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Absence of *Escherichia coli* O157 in a Survey of Wildlife from Trinidad and Tobago

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ABSTRACT: Fecal, cloacal, or rectal swabs of free–ranging and captive mamalian and avian wildlife in Trinidad and Tobago were cultured for non-sorbitol fermenting *Escherichia coli* and tested for O157:H7 strains. Ability of *E. coli* strains to produce hemolysin and mucoid colonies also was investigated. Of 271 free-ranging mammals tested, 158 (58%) yielded *E. coli*; only one (<1%) bacterial isolate was a non-sorbitol fermenter which was not agglutinated by O157 antiserum. All isolates were negative for hemolysin production and mucoid colonial growth. Two hundred and sixty-three (90%) of 293 free-flying birds were positive for *E. coli* and all isolates were sorbitol fermenters and negative for production of hemolysin and mucoid growth. Of 175 captive wild animals from individual backyard farms and a government demonstration farm, 145 (83%) yielded *E. coli* with four (2%) non-sorbitol fermenters; all were negative for O157 strains, hemolysin production, and mucoid colonial growth. Of 373 animals in a zoo, 250 (67%) were positive for *E. coli* with only two (0.5%) non-sorbitol fermenters. All strains were non-hemolytic and non-mucoid farms. It appears that free-ranging and captive avian and mammalian wildlife are not important reservoirs of O157:H7 strains of *E. coli* in Trinidad and Tobago.

Key words: *Escherichia coli*, capture wildlife, free-ranging wildlife, survey.

Escherichia coli is a normal inhabitant of the gastrointestinal tract of humans and animals (Sojka, 1971). Virulence and pathogenicity of *E. coli* strains are expressed by invasiveness, enterotoxigenicity and enteropathogenicity, and hemolysin production (Levine, 1987 Marques et al., 1995).

Recently, verocytotoxin-producing *E. coli* (VTEC) strains were documented and these strains have been responsible for foodborne outbreaks and hemolytic uremic syndrome (HUS) particularly in children (Karmali, 1989; Ostroff et al., 1990). Verocytotoxigenic *E. coli* strains are mostly non-sorbitol fermenters (March and Ratnam, 1986; Kleanthous et al., 1988) and are agglutinated by *E. coli* O157 antiserum and are hemolytic (Karmali, 1989).

Cattle are considered the most important animal reservoir of VTEC strains for human infection although these strains have been isolated from other livestock species (Mohammed et al., 1985; Karmali et al., 1989; Adesiyun and Kaminjolo, 1994). In Trinidad and Tobago, VTEC strains have been isolated from cattle, sheep, goats and pigs (Adesiyun and Kaminjolo, 1994), cattle feces, and bulk milk (Adesiyun, 1994; Adesiyun et al., 1997) and from black pudding or 'boundin noir' a local delicacy (Adesiyun and Balbirsingh, 1996). However, there is little information on the prevalence of VTEC strains in wildlife.

The present study was conducted to determine the prevalence of *E. coli* in free-ranging or captive mammalian and avian wildlife and to characterize the isolates as to their ability to ferment sorbitol, to be agglutinated by *E. coli* O157 antiserum and to produce hemolysin and mucoid colonies. The sources of avian and mammalian wildlife and the protocol used for sample collection are described by Adesiyun et al. (1998). The Emperor Valley Zoo, the only Zoo in Trinidad, served as the source of captive wildlife. For each species with less than five individuals, all animals were sampled. However, if more than five individuals were in the facility, a maximum of five samples was obtained per species.

For animals that could be safely handled, sterile swabs were inserted into their rectums or cloacae and gently rotated in a circular motion to obtain fecal materials. For dangerous caged animals, freshly voided feces were collected into sterile plastic containers using wooden spatula. Swabs in Amies Transport Medium (ATM) (Difco,

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TABLE 1. Prevalence and characteristics of *Escherichia coli* strains in feces of free-ranging wildlife from Trinidad and Tobago.

Common name	Scientific name	Number tested	Number positive (%)	
			E. coli[a]	Non-sorbitol fermenters
Agouti	*Dasyprocta leporina*	232	129 (56)	1 (≤1)
Opossum	*Didelphis marsupialis insularis*	17	9 (53)	0
Deer	*Mazama americana trinitatis*	15	8 (53)	0
Lappe	*Agouti paca*	15	7 (47)	0
Armadilo	*Dasypus novemcinctus*	6	3 (50)	0
Peccary	*Tayassu tajacu*	6	2 (33)	0
Total		271	158 (58)	1 (<1)[b]

[a] All strains of *E. coli* were negative for hemolysin production and formed non-mucoid colonies on blood agar.
[b] A non-0:157 *E. coli* strain.

Detroit, Michigan, USA) were transported to the laboratory within hours of collection. For fish pond samples, 100 ml of water was centrifuged at 10,000 rpm in universal bottles and pellets were inoculated into selective media. The procedure for collecting samples of hunted free-ranging animals is described by Adesiyun et al. (1998).

Swabs of feces, rectum, or cloaca were subcultured onto eosin methylene blue (EMB) agar and plated for *E. coli* isolation as described by Adesiyun et al. (1998). All *E. coli* isolates were further inoculated onto sorbitol MacConkey agar (Difco) to determine sorbitol utilization (March and Ratnam, 1986), and on blood agar plates to detect hemolysis and mucoid colonies. Inoculated plates were incubated at 37 C for 18 hr. Strains which produced complete clearing around colonies on blood agar plates were considered hemolytic, mucoid colonies also were noted. The slide agglutination test was used to detect O157 strains using *E. coli* O157 antiserum (Difco). The colonies which were agglutinated by the antiserum were considered positive for the O157 strain of *E. coli*. The prevalences of *E. coli* in fecal samples or rectal/cloacal swabs of various animal species were compared using the chi-square test for independence, with one degree of freedom.

The frequency of isolation of *E. coli* and non-sorbitol fermenting bacteria from free-ranging wildlife is shown in Table 1. Of 271 animals tested, 158 (58%) were positive for *E. coli* but only one (<1%) isolate was a non-sorbitol fermenter. None of the isolates were agglutinated by *E. coli* O157 antiserum and all were non-hemolytic and non-mucoid.

For captive wildlife, 145 (83%) of 175 animals tested yielded *E. coli* but only four (2%) isolates were non-sorbitol fermenters and all were negative for O157, hemolytic, or mucoid strains (Table 2).

Prevalence of *E. coli* in free-ranging birds and captive racing pigeons is shown in Table 3. All pigeons sampled were positive for *E. coli* compared to only 50% of other wild birds which yielded the microorganism. The difference was statistically significant (χ^2, $P \leq 0.05$). Overall, 263 (90%) of 293 birds yielded *E. coli*. No non-sorbitol fermenters, *E. coli* O157 strains, hemolytic, or mucoid strains were identified.

The prevalence of *E. coli* in zoo animals was 67% (250 of 373) but only two (1%) animals were positive for non-sorbitol fermenters. All isolates were non-hemolytic and these were not O157 strains. Avian species yielded two non-sorbitol fermenting *E. coli* strains. Among mammals, 113 (83%) of 137 were positive for *E. coli* compared to 37% (28 of 75) of reptiles and amphibians, 78% (107 of 137) of birds,

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TABLE 2. Prevalence and characteristics of *Escherichia coli* strains isolated from feces of captive wildlife in Trinidad and Tobago.

Common name	Scientific name	Number tested	Number positive (%)	
			E. coli[a]	Non-sorbitol fermenters
Agouti	*Dasyprocta leporina*	88	81 (92)	4 (5)[b]
Snakes	Boa constrictor and others[c]	23	7 (30)	0
Deer	*Mazama americana trinitatis*	19	17 (90)	0
Lappe	*Agouti paca*	10	6 (60)	0
Pigeon	*Columba* spp.	8	8 (100)	0
Parrot	*Amazona amazonica*	6	6 (100)	0
Peccary	*Tayassu tajacu*	5	4 (80)	0
Porcupine	*Coendou prehensilis*	5	5 (100)	0
Morocoy	*Geochelone denticulata*	4	4 (100)	0
Turtle	*Chelydra serpentina*	4	4 (100)	0
Caiman	*Caiman crocodilus*	1	1 (100)	0
Macaw	*Ara chloroptera*	1	1 (100)	0
Toucan	*Ramphastos tucanas*	1	1 (100)	0
Total		175	145 (83)	4 (2)

[a] All *E. coli* strains were negative for hemolysin and formed non-mucoid colonies on blood agar.
[b] Four isolates were non-O157 strains of *E. coli.*
[c] Consisted of snakes in other families including Colubridae, Elapidae, Amphisbaenidae, and Viperidae.

and only 8% (two of 24) of fish tanks. The differences in prevalences of *E. coli* infection were statistically significant ($P \leq 0.001$).

The prevalences of *E. coli* in free-ranging (Table 1) and captive mammals (Table 2) were significantly lower ($P \leq 0.05$) in the former than the latter; the difference across the agouti (*Dasyprocta leporina*) was 56 versus 92%, for deer (*Mazama americana trinitalis*) it was 53 versus 90%, and for the peccary (*Tayassu tajacu*) it was 33 versus 80%. This may be explained in part, by a difference in diet and exposure, which may result from more crowded conditions in captivity. The prevalences of *E. coli* detected in captive wildlife were similar to those found in confined or semi-confined livestock in the same environment (Adesiyun and Kaminjolo, 1994; Adesiyun et al., 1998).

The fact that only eight (<1%) of 1,112

TABLE 3. Prevalence and characteristics of *Eschericheria coli* strains isolated from wild birds in Trinidad and Tobago.

Common name	Scientific name	Number tested	Number positive (%)	
			E. coli[a]	Non-sorbitol fermenters
Racing pigeons	*Columba* sp.	174	174 (100)	0
Free-flying pigeons	*Columba livia*	59	59 (100)	0
Tanagers	*Ramphacelus carbo*	30	16 (53.3)	0
Doves	*Geopelia cuneata* and *Streptopelia decaocto*	14	6 (42.8)	0
Yellow-hooded blackbird	*Agelaius icterocephalus*	8	4 (50.0)	0
Thrush	*Turdus nudigenis*	5	3 (60.0)	0
Banaquit	*Coereba flaveola*	3	1 (33.3)	0
Total		293	263 (90)	0

[a] All strains of *E. coli* were non-hemolytic and non-mucoid.

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TABLE 4. Characteristics of *Escherichia coli* strains isolated from zoo animals in Trinidad and Tobago

Common name	Scientific name	Number tested	Number positive (%)	
			E. coli[a]	Non-sorbitol fermenters[b]
Mammals				
Monkey	*Cercopithecus aethiops* and others[c]	40	39 (98)	0
Raccoon	*Procyon cancrivorus cancrivorus* and others[d]	14	13 (93)	0
Deer	*Mazama americana trinitatis* and *Cervus elaphus*	10	10 (100)	0
Opossum	*Didelphis marsupialis insularis* and others[e]	6	2 (33)	0
Tayra	*Eira barbara trimitatis* and others[f]	6	5 (83)	0
Ocelot	*Felis pardalis*	5	1 (20)	0
Peccary	*Tayassu tajacu*	5	5 (100)	0
Porcupine	*Coendou prehensilis*	5	5 (100)	0
Bat	*Corollia perspicillata perspicillata*	5	3 (60)	0
Guinea pig	*Civia porcelius*	5	1 (20)	0
Rabbit	*Dryctolagus cuniculus*	5	5 (100)	0
Capybara	*Hydrochoerus hydrochaeris*	5	2 (40)	0
Agouti	*Dasyprocta leporina*	5	2 (40)	0
Otter	*Lutra longicaudis*	4	4 (100)	0
Lion	*Felis concolor*	4	4 (100)	0
Jaguar	*Panthera tigris*	3	3 (100)	0
Tiger	*Panthera onca, P. sumatrae*	2	2 (100)	0
Tapir	*Tapirus terrestris*	2	2 (100)	0
Squirrel	*Sciurus grantensis*	2	2 (100)	0
Marmoset	*Callithrix jacchus*	1	1 (100)	0
Tattoo	*Dasypus novemcinctus*	1	1 (100)	0
Lappe	*Agouti paca*	1	1 (100)	0
Mongoose	*Herpestes auropunctatus*	1	0	0
Reptiles/Amphibians				
Snakes	*Amphisbaena alba* and others[g]	38	17 (44)	0
Tortoise	*Geochelone denticulata, G. sulcata, G. carbonaria*	14	6 (43)	0
Turtles	*Podocnemis expanse*	9	2 (22)	0
Iguana	*Iguana iguana*	5	3 (50)	0
Galap	*Kinosternon scorpionides*	5	0	0
Caiman	*Caiman crocodilus*	2	0	0
Toad	*Pipa pipa*	1	0	0
Slider	*Trachemys scriptaelegans*	1	0	0
Avian				
Birds	White-lined Tanager and others[h]	110	88 (80)	2
Parrot	*Amazona amazonica, Amazona ochrocephala, Psittacus erithacus*	14	8 (57)	0
Macaw	*Ara araraura, A. chloroptera, A. macao*	11	9 (82)	0
Mountain chicken	*Leptodactylus pentadactylus pentadactylus*	2	2 (100)	0
Fish				
Fish	*Astyanax bimaculatus* and others[i]	24	2 (8)	0 (0)
TOTAL		373	250 (67.0)	2 (1)

[a] All strains were non-hemolytic and non-mucoid.
[b] Both were O157 *E. coli* strains.
[c] Consisted of tufted capuchin (n = 6), brown spider monkey (5), white-fronted capuchin (6), red howler (5), mona monkey (2), patas monkey (3), green monkey (5), mandrill (5) and chimpanzee (3).
[d] Consisted of other members of the family Procyonidae which were 5 kinkajous (*Potos falvus*), and 4 coati mundi (*Nasua nasua*).
[e] Consisted of another member of the family Didelphidae which was the greater Trinidad murine opossum (*Maraosa robinson*).
[f] Consisted of an additional otter (*Lutra longicaudis*).
[g] Other families are Boidae, Colubridae, Elapidae and Viperidae. Consisted of cascabel (n = 9), anaconda (4), tegu (4), rainbow boa (3), boa constrictor (2), red spitting cobra (2), ratonel (2), machette (2) and others (10).
[h] Numerous species of birds in 28 families.
[i] Various species in the families Cabridae, Pomacentridae, Muraenidae, Palinuridae and Pomacanthidae.

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wild animals were positive for non-sorbitol fermenting (NSF) *E. coli* is a good indication that wildlife in the environment of Trinidad are not important reservoirs of VTEC strains. Sorbitol MacConkey agar has been reported to have a sensitivity of 100%, specificity of 85%, and an accuracy of 86% (i.e., the proportion of all tests, both negative and positive correctly classified) when used to detect *E. coli* O157:H7 strains (March and Ratnam, 1986). Use of O157 antiserum in conjunction with plating on sorbitol MacConkey agar has been reported to increase the specificity of the plating medium in detecting *E. coli* O157:H7 strains from 45–52% to 100% (Kleanthous et al., 1988). Although it is recognized that non-O157:H7 strains of *E. coli* also elaborate verocytotoxin, most of which are also non-sorbitol fermenting strains (Karmali, 1989; Marques et al., 1995).

All *E. coli* strains were isolated from apparently healthy, wild animals and these were non-hemolytic and did not produce mucoid colonies. The phenotypic characteristics, particularly the production of hemolysins have been considered to be virulence markers (Marques et al., 1995). *Escherichia coli* is documented to cause health problems in wildlife (Janovski, 1966), but this is considered rare. Adesiyun et al. (1998) reported that of the 313 isolates of *E. coli* from the feces of apparently healthy dairy cows, eight (3%) and 19 (6%) were mucoid and hemolytic strains, respectively. Therefore the difference between wild and domestic animals may be a reflection of species susceptibility, rather than environmental conditions.

In conclusion, wildlife in Trinidad and Tobago are not important reservoirs of *E. coli* O157:H7 strains. Therefore, the health risk of hemorrhagic uremic syndrome to consumers of wild meat is minimal.

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