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Serovars and antibiotic sensitivity of *Salmonella* spp. isolated from non-diarrhoeic cats in Trinidad

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ABSTRACT

The prevalence of *Salmonella* spp. in non-diarrhoeic cats across Trinidad was determined. The serovars of *Salmonella* spp. isolated were identified and the resistance to eight antimicrobial agents was determined. Of the 94 cats sampled 2 (2.1%) were positive for *Salmonella* spp. with two serovars identified: *S*. Johannesburg and a serovar belonging to Group C_1. Only 1 isolate was resistant. It was concluded that the isolation of *Salmonella* spp. from apparently healthy cats poses a health hazard to their owners, since most serovars are known to be potentially pathogenic. Furthermore, the existence of resistance to antimicrobial agents amongst *Salmonella* isolates from cats could cause chemotherapeutic consequences to their human owners.

Key words: prevalence, antibiotic sensitivity, *Salmonella*, non-diarrhoeic cats

Introduction

Salmonella has long been recognized as an important zoonotic pathogen of worldwide economic significance (HUMPHREY, 2000). The organism inhabits the intestinal tract of vertebrate and invertebrate animals worldwide and its excretion results in the contamination of food, water and the environment (TURNBULL, 1979).

It has been documented that the faecal *Salmonella* carrier state in most cats is clinically inapparent and prevalence of carriers was found to be variable, with isolation rates of *Salmonella* serovars ranging from 0.0% to 14.0% (IKEDA et al., 1986). It has been noted

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that stray cats and shelter cats are more likely to excrete *Salmonella* in their faeces than pet household cats (SIEGAL, 1989). HILL et al. (2000) in a study on the prevalence of enteric zoonotic organisms in cats found that a higher proportion of shelter cats (18.2%) compared with client-owned cats (10.1%) had enteric organisms.

Bacteria with potentially transferable antimicrobial resistance determinants have been reported to be isolated from rectal swabs taken from healthy cats in urban and rural environments (NORMAND et al., 2000). DAVIES and STEWART (1978) reported the interrelationship of antimicrobial resistance in the flora of humans and domestic pets, concluding that resistance plasmids in the two populations were similar. This may be due to the fact that the antimicrobial agents used in companion animals are almost identical to those used in humans (STERNBERG, 1999). Heavy use of antibiotics in settings such as hospitals may increase the level of bacteria-carrying resistance genes (LEVY, 1998).

Infections with *Salmonella* spp. do not generally require antibiotic therapy, as antimicrobials are not indicated for uncomplicated gastroenteritis which may be caused by the organisms (CLARKE and GYLES, 1993). Treatment has, however, been reported not to reduce the duration or severity of the symptoms of salmonellosis, but may also prolong convalescence and the carrier state and may result in the emergence of resistant strains (COHEN and TAUXE, 1986).

The objectives of the study were to determine the prevalence of *Salmonella* spp. in non-diarrhoeic cats from various sources in Trinidad, and to determine the susceptibility of *Salmonella* spp. isolated to various antimicrobial agents.

Materials and methods

Source of samples. For household cats, Trinidad was divided into four geographical regions, North-West, North-East, Central and South. Systematic random sampling was employed where cats were sampled from every third household on a selected street in the area identified. However, a maximum of two cats per household (because of the scarcity of cats) in the sampling areas identified, and whenever such animals were encountered they were sampled.

Additionally, the Trinidad and Tobago Society for the Prevention of Cruelty to Animals (TTSPCA) was sampled, where a maximum of five cats were sampled from each kennel.

Cats sampled from veterinary establishments were also included in the study. Six veterinary Hospitals (in-patients) were identified using the telephone directory in the four regions mentioned above: North-West, North-East (no hospital facilities were available in the north-east region), Central and South.

The Quarantine Station, where cats from rabies-endemic countries was used as a source of imported cats. These animals are housed in quarantine for 6 months. For a period of 24

months all cats that were admitted to the station were sampled on entry, after 3 months, and immediately prior to release at the 6- month point.

Regardless of source, all cats sampled were apparently healthy and non-diarrhoeic.

Sample collection and transportation. Types of sample collected were either rectal swabs or fresh faeces. All samples were taken aseptically. All samples were taken originated from apparently healthy animals, without diarrhoea. Samples taken as rectal swabs were obtained with sterile cotton-tipped applicators. Commercially available cotton swabs were moistened in sterile saline before being inserted in the rectum. Swabs with faecal materials were then dipped in tubes containing 10 ml of Amies Transport Medium (ATM) (Difco, Detroit, U.S.A).

Samples taken in the form of fresh faeces were collected with sterile wooden tongue depressors into sterile plastic faecal cups. All samples, regardless of source, were transported ice-cooled to the laboratory within 24 h of collection.

Questionnaires. Questionnaires were specifically prepared for each source of samples studied. Information on age of animal sampled was obtained.

Isolation of Salmonella spp. Isolation of *Salmonella* used two enrichment broths, tetrathionate (TT) broth (Oxoid, Basingstoke, U.K.) and selenite cystine (SC) broth (Oxoid, Basingstoke, U.K.). Approximately one gram each of fresh faeces or rectal swabs was inoculated into 9 ml of tt and 9 ml of sc broths. Inoculated broths were incubated for 18-24 h at 37 ˚C and 42 ˚C, respectively for tt and sc broths. Both enrichment broths were then inoculated onto xylose lysine desoxycholate (XLD) agar, (Oxoid, Basingstoke, U.K.) and streaked for isolation. Presumptive *Salmonella* were subjected to biochemical tests (MACFADDIN, 1980), and a slide agglutination test was employed thereafter, using a commercially available *Salmonella* polyvalent O antiserum (A - I α Vi) (Difco Laboratories, Detroit, U.S.A) to further identify the organism as *Salmonella* spp.

Serotyping of Salmonella spp. Salmonella isolates were confirmed and serotyped with the kind cooperation of the Caribbean Epidemiology Center, Federation Park, Port-of-Spain, the Regional Reference Laboratory for *Salmonella*.

Determination of antibiotic resistance of Salmonella isolates. The Kirby Bauer disc diffusion method (BAUER et al., 1966) was employed to determine the resistance of *Salmonella* isolates to antimicrobial agents. Antimicrobial agents and concentrations used in the study included cephalothin (KF, 30 μg), ampicillin (AMP, 10 μg), streptomycin (S, 10 μg), chloramphenicol (C, 30 μg), neomycin (N, 30 μg), gentamicin (CN, 10 μg), sulphamethoxazole/trimethoprim (SXT, 1.75 μg/23.25 μg) and norfloxacin (NOR, 10 μg) (Oxoid, Basingstoke, U.K.).

Statistical analysis. Where possible, the chi-square test for independence was employed to compare prevalence and the type 1 error was set at 0.05 for all tests. Epi-Info (Center

for Disease Control and Prevention, Atlanta, Georgia, U.S.A.; Version 6.02) was used to determine whether there were any statistically significant differences in the parameters investigated.

Results

Overall, of 94 cats sampled from all sources, only 2 (2.1%) were positive for *Salmonella* spp. (Table 1). The two serovars detected in cats were Johannesburg (3 isolates) and a *Salmonella* serovar belonging to Group C_1 (1 isolate).

The prevalence of *Salmonella* spp. in animals by age is shown in Table 2. The frequency of isolation of *Salmonella* infection was comparatively higher in animals less than one year old compared with those older than one year.

Table 1. Prevalence of *Salmonella* spp. in cats by source

Source of samples	N° of samples tested	N° (%) positive for *Salmonella* spp.	N° of *Salmonella* isolates[a]	Serovar (N°)[d]
Quarantine	16	0 (0.0)	0	
Households	37	1 (2.7)	3	*S*. Johannesburg (3)
Hospitals[b]	12	0 (0.0)	0	
TTSPCA[c]	29	1 (3.5)	1	Group C1 (1)
Total	94	2 (2.1)	4	

a = an animal may have yielded more than one *Salmonella* isolate; b = in-patients; c = Trinidad and Tobago Society for the Prevention of Cruelty to Animals; d = number of isolates

Table 2. Prevalence of *Salmonella* spp. in cats by age

Animal type	Age category	N° of animals[a] tested	N° (%) positive[b] for *Salmonella* spp.
Cats	< 1 yr.	22	1 (4.6)
	> 1 yr.	72	1 (1.4)
Total		94	2 (2.1)

a = takes into account age at which animals tested positive with reference to quarantine cats at different samplings; b = only done for cats where ages could be determined

Of the 4 isolates tested for antimicrobial resistance from cats, only 1 was resistant to one antimicrobial agent, streptomycin. All 4 isolates of *Salmonella* spp. were sensitive to sulphamethoxazole/trimethoprim, cephalothin, neomycin, gentamicin, ampicillin, norfloxacin and chloramphenicol.

Table 3. Antibiograms of *Salmonella* spp. isolates from cats

Animal type	Source of samples	N° of *Salmonella*[a] isolates	N° (%) of isolates resistant[b]	N° (%) of *Salmonella* spp. resistant to:			
				S[c]	KF	N	CN
Cats	Households	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	TTSPCA[d]	1	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Total	4	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)

a = all 4 isolates of *Salmonella* spp. were sensitive to Ampicillin, Norfloxacin, Chloramphenicol and Sulphamethoxazole/Trimethoprim, Cephalothin, Neomycin and Gentamicin; b = resistant to one or more antimicrobial agents; c = S-Streptomycin, KF-Cephalothin, N-Neomycin, CN-Gentamicin; d= Trinidad and Tobago Society for the Prevention of Cruelty to Animals

Discussion

The prevalence of 2.1% for *Salmonella* spp. in cats found in the current study is higher than the 0.8% in cats in New York (SPAIN et al., 2001) and in 1.0% in cats in Colorado, U.S.A (HILL et al., 2000). Conversely, a higher prevalence of *Salmonella* in cats has been documented by FOX and BEAUCAGE (1979) who recovered *Salmonella* spp. from 10.6% of randomly sourced cats, whilst SHIMI and BARIN (1977) detected the pathogen in 29 (10.8%) of 269 apparently healthy cats in Iran.

A prevalence of 2.7% for *Salmonella* spp. detected in household cats was considerably lower than the prevalence of 10.5% (KHAN, 1970), 12.1% (MACKEL et al., 1952) and 12.8% (SHIMI and BARIN, 1977) reportedly detected in household cats in the Sudan, Florida, U.S.A and Iran, respectively. On the other hand, no *Salmonella* spp. was detected in 52 household cats in Colorado, U.S.A. as reported by HILL et al. (2000). The difference in the prevalence of *Salmonella* in cats from various sources may reflect types of feed and contaminated environment. Cats allowed to roam or hunt and to feed on rodents or wildlife species are known to have a high exposure to *Salmonella* infection, thus playing a significant role in the epidemiology of salmonellosis in man and animals (SHIMI and BARIN, 1977). Any isolation of *Salmonella* spp. in household cats would be of public health significance, as many cats spend most of their lives indoors in close contact with human beings.

The finding of a higher prevalence of *Salmonella* in kennelled cats compared to household cats is in agreement with HILL et al. (2000), who found 1.9% (1 of 53) of shelter cats to shed *Salmonella* spp. as compared with a failure to isolate *Salmonella* from household cats. BALL (1951) also detected *Salmonella* spp. in 4 (2.3%) of 175 shelter cats compared to a zero prevalence in household cats. The relatively higher prevalence detected in TTSPCA cats compared to households may be attributed to confinement, as documented elsewhere (FOX and BEAUCAGE, 1979; CARTER and QUINN, 2000). The presence of *Salmonella*

spp. in TTSPCA cats is of zoonotic significance as there is a possibility of spread of the infection to new owners if adopted. Contacts with cats have been previously reported to cause human salmonellosis (MADEWELL and MCCHESNEY, 1975; ANONYMOUS, 2001). ANONYMOUS (2001) reported that the owner of two asymptomatic cats acquired from a shelter fell ill 77 days after adoption. In the same report, an outbreak strain of *Salmonella* Typhimurium was recovered from another shelter cat after 115 days of adoption, and the isolates from the cats and the humans affected were indistinguishable by pulse field gel electrophoresis and antibiotic resistance patterns, suggesting transmission from the shelter cat to humans.

There is no host-adapted serovar described for cats (CARTER AND QUINN, 2000). Only two serovars were isolated from cats in the study, serovar Johannesburg from a household cat and a *Salmonella* serovar belonging to Group C_1 from the TTSPCA. Johannesburg has previously been isolated from cats in Sudan (KHAN, 1970) whilst a study on wildlife in Trinidad (EVERARD et al., 1979) yielded the serovar from a mongoose (*Herpestes auropunctatus*). According to CAREC annual reports (ANONYMOUS, 1990-2002), no report of human salmonellosis caused by a *Salmonella* Johannesburg infection has ever been documented. Since many serovars of the microorganism are considered potentially pathogenic for man and animals (CLARKE and GYLES, 1993), in the absence of information in Trinidad regarding the serotype, the presence of serovar Johnnesburg in a household cat could be considered a health hazard to its owner.

Overall, only one isolate of *Salmonella* spp. from cats exhibited resistance to one of the eight antimicrobial agents tested. The prevalence of resistance (25.0 %) observed in the present study is lower than that observed in studies previously conducted in Trinidad on *Salmonella* isolates from livestock (50.0%) (ADESIYUN et al., 1993) and captive wildlife (98.0%) at the zoo (GOPEE et al., 2000), but higher than the prevalence of resistance observed for pet dogs sampled in a clinic (0.0%) (ADESIYUN et al., 1997) and dairy farms (0.0%) (ADESIYUN et al., 1996). This isolate exhibited resistance of *Salmonella* to only streptomycin (25.0%). It has been documented by THRELFALL et al. (1986) that the streptomycin resistance genes were carried on plasmids in a strain of *Salmonella* Typhimurium. Any misuse of antibiotic administration may cause an infecting organism or even commensals to acquire transferable (plasmid-mediated resistance) resistance, which is of public health significance as such resistance has been demonstrated amongst *Salmonella* isolates from dogs to humans (GREENE, 1998). Therapeutic failure could therefore result when the antibiotic is used in infected individuals. However, the fact that all *Salmonella* isolates were sensitive to cephalothin, neomycin, gentamicin, sulphamethoxazole/trimethoprim, ampicillin, norfloxacin and chloramphenicol indicates that these agents could be successfully used in the treatment of clinical salmonellosis in these animals.

In conclusion, although with a low prevalence, the isolation of *Salmonella* spp. from apparently cats poses a health hazard to their owners, since many serovars are considered to be potentially pathogenic. Although *Salmonella* was not detected in quarantine pets in the present study, the potential for imported cats to serve as sources of introducing new serovars of *Salmonella* into the country cannot be ignored. The existence of resistance to antimicrobial agents amongst *Salmonella* isolates from cats could have chemotherapeutic implications to their human owners and contacts should they become infected by serovars of *Salmonella* from their pet animals.

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SAŽETAK

U Trinidadu je istražena proširenost *Salmonella* spp. u klinički zdravih mačaka. Izdvojeni serovarovi *Salmonella* spp. bili su identificirani te im je određena otpornost na osam antimikrobnih sredstava. Od 94 mačke, dvije (2,1%) su bile pozitivne na salmonele s dva identificirana serovara: *S*. Johannesburg i serovar koji pripada skupini C_1. Jedan izolat bio je rezistentan. Može se zaključiti da izdvajanje *Salmonella* spp. iz zdravih mačaka predstavlja zdravstvenu opasnost za njihove vlasnike, jer je poznato da je većina serovarova potencijalno patogena. Nadalje, rezistencija izolata iz mačaka na antimikrobna sredstva može imati kemoterapeutske posljedice na njihove vlasnike.

Ključne riječi: osjetljivost na antibiotike, salmonele, mačke