Prevalence and antimicrobial resistance of Salmonella *serotypes isolated from cats and dogs in Tripoli, Libya*

Hiam R. Elnageh[1], Murad A. Hiblu[2], Mohamed S. Abbassi[3], Yousef M. Abouzeed[1] and Mohamed O. Ahmed[1*]

[1]*Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Libya.*
[2]*Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tripoli, Libya.*
[3]*Université de Tunis El Manar, Institut de la Recherche Vétérinaire de Tunisie, 20 rue Jebel Lakhdhar, Bab Saadoun, Tunis 1006, Tunisie.*

[*]*Corresponding author at: Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Libya. E-mail: a.mo@live.com.*

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Summary
The prevalence of *Salmonella* in dogs and cats was investigated and further characterized with serotyping, antimicrobial susceptibility and risk factor analysis. In total, 151 faecal samples from 103 and 48 healthy and nonhealthy (diarrheic) cat and dogs, respectively were examined. Salmonellae were confirmed by laboratory and biomedical characteristics including serotyping and antimicrobial susceptibility tests. Risk factors typically associated with salmonellae shedding were identified using Fisher's exact tests. *Salmonella* was detected in 18% (n = 27/151) of pets. Most of the positive samples 85% (n = 23/27) were from healthy cats, 7.4% (n = 2/27) from healthy dogs and 7.4% (n = 2/27) from a diarrhoeic cat and diarrhoeic dog. In total, 25 salmonellae (93% of strains) were serotyped as *S.* Thompson mostly originated form healthy cats (n = 23/25). All isolates were resistant to tetracycline and trimethoprim-sulfamethoxazole and expressed an overall intermediate susceptibility patterns to ciprofloxacin. Also, multidrug resistant *S.* Kentucky and *S.* Minnesota were identified from a diarrhoeic and a healthy dog, respectively. This is the first isolation report of *Salmonella* from cats and dogs in Libya. It indeed represents a public health concern which requires further monitoring.

Introduction

Salmonella is one of the most prevalent zoonotic foodborne pathogens colonizing different animal species capable of spreading rapidly and causing significant morbidity and mortality (WHO 2016, EFSA 2018, Majowicz *et al.* 2010). The incidences of human salmonellosis have been reported worldwide; however, a substantial proportion of *Salmonella* infections are either not recognized or sporadically classified as atypical *Salmonella* (Dotto *et al.* 2017, Fisher *et al.* 2009, Graziani *et al.* 2015, WHO 2018). To date, over 2,500 *Salmonella* have been identified with a variety of serotypes affecting both humans and animals (Fernández *et al.* 2018, Gossner *et al.* 2016, WHO 2018, Afema *et al.* 2016).

Salmonella enterica serovars are responsible for typhoidal and non-typhoidal infections (Lokken *et al.* 2016, Phu Huong Lan *et al.* 2016). Unlike typhoidal salmonellae, which can be carried by humans, non-typhoidal *Salmonella* (NTS) are frequently associated with animal carriers. These can be transmitted to humans causing gastroenteritis illnesses and invasive (iNTS) bacteraemia (Uche *et al.* 2017, Lokken *et al.* 2016, Afema *et al.* 2016). NTS can be transferred to humans by various routes such as the consumption of contaminated food products (i.e. eggs, poultry, undercooked meat), or through contact with animals or environment sources (i.e. infecting sewage waste and contaminating vegetables) (Nair *et al.* 2015, Uche *et al.* 2017). Pet animals are usually fed with discarded raw animal parts and treats which can be frequently contaminated with salmonellae (Leonard *et al.* 2011, CDC 2006, CDC 2008, Marks *et al.* 2011, EFSA 2018).

Non-typhoidal *Salmonella* are among the leading

causes of food-borne illnesses in humans particularly in Africa affecting mainly children aged under five year old (Uche *et al.* 2017, Nair *et al.* 2015, Le Hello *et al.* 2013a, Fernández *et al.* 2018). NTS strains, expressing multidrug resistances, including to or against quinolones and beta-lactamase inhibitors, have been increasingly reported worldwide. This is mainly attributed to inappropriate use of antibiotics in food animals (Bangera *et al.* 2019). In Africa, various salmonellae have been reported from various animal species. Among these, *Salmonella* Typhimurium is the most commonly identified serovars (Al-Rifai *et al.* 2019, Thomas *et al.* 2019). In North Africa, *Salmonella* is a common foodborne pathogen. Serovars Enteritidis and Typhimurium have been increasingly reported (Al-Rifai *et al.* 2019). In Libya, *Salmonella* expressing resistance to critically important antibiotics such as fluoroquinolones has been reported in 18% of hospitalized patients mainly children (Altayyar *et al.* 2016, Ghenghesh *et al.* 2013); the source of these outbreaks or the role played by animals has never been investigated.

Recent global reports from North Africa have documented the role of pet animals as a potential reservoir of drug-resistant salmonellae (e.g. ESBL-producers and quinolone resistance isolates) (Stull *et al.* 2015, Damborg *et al.* 2015, Srisanga *et al.* 2017, Chipangura *et al.* 2017, Ahmed *et al.* 2017, Ezenduka 2019, Elnageh *et al.* 2017). Nevertheless, data or studies estimating pet salmonellosis and the associated risk factors are scarse (Giacometti *et al.* 2017). No information is available about the occurrence and characteristics of *Salmonella* in animals and their zoonotic role in the spread of AMR in Libya. In the current study, the prevalence and the antimicrobial susceptibility of *Salmonella* serotypes isolated from faecal samples from cats and dogs collected between March and July 2018 were investigated.

Materials and methods

Criteria of selection and sampling

This study obtained the approval from the Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Libya. Prior to including pets in this study, owners were informed about its purpose and animals were included on the basis of the availability and acceptance of their owners. Samples from nonhealthy pets were collected on admission at three local veterinary clinics. Non-healthy pets were involved on the basis of typical gastrointestinal (GI) symptoms (e.g. diarrhoea) and referred to as the GI group throughout the study. Healthy pets were included from their households and were not showing signs of any illnesses or being under any medication including antimicrobial drugs at least three months prior to sampling.

Faecal samples and data collection

A fresh faecal sample was taken from the rectum of each animal using a small cotton-tipped swap, and rolling the swab inside the rectum. The sample was then placed in a Stuart transport medium and kept in cool place. Samples were transported to the laboratory of the department of microbiology, faculty of veterinary medicine, University of Tripoli, within four hours. After the collection process, data were obtained on each animal using a simple constructed questionnaire to examine potential risk factors that were frequently associated with *Salmonella* shedding. The chosen variables and recorded data included information on age, sex, purpose of ownership (i.e. caring, security and breeding), type and source of food and diet, history of diarrhoea, and history of antibiotic therapy, particularly at the time of sampling. Each faecal sample was recorded as the unit of analysis.

Identification and serotyping of salmonellae

Faecal samples were initially mixed in a 10 ml solution of brain heart infusion broth containing 5% glycerol and homogenized using a vortex mixer. A volume of 2 ml from the faecal mixture was transferred into 10 ml of sterile buffered peptone water (BPW) broth and incubated for 48 h at 37 °C. A loopful from each BPW broth was spread onto Xylose Lysine Decarboxylase (XLD) agar and incubated at 37 °C for 24 h. Plates were then checked for presumptive colonies of *Salmonella* (i.e. red colonies with black centre) and a single typical colony from each plate was transferred onto a nutrient agar and incubated at 37 °C for 24 h. Suspected isolates were initially subjected to Gram stain examination and then further confirmed by the API 20E system (Bio-Mérieux). Afterwards, confirmed strains were further serotyped according to the Kauffmann-White scheme (Bio-Rad, Marnes-la-Coquette, France).

Antibiotic susceptibility tests

The confirmed *Salmonella* serotypes were subjected to antimicrobial susceptibility test using the disc diffusion method following clinical and laboratory standards institute (CLSI) guidelines (CLSI 2015). Isolates were tested against eleven antibiotic classes including ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), azithromycin (15 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg),

chloramphenicol (30 µg), cefotaxime (30 µg) and ceftazidime (30 µg). Plates were incubated at 37 °C under aerobic conditions for 24 hours. Resistance was indicted by growth reaching the antibiotic disc within the acceptable range based on CLSI guidelines. Isolates that expressed resistance to ampicillin based on disc diffusion were further tested against imipenem (10 µg) disks for the initial assessment of carbapanemase producers.

Isolates expressing multidrug resistance phenotypes (≥ 3 antibiotic classes) including ciprofloxacin, ampicillin and imipenem were further subjected to antimicrobial susceptibility dilution method for the determination of the minimum inhibitory concentration (MIC) range using designated susceptibility testing plates (Thermo-Sensititre). The MICs was obtained by implementing the microdilution Sentitre™ GNX2F plate (Trek Diagnostic Systems, thermofisher/GNX2F) according to the manufacturing instructions and interpreted using the 2019 criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Risk factors analysis

Risk factors analysis was carried out on the collected data information to examine potential factors that are significantly associated with *Salmonella* shedding using the Fisher's exact tests at $p \leq 0.05$.

Results

One hundred and fifty one pets, 103 cats (62 healthy and 41 nonhealthy) and 48 dogs (37 healthy and 11 nonhealthy), were enrolled in this study. *Salmonella* was detected in 18% (n = 27/151) of the pet faecal samples. Of these, 23% (n = 24/103) were from cat and 6% (n = 3/48) from dog. Healthy cats were the main reservoir of salmonellae representing 22% (n = 23/103) and 85% (n = 23/27) of the cat and the total positive samples, respectively (Table I).

Of the 103 cats, 40% (n = 41) had diarrhoea. Of these, only 2.4% were positive for *Salmonella* compared to 37% *Salmonella*-positive non-diarrhoeic cats ($p > 0.05$). In addition of the 9 cats that had been fed a raw diet at the time of sampling, 4 (44%) were found positive to *Salmonella* while only 21% (n = 20) of cats which did not have raw diet food were positive to *Salmonella*. Among the cat group, 40% (41/103) had been under antibiotic therapy at the time of sampling. Of these, only 2% were *Salmonella* positive comparing to 37% *Salmonella* positive nonantibiotics-intake (ABs-) samples. However, these differences as well as those found when analysing the same variables in dogs were not significant ($p > 0.05$).

Serotyping identified 25 *S.* Thompson. Most of them were from healthy cats (n = 23/25), one from a diarrhoeic cat and one from healthy dog. The remaining two salmonellae obtained from a healthy and one diarrhoeic dog were serotyped as *S.* Minnesota and *S.* Kentucky, respectively (Table II). Most of the *S.* Thompson serotypes expressed intermediate susceptibility to ciprofloxacin and resistance to tetracycline and trimethoprim-sulfamethoxazole. The *S.* Minnesota serotype showed resistance only to ciprofloxacin and tetracycline whereas the *S.* Kentucky serotype expressed a multidrug resistant phenotype, including to ciprofloxacin. The MICs for the *S.* Kentucky serotype revealed further resistance to ampicillin, fluoroquinolones (ciprofloxacin and levofloxacin), tetracyclines (doxycycline minocycline, and tigecycline), and aminoglycosides (gentamicin and tobramycin) but susceptible to azithromycin, cephalosporins and carbapenems (Table III).

Discussion

Salmonella is a colonizing bacterium of cats and dogs. Most of these animals may become asymptomatic carriers but may also show clinical signs associated with opportunistic infections and host immunosuppression (Giacometti *et al.* 2017, Marks *et al.* 2003, Finley *et al.* 2007). Dogs may shed *Salmonella* at a prevalence range between 1% to 44% depending on location, population and the applied laboratory methodology (Greene *et al.* 1998, Finley *et al.* 2007, Reimschuessel *et al.* 2017). Risk factors including raw food diet, diarrhoea, home-made diets and antibiotics might influence the shedding of *Salmonella* either in healthy or diarrhoeic dogs (Hackett and Lappin 2003, Marks *et al.* 2011, Westermarck 2016, Reimschuessel 2017, Leonard *et al.* 2011, Stavisky *et al.* 2011). In Africa, recent reports have documented various *Salmonella* serovars in apparently healthy dogs with previous diarrhoea at a prevalence of 12% expressing a high rate of multi drug resistance (MDR) (Kiflu *et al.* 2017).

In cats, the prevalence of *Salmonella* is less reported

Table I. *Serotypes of* Salmonella *species from faecal samples of Libyan pets.*

Animal species	Proportion of carriage within each group	No. of Identified *Salmonella* serotypes within each group
Cats (n = 103)	23% (n = 24)	†S. Thompson (n = 23) ‡S. Thompson (n = 1)
Dogs (n = 48)	6% (n = 3)	†S. Thompson (n = 1) †S. Minnesota (n = 1) ‡S. Kentucky (n = 1)

N = number; † Healthy (control) origin; ‡ Nonheathy (diarrheic) origin.

Table II. *Antimicrobial resistance features of the collection of* Salmonella *strains isolated from faecal samples of Libyan pets.*

	Animal	Serotype	Health status	Antimicrobial susceptibility profiling		
				Susceptible	Intermediate	Resistance
1	Cat	Thompson	Diarrhoea	AMP, AMC, GMN, AZM, CHL	CIP	TET, STX
2	Cat	Thompson	Healthy	AMP, AMC, GMN, AZM, CHL	CIP	TET, STX
3	Cat	Thompson	Healthy	AMP, AMC, STX, AZM, CHL	CIP	TET, GMN
4	Cat	Thompson	Healthy	AMP, AMC, STX, GMN, AZM, CHL	CIP	TET
5	Cat	Thompson	Healthy	AMP, AMC, GMN, CHL	CIP	TET, STX
6	Cat	Thompson	Healthy	AMP, AMC, STX, GMN, AZM, CHL	CIP	TET
7	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
8	Cat	Thompson	Healthy	AMP, AMC, GMN, AZM, CHL	TET, CIP	STX
9	Cat	Thompson	Healthy	AMP, AMC, GMN, AZM, CHL	CIP	TET, STX
10	Cat	Thompson	Healthy	AMP, AMC, TET, CIP, STX, GMN, AZM, CHL	-	-
11	Cat	Thompson	Healthy	AMP, AMC, STX, GMN, AZM, CHL	CIP	TET
12	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
13	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
14	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
15	Cat	Thompson	Healthy	AMP, AMC, TET, CIP, STX, GMN, AZM, CHL	-	-
16	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
17	Cat	Thompson	Healthy	AMP, AMC, GMN, AZM, CHL	TET, CIP	STX
18	Cat	Thompson	Healthy	AMP, AMC, CIP, STX, GMN, AZM, CHL	-	TET
19	Cat	Thompson	Healthy	AMP, AMC, TET, CIP, STX, GMN, AZM, CHL	-	-
20	Cat	Thompson	Healthy	AMP, AMC, STX, GMN, AZM, CHL	CIP	TET
21	Cat	Thompson	Healthy	AMP, AMC, STX, GMN, AZM, CHL	TET, CIP	-
22	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
23	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
24	Cat	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
25	Dog	Thompson	Healthy	AMP, AMC, TET, STX, GMN, AZM, CHL	CIP	-
26	Dog	Minnesota	Healthy	AMP, AMC, STX, GMN, AZM, CHL	-	TET, CIP
27	Dog	Kentucky	Diarrhoea	AMC, AZM, CTX, TEX, CAZ	IMP	AMP, TET, CIP, STX, GMN, CHL

AMP = Ampicillin; AMC = amoxicillin-clavulanate; TET = tetracycline; CIP = ciprofloxacin; STX = trimethoprim-sulfamethoxazole; GMN = gentamicin; AZM = azithromycin; CHL = chloramphenicol; CAZ = ceftazidime; CTX = cefotaxime; IMP = imipenem,.

than dogs and the available salmonellosis feline reports refer mainly to serious clinical cases (Stiver *et al.* 2003, Reimschuessel 2017). In diarrheic cats, the prevalence of *Salmonella* can range from 0 to 8.6% (Marks *et al.* 2011, Giacometti *et al.* 2017) whereas healthy house cats, because of housing hygiene, can harbour *Salmonella* in their faeces at a low prevalence (< 1%) (Van Immerseel *et al.* 2004). The prevalence in non-diarrhoeic cats was also reported and ranged between 0 to 14% (Giacometti *et al.* 2017). In the current study, *Salmonella* was identified in 23% of the cat samples, a percentage much higher than that found in dogs. Most of these cats were healthy. Also, no significant differences were found in relation to the studied selected variables for cats and dogs which contradicts other published reports worldwide. This might be attributed to sample size of the current study but also could indicate the high colonization status of *Salmonella* among a healthy population of cats in Tripoli. This issue together with the possible source of infection requires further investigations.

In the current study, 25 *Salmonella* isolates were characterized as *S.* Thompson serotype. These isolates expressed intermediate susceptibility to ciprofloxacin and were mostly resistant to tetracycline and trimethoprim-sulfamethoxazole (Table II). However, these serotypes were susceptible to azithromycin which is a recommended treatment antimicrobial class against fluoroquinolone resistant salmonellae (Crump *et al.* 2015). Generally, limited information is available on the epidemiology of *S.* Thompson worldwide. However, outbreak in humans directly linked to the handling of contaminated dog food and treats have been described (CDC 2006, CDC 2008). In the last decade, this serotype was listed within the most

Table III. *The determination of minimum inhibitory concentration range of S. Kentucky using antimicrobial susceptibility dilution method.*

Antibiotic agent	Dilution (MICs) range (mg/L)	Results (mg/L)	Interpretation
Ampicillin	8-16	8	R
Azithromycin	2-16	2	S
Ceftazidime/ clavulanic acid	0.12/4-128/4	0.12/4	S
Cefotaxime/ clavulanic acid	0.12/4-64/4	0.25/4	S
Ticarcillin/ clavulanic acid	16/2-128/2	32/2	S
Piperacillin/tazobactam	4/4-64/4	4/4	S
Cefazolin	8-16	8	NA
Cefoxitin	4-64	8	NA
Cephalothin	8-16	8	NA
Cefotaxime	0.25-64	0.5	S
Ceftriaxone	1-128	1	S
Ceftazidime	0.25-128	0.25	S
Cefepime	1-16	1	S
Cefpodoxime	0.25-32	0.25	S
Ciprofloxacin	0.25-2	-	R
Levofloxacin	1-8	8	R
Doxycycline	2-16	16	R
Minocycline	2-16	8	R
Tigecycline	0.25-8	0.5	R
Ertapenem	0.25-4	0.25	S
Imipenem	0.5-16	0.5	S
Meropenem	1-8	1	S
Gentamicin	1-16	4	R
Tobramycin	1-8	4	R
Amikacin	4-32	4	S
Trimethoprim/ sulfamethoxazole	0.5/9.5-4/76	-	S
Colistin	0.25-4	1	S
Polymyxin B	0.25-4	1	S

NA = Not available; - = no growth; R = resistance; S = susceptible.

frequently isolated salmonellae responsible for human infections in the United States (CDC 2012). In addition, MDR *Salmonella* serotypes including *S.* Kentucky, *S.* Thomson, and *S.* Enteritidis have been increasingly reported posing significant public health challenges (Shah *et al.* 2017). A recent genomic study of *S.* Thompson strains isolated from an outbreak of contaminated food products revealed distinct genomic characteristics (Parker *et al.* 2015).

Pet owners are often unaware and/or underestimate the health risks that their pets may present (Chipangura *et al.* 2017, Damborg *et al.* 2015, Damborg *et al.* 2016, Stull *et al.* 2015, Halsby *et al.* 2014). Household pets are usually kept for specific purposes and the level of interaction with owners, feeding, caring and health services varies between regions and countries (Selmi *et al.* 2011). In the low-income regions including Libya, cats and dogs are frequently fed on non-commercial food particularly remaining raw animal parts of poultry meat (personal communication). Feeding on raw food diets and *Salmonella*-contaminated raw food diets are widely reported to be highly associated with a high prevalence of *Salmonella* shedding in cats and dogs; i.e. up to 23 times greater shedding comparing to commercial diets (Giacometti *et al.* 2017, Finley *et al.* 2007). Also, cats compared to dogs can roam more freely between indoor and outdoor environments, increasing the risk of contacting contaminated foods with *Salmonella* from environmental sources such as rodents and birds (Giacometti *et al.* 2017). Poultry frequently act as carrier of various *Salmonella* serotypes and represent the most important source of human salmonellosis worldwide including Africa (Thomas *et al.* 2019, Mshelbwala *et al.* 2017). Recent studies investigating meat and intestinal samples of poultry have reported different NTS serovars mainly belonging to the *S.* Kentucky and *S.* Thompson serotypes showing high resistance rates to important drugs including ciprofloxacin (Bangera *et al.* 2019, Carli *et al.* 2001). These reports have attributed such isolations and documentations to the introduction of imported feed or new flocks from other countries such as the United States and Turkey (Carli *et al.* 2001). This may explain the results of the current study particularly the high prevalence of the *S.* Thompson in cats and also highlights the need to investigate other sources of infection.

In the current study, the isolated *S.* Kentucky serotype originated from a diarrheic dog. It expressed a multidrug resistance phenotype (Table II). The determination of MICs revealed further resistance to ampicillin, fluoroquinolones, tetracyclines, and aminoglycosides but susceptibility to azithromycin, cephalosporins, and carbapenems (Table III). In contrast, *S.* Kentucky has been recently reported in Africa, isolated from apparently healthy dogs representing 9.5% of the collected *Salmonella* isolates but susceptible to ciprofloxacin (Kiflu *et al.* 2017). This serotype was isolated from human, environmental sources and animals from different African countries expressing very high resistance to antimicrobial agents (Afema *et al.* 2016, Le Hello *et al.* 2013b). Global reports have associated the emergence of multidrug resistance salmonellae to the intensive animal husbandry particularly in poultry (OIE 2008) as well as travel history in Europe, Africa and the Middle East (Le Hello *et al.* 2013b, Mulvey *et al.* 2013, Rickert-Hartman and Folster 2014, Westrell *et al.* 2014). For instance, OXA-48-producing

S. Kentucky (ST198) was isolated from a traveller patient transferred from North Africa to Europe expressing MDR resistance to cephalosporins, ciprofloxacin, and reduced susceptibility to imipenem attributed to both the transposon genes and the chromosomal genetic determinants (Seiffert *et al.* 2014).

The emergence of MDR salmonellae (including the fluoroquinolone non-susceptible *S.* Kentucky) is mainly attributed to the acquisition of various genetic mechanisms such as the chromosomal resistance determinants (mutations in genes encoding DNA gyrase) and the plasmid-mediating mechanisms (Le Hello *et al.* 2013b, Crump *et al.* 2015). In Africa, the heterogeneity of fluoroquinolones-resistance patterns among the clinical *Salmonella* of food-animal origins is reported to be largely associated with chromosomal point mutations i.e. *gyr*A mutations (Tadesse *et al.* 2018, Al-Gallas *et al.* 2013). These genotypic and phenotypic expressions have been largely adopted as a useful distinguishing trait to characterize certain *Salmonella* serotypes such as ciprofloxacin non-susceptible *S.* Kentucky clone ST198 (Le Hello *et al.* 2013b). Such global emergence and geographic variation are largely attributed to intensive farm production systems and associated with the use of antimicrobial agents particularly fluoroquinolones and cephalosporin classes (Le Hello *et al.* 2011). Fluoroquinolone resistance in NTS remains low; however, increasing reports have linked ciprofloxacin intermediately-susceptible salmonellae to treatment failure associated mainly to mutations with the *gyr*A and to plasmid mediated systems (Parry and Threlfall 2008, Sjölund-Karlsson *et al.* 2009).

In conclusion, this is the first study that provides novel information on *Salmonella* isolated from cats and dogs. Cats and dogs can carry and be colonized with different salmonellae of public health concern requiring preventative measures and good hygienic practices. The current findings warrant further attention, and epidemiological and surveillance investigations are required to limit the dissemination of this pathogen in Libya.

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