

Prevalence and drug resistance of *Salmonella* in dogs and cats in Xuzhou, China

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Abstract

Introduction: Salmonellosis is a zoonotic disease, and *Salmonella* spp. can sometimes be found in dogs and cats, posing a risk to human health. In this study, the prevalence and antimicrobial susceptibility of faecal *Salmonella* were investigated in pet dogs and cats in Xuzhou, Jiangsu Province, China. **Material and Methods:** Faecal samples from 243 dogs and 113 cats, at seven pet clinics, were tested between March 2018 and May 2019. Each *Salmonella* isolate was characterised using serotyping and antimicrobial susceptibility tests. **Results:** The prevalence of *Salmonella* was 9.47% in dogs and 1.77% in cats. Among the 25 isolates, eight serotypes of *Salmonella enterica* subsp. *enterica* were detected, *S. Kentucky* (n = 11), *S. Indiana* (n = 5), and *S. Typhimurium* (n = 4) predominating. *S. Derby*, *S. Toucra*, *S. Sandiego*, *S. Newport*, and *S. Saintpaul* all occurred singly. The 23 *Salmonella* strains found in dogs were from seven different serovars, while the two strains in cats were from two. The highest resistance rates were found for tetracycline (92%), azithromycin (88%), cefazolin (84%), nalidixic acid (80%), ampicillin (80%), ceftriaxone (80%), and streptomycin (76%). Resistance to three or more antimicrobial agents was detected in 24 (96%) isolates. Most of the *S. Kentucky* and *S. Indiana* isolates were multi-drug resistant to more than 11 agents. **Conclusion:** The carriage rate was far higher in dogs than in cats from Xuzhou. Some isolated strains were highly resistant to antimicrobials used to treat infections in humans and pets, which may raise the risk of humans being infected with multi-drug resistant *Salmonella* via close contact with pets.

Keywords: dogs, cats, *Salmonella*, prevalence, serotype, antimicrobial resistance.

Introduction

Salmonellosis is a significant infectious disease that can be transmitted in both humans and animals (23). There are an estimated 93.8 million human cases of gastroenteritis and 155,000 deaths caused by *Salmonella* worldwide annually, of which around 80 million are food-borne. It has been estimated that about 9% are due to direct contact with animals (9, 17). When the animals are pets, the incidence of salmonellosis due to direct contact is low, accounting for approximately 1% per year (28). Although the rate is low, healthy pets may intermittently excrete *Salmonella*, which can

infect other animals and humans through the environment (34). The possibility of being infected with *Salmonella* increases when humans are in contact with pets that carry the bacteria or when they are exposed to the environments that these animals live in (32). This may pose risks to the elderly, children, and people with low immunity status (13, 18, 25).

In 2018, the number of pet dogs and cats in China was 200 million (10). Pets are our spiritual partners and can bring us happiness and reduce loneliness and anxiety (1). In order that nothing avoidable detract from these benefits, it is necessary for the public to understand the risks of zoonotic diseases, such as

salmonellosis. Accordingly, several studies of the transmission of *Salmonella* from dogs or cats to people have been conducted to date (5, 13, 24).

Salmonella has been found in pet dogs worldwide; however, the prevalence rates differed among areas. For example, in 1951, 1,741 strains of *Salmonella* were isolated from 3,072 samples from hunting dogs in Florida, with a rate of 45.25% (29). In Copenhagen, 103 strains of *Salmonella* were isolated from 2,985 diarrhoeal canine faecal samples from 1975 to 1994 (33). Cantor *et al.* (4) isolated *Salmonella* from 26 asymptomatic dogs and 30 diarrhoeal dogs, with rates of 69% and 63%, respectively. These surveys revealed a high prevalence of *Salmonella* in dogs in some countries; however, fewer studies have investigated cats. Therefore, the present study was conducted to investigate the occurrence of *Salmonella* in dogs and cats from Xuzhou, to detect the resistance of the strains to various antimicrobial agents and to estimate the potential for pets to be a source of *Salmonella* infection to people.

Material and Methods

Sample collection and *Salmonella* isolation.

A total of 356 faecal samples were obtained from seven separate pet clinics in Xuzhou, Jiangsu Province, China, from March 2018 to May 2019 (243 from dogs and 113 from cats). Both sick (130 dogs and 48 cats) and apparently healthy animals (113 dogs and 65 cats) were included in the study. Faecal samples from each pet were accompanied by a detailed questionnaire to record its breed, age, and clinical conditions.

Each faecal sample was collected into a sampling tube, stored at 4°C, and then transported in a cooler to the laboratory for further analysis within 6 h. A 1–5 g sample was randomly taken from the pet's rectum or faeces and added to 10 mL of buffered peptone broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), after which the samples were shaken and incubated at 37°C for 16–24 h. Next, 800 µL of this broth was subcultured in 15 mL of modified semisolid Rappaport–Vassiliadis (MSRV) medium (Becton, Dickinson and Company) at 42°C for 24 h. Subsequently, loopfuls of MSRV cultures were streaked onto xylose lysine tergitol 4 (XLT4) selective agar plates (Becton, Dickinson and Company) and incubated for 24 h at 37°C. The presumptive *Salmonella* isolates were selected from each plate, then stabbed into triple sugar iron slants and lysine decarboxylase agar (Hangzhou Microbial Reagent Co, Hangzhou, China) and incubated for 24 h at 37°C for biochemical analysis. Isolates with typical phenotypes were further confirmed to be *Salmonella* using API 20E test strips (bioMérieux, Marcy-l'Étoile, France).

Salmonella identification and serotyping.

Strains with *Salmonella* biochemical properties were subjected to PCR for the *invA* gene, using forward

primer 1 (5'-GTGAAATTATGCCACTGTCGGG CAA-3') and reverse primer 2 (5'-TCATCGCAC CGTCAAAGGAACC-3') to confirm the isolates at gene level (20). All strains were serotyped according to the White–Kauffmann–Le Minor scheme by slide agglutination using commercial somatic (O) and flagellar (H) antisera (Ningbo Tianrun Biopharmaceutical Co., Ningbo, China). Isolates that were difficult to serotype were sent to Sangon Biotech (Shanghai, China) for whole-genome sequencing to determine their serotypes.

Antimicrobial susceptibility testing.

Antimicrobial susceptibility was tested using the Kirby–Bauer disk diffusion method with 16 antimicrobial agents, in accordance with the guidelines of the Clinical Laboratory Standards Institute (6). The antimicrobial agents were amoxicillin/clavulanic acid (AMC, 30 µg), ampicillin (AMP, 10 µg), amikacin (AMK, 30 µg), aztreonam (ATM, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TET, 30 µg), norfloxacin (NOR, 10 µg), sulphamethoxazole/trimethoprim (SXT, 1.25/23.75 µg), streptomycin (STR, 10 µg), gentamicin (GEN, 10 µg), meropenem (MEM, 10 µg), nalidixic acid (NAL, 30 µg), azithromycin (AZM, 15 µg), cefazolin (CZO, 30 µg), and ceftriaxone (CRO, 30 µg) (Oxoid, Basingstoke, UK).

Results

Occurrence of *Salmonella* in dogs and cats.

Only two cats were positive for *Salmonella* (prevalence 1.77%). Of these, one was apparently healthy but carried *S. Derby*. The other one was sick and suffered from cat distemper virus and carried *S. Indiana*. Overall, the prevalence of *Salmonella* shedding in sick and apparently healthy cats was 2.08% (1/48) and 1.54% (1/65).

Salmonella was isolated from 23 dogs (9.47%); the prevalence of *Salmonella* shedding in sick and apparently healthy dogs was 10% (13/130) and 8.85% (10/113), respectively. The dogs were infected with seven different serotypes (*S. Kentucky*, *S. Indiana*, *S. Typhimurium*, *S. Toucra*, *S. Sandiego*, *S. Newport*, and *S. Saintpaul*). Among the 23 dogs that tested positive for *Salmonella*, 10 were apparently healthy, 6 suffered from canine parvovirus, and the remainder suffered from other diseases.

Salmonella serotype distribution. Overall, eight different *Salmonella* serotypes were identified, the predominant ones being *S. Kentucky* (n = 11), *S. Indiana* (n = 5), and *S. Typhimurium* (n = 4). Other serotypes such as *S. Derby* (n = 1), *S. Toucra* (n = 1), *S. Sandiego* (n = 1), *S. Newport* (n = 1), and *S. Saintpaul* (n = 1) were also identified. The overall results are provided in Table 1.

Antimicrobial resistance. Table 2 shows the resistance and susceptibility of 25 isolates to 16

antimicrobial agents. In the current study, resistance to tetracycline was the most prevalent in 25 strains (92%), followed by resistance to azithromycin (88%), cefazolin (84%), ampicillin (80%), nalidixic acid (80%), and ceftriaxone (80%). Among the 23 multidrug-resistant isolates, resistance to tetracycline, azithromycin, and cefazolin was most often observed (Table 3). All 25 isolates were susceptible or moderately susceptible to meropenem and amoxicillin/clavulanic acid. In total, more than 64% (16/25) of the *Salmonella* isolates were resistant to at least 11 antimicrobial agents.

Different serotypes showed different sensitivities and resistance to various antibiotics (Table 3). For

instance, only *S. Toucra* was resistant to as few as two antibiotics (streptomycin and azithromycin), while 96% of the isolates (24 out of 25) were resistant to at least three. However, all 11 isolates of *S. Kentucky* displayed resistance to tetracycline, nalidixic acid, ciprofloxacin, norfloxacin, cefazolin, and ceftriaxone. Moreover, they were resistant to at least nine antibiotics, and two of them exhibited resistance to 14. However, three isolates of *S. Indiana* were resistant to 12 antibiotics, and two were resistant to 13. The resistance of four *S. Typhimurium* strains to the 16 antibiotics tested was not uniform, and the number of antibiotics resisted by these strains ranged from 4 to 12.

Table 1. Serotype distribution of 25 *Salmonella* strains

Serotype	O-antigen	H-antigen	Number of isolates
<i>S. Kentucky</i>	8, 20	i; z ₆	11
<i>S. Indiana</i>	1, 4, 12	z; 1, 7	5
<i>S. Typhimurium</i>	1, 4, 5, 12	i; 1, 2	4
<i>S. Derby</i>	4, 12	f, g	1
<i>S. Toucra</i>	48	z; 1, 5	1
<i>S. Sandiego</i>	1, 4, 5, 12	e, h; e, n, z ₁₅	1
<i>S. Newport</i>	6, 8, 20	e, h; 1, 2	1
<i>S. Saintpaul</i>	1, 4, 5, 12	e, h; 1, 2	1

Table 2. Distribution of susceptibility to 16 antimicrobial agents in 25 *Salmonella* strains

Antimicrobial agent	Number of susceptible strains (%)	Number of moderately susceptible strains (%)	Number of resistant strains (%)
Amikacin (AMK)	12 (48)	7 (28)	6 (24)
Gentamicin (GEN)	9 (36)	0 (0)	16 (64)
Streptomycin (STR)	2 (8)	4 (16)	19 (76)
Amoxicillin/clavulanic acid (AMC)	16 (64)	9 (36)	0 (0)
Aztreonam (ATM)	5 (20)	4 (16)	16 (64)
Meropenem (MEM)	25 (100)	0 (0)	0 (0)
Chloramphenicol (CHL)	11 (44)	2 (8)	12 (48)
Ampicillin (AMP)	2 (8)	3 (12)	20 (80)
Ciprofloxacin (CIP)	6 (24)	2 (8)	17 (68)
Nalidixic acid (NAL)	1 (4)	4 (16)	20 (80)
Norfloxacin (NOR)	7 (28)	1 (4)	17 (68)
Sulphamethoxazole(trimethoprim) (SXT)	15 (60)	1 (4)	9 (36)
Tetracycline (TET)	2 (8)	0 (0)	23 (92)
Azithromycin (AZM)	3 (12)	0 (0)	22 (88)
Cefazolin (CZO)	1 (4)	3 (12)	21 (84)
Ceftriaxone (CRO)	5 (20)	0 (0)	20 (80)

Table 3. Multi-drug resistance profiles of 25 *Salmonella* strains

Serotype	Number of isolates	Antibiotic resistance profile
<i>S. Kentucky</i>	1	TET, STR, AMP, NAL, CIP, NOR, GEN, CZO, CRO
<i>S. Kentucky</i>	6	TET, STR, AMP, NAL, ATM, CIP, NOR, GEN, AZM, CZO, CRO
<i>S. Kentucky</i>	1	TET, STR, AMP, NAL, ATM, CIP, NOR, AMK, AZM, CZO, CRO
<i>S. Kentucky</i>	1	TET, AMP, NAL, CHL, ATM, CIP, NOR, GEN, AMK, AZM, CZO, CRO
<i>S. Kentucky</i>	2	TET, STR, AMP, NAL, CHL, ATM, CIP, NOR, GEN, SXT, AMK, AZM, CZO, CRO
<i>S. Indiana</i>	1	TET, AMP, NAL, CHL, ATM, CIP, NOR, GEN, SXT, AZM, CZO, CRO
<i>S. Indiana</i>	2	TET, STR, AMP, NAL, CHL, CIP, NOR, GEN, SXT, AZM, CZO, CRO
<i>S. Indiana</i>	2	TET, AMP, NAL, CHL, ATM, CIP, NOR, GEN, SXT, AMK, AZM, CZO, CRO
<i>S. Typhimurium</i>	1	TET, STR, AMP, CHL
<i>S. Typhimurium</i>	1	TET, STR, AMP, NAL, CZO, CRO
<i>S. Typhimurium</i>	1	TET, STR, AMP, NAL, CHL, ATM, AZM, CZO, CRO
<i>S. Typhimurium</i>	1	TET, STR, AMP, NAL, CHL, CIP, NOR, GEN, SXT, AZM, CZO, CRO
<i>S. Derby</i>	1	TET, STR, NAL, AZM
<i>S. Toucra</i>	1	STR, AZM
<i>S. Sandiego</i>	1	STR, AZM, CZO, CRO
<i>S. Newport</i>	1	TET, CHL, ATM, SXT, AZM, CZO, CRO
<i>S. Saintpaul</i>	1	TET, ATM, AZM

Antibiotics denoted as in Table 2

Discussion

Apparently healthy or sick pets can harbour *Salmonella*, and may thus be a potential source of human infection. Consequently, it is important for every person to understand the risk of transmission of zoonotic infections. In our study, the *Salmonella* prevalence rates were low in dogs (9.47%) and cats (1.77%) located in Xuzhou, China.

It has been reported that the prevalence of *Salmonella* in faecal samples from healthy dogs was between 1% and 36%, and that in samples from healthy cats, it ranged from 1% to 18% (24). The results of our study were all within these ranges. Cases of *Salmonella* shedding from pets are common in many countries. A study of 360 dogs from four sub-cities in Addis Ababa, Ethiopia, revealed that 11.7% (42 dogs) were positive for *Salmonella* (12). A study of 138 domestic dogs in Ontario, Canada, reported *Salmonella* in 23% of them (15). A study of 38 clinically healthy mixed-breed shepherd dogs from Garmas, Iran, revealed a prevalence of 10.5% (nine dogs) (22). However, studies in some countries revealed lower rates of *Salmonella* carriage compared to this study, such as 0.23% in the UK (16), 2.5% in the United States (21),

and 1% in Turkey (2). Moreover, a study of 59 apparently healthy cats in Mosul, Iraq, showed *Salmonella* in six of them (35). A study conducted in South Africa reported a prevalence of 0.5% (six cats) (8), while a study of 542 cats in the United States revealed that 0.6% (three cats) were positive for *Salmonella* (21). In the current study, the prevalence of *Salmonella* in both dogs and cats was at variance with that of other countries, probably because of differences in sample size, pet feeding habits, pet sanitary practices, sampling strategies, and pet owners' awareness of zoonosis. In addition, seasonal, geographical, and regional differences among studies probably led to some disparities (26).

The prevalence of *Salmonella* may be affected by the clinical condition of the pet being tested. The prevalence of *Salmonella* in cats with diarrhoea and healthy cats was 8.33% (1/12) and 1.54% (1/65), respectively. In 1977, Shimi and Barin (27) reported that diarrhoea was more prevalent in the *Salmonella*-carrying population, and the fact that healthy cats can also carry *Salmonella* was confirmed by Zened *et al.* (35) in Iraq. In addition, our study also revealed that the prevalence of *Salmonella* in dogs infected with canine parvovirus and healthy dogs was 15% (6/40) and

8.85% (10/113), respectively. In South Africa, Botha *et al.* (3) investigated a group of 74 dogs with canine parvovirus from 2015 to 2017 and found the prevalence of *Salmonella* shedding to be 22%. There is the possibility that dogs with canine parvovirus suffer from a greater degree of dysbiosis compared to healthy individuals, which would explain why dogs with canine parvovirus were more easily infected by *Salmonella* (3).

Among the 25 isolates seen in the current study, eight serotypes were found, with *S. Kentucky*, *S. Indiana*, and *S. Typhimurium* being most common. Similarly, Wang (32) reported the isolation of *S. Enteritidis*, *S. Derby*, and *S. Indiana* from dogs in Hefei, Anhui Province, China. Lefebvre *et al.* (14) reported that *S. Typhimurium* and *S. Kentucky* were the most common serovars in dogs from Canada. Additionally, Kiflu *et al.* (12) showed that some non-typhoidal *Salmonella* could also be found in dogs from Ethiopia, such as *S. Bronx*, *S. Indiana*, and *S. Kentucky*. In Canada, Leonard *et al.* (15) isolated six strains of *S. Kentucky* and one strain of *S. Indiana* from dogs. For context, *S. Typhimurium* is considered the most widely distributed serotype because it is frequently associated with diseases in numerous species, such as humans, domestic fowl, rodents, and birds (19).

Of the 25 *Salmonella* strains examined, most were resistant to at least three antimicrobial agents. Tetracycline, azithromycin, cefazolin, nalidixic acid, and ceftriaxone were found to be resisted more frequently than other antimicrobial agents. Drug resistance of *Salmonella* is becoming increasingly serious, although the susceptibility of *Salmonella* to antimicrobial drugs varies among countries and regions. In Thailand, among 86 strains of *Salmonella* from pigs, the resistance rates to tetracycline and ampicillin were more than 81% (30). A study conducted in Guangdong Province, China, found that in 1,764 strains of *Salmonella* isolated from humans, over 90% showed resistance to ceftazidime, cefotaxime, cefepime, and ciprofloxacin (11). In Taiwan, the resistance rates of *Salmonella* strains from dogs to sulfamethoxazole/trimethoprim (37.5%) and tetracycline (77.5%) were similar to those observed in the present study, but the resistance to other antimicrobial agents such as ampicillin/clavulanic acid, ceftriaxone, and gentamicin was lower than that in the present study (31). The differences in the resistance to various antimicrobial agents may have been observed because of differences in serotypes and the use of antibiotics in humans, livestock, and pets in different countries and regions. Quinolones and cephalosporins have become common in clinical use drugs for the treatment of *Salmonella*, and their frequent use is an important cause of the high resistance to ciprofloxacin, nalidixic acid, norfloxacin, cefazolin, and ceftriaxone that was observed.

S. Kentucky was found to be resistant to many antimicrobial agents in this study, a finding similar to

that for two strains previously isolated from human faeces (7). *S. Typhimurium* and *S. Indiana* may also have a high rate of resistance to many antimicrobial agents because they are commonly isolated from animal products such as those from poultry and other livestock. A possible consequence could be the transfer of the resistance of these strains to the *Salmonella* strains common in pets.

This study indicated that *Salmonella* had a significantly higher carriage rate in dogs than in cats from Xuzhou, Jiangsu, China and that the carriage rate in cats was low. Most *Salmonella* serotypes isolated in this study have been found in humans, other animals, and animal products. In addition, some isolated strains were highly resistant to antimicrobial agents used in the treatment of bacterial infections in humans and pets, which may increase the risk of humans being infected with multi-drug resistant *Salmonella* via close contact with pets, especially for children and the elderly. Thus, it is important to raise public awareness of zoonotic diseases and develop good hygiene habits. Pet owners also need to pay attention to the food their pets eat every day and preferably provide cooked food or pet food to reduce the infection of pets with *Salmonella* and the potential for its transfer to humans.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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