Occurrence of phenotypic virulence markers, enteropathogenic serotypes and verocytotoxin production amongst strains of *Escherichia coli* isolated from non-diarrhoeic dogs in Trinidad

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ABSTRACT

The study was conducted to determine the prevalence and selected characteristics of *Escherichia coli* in non-diarrhoeic dogs from Trinidad. Rectal swabs or faecal samples were collected. Primary isolation of *E. coli* was done on eosin methylene blue (EMB) agar, haemolysin and mucoid production were detected on blood agar (BA) and sorbitol fermentation was assayed on sorbitol MacConkey agar (SMAC) agar. Agglutination tests, using commercially available antisera, determined O157-positive and enteropathogenic (EPEC) strains of *E. coli* while the vero cell assay detected verocytotoxigenic *E. coli* (VTEC) strains. Overall, of a total of 1,391 dogs sampled, *E. coli* was isolated from 1,266 (91.0%) dogs. Differences were statistically significant amongst *E. coli* isolated from dog sources (P < 0.05; χ^2) with the highest (96.0%) and lowest (81.3%) prevalence detected in hunting dogs and pound dogs, respectively. Of the 1,900 *E. coli* isolates tested, 100 (5.3%), 81 (4.3%) and 133 (7.0%) were mucoid, haemolytic and non-sorbitol fermenting (NSF) respectively. The difference was statistically significant (P < 0.05; χ^2). For EPEC strains, of the 333 *E. coli* isolates tested, 189 (56.8%) belonged to 'enteropathogenic' serogroups. The difference in prevalence across the various dog sources was statistically significant (P < 0.05; χ^2). Of the 558 *E. coli* strains tested, 74 (13.3%) were positive for verocytotoxin production. It was concluded that asymptomatic dogs in Trinidad carry virulent strains of *E. coli*, posing a threat as reservoirs of infections to human handlers and their owners and therefore are of significance for public health.

Key words: E. coli, non-diarrhoeic dogs, Trinidad

Introduction

Escherichia coli is considered a normal inhabitant of the gastrointestinal tract of animals and human beings (DUFOUR, 1987; GYLES, 1993). Although most strains of E.

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coli in humans and animals are harmless commensals, the barrier that exists between commensalism and virulence relies on a complex balance between the status of the host and the expression of virulence factors in the bacteria (PICARD et al., 1999).

A number of phenotypic virulence markers including haemolysin production, mucoid colonies and non-sorbitol fermenting ability of strains of *E. coli* have been documented in the literature (CAVALIERI et al., 1984; OJEDA et al., 1995; ROBBINS et al., 1974). These virulence markers have also been associated with pathogenicity (CAVALIERI et al., 1984; OJEDA et al., 1995; ROBBINS et al., 1974). *E. coli* strains have been recovered from pet animals including dogs, cats and birds (BEUTIN et al., 1993; DORRESTEIN et al., 1985; GYLES, 1993).

Generally, there are several categories of *E. coli* which are considered of aetiological importance, these include enteropathogenic *E. coli* (EPEC) and verocytotoxigenic/Shigalike toxin *E. coli* (VTEC/STEC) (BEUTIN et al., 1993).

In pet animals, the degree of human contact with their pets has been demonstrated to be a risk factor for exposure to zoonotic agents (SCARLETT-KRANZ, 1983). Studies have reported the exposure of humans to *Campylobacter* spp., *Salmonella* spp., and *Yersinia* spp. following contact with their diarrhoeic pet animals (CROTT, 1993; MORENO et al., 1993; STONE et al., 1993). There are also reports of apparently healthy pet animals being sources of infection to their owners (SANYAL et al., 1997).

In Trinidad and Tobago, *E. coli* with virulence markers have been isolated from diarrhoeic and non-diarrhoeic livestock (ADESIYUN and KAMINJOLO, 1994), apparently healthy wildlife that are free-ranging (ADESIYUN and DOWNES, 1999) and confined (GOPEE et al., 2000), ready-to-eat foods (ADESIYUN, 1995; RAMPERSAD et al., 1999) and dairy cows and milk (ADESIYUN et al., 1997b; NGELEKA et al., 1998). There is however a dearth of information on the status of *E. coli* and their virulence in pet animals in Trinidad and Tobago.

The study was therefore conducted to determine the prevalence of *E. coli* in dogs animals from various sources across the country as well as to determine the frequency of occurrence of virulence markers in strains isolated.

Materials and methods

Source of samples. Rectal swabs or fresh faeces were the types of samples collected. All samples were taken aseptically. All samples taken originated from apparently healthy dogs, without diarrhoea.

For household samples, Trinidad was divided into four geographical regions, namely North West, North East, Central and South, where because of the unavailability of

information on the dog population, a convenient sampling procedure was employed. The areas visited for sampling are shown in Fig. 1.

One hundred (100) dogs each were sampled from North West, Central and South while fifty (50) dogs were tested from North East. Sampling covered both urban and rural areas. Systematic random sampling was employed where dogs were sampled from every third household on a selected street in the area identified. A maximum of five dogs were sampled per selected household.

The Dog Pound operated by the Ministry of Health and located in Port-of-Spain, the capital of Trinidad and Tobago, represented the source of stray dog population. Weekly visits were made to the pound where all kennels were sampled. All dogs were sampled from each kennel over the period of one year. No dog was sampled twice during the study period since dogs were euthanised within five days of arrival in the pound.

The Trinidad and Tobago Society for the Prevention of Cruelty to Animals (TTSPCA) also located in Port-of-Spain served as the source of samples from an animal shelter. A maximum of five dogs was sampled from all kennels.

Samples from in-patient pet animals were obtained from hospitalized dogs in veterinary hospitals which were identified, using the telephone directory in three regions mentioned earlier as North East had no hospital facilities. These hospitals served as sources of animals housed in a hospital environment.

Veterinary Clinics were identified in the four regions mentioned and served as sources of dogs presented at walk-in clinics.

The Quarantine Station located at Curepe served as the source of dogs imported from foreign countries. For a period of 24 months, dogs were sampled on entry to the station, after three months and at six months prior to release.

Two major dairy farming areas (Waller Field and Carlsen Field) served as sources of dogs living on farms with exposure to dairy animals. A total of twenty-five farms were sampled from each area with a maximum of five dogs sampled per farm.

Hunting dogs were sampled from each of the four regions earlier mentioned. Hunting dogs represented animals exposed to wildlife in their activities. Overall, a total of 100 dogs were studied with 25 originating from each region.

Other samples included puppies from pet shops.

Sample collection and transportation. Samples taken as rectal swabs used sterile cotton-tipped applicators. Swabs were inserted into the rectum of the animal, rotated gently and then placed in tubes containing sterile Amies Transport Medium (ATM) (Difco, Detroit, and U.S.A). Samples taken as fresh faeces were put in sterile plastic faecal cups using sterile tongue depressors. Faecal material, not in contact with any environmental surface, was collected and transferred to the faecal cup.

Questionnaires. Questionnaires were specifically prepared for each source of dogs studied. Information including age, breed, sex, source of acquisition, diet, purpose of keeping animal, length of time the animal was kept, the number of animals kept as well as amount of exposure to owners, were some of the questions asked for household pets.

Isolation and identification of E. coli. Rectal or fresh faeces were streaked for isolation on eosin methylene blue (EMB) (Oxoid, Basingstoke, U.K.) agar plates for primary isolation of E. coli. Morphologically different colonies exhibiting a metallic green sheen were then picked for biochemical identification using standard methods (MACFADDIN, 1980). Isolates identified as E. coli were inoculated onto blood agar plates (BAP) (Oxoid, Basingstoke, U.K.) and incubated at 37 °C overnight to determine ability to produce haemolysins and mucoid colonies. Colonies from BAP were also subcultured onto Sorbitol MacConkey (SMAC) (Oxoid, Basingstoke, U.K.) and incubated at 37 °C overnight. Pale or colourless colonies were classified as non-sorbitol fermenters (NSF) while pink colonies were classified as sorbitol fermenters (SF) as earlier recommended (MARCH and RATNAM, 1986).

Detection of O157 strains of E. coli. E. coli isolates that were non-sorbitol fermenting (NSF) on SMAC were subjected to a slide agglutination test using commercially prepared O157 E. coli antiserum (Difco, Detroit, U.S.A). In addition, all NSF strains were subjected to the tube test to detect the O157 strain. In brief, the isolate was inoculated into 5 mL of sterile brain heart infusion (BHI) broth (Oxoid, Basingstoke, U.K.) and incubated overnight in a shaker bath at 37 °C. The bacterial growth was then centrifuged at high speed (2500 rpm) for 15 minutes after which the supernatant was decanted. The resulting pellet was then suspended in 5 mL of sterile saline. The bacteria suspension was then centrifuged again at high speed for 15 minutes and the supernatant decanted. The pellet was finally suspended in 1 mL of sterile saline and the suspension boiled in a water bath for 2 hours. After boiling, the cooled suspension was then subjected again to the slide agglutination test using the commercially prepared O157 E. coli antiserum (ØRSHOV and ØRSHOV, 1984).

Detection of enteropathogenic strains of E. coli. The slide agglutination test was used to determine E. coli isolates which belonged to enteropathogenic serogroups using E. coli polyvalent antisera A, B and C (S.A. Scientific, Texas, and U.S.A.). A total of 333 isolates of E. coli was tested from the various dog sources. The number of isolates selected was proportional to the total number of E. coli isolates recovered from each source. Random selection was thereafter used to determine the allotted number of isolates to be tested within each source.

Detection of verocytotoxigenic strains of E. coli. A cell culture technique employing vero cells from the kidney of the African Green Monkey was used to determine the verocytotoxigenicity of E. coli strains (KONOWALCHUK et al., 1997). Verocytotoxin

(VT) -positive and negative control strains were used for each assay. Overall, a total of 558 isolates of *E. coli* were tested for verocytotoxin production, having been selected proportional to the total number of isolates from each source. Similarly random selection was used to determine the number of isolates to be tested from each source. Isolates tested were both sorbitol and non-sorbitol fermenting *E. coli*.

Statistical analysis of data. 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

Table 1 shows the number and sources of dogs studied as well as the frequency of isolation of *E. coli*. Of the 1,391 dogs sampled, regardless of source, 1,266 (91.0%) were positive for *E. coli*. The difference in prevalence of *E. coli* amongst the sources was statistically significant (P<0.05; χ^2) with the highest isolation rate detected in hunting dogs (96.0%) and the lowest from dogs sampled at the pound (81.3%).

Source of samples	No of animals tested	N° of (%) of animals positive for <i>E. coli</i>
Households	350	327 (93.4)
Pound	252	205 (81.3)
TTSPCA ^a	119	110 (92.4)
Hospitals ^b	181	164 (90.6)
Clinics ^b	146	134 (91.8)
Pet shops ^c	8	7 (87.5)
Quarantine	125	118 (94.4)
Hunting dogs	100	96 (96.0)
Dairy farms ^d	110	105 (95.5)
Total	1391	1266 (91.0)

Table 1. Prevalence of *E. coli* in pet animals from various sources

"Trinidad and Tobago Society for the Prevention of Cruelty to Animals; bMt. Hope Vet. Hospital; Jones Animal Clinic; North Western Veterinary Clinic; West Park; St. Augustine Clinic; Aziz Veterinary Clinic; O' Meara Clinic; Lalla's Clinic; cAli's Pet Shop - San Juan, El Dorado, POS; Pantin's Pet Shop; Nature's World; Central Chem. and Agri. Supplies; Discount City Pet Shop; Paws and Claws; Pet Shop Boys; St. Augustine Pet Shop; Ideal Pet Supplies; Wild World; Pet Shop; Seepay Pet and Variety Store; Ideal Supplies; Georges Pet Shop; Motee and Son Garden Shop; Sangre; Grande Pet Supplies; Pet Plus; Galley Pet Shop; The Dog House; Fyzabad Pet Shop; Goldys Aquarium and Flowers; Tropical Pets; Rick's Pet Shop; Tenkel's Pet Shop; Acme Building Pet Shop; Acme Pet World; Champion Pet Shop; Cunapo Garden Shop; Tropical Pet Shop; "Dairy farms in Wallerfield (25) and Carlsen Field (25)

N° (%) of isolates with phenotypic Nº of (%) virulence markers Nº of (%) of dogs Source of N^o of E. of positive positive for coli isolates Mucoid NSF^a samples for E. coli E. coli Haemolytic Households 350 327 (93.4) 429 10 (2.3) 17 (4.0) 33 (7.7) Pound 252 205 (81.3) 282 5 (1.8) 16 (5.7) 10(3.5)TTSPCAb 110 (92.4) 119 176 10 (5.7) 7 (4.0) 10 (5.7) Hospitals^c 181 164 (90.6) 239 34 (14.2) 7 (2.9) 16 (6.7) Clinics^d 146 134 (91.8) 175 8 (4.6) 6(3.4)18 (10.3) Pet shops 8 6 7 (87.5) 0(0.0)0(0.0)0(0.0)**Ouarantine** 125 118 (94.4) 311 16 (5.1) 23 (7.4) 21 (6.8) Hunting dogs 96 (96.0) 134 11 (8.2) 3 (2.2) 100 11 (8.2) 105 (95.5) Dairy farmse 110 148 6(4.1)2(3.4)14 (9.5)

Table 2. Frequency of phenotypic virulence markers amongst *E. coli* from dogs

100 (5.3)

81 (4.3)

133 (7.0)

1391

1266 (91.0)

The prevalence of phenotypic virulence markers amongst E. coli strains isolated from dogs from various sources is shown in Table 2. Of the 1900 E. coli isolates tested from 1.391 dogs, 100 (5.3%), 81 (4.3%) and 133 (7.0%) were mucoid, haemolytic and non-sorbitol fermenting (NSF) respectively. The difference was statistically significant (P<0.05; γ^2). Dogs from the quarantine yielded the highest prevalence of haemolytic isolates (7.4%) whilst clinic dogs yielded the highest prevalence of non-sorbitol fermenting strains (10.3%). Of the NSF strains tested from 1,391 dogs, 12 (0.9%) tested positive for belonging to the O157 serogroup.

Table 3 shows the frequency of 'enteropathogenic' and verocytotoxigenic strains of E. coli from dogs from various sources. Of the 333 E. coli isolates tested, 189 (56.8%) belonged to the 'enteropathogenic' serogroups of which 24 (7.2%), 161 (48.4%) and 5 (1.5%) belonged to serogroup A, B and C respectively. The difference was statistically significant (P<0.05; γ^2). Overall, 184 (57.1%) of 322 dogs tested and 189 (56.8%) of 333 E. coli isolates tested were positive for EPEC strains. The difference in prevalence across the various sources was statistically significant (P<0.05; χ^2). EPEC strains were present throughout all the sources sampled, with prevalence that ranged from 34.8% for isolates from hunting dogs to 79.3% for isolates from dairy farm dogs.

Total

¹⁹⁰⁰ aNon-sorbitol fermenter; hTrinidad and Tobago Society for the Prevention of Cruelty to Animals; aIn-patients; ^dOut-patients; ^eInclude Waller field (25) and Carlsen field (25)

Table 3. Frequency of 'enteropathogenic' and verocytotoxigenic isolates of E. coli from dogs

Virulence markers:									
	Enteropathogenic serogroups ^a						VTEC ^b		
Source of	Nº of animals	Nº of E. coli isolates	N° (%) of	Nº (%) of isolates in serogroups ^d			Nº of	E. coli	*
samples	tested	tested	isolates	A	В	C	tested	tested	tested
Households	74	74	27 (36.5)	4 (5.4)	23 (31.1)	0 (0.0)	119	128	16 (12.5)
Pound	48	48	37 (77.1)	5 (10.4)	32 (66.7)	0 (0.0)	69	73	8 (10.9)
TTSPCAe	30	30	22 (73.3)	0 (0.0)	20 (66.7)	2 (6.7)	43	49	14 (28.6)
Hospitals ^f	43	43	26 (60.5)	5 (11.6)	19 (44.2)	2 (4.8)	63	69	14 (20.3)
Clinicsg	30	30	14 (46.7)	2 (6.7)	12 (40.0)	0 (0.0)	52	58	12 (20.7)
Pet shops	2	2	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2	2	0 (0.0)
Quarantine	43	54	31 (57.4)	0 (0.0)	31 (57.4)	0 (0.0)	66	88	4 (4.6)
Hunting dogs	23	23	8 (34.8)	2 (8.7)	6 (26.1)	1 (4.4)	36	41	6 (14.6)
Dairy farms ^h	29	29	23 (79.3)	5 (12.4)	18 (62.1)	0 (0.0)	43	49	0 (0.0)
Total	322	333	189 (56.8)	24 (7.2)	161 (48.4)	5 (1.5)	494	558	74 (13.3)

^aIsolates of *E. coli* which belonged to enteropathogenic serogroups tested; ^bVerocytotoxigenic *E. coli*; ^cAgglutinated by *E. coli* polyvalent antisera A, B or C; ^aPolyvalent antisera with *E. coli* serogroups A - O55: K59 (B5); O111:K58 (B4); O127:K63 (B8) & O26:K60 (B26); B - O86:K61 (B7); O119:K69 (B14); O124:K72 (B17); O125:K70 (B15); O126:K71 (B16) & O128:K67 (B12) and C - O18:K77; O20:K61; O20:K84 (B); O28: K73 and O44:K74 (L); ^cTrinidad and Tobago Society for the Prevention of Cruelty to Animals; ⁱIn-patients; ^aMt. Hope Vet. Hospital; Jones Animal Clinic; North Western Veterinary Clinic; West Park; St. Augustine Clinic; ^bInclude Wallerfield (25) and Carlsen field (25)

Of the 558 *E. coli* strains tested from various sources, 74 (13.3%) tested positive for verocytotoxin production as detected by the vero cell assay. Overall, 72 (14.6%) of the 494 dogs sampled harboured VTEC strains. Verocytoxigenic *E. coli* were isolated from all sources with the exception of dairy farms and pet shop dogs. Prevalence ranged from 4.6% in isolates from quarantine dogs to 28.6% in isolates from TTSPCA dogs. Clinic dogs yielded a prevalence of 20.7% for VTEC strains followed closely by dogs kept in hospitals (20.3%).

Discussion

The fact that *E. coli* was isolated from dogs from all the various sources sampled was not unexpected, since the microorganism is considered a part of the normal flora of animals and humans (GYLES, 1993). BATT et al. (1996) have reported a wide variation in

the bacterial flora amongst normal individuals, with concentrations of bacteria affected by a variety of circumstances inclusive of environment, diet, scavenging and coprophagy. The comparatively lower prevalence of 81.3% for *E. coli* observed in the present study for pound dogs may therefore be explained, in part, by their indiscriminate eating habits inclusive of scavenging and coprophagy, which may have contributed to an alteration of the microflora in their intestinal tracts.

The overall prevalence (91.0%) of *E. coli* amongst dogs from all the sources is however comparable to an earlier study (DUFOUR, 1987) who also reported a prevalence of 91.0% for *E. coli* in dogs. In Trinidad, the only published report on the prevalence of *E. coli* in dogs (ADESIYUN and DOWNES, 1999), detected the organism in 49 (75.4%) of 65 non-diarrhoeic dogs presented at a walk-in clinic, which is considerably lower than the prevalence of 91.8% found in veterinary clinics in the present study. This disparity in prevalence may be explained in part, by the difference in number of clinics studied, with eight clinics sampled in the present study compared with one clinic in the previous study. Additionally, the sample size in the previous study was 65 non-diarrhoeic dogs compared with 146 apparently healthy dogs sampled in the present study.

The prevalence (91.0%) of *E. coli* detected in dogs from all sources in the present study is considerably higher than reported for other animal types in the same environment in Trinidad. Prevalence varied from 58% in free-ranging wildlife (ADESIYUN, 1999) to 62.5% in dairy cows (ADESIYUN et al., 1997b) and 67% in zoo animals (ADESIYUN, 1999) to 83% in captive wildlife (ADESIYUN, 1999). The close association experienced by most dogs with humans and their environment makes the potential for transmission high between the two species.

The prevalence (5.3%) of mucoid strains of *E. coli* detected in dogs in the present study is comparable to the 3% of mucoid strains recovered from the recta of healthy dogs in a study conducted by WADÅS et al. (1996). The authors had opined that faecal *E. coli* isolates from healthy dogs were seldom of the mucoid types. The significantly higher prevalence (14.2%) of mucoid strains in isolates recovered from hospitalized dogs found in the study may be a result of production of extracellular polysaccharides by these strains because of the environment as earlier suggested by GYLES (1993). It has been reported that *E. coli* may have developed a survival strategy by producing mucous to prevent it from being phagocytosed (NGELEKA et al., 1998).

The fact that as high as 7.4% of quarantine dogs harboured haemolytic strains of *E. coli* is an indication that transmission of these strains may result in health problems for pet owners or personnel working at the quarantine station, since haemolysin production has been associated with pathogenicity of *E. coli* strains (CAVALIERI et al., 1984).

Clinic dogs had the highest recovery rate (10.3%) of NSF strains of *E. coli* which is higher than the prevalence of NSF (6.1%) observed in non-diarrhoeic clinic dogs in

the study conducted by ADESIYUN et al. (1997a). Walk-in clinics are outpatient facilities that provide veterinary health services to animals from the community. The finding of NSF strains in outpatient dogs indicates of what exists in the community as the infection or carriage more than likely may have been community-acquired than contracted at the clinic. The NSF phenotype has been typically associated with the O157:H7 serotype of *E. coli* which has been implicated in cases of haemolytic uremic syndrome (HUS) and haemorrhagic colitis in humans (GRIFFIN and TAUXE, 1991). Cattle are considered the most important reservoir of O157:H7 strain (TANAKA et al., 1992). In Trinidad, NGELEKA et al. (1998) detected the O157 *E. coli* strain in water, bulk and composite milk on dairy farms while in the present study however, no O157 strains were detected in dogs living on dairy farms.

The relatively high prevalence (56.8%) of EPEC strains in all dogs sampled across sources in Trinidad has potential public health significance because these strains have been known to cause diarrhoea in humans (NATARO and KAPER, 1998). Most of the *E. coli* isolates that belonged to 'enteropathogenic' serogroups were in serogroup B (48.5%) which includes serotypes O86, O119, O124, O125, O126, and O128. These serogroups have been found to be virulent in disease associated with infantile diarrhoea (NATARO and KAPER, 1998). The finding therefore indicates a significant zoonotic risk to dog owners. Equally of zoonotic importance is the fact that 36.5% of household dogs were carriers of EPEC strains when consideration is given to the close contact that exists between pet dogs and their owners in households. Asymptomatic carriage of EPEC has been reported in dogs (GOFFAUX et al., 2000).

Verocytotoxigenic E. coli is a recognized human pathogen (BETTELHEIM, 1996). Several studies elsewhere have documented the pathogen in sporadic, general and family outbreaks (ANONYM., 1993). VTEC strains have been isolated from animal faeces, meat, dairy products and water in other countries (WELLS et al., 1991). It was significant that VTEC strains were isolated from dogs sampled from all sources with the unexpected exception of dogs from dairy farms and pet shops. This is surprising as dairy cows are considered as important reservoir of VTEC strains (BORCZYK et al., 1987) and may enjoy close contact with dogs on farms, and the fact that VTEC strains have been isolated from dairy farms and cow's milk in Trinidad (ADESIYUN et al., 1997b). The finding that dogs from sources like households, TTSPCA, clinics and hospital dogs, all with potential human contact had prevalence for VTEC ranging from 12.5% to 28.6% poses a zoonotic threat to their owners and members of the household. Household dogs that are carriers of VTEC strains (12.5%) appear to pose the greatest risk to their owners. The O157:H7 strain of E. coli has been documented to be typically verocytotoxigenic (GRIFFIN and TAUXE, 1991). Similarly, the finding that 12 (0.9%) of the 1391 isolates of E. coli were O157 strains from dogs from various sources is of zoonotic relevance, as this strain has been reported to cause life threatening diseases in humans (GRIFFIN and TAUXE, 1991). In Trinidad, O157 strains have previously been isolated from dairy farms (NGELEKA et al., 1998), zoo animals (GOPEE et al., 2000), household drinking water (OJEDA et al., 1995) and oysters (RAMPERSAD et al., 1999).

In conclusion, asymptomatic pet dogs in Trinidad carry virulent strains of *E. coli* which may serve as reservoirs of infection to human handlers and owners and therefore of public health significance.

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References

- ADESIYUN, A. A. (1999): Absence of *Escherichia coli* O157 in a survey of wildlife from Trinidad and Tobago. J. Wild. Dis. 35, 115-120.
- ADESIYUN, A. A. (1995): Bacteriological quality of some Trinidadian ready-to-consume foods and drinks and possible health risks to consumers. J. Food. Prot. 58, 253-261.
- ADESIYUN, A. A., M. CAMPBELL, J. S. KAMINJOLO (1997a): Prevalence of bacterial enteropathogens in pet dogs in Trinidad. J. Vet. Med. B 44, 19-27.
- ADESIYUN, A. A., M. DOWNES (1999): Antibiograms of *E. coli* isolates from wildlife in Trinidad and Tobago. Vet. Arhiv. 69, 335-347.
- ADESIYUN, A. A., J. S. KAMINJOLO (1994): Prevalence and epidemiology of selected enteric infections of livestock in Trinidad. Prev. Vet. Med. 19, 151-165.
- ADESIYUN, A. A., L. A. WEBB, H. ROMAIN, J. S. KAMINJOLO (1997b): Prevalence and characteristics of strains of *Escherichia coli* isolated from milk and faeces of cows on dairy farms in Trinidad. J. Food Prot. 60, 1174-1181.
- ANONYMOUS (1993): Centre for Disease Control (CDC) -Multi-state outbreak of *E. coli* O157: H7 infections from hamburgers, Western United States. 1992-1993. MMWR 42, 258-263.
- BATT, R.M., H. C. RUTGERS, A. A. SANCAK (1996): Enteric bacteria: Friend or foe? J. Small Anim. Pract. 37, 261-267.
- BETTELHEIM, K. A. (1996): Enterohaemorrhagic *Escherichia coli*: A new problem, an old group of organisms. Aust. Vet. J. 73, 20-26.
- BEUTIN, L., D. GEIER, H. STEINRÜCK, S. ZIMMERMAN, F. SCHEUTZ(1993): Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *E. coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31, 2483-2488.
- BEUTIN, L. (1999): Escherichia coli as a pathogen in dogs and cats. Vet. Res. 30, 285-298.

- BORCZYK, A. A., M. A. KARMALI, H. LIOR, L. M. DUNCAN (1987): Bovine reservoir for verotoxin producing O157:H7. Lancet I, 98.
- CAVALIERI, S. J., G. A. BOHACH, I. S. SNYDER (1984): *Escherichia coli* α-haemolysin: characteristics and probable role in pathogenicity. Microbiol. Rev. 48, 326-343.
- CROTT, D. (1993): Characterization of *Yersinia* species isolated from a kennel and from cattle and pig farms. Vet. Rec. 132, 532-534.
- DORRESTEIN, G. M., M. N. BUITELAAR, M. H. VAN DE HAGE, P. ZWART (1985): Evaluation of a bacteriological and mycological examination of psittacine birds. Avian Dis. 29, 951-962.
- DUFOUR, P. (1987): *Escherichia coli*. The faecal coliform. Special Technical Publication. American Society for Testing and Materials 65, 48-58.
- GOFFAUX, F., B. CHINA, L. JANNSEN, J. MAINIL (2000): Genotypic characterization of enteropathogenic *Escherichia coli* isolated in Belgium from dogs and cats. Res. Microbiol. 151, 865-871.
- GOPEE, N. V., A. A. ADESIYUN, K. CAESAR (2000): A longitudinal study of *Escherichia coli* strains isolated from captive mammals, birds and reptiles in Trinidad. Zoo Wildl. Med. 31, 353-360.
- GRIFFIN, P. M., R. V. TAUXE (1991): The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13, 60-98.
- GYLES, C. L. (1993): *Escherichia coli*. In: Pathogenesis of Bacterial Infections in Animals Gyles CL, Thoen CO. editors. 2nd ed. Iowa State University Press. p. 164-187.
- KONOWALCHUK, J., J. I. SPEIRS, S. STAVRIC (1977): Veroresponse to a cytotoxin of *Escherichia coli*. Infect. Immun. 18, 775-779.
- MACFADDIN, J. F. (1980): Biochemical Tests for Identification of Bacteria. Williams and Wilkins, New York.
- MARCH, S. B., S. RATNAM (1986): Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with haemorrhagic colitis. J. Clin. Microbiol. 23, 869-872.
- MORENO, G. S., P. S. GRIFFITHS, I. F. CONNERTON, R. W. A. PARK (1993): Occurrence of campylobacters in small domestic and laboratory animals. J. Appl. Bacteriol. 75, 49-54.
- NATARO, J. P., J. B. KAPER (1998): Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11, 142 -201.
- NGELEKA, M., A. A. ADESIYUN, H. ROMAIN (1998): Occurrence of selected phenotypic virulence markers and antibiotic resistance of *E. coli* strains isolated from milk, faeces of dairy cows and water in dairy farms in Trinidad. Proc. 4th Wld. Cong. Foodborne Infections and Intoxications, July 7-12, p. 362-367.
- OJEDA, A, V. PRADO, J. MARTINEZ, C. ARELLANO, A. BORCZYK, W. JOHNSON, H. LIOR, M. M. LEVINE (1995): Sorbitol-negative phenotype among enterohaemorrhagic *Escherichia coli* strains of different serotypes and from different sources. J. Clin. Microbiol. 33, 2199-2201.

- ØRSHOV, I., F. ØRSHOV (1984): Serotyping of *Escherichia coli*. In: Methods in Microbiology. (Bergan, T., Ed.), Vol.14. London, Academic Press. p. 43-112.
- PICARD, B., J. SEVALI GARCIA, S. GOURIOU, P. DUIREZ, N. BRAHIMI, E. BINGEN, J. ELION, E. DENAMUR (1999): The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. 67, 546-553.
- RAMPERSAD, F. S., S. LALOO, A. LA BORDE, K. MAHARAJ, L. SOOKHAI, J. TEELUCKSINGH, S. REID, L. MCDOUGALL, A. A. ADESIYUN (1999): Microbial quality of oysters sold in western Trinidad and potential health risks to consumers. Epidemiol. Infect. 123, 241-250.
- ROBBINS, J. B., G. H. McCRACKEN JR., E. C. GOTSCHLICH, F. ØRSHOV, I. ØRSHOV, L. A. HANSEN (1974): A. Escherichia coli K-1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. 290, 1216-1221.
- SANYAL, D., T. DOUGLAS, R. ROBERTS (1997): *Salmonella* infection acquired from reptilian pets. Arch. Dis. Child. 77, 345-346.
- SCARLETT-KRANZ, J. M. (1983): Defining and measuring exposure in epidemiologic studies of potential zoonoses. J. Am. Vet. Med. Assoc.183, 1454-1458.
- STONE, G. G., M. M. CHENGAPPA, R. D. OBERST, N. H. GABBERT, S. MCVEY, K. J. HENNESSY, M. MUENZENBERGER, J. STAATS (1993): Application of polymerase chain reaction for the correlation of *Salmonella* serovars recovered from greyhound faeces with their diet. J. Vet. Diagn. Invest. 5, 378-385.
- TANAKA, H., R. KONDO, C. NISHIUCHI, H. MATSUDA, M. KIMURA, K. MEMIDA, M. KIKUCHI, T. TSUKAMOTO, S. YAMASAKI, Z. LIN (1992): Isolation of verocytotoxin-producing *E. coli* from cattle and pets. J. Jpn. Assoc. Infect. Dis. 66, 448-455.
- WADÅS, B., I. KÜHN, A. S. LAGERSTEDT, P. JONSSON (1996): Biochemical phenotypes of Escherichia coli in dogs: Comparison of isolates isolated from bitches suffering from pyometra and urinary tract infection with isolates from faeces of healthy dogs. Vet. Microbiol. 52, 293-300.
- WELCH, P., J. DAVID, W. CLARKE, A. TRINIDADE, D. PENNER, S. BERNSTEIN, L. MCDOUGALL, A. A. ADESIYUN (2000): Microbial quality of water in rural communities of Trinidad. Pan Am. J. Public Health 8, 172-179.
- WELLS, J. G., L. D. SHIPMAN, K. D. GREENE, E. G. SOWERS, J. H. GREEN, D. N. CAMERON, F. P. DOWNES, M. L. MARTIN, P. M. GRIFFIN, S. M. OSTROFF (1991): Isolation of *E. coli* serotype O157:H7 and other shiga-like-toxin producing *E. coli* from dairy cattle. J. Clin. Microbiol. 29, 985-989.

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SAŽETAK

Istraživanje je poduzeto radi određivanja prevalencije i određenih značajki bakterije *Escherichia coli* u pasa bez proljeva na Trinidadu. Pretraženi su bili uzorci obrisaka rektuma i izmeta. *E. coli* bila je izdvojena na eozinmetilensko plavilo agaru. Tvorba hemolizina i sluzi dokazivana je na krvnomu agaru, a fermentacija sorbitola na MacConkey sorbitolskom agaru. Serološka skupina O157 i enteropatogenost dokazani su aglutinacijskim testom pomoću komercijalnih antiseruma, dok su verocitotoksigeni sojevi dokazani testom na staničnoj kulturi VERO. *E. coli* bila je izdvojena iz 1266 (91,0%) od pretraženih 1391 psa. Statistički značajne razlike ustanovljene su u izdvajanju *E. coli* (P<0,05; χ2) s najvećom prevalencijom (96,0%) u lovačkih pasa i najmanjom (81,3%) u tornjaka. Od analiziranih 1900 izolata *E. coli*, 100 (5,3%) je imalo sluzave kolonije, 81 (4,3%) je bio hemolitičan, a 133 (7,0%) izolata nisu fermentirala sorbitol. Razlika je bila statistički značajna (P<0,05; χ2). Od 333 pretražena izolata, 189 (56,8%) ih je pripadalo enteropatogenim (EPEC) serološkim skupinama. Razlika je bila statistički značajna s obzirom na različito podrijetlo pretraženih pasa (P<0,05; χ2). Od pretraženih 558 izolata *E. coli*, 74 (13,3%) tvorila su verocitotoksin. Može se zaključiti da psi na Trinidadu u kojih nije ustanovljen proljev mogu biti nositelji patogenih sojeve *E. coli* što predstavlja prijetnju za njihove vlasnike, a taj je nalaz od javnozdravstvenog značenja.

Ključne riječi: E. coli, pas, Trinidad