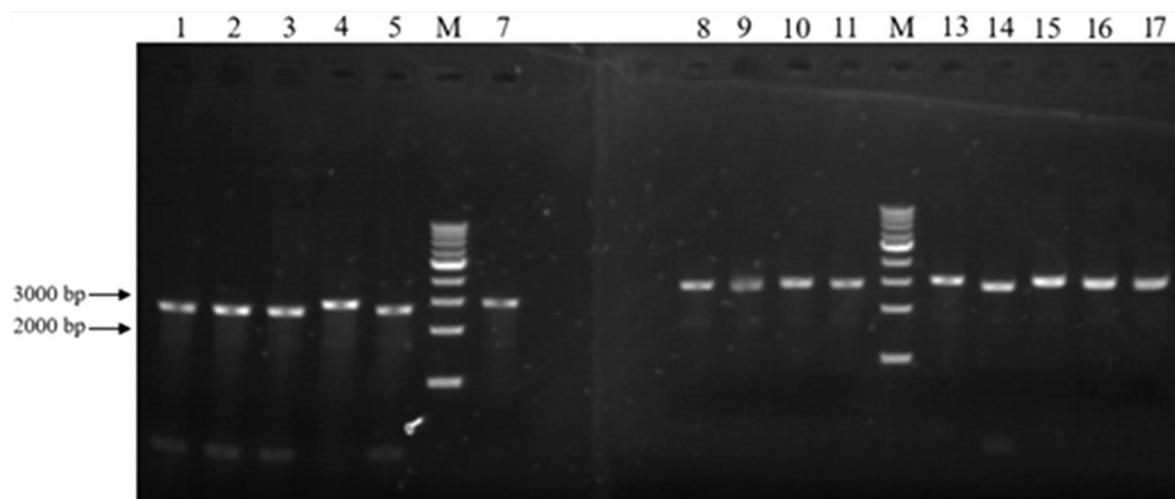


Fig-2: Gel electrophoresis of recombinant plasmids after restriction digest by HindIII enzyme.

The plasmid vector was 2700bp. The two inserts used were ipaB gene at 314bp and ITS gene at 375bp.

Lane M: Marker (1Kbp ladder); Lanes 1-5: DK4 (ipaB gene); 7-11: FA6 (ipaB gene); 13-17: FA9 (ITS gene).

D = Domestic; K = Kitten; F = Feral; A = Adult cat

Discussion

At the beginning of our study, we hypothesized that zoonotic agents would be more prevalent in kittens than adult cats, and specifically more kittens would be infected with parasites due to a relatively weaker immune system. Our findings revealed that 60% of the kittens' stools contained enteric zoonoses while 30% of the adult cats' stools contained enteric zoonoses. This supports our hypothesis, given the immune systems of kittens are still less immunocompetent, as they have not been fully developed, thereby increasing their susceptibility to infections [24]. Similarly, immunocompromised populations such as pregnant women, young children, HIV/AIDS patients and transplant patients on immunosuppressive therapy are reported to suffer from debilitating symptoms when infected with, for instance, *T. gondii* [25,26]. Our results (Fig-1) also showed a higher prevalence of bacteria (3 out of 4) than parasites. This could be attributed to factors such as: geographical locations and the unique life cycles of parasites. As our study was conducted in Shanghai, an urban city, the prevalence of parasites would be comparatively lower than suburbs and suburban areas where the presence of intermediate hosts (birds, rodents and rabbits) is more abundant [3,7]. Furthermore, despite the fact that cats are definitive hosts of the parasite *Toxoplasmosis*, studies have shown that infected cats are limited to excreting *T. gondii* oocysts in stools up to 21 days post-infection [25,27]. After the initial infection, they develop lifelong immunity to further excretion of oocysts unless immunocompromised [25,28]. Given the brief window period of oocyst excretion, it is uncommon to detect oocysts in stools of infected cats, as reported by this study involving 563 feral cats, where the average rate was 0.9% over a three year period [28].

As seen in Table-2, samples that are positive for pathogenic *E. coli* and *S. enterica* on PCR and gel electrophoresis were 96%-99% accurate to their respective species using BLAST. The Entam primer was designed to amplify PCR products at 400-500bp for *E. histolytica* (Table-1). However, samples DA1 and DA2 did not produce bands at the intended base pair level - a 600bp sequence was amplified instead. Results from BLAST revealed that the sequence in

sample DA1 belongs to a plant from the *Euphorbiaceae* family while the sequence in sample DA2 belongs to a parasite species, *Gregarina blattarum* (Table-2), which belongs to a group of Apicomplexan alveolates closely related to *Toxoplasma* and *Plasmodium*. However, *G. blattarum* is non-zoonotic; it is an endoparasite that only infects invertebrates, with its host specificity being domiciliary cockroaches. From these findings, *Euphorbiaceae* and *G. blattarum* most likely originated from the cats' diet of fiber and insects respectively.

Fig-2 and Fig-3 indicated successful DNA ligation for samples DK4, FA6, FA9 and FA10 where DK4 and FA6 carried the ipaB gene (314bp) for *Salmonella spp*, while FA9 and FA10 carried the ITS gene (375bp) for *Toxocara cati*. The original plasmid DNA was 2700bp. The expected molecular weight of the ligated DNA is around 3000bp, which was seen in the gel electrophoresis result. Despite evidence supporting samples FA9 and FA10 containing *T. cati*, repeated sequence analyses after DNA amplification did not yield any pathogen sequence using BLAST (Table-2). A Neighbor-Joining (NJ) phylogenetic tree (Fig-4) was created to further investigate samples FA9 and FA10, which revealed the two samples sharing a common ancestor with *Schistosoma japonicum*, a trematode in clade 12. The nematode, *T. cati*, is located in clade 11 within this phylogenetic tree. This verifies that samples FA9 and FA10 are more related to *S. japonicum* than *T. cati*.

The NJ phylogenetic tree in Fig-5 indicates that DNA sequences found in samples DK4 and FA6 are most closely related to each other compared to other *Salmonella* species. They share a most recent common ancestor (MRCA) with the enteric pathogen *Salmonella enterica* subspecies *Enterica* (*S. Enterica*) as evidenced by node 15. Infections in humans can involve all species of *Salmonella*. There are around 2610 different serotypes of *Salmonella enterica* subspecies *enterica* and the most commonly known serotypes include *Typhimurium*, *Enteritidis*, *Typhi*, *Paratyphi* (further subdivided into *Types A, B and C*) and *Cholerae-suis* [29]. There are 3 surface antigens in *Salmonella* serotypes: the flagellar H antigen, oligosaccharide O antigen and polysaccharide Vi antigen (found in *Typhi* and *Paratyphi* serotypes). These surface antigens form

the basis for serotyping *Salmonella* [30].

S. Typhimurium and *S. Enteritidis* are non-typhoidal whereas *S. typhi* and *S. Paratyphi* are typhoidal. Developed countries have a higher prevalence of non-typhoidal salmonellosis whereas typhoid fever is more prevalent in developing countries (with the most cases in Asia) [30,31]. Approximately 1.3 billion cases of non-typhoidal salmonellosis occur annually worldwide and it has been estimated by WHO that there are 17 million cases and over 500,000 deaths per year caused by typhoid fever [30,31]. In **Fig-5**, *S. Typhi* and *S. Paratyphi* serotypes are non-zoonotic, while the rest are zoonotic, with *S. Enteritidis* and *S. Typhimurium* being the most prevalent serotypes infecting humans worldwide [24]. The latter serotype has been identified as an emerging pathogen since 1990, being responsible for numerous outbreaks associated with contaminated food sources as reported in these studies [32-34].

Interestingly, despite identifying *S. enterica* in two of our stool samples (**Table-2**) from one feral adult cat, FA6, and one domestic kitten, DK4 (**Fig-1**), both stool samples were non-diarrheic at time of collection. Furthermore, none of the cats and kittens in our study displayed signs of septicemia or gastroenteritis such as anorexia and diarrhea, which are characteristic presentations of clinical salmonellosis; [10] this observation is also seen in studies where a significant majority of cats and dogs infected with *Salmonella* were asymptomatic carriers that continued to excrete *Salmonella* in their stools up to 4 weeks [35-37]. The pathogen resides within the host's macrophages in the liver, spleen and intestinal lymph nodes, which become recrudesced and excreted in stools during periods of illnesses or stress in the presence of elevated catecholamines and glucocorticoids [38,39]. Increased frequency of *Salmonella* excretion under these circumstances can be explained by the pathogen's response to stress hormones, resulting in proliferation and virulence gene expression, *scsA* gene [39]. This ability of proliferation and excretion is seen as an evolutionary adaptation for the pathogen to propagate by infecting novel hosts upon sensing the current host has seemingly become less healthy. Outside the host, *Salmonella* is capable of surviving for extended periods of time – this strongly suggests a role in indirect

transmission to humans and other animals [36,37].

The ingestion of raw meat via hunting for prey and feeding on commercial raw diets was also hypothesized early in our study as a potential risk factor for cats to acquire enteric zoonoses. This dietary factor predisposes feral cats to have a higher prevalence of enteric pathogens, which is observed in **Fig-1**. This is especially true for *Salmonella*, where the highest prevalence (51%) was reported in shelter and feral cats and dogs [24]. In a 2017 case report, two domestic cats of the same household developed symptoms of gastroenteritis. A stool analysis revealed *Salmonella spp.* via PCR. Both cats were fed with commercial raw diets, where *S. Typhimurium* was cultured and isolated using real-time PCR. Both cats were also fed with dry pet feed, where no pathogens were isolated in the investigation [40]. This report emphasizes the insidious risk of transmission to humans, which is particularly the case in asymptomatic cats acting as vectors by excreting *Salmonella* in their stools [35-37].

Furthermore, the recent growing trend of introducing raw meat into the diets of pet cats (either via commercial raw diets or home recipes) may indirectly pose a hazard to human health as the handling of *Salmonella*-contaminated food source is an established risk for human salmonellosis [8,41]. A comprehensive review explored the prevalence of bacterial pathogens in raw commercial pet foods in North America reported that 7.1% to 21% of the samples contained *Salmonella* [8]. Other countries such as Egypt and the United Kingdom also reported similar values [9,42]. In 2018, two children of the same household were reported with clinical salmonellosis. Investigation revealed identical strains of *Salmonella* in both children and a commercial raw dog feed that was newly introduced to their pet's diet [43]. Although no further investigation was done to determine if the infection was a result of direct contact with the commercial raw feed or via contact with the pet's stool, the transmission of *Salmonella* between raw pet feed and humans remained a strongly suggested finding.

According to case-control and cohort studies, immunocompromised groups of people are most prone to contracting zoonotic pathogens and develop debilitating symptoms [44,45]. Hence, they are advised against rearing exotic pets such as reptiles, which are natural reservoirs for salmonellosis [46,47]. As this population is at an increased risk of zoonotic diseases, the practice of feeding raw meats to their pets is strongly discouraged. Additionally, they should follow proper pet husbandry techniques that not only minimize stress on their pets, but also reduce high risk activities that may predispose potential zoonotic infections; this includes keeping cats indoors, attending regular vet visits, proper storage of pet feed out of reach from children, placing litter boxes away from the kitchen, etc. Strict hand hygiene practices as well as the routine disinfection of pet and pet feed contact surfaces are also recommended [47,48].

Conclusion

From the present study, age and dietary factors of cats influence the prevalence of enteric zoonotic pathogens in their stools, where kittens are more susceptible to zoonotic infections and a diet of raw meat is significantly related to a higher prevalence of enteric zoonoses. PCR, when used with diagnostic primers under optimal conditions, followed by sequence analysis, yield adequate results in the detection of zoonotic pathogens to investigate the prevalence of enteric zoonoses in animal stools, and their impact on public health and safety. With the recent trend of commercial raw meat diet for pets, *Salmonella* has become an increasing concern to public health. Furthermore, as previously discussed, pets remain an insidious source of infection to the immunocompromised population. Aside from taking proper precautions when handling pets, healthcare workers should consider inquiring the pet ownership status of immunocompromised patients during history taking as a routine assessment.

Limitations and Improvements

I would like to address the limitations encountered in this study and potential improvements to increase the reliability of our findings as well as its future prospects.

Firstly, to increase the sample size from thirty to at least a hundred and classify them by location (e.g., urban, suburban and rural) to explore the significance in geography and zoonoses distribution. Secondly, implement qPCR with species specific primers to obtain reliable results for quantification of pathogen load and ascertain the clinical manifestation of disease [49]. Thirdly, serotyping all *Salmonella* positive samples to compare with literatures to discern if the serotypes in our study are previously involved in human salmonellosis outbreaks. Fourthly, implement the serotyping results to investigate for antimicrobial resistance and compare their resistance genes, especially in *Salmonella* positive samples due to a growing concern regarding multi-resistant *Salmonella* strains causing infected pets to profusely excrete resistant *Salmonella* in their stools [50]. Finally, from a public health perspective, recognize the need to establish diet strategies to feed feral cats present on university campuses in Shanghai as a means of minimizing zoonotic transmission to the target population of students, who have been observed throughout this study, to routinely engage close contacts with feral cats.

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Conflict of Interest

All authors have read and approved the final version of the manuscript. The authors have no conflicts of interest to declare.

Footnote

Alice Halim and Abhijeet Kumar Bhekarhee both contributed equally to this research.

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