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SOME BACTERIAL ENTEROPATHOGENS IN WILDLIFE AND RACING PIGEONS FROM TRINIDAD

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ABSTRACT: Fecal and cloacal swabs or feces of wild mammalian, avian and reptilian species, either farmed or free-ranging, and of racing pigeons (*Columba livia*) kept in lofts were cultured for *Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp. Of 291 free-ranging mammals tested, 6 (2%) and 1 (<1%) yielded positive cultures of *Salmonella* spp. and *Campylobacter* spp., respectively. *Salmonella newport* was the predominant serotype isolated and the opossum (*Didelphis marsupialis insularis*) had the significantly highest prevalence (29%) of *Salmonella* spp. infection compared to other species such as deer (*Mazama americana trinitatis*), lappe (*Agouti paca*), tattoo (*Dasypus novemcinctus*), agouti (*Dasyprocta leporina*), and wild hog (*Tayassu tajacu*). Among 14 species of farmed wildlife studied, 13 (7%) and 10 (5%) of 184 fecal or cloacal samples tested were positive for *Salmonella* spp. and *Campylobacter* spp., respectively. *Salmonella javiana* accounted for 50% of the *Salmonella* spp. isolates and *C. jejuni* represented 90% of the *Campylobacter* spp. cultured. Only 1 (1%) of 124 cloacal swabs of free-flying avian species yielded *Salmonella* spp. compared to 21 (17%) samples positive for *Campylobacter* spp. Of 171 racing pigeons which originated from 8 fanciers, 8 (5%) yielded *Salmonella* spp. all of which were serotype *typhimurium* while only 1 (1%) was positive for *Campylobacter* spp. Seven (88%) of 8 *Salmonella* spp. isolates were recovered from one fancier. *Yersinia* spp. was not cultured from any of the above samples. Although the prevalences of *Salmonella* spp. and *Campylobacter* spp. in wildlife in Trinidad are low, the practice of wildlife farming and the increased consumption of meat from wildlife may increase the health risk to human consumers.

Key words: Bacterial enteropathogens, *Campylobacter* spp., mammals, racing pigeons, *Salmonella* spp., survey, wild birds, *Yersinia* spp., zoonoses.

INTRODUCTION

Free-ranging or captive wildlife species are asymptomatic carriers of enteric pathogens such as *Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp. (Kapperud and Rosef, 1983; Fukata et al., 1986; Euden, 1990; Aguirre et al., 1991). Mortalities in various species also have been attributed to these enteric zoonoses (Shima and Osborn, 1984; Blake et al., 1991; Jacobson et al., 1992). The potential for free-ranging avian or mammalian species to contaminate the environment, particularly waterways, with waterborne zoonoses is well known (Kapperud, 1977; Mentzing, 1981; Kapperud and Rosef, 1983). The consumption of improperly cooked meat from these hosts also may lead to gastroenteritis in consumers (Prescott and Monroe, 1982; Ho et al., 1986; Anonymous, 1988; Oboegbulem and Okoronkwo, 1990).

There is a dearth of information on the prevalence of enteric zoonoses in the wildlife population of Trinidad. Everard et al. (1979) is the only report documenting the isolation of *Salmonella* spp. from free-living host species in Trinidad and Grenada. However, since that study, the government of Trinidad and Tobago has embarked on a policy to encourage wildlife farming. The potential health risk to such farmers presently is unknown. Secondly, pigeon racing is becoming a popular sport on the island, with pigeons (*Columba livia*) flying between islands in the region. The prevalence of enteropathogens in these pigeons is unknown. Finally, during the annual hunting season from October to February, licensed hunters are allowed to kill wildlife for their consumption or sale to the population at certain outlets. It is well documented that during evisceration under poor sanitary practices, enteric pathogens may contaminate carcasses (Grizmek, 1979; Grau, 1988; Oboegbulem and Okoronkwo, 1990; Adesiyun and Krishnan,

1995). Enteric pathogens such as *Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp. have been isolated in Trinidad from domestic livestock on farms (Adesiyun and Kaminjolo, 1994), slaughtered pigs (Adesiyun and Krishnan, 1995), and from market meat (Adesiyun, 1993). Therefore, this study was designed to determine the prevalence of important enteric zoonoses (salmonellosis, campylobacteriosis, and yersiniosis), in free-living and captive wildlife and in racing pigeons from Trinidad.

MATERIALS AND METHODS

Free-ranging bird and mammal samples were collected from licensed hunters during the 1995–96 hunting season (1 October 1995 to 29 February 1996) in the island nation of Trinidad (10°30′N, 61°30′W). Groups of hunters across the country were contacted, prior to the opening of the hunting season, and their support solicited. Samples from captive wildlife species were obtained from individual farmers across the country registered with the Wildlife Section of the Ministry of Food Production (Marine Exploitation, Forestry and Environment, St. Joseph, Trinidad). Free-flying small avian species were trapped in forest areas and near a liquid sewage dump site using mist nets. Samples from vultures (*Coragyps atratus*) were obtained from a solid waste dump site. Samples from free-flying pigeons were collected from a flour mill (Port of Spain, Trinidad). Racing pigeons were sampled from the lofts of eight fanciers in Diego Martin, San Juan and Valsayn areas of Trinidad.

For wildlife species that were hunted, the hunters were requested to tie up a 5 cm section of the small intestine of the respective specimen during evisceration and to put the sample into sterile plastic bags that were provided. Information on the type of wildlife, location of the hunt and whether the animals were adults or a young specimen were indicated on the bags. Samples were kept at 4 C and sent to the laboratory as soon as possible. When the hunting group was in the field for a day or a weekend, they were provided with sterile swabs and tubes containing 9 ml of Amies transport medium (ATM) (Difco, Detroit, Michigan, USA) to collect swab samples of the intestinal contents. When intestinal contents could not be sent to the laboratory within 1 day following collection, they were stored frozen at −20 C.

Fecal samples were obtained from captive or farmed animals after restraint. Where it was impossible to collect fecal samples, rectal swabs or cloacal swabs were obtained and transported to the laboratory in ATM. On a few occasions, freshly voided feces were collected with a clean wooden spatula, sampling only materials that had not made any contact with the floor or other surface.

To collect samples of small avian species, mist nets were set at selected sites. Fecal swabs or cloacal swabs were immediately obtained from trapped birds and dipped into ATM. The species of the birds were identified and immediately released. Occasionally, when many birds were trapped simultaneously, some were put into well-ventilated cardboard boxes, the cloacal swab samples were collected as soon as possible, and the birds subsequently were released.

Cloacal swabs or fecal samples were collected from feral pigeons at the rooftop of a flour mill in Port of Spain. The pigeons were enticed with grain into a wire-gauzed enclosure. Swabs of freshly voided feces of pigeons or their cloacal swab samples were obtained and dipped into ATM. The pigeons were released immediately.

At a solid waste dump, vultures were captured with the aid of a nylon string and their cloacal swab samples collected and put into ATM. The vultures were immediately released after sampling.

Crane (*Ardea cocoi*) and duck (*Dendrocygna bicolor*) samples were obtained from frozen intestinal contents provided by hunters. In the laboratory, the samples were thawed to obtain material for processing.

Finally, eight fanciers associated with pigeon racing participated in the study. Representative pigeons from each loft were sampled by collecting cloacal swabs or freshly voided feces and subsequently transported to the laboratory in ATM.

To culture for *Salmonella* spp., approximately 1 g of feces, rectal or cloacal swab samples was pre-enriched in 9 ml of selenite broth (Difco) at 42 C overnight. Thereafter, 1 ml each of inoculated selenite broth sample was enriched in 10 ml of tetrathionate broth (Difco) and 10 ml selenite cystine broth (Difco) and incubated at 37 C. Colonies on xylose lysine desoxycholate (XLD) agar (Difco) with black centers were inoculated into biochemical media using standard methods (Macfaddin, 1980; Food and Agricultural Organization, 1992). Polyvalent antisera A-I ∝ Vi for *Salmonella* spp. were used to identify all isolates biochemically identified as *Salmonella* spp. The confirmation and complete serological typing of the *Salmonella* spp. isolates were kindly done at the Caribbean Epidemiology Centre (Port of Spain, Trinidad), us-

TABLE 1. Prevalence of *Salmonella* spp. and *Campylobacter* spp. in free-ranging mammals from Trinidad.

Common name	Scientific name	Number tested	Number (%) of animals positive[a]	
			Salmonella spp.	*Campylobacter* spp.
Agouti	*Dasyprocta leporina*	232	1 (<1)	0 (0)
Opossum	*Didelthis marsupialis insularis*	17	5 (29)	1 (6)[b]
Deer	*Mazama americana trinitatis*	15	0 (0)	0 (0)
Lappe	*Agouti paca*	15	0 (0)	0 (0)
Armadillo	*Dasypus novemcinctus*	6	0 (0)	0 (0)
Wild hog	*Tayassu tajacu*	6	0 (0)	0 (0)
Total		291	6 (2)[c]	1 (<1)

[a] All samples were negative for *Yersinia* spp.
[b] *C. jejuni*.
[c] 11 strains of the genus *Salmonella* consisting of serotypes *S. newport* (4), *S. chameleon* (2), *S. albany* (1) and *S. oranienburg* (4).

ing standard methods (Food and Agricultural Organization, 1992) and specific somatic (O) and flagella (H) antisera.

To isolate *Campylobacter* spp. swabs of feces, cloaca or rectums were inoculated onto blood-free *Campylobacter* sp. agar containing CCDA supplement (Oxoid, Basingstoke, U.K.) and streaked for isolation. Inoculated plates were incubated at 42 C in a CO_2 incubator (Forma Scientific Inc., Marietta, Ohio, USA) with 8 to 10% CO_2 for 24 to 48 hr. Greyish, running colonies were Gram-stained and all Gram-negative, slender, comma-shaped, curved or sea gull-appearing isolates subcultured onto sheep blood agar and incubated at 42 C overnight in 8 to 10% CO_2. Biochemical identification of isolates was done as described by Lior (1984). Hippurate hydrolysis (Lior, 1984) was used to distinguish between *C. jejuni* and *C. coli*.

To culture for *Yersinia* spp. in samples, 0.5 g of feces or swab samples were enriched in 4.5 ml of 0.067 M phosphate buffered saline (PBS) at pH 7.6 for 3 wk at 4 C. Subcultures were made after 1 wk and 3 wk of enrichment onto *Yersinia* spp. agar with selective supplement (Oxoid) and streaked for isolation. Inoculated plates were incubated at room temperature for 24 to 48 hr. Typical "bull-eye"-appearing colonies were Gram-stained and the isolates that were Gram-negative coccobacilli were subjected to biochemical tests which included inoculation into triple sugar iron (TSI) agar, urea agar, sulphide iron motility (SIM) agar, methyl-red broth, Voges-Proskauer broth and testing for oxidase activity and motility. All tests, with the exception of oxidase activity detection, were done at 25 and 37 C. Identification procedures used were those suggested by Schiemann and Fleming (1981).

Statistical comparisons across prevalence in the pipes utilized chi-squared analyses (Cotton, 1994). Significance was established at $P \leq 0.05$.

RESULTS

The results of isolation attempts for agents of salmonellosis, campylobacteriosis, and yersiniosis from free-ranging mammals are presented in Table 1. Of the 291 fecal or rectal swab samples processed, 6 (2%) and 1 (<1%) were positive for *Salmonella* spp. and *Campylobacter* spp., respectively. The prevalence of *Salmonella* spp. in opossums (*Didelphis marsupialis insularis*) (29%) was significantly ($P \leq 0.001$; χ^2-analysis) higher than for the other 5 infected species including lappe (*Agouti paca*), deer (*Mazama americana trinitatis*), agouti (*Dasyprocta leporina*), tattoo (*Dasypus novemcinctus*) and wild hog (*Tayassu tajacu*). The predominant serotype of the genus *Salmonella* was *newport* accounting for 4 (36%) of 11 isolates. The only *Campylobacter* spp. isolate was *C. jejuni* collected from an opossum. All tested samples were negative for *Yersinia* spp.

Table 2 shows the prevalence of agents for salmonellosis and campylobacteriosis in farmed wildlife. Of a total of 14 host species tested, 13 (7%) and 10 (5%) of 184 fecal or cloacal samples cultured yielded *Salmonella* spp. and *Campylobacter* spp., respectively. *Salmonella* spp. infection ranged from 0% to 50% (turtles), but the

TABLE 2. Prevalence of enteropathogens in farmed wildlife from Trinidad.

Common names	Scientific names	Number tested	Number (%) of animals positive[a] for: *Salmonella* spp.	Number (%) of animals positive[a] for: *Campylobacter* spp.
Agouti	*Dasyprocta leporina*	88	2 (2)	8 (9)[b]
Snake[c]	*Amphisbaena alba*	23	4 (17)	0 (0)
Deer	*Mazama americana trinitatis*	19	0 (0)	0 (0)
Lappe	*Agouti paca*	10	2 (20)	0 (0)
Monkey	*Alouata seniculus*	9	1 (11)	0 (0)
Pigeon	*Columba* spp.	8	0 (0)	0 (0)
Parrot	*Amazona amazonica*	6	0 (0)	0 (0)
Wild hog	*Tayassu tajacu*	5	2 (40)	1 (20)[e]
Porcupine	*Coendou prehensilis*	5	0 (0.0)	1 (20)[b]
Marocoy	*Geochelone denticulata*	4	0 (0.0)	0 (0)
Turtle	*Chelydra serpentina*	4	2 (50)	0 (0)
Toucan	*Ramphastos tucanas*	4	0 (0)	0 (0)
Macaw	*Ara macao*	1	0 (0)	0 (0)
Caiman	*Caiman crocodilus*	1	0 (0)	0 (0)
Total		184	13 (7)[d]	10 (5)

[a] All samples were negative for *Yersinia* spp.

[b] *C. jejuni.*

[c] Others are: Macajuel (*Boa constrictor*), Tigre (*Spilotes pullatus*), Horsewhip snake (*Oxybelis aeneus*), Red-spitting cobra (*Naja pallida*) and South American rattlesnake (*Crotalus durissus*).

[d] *C. coli.*

[e] A total of 16 strains of the genus *Salmonella* were isolated consisting of serotypes *S. javiana* (8), *S. gaminara* (3), *S. typhimurium* (2), *S. nigeria* (1), *S. parera* (1) and *S. oranienburg* (1).

difference was not statistically significant ($P \geq 0.05$; χ^2-analysis). *Salmonella* spp. serotype *javiana* accounted for 50% of all *Salmonella* spp. isolates. Six (43%) of 14 host species yielded *Salmonella* spp. isolates. Agoutis yielded 8 (80%) of the 10 *Campylobacter* isolates. Nine (90%) of the 10 *Campylobacter* spp. were *C. jejuni.* Only 3 (21%) of 14 animal hosts yielded *Campylobacter* spp.; these included the agouti, porcupine (*Coendou prehensils*) and wild hog.

A comparison of free-ranging (Table 1) and captive (Table 2) agouti, deer, lappe and wild hog showed significant differences in prevalences of *Salmonella* spp. and *Campylobacter* spp. Of 268 samples of free-ranging agouti (n = 232), deer (n = 15), lappe (n = 5) and wild hog (n = 6), only 1 (<1%) agouti was positive for *Salmonella* spp. compared to a prevalence of 5% (6 of 132) found in farmed agouti (n = 88), deer (n = 19), lappe (n = 20) and wild hog (n = 5). The difference was statistically significant ($P \leq 0.01$; χ^2-analysis). Similarly, the prevalences of *Campylobacter* spp. in free-ranging wildlife, 0% (0 of 268) was statistically significantly lower ($P \leq 0.001$; χ^2-analysis) than the 7% (9 of 132) found for captive (farmed) wildlife species.

Of 124 cloacal swabs of free-ranging birds that were cultured, 1 (<1%) and 21 (17%) were positive for *Salmonella* spp. and *Campylobacter* spp., respectively (Table 3). The difference in prevalences of *Salmonella* spp. and *Campylobacter* spp. was statistically significant ($P \leq 0.001$; χ^2-analysis). One vulture yielded the only *Salmonella* spp. (serotype Group B) isolate while all 21 isolates of *C. jejuni* originated from pigeons in the limits of Port of Spain. The prevalence (36%) was statistically significantly ($P \leq 0.001$; χ^2-analysis) higher than the 0% found in other avian species.

Table 4 shows the prevalence of *Salmonella* spp. and *Campylobacter* spp. in racing pigeons. Of 171 pigeons originating

TABLE 3. Prevalence of enteropathogens from free-flying birds in Trinidad.

Species	Number tested	Number (%) positive[a]	
		Salmonella	*Campylobacter*
Free-flying birds[b]	60	0 (0)	0 (0)
Free-flying pigeons[c] (*Columba livia*)	59	0 (0)	21 (36)[e]
Vulture (*Coaragyps atratus*)	3	1 (33)[d]	0 (0)
Crane (*Ardea cocoi*)	1	0 (0)	0 (0)
Duck (*Dendrocygna bicolor*)	1	0 (0)	0 (0)
Total	124	1 (1)	21 (17)

[a] All samples were negative for *Yersinia* spp.
[b] Consisted predominantly of 15 Tanagers (*Ramphacelus carbo*), 14 Doves (*Geopelia cuneata* and *Streptopelia decaocto*), 8 Yellow-hooded blackbird (*Agelaius icterocephalus*), 5 Thrush (*Turdus nudigenis*) and 3 Banaquit (*Coereba flaveola*).
[c] Pigeons around a flour mill located in Port of Spain.
[d] *Salmonella* sp. Group B.
[e] All isolates were *C. jejuni*.

TABLE 4. Prevalence of enteropathogens in racing pigeons from Trinidad.

Racing fancier loft	Number sampled	Number (%) positive[a]	
		Salmonella spp.	*Campylobacter* spp.
I	28	0 (0)	0 (0)
II	28	7 (25)	0 (0)
III	19	0 (0)	0 (0)
IV	21	0 (0)	1 (5)
V	29	0 (0)	0 (0)
VI	16	0 (0)	0 (0)
VII	11	0 (0)	0 (0)
VIII	19	1 (5)	0 (0)
Total	171	8 (5)[b]	1 (1)[c]

[a] All samples were negative for *Yersinia* spp.
[b] Fourteen strains of *Salmonella typhimurium* were isolated.
[c] *C. jejuni*.

from 8 fanciers, 8 (5%) were positive for *Salmonella* spp. compared to only 1 (<1%) positive that was for *Campylobacter* spp. The difference was statistically significant ($P \leq 0.05$; χ^2-analysis). All 14 strains of *Salmonella* spp. isolated were of serotype *typhimurium*. Pigeons from lofts in fancier II yielded 7 (88%) of the 8 isolates of *Salmonella* spp. while pigeons from the lofts of 6 fanciers were negative for the microorganism.

Yersinia spp. was not isolated from the processed samples of any of the sources (Tables 1–4).

DISCUSSION

Meat originating from mammalian, avian and reptilian species is a rapidly growing delicacy among consumers in Trinidad and Tobago. Thus, it is imperative to assess its potential health risk. Meatborne epidemics of salmonellosis, campylobacteriosis and yersiniosis are well documented (Checko et al., 1977; Prescott and Monroe, 1982; Centers for Disease Control, 1990).

Although the prevalence of *Salmonella* spp. infection found in free-living mammals in the present study is relatively low (2%), the finding agrees with published reports (Taylor, 1969; Everard et al., 1979; Howerth et al., 1994). However, it is difficult to ignore the health risk these wildlife meats may pose to the consumer because opossum, a popular delicacy, had a significantly higher prevalence of *Salmonella* spp. infection (29%) compared to other wild mammals. Because the opossum is a carrion-eating animal, it may have a higher exposure potential to *Salmonella* spp. compared to herbivorous species. Similar prevalences of *Salmonella* spp. infection in the opossum are reported and these range from 12 to 20% (Kourany et al., 1976; Everard et al., 1979). Badgers (*Meles meles*) which have similar feeding habits to those of opossums were found to be more frequently infected with *Salmonella* spp. than other wildlife in the same environment in the United Kingdom (Euden, 1990).

Of epidemiological significance was the comparison of species of free-ranging with farmed mammals which indicated that the prevalence of *Salmonella* spp. in captive species was significantly higher. Confinement, diet, and closeness to humans are some factors that may be responsible for this difference. It also was of interest that the predominant serotypes of the genus

Salmonella isolated from free-living and captive hosts were different, an indication of different sources of exposure. However, it is of zoonotic relevance from the view point that a majority of the serotypes of the genus *Salmonella* from both sources have been isolated from human gastroenteritis cases in Trinidad (Caribbean Epidemiology Centre, 1983–90). Also, a number of these serotypes have been isolated from domestic livestock in this environment (Adesiyun et al., 1993). Contamination of carcasses and improper cooking of meat and meat products have been known to cause gastroenteritis in consumers (Grizmek, 1979; Ho et al., 1986; Oboegbulem and Okoronkwo, 1990; Adesiyun and Krishnan, 1995).

The relatively higher prevalences of *Salmonella* spp. in apparently healthy snakes (17%) and turtles (50%) were not unexpected as similarly high prevalences have been reported in other studies (Harvey and Price, 1983; MacNeill and Dorward, 1986; Zwart, 1986). Reptiles are known to have asymptomatic infections of salmonellae (Zwart, 1986; Obwolo and Zwart, 1993).

The prevalence of *Salmonella* spp. infections in avian hosts was generally low with the isolations made only from a vulture and from semi-captive racing pigeons. *Salmonella* spp. in apparently healthy birds have been reported to be low (Locke et al., 1973; Kapperud and Rosef, 1983; Brittingham et al., 1988). However, it is well documented that the feeding habits of birds affect the prevalence of infection by *Salmonella* spp. Omnivorous seagulls (*Larus atricilla*) are known to be more frequently infected than herbivorous avian species such as pigeons (Kapperud and Rosef, 1983; Quessy and Messier, 1992). Seagulls, because of their feeding habits, have been associated with occurrences of human and animal salmonellosis (Reilly et al., 1981; Williams et al., 1977). The comparatively higher prevalence of *Salmonella* spp. infection in racing pigeons from one fancier could be attributed to poor management practices. Finally vultures, because of their carrion-eating practices, have been reported to be asymptomatic carriers of several enteric pathogens including *Salmonella* spp. (Houston and Cooper, 1975).

Campylobacter spp. prevalence in free-living or captive mammals, while generally low, had higher prevalences in farmed species; this may reflect the feed and contact with human handlers. Comparatively lower prevalences of *C. jejuni* have been found elsewhere, as well as a predominance of *C. jejuni* over *C. coli* in infected animals (Skirrow and Benjamin, 1980). However, birds are known to be important reservoirs of *Campylobacter* spp. while they only act as sporadic carriers of *Salmonella* spp. and *Yersinia* spp. (Kapperud and Rosef, 1983). Variations in the diet of the respective species, as well as whether they feed in forested or urban areas, may significantly affect the prevalence of *C. jejuni* (Kapperud and Rosef, 1983; Quessy and Messier, 1992). Water borne campylobacteriosis in humans has been associated with avian contamination (Mentzing, 1981). Our findings that pigeons sampled in Port of Spain had a higher prevalence of *C. jejuni* infection may be attributed to their exposure in an urban setting as previously reported by Kapperud and Rosef (1983) and Fukata et al. (1986).

The fact that *Yersinia* spp. was not isolated from all the potential host species studied was not unexpected because in tropical environments like Trinidad and Tobago, yersiniosis in domestic livestock was found to be very low (Adesiyun and Kaminjolo, 1994). Pigs, which are natural reservoirs of *Yersinia* spp., had a prevalence of 2% (6 of 296), cattle had <1% (1 of 30) and all the sheep tested were negative for the infection (Adesiyun and Kaminjolo, 1994). Similarly in temperate countries, prevalence of yersiniosis in wildlife is generally low. Kapperud and Rosef (1983) reported that only 1% of 504 wild birds in Norway were positive. Kwaga and

Iversen (1993) found only 1 of 201 snakes in Canada was infected with *Yersinia* spp.

While it is evident from our study that the prevalences of *Salmonella* spp. and *Campylobacter* spp. in wildlife from Trinidad are relatively low, their potential to contaminate the environment cannot be ignored. The practice of wildlife farming and the increased consumption of meat from wildlife may enhance the health risk to human consumers.

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