

Annual Research & Review in Biology 7(2): 91-99, 2015, Article no.ARRB.2015.111 ISSN: 2347-565X



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Absence of *Escherichia coli* O157:H7 Serotype in Small Indian Mongooses (*Herpestes auropunctatus*) in Grenada and Antimicrobial Drug Resistance of the non-O157 Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VAA, UZ and HH designed the study. Author UZ managed the collection of all the samples. Authors VAA, OAO and VMB managed the analyses and literature searches. Authors VAA and HH wrote the protocol. Author VAA wrote the first draft of the manuscript. Authors VAA, UZ, RS and HH wrote the final draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2015/18143 <u>Editor(s):</u> (1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA. <u>Reviewers:</u> (1) Anonymous, Universidad Autónoma Metropolitana Unidad Xochimilco, México. (2) Salama Ahmed Osman, Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. Complete Peer review History: <u>http://www.sciencedomain.org/review-history.php?iid=974&id=32&aid=9139</u>

Original Research Article

Received 7th April 2015 Accepted 22nd April 2015 Published 7th May 2015

ABSTRACT

Aim: To determine the occurrence of the *E. coli* including the O157:H7 serotype in mongooses and their antimicrobial drug resistance in Grenada.

Study Design: Experimental based study of feces of mongooses captured from six parishes of Grenada from April 2011 to March 2013 during an active rabies surveillance program.

Methodology: Fecal samples from 156 mongooses were cultured for *E. coli* and tested for O157:H7 serotype by the presence of non-sorbitol fermenting colonies and a positive reaction to

O157-agglutination latex kits.

Results: Of the 156 mongooses, 71 (46%) were culture positive for *E. coli*. A total of 213 *E. coli* isolates were recovered and examined for the presence of non-sorbitol fermenting colonies and O157-agglutination. Of the 213 *E. coli* isolates, only 8 (4%) were non-sorbitol fermenters. However, none of the 213 isolates gave a positive reaction (O157-agglutinating) to the two *E. coli* O157:H7 latex kits. Antimicrobial susceptibility tests against 12 drugs revealed a low resistance rates to ampicillin (8%), amoxicillin-clavulanic acid (0.5%), ciprofloxacin (1.4%), enrofloxacin (2.3%), gentamicin (0.5%), nalidixic acid (3.3%), and trimethoprim-sulfamethoxazole (5.6%). High resistance rates to streptomycin (38%) and tetracycline (36%) was observed among the *E. coli* isolates. The susceptibility rate to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ciprofloxacin, enrofloxacin, gentamicin, imipenem, nalidixic acid and trimethoprim-sulfamethoxazole ranged from 86.9 to 100%. Resistance to two or more antibiotics was observed in 57 (27%) of the *E. coli* isolates recovered.

Conclusion: This study showed that presently, mongooses in Grenada are neither a reservoir for the *E. coli* O157:H7 serotype nor for multiple drug resistant *E. coli* strains. Among the 213 non-O157:H7 *E. coli* isolates, the resistance rate to drugs other than streptomycin and tetracycline was very low.

Keywords: Mongooses; O157:H7; Escherichia coli; drug resistance; Grenada.

1. INTRODUCTION

Small (Herpestes Indian mongooses auropunctatus) were introduced from Asia into the West Indies including Grenada in the late 1800s to control rats in sugar cane plantations [1,2]. Mongoose populations in the West Indies expanded rapidly due to their high reproductive capacity, year-around availability of food and the lack of natural predators [3]. In Grenada, mongooses are now among the most abundant mammals on the island with an estimated population density of 6.6 mongooses/ha [3]. They frequently den in proximity to human settlements and livestock farms [3] and tend to prey on poultry. Mongoose also feed on a variety of prev including small mammals, birds, reptiles, amphibians, insects and other invertebrates [3,4], many of which are known reservoirs for bacteria pathogenic to humans.

Worldwide, mongooses are known as reservoir hosts for a wide variety of human pathogens including those causing rabies [1,5-9], hepatitis E [10] and tuberculosis [11]. In Barbados, Salmonella and Campylobacter species were isolated from the small Asian mongoose [12]. In Grenada, mongooses have been shown to harbor multiple human pathogens including those causing rabies [2,6,7], salmonellosis [13] and campylobacteriosis [14]. Fresh fruits, nuts, and vegetables grown in open fields and orchards in Grenada are vulnerable to pre-harvest microbial contamination; a wild animal such as mongoose can contaminate plants directly through fecal deposition or indirectly via fecal contamination of water or soil.

E. coli serotype O157:H7 is the most important food-borne, zoonotic pathogen in humans [15,16]. It has also emerged as the most important enterohaemorrhagic (EHEC) serotype associated with human disease [16]. Its importance as a public health problem was recognized in 1982, following an outbreak in the United States of America [16]. Besides gastrointestinal disease, E. coli 0157:H7 can cause hemolytic uremic syndrome (HUS) in humans [17,18]. WHO [16] estimated that more than nine percent of patients with EHEC infection may develop HUS, with a case-fatality rate of three to five percent. E. coli O157:H7 is transmitted to humans mostly through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk and contaminated raw vegetables and sprouts [16,19,20] and less frequently through contact with manure, animals, or infected people [21]. Unlike most E. coli strains, serotype O157:H7 does not ferment sorbitol, thus, testing for sorbitol fermentation has been proposed as a means to screen for this serotype [22-24].

To date, antibiotic resistance is recognized as a global problem in human and veterinary medicine [25] and the spreading of resistance genes among bacterial strains still remain an increasing problem in infectious diseases [26]. The intestines of animals serve as bacterial reservoirs and provide an ideal environment for the selection and transfer of antimicrobial resistance genes. Studies have shown that *E. coli* can serve as reservoirs of antibiotic resistance genes [27] which have been effectively transferred not only

to other *E. coli* strains but also to other enteric pathogens of humans and animals [28]. As *E. coli* is a common inhabitant of the intestines of variety of animals, these animals can easily shed the antibiotic resistant *E. coli* into the environment. Previous studies carried out in Grenada have shown that both wild and domesticated animal species may serve as reservoirs for antibiotic resistant bacteria including chicken [29-32], dogs [33], cats [34], cane toads [35] and green iguanas [36].

The importance of mongooses for the epidemiology of *E. coli* O157:H7 is unknown in Grenada. In addition, there have been no published surveys on the prevalence and antimicrobial susceptibility of *E. coli* recovered from mongooses in Grenada. The present study was conducted to determine: the occurrence of the *E. coli* including the O157:H7 serotype in mongooses and their antimicrobial drug resistance in Grenada.

2. MATERIALS AND METHODS

Mongooses were sampled from April 2011 to March 2013 during an active rabies surveillance program under the approval of St. George's University Institutional Animal Care and Use Committee (IACUC 10003-R). A total of 156 mongooses were collected using Tomahawk catlive traps (63 cm L × 18 cm W × 18 cm H, Tomahawk, Hazel-hurst, WI, USA) from all six parishes of Grenada: 59 from St. George's, 33 from St. Andrew's, 23 from St. Patrick's, 16 from St. Mark's. 14 from St. David's. and 11 from St. John's. The methods used for anesthesia and euthanasia of the animals were previously described by Zieger et al. [6]. The animals were anesthetized within two hours of collection via intra-muscular injection with ketamine (10 mg/kg) (Ketamine hydrochloride, Rotexmedica[®], Trittau, Germany) and xylazine (0.2 mg/kg) (AnaSed®, Decatur, IL) and then euthanized via cardiac injection of potassium chloride (1-2 mmol/kg) (FisherScientific, Fair Lawn, NJ). A section of large intestine including luminal contents was removed between the ileo-cecal junction and the rectum and stored in a stomacher bag at -80°C till tested. All samples were processed within 18 months of collection.

For the isolation of *E. coli*, each intestinal section (approximately 6–7 g including contents) was thawed and 10 mL tryptic soy broth (TSB) (BBL, Becton, Dickinson and Company, Sparks, MD, USA) were added to the stomacher bag. The contents were blended using a stomacher machine (Seward Limited, Worthing, West Sussex, UK). The entire suspension was transferred to a capped culture test tube (polystyrene with polyethylene cap, Fisher Scientific, Fair Lawn, NJ, USA) and incubated at 37°C for 24 hours. After incubation, an aliquot was streaked onto MacConkey (MAC) agar (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 hours. To increase the chances of identifying E. coli O157:H7 in a sample, three (3) pink to red color colonies with or without a zone of precipitated bile morphologically representing E. coli were subcultured via streaking onto individual MAC agar and incubated at 37°C for 24 hours for isolation of pure colonies. Colonies from the second MAC agar plate were Gram stained and further tested using the API20E (Analytical Profile Index; BioM6rieux, Hazelwood, MO) bacterial identification strips for confirmation as E. coli. Non-lactose fermenting isolates identified as E. coli by API20E were also added in the study despite the fact that they were nonlactose fermenting variants.

For detection of E. coli serotype O157:H7, the pure colonies were first plated on sorbitol-MacConkey agar (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 hours. After incubation, the sorbitol-MacConkey agar plates were examined for the presence of nonsorbitol fermenting colonies. All the colonies (both the sorbitol and the nonsorbitol fermenting colonies) were then subjected to slide agglutination using two (2) E. coli O157:H7 latex kits: Remel Wellcolex* E. coli O157 Rapid Latex Test (Remel Europe Ltd, Kent, UK) and Prolex[™] E. coli O157 Latex Kit (Pro-lab Diagnostics, Toronto, Canada). Two latex kits were used to reduce the possibility of obtaining a false positive result. An isolate giving a positive reaction to both latex test kits was considered to be E. coli O157:H7 positive.

The antimicrobial susceptibility tests were carried out using the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) using Mueller Hinton agar (BBL), and the inhibition zone sizes were interpreted as per CLSI guidelines [37]. The antibiotics discs used were ampicillin, amoxicillinclavulanic acid. cefotaxime. ceftazidime. ciprofloxacin. enrofloxacin. gentamicin. imipenem, nalidixic acid. streptomycin. tetracycline and trimethoprim-sulfamethoxazole (Becton, Dickinson and Co., Sparks, MD, USA).

3. RESULTS

Of the 156 mongooses examined in this study, 71 (46%) were culture positive for *E. coli*. Of the 213 *E. coli* isolates, only 8 (4%) were nonsorbitol fermenters. However, none of the 213 isolates gave a positive reaction (O157agglutinating) to the two *E. coli* O157:H7 latex kits.

The results of the antimicrobial susceptibility test of the 213 non-O157 E. coli isolates are shown in Table 1. The results revealed a low resistance rate (0.5 to 8%) for all the 213 E. coli isolates to seven of the tested antibiotics except to streptomycin (38%) and tetracycline (36%). However, a number of the non-O157 E, coli isolates revealed a moderate to high intermediate resistance rate (1.4 to13.6%) to some of the tested antibiotics. The E. coli isolates recovered in this present study revealed a high susceptibility rate (86.9 to 100%) to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime. ciprofloxacin, enrofloxacin. gentamicin, imipenem, nalidixic acid and trimethoprim-sulfamethoxazole. Twenty seven percent (57 out of 213) of the E. coli isolates in this present study showed resistance to two or more antibiotics.

4. DISCUSSION

The most important reservoir of *E. coli* O157:H7 serotype is considered to be ruminants, particularly cattle [19,20,38-40]. Nevertheless, this serotype has been isolated from a variety of

other wild and domestic animals including sheep, goats, pigs, cats, dogs, deer, wild rabbits, birds, and rats [41-49]. Studies have shown that wildlife does not serve as a major reservoir of *E. coli* O157:H7. However, sporadic isolation of the bacteria from wildlife such as deer and synanthropic rodents and birds perhaps reflects environment-mediated transmission from humans and other animal reservoirs [21].

The results of our study showed that mongooses in Grenada harbor *E. coli* in their gastrointestinal tract, but do not harbor *E. coli* O157:H7 serotype. No information was found concerning the occurrence of *E. coli* O157:H7 in mongooses in other countries. Nevertheless, the observation in our study is not surprising as generally, wildlife with the exception of deer, does not constitute a significant source of *E. coli* O157:H7 serotype [21,47,49]. Rice et al. [47] reported a low prevalence rate of *E. coli* O157 in white-tailed deer (<1%, 5/630), pooled bird of unknown species (<1%, 1/296), and pooled flies (3.3%, 2/60).

The absence of *E. coli* O157:H7 serotype observed in this current study concurred with the results of other researchers who did not find *E. coli* O157:H7 in wildlife: the study of Rice et al. [47] revealed the absence of *E. coli* O157:H7 in the following wild animals: elk (n = 244), bison (n = 57), bighorn sheep (n = 32), antelope (n = 1), Canada goose (n = 121), trumpeter swan (n = 67), gull (n = 150), duck (n = 20), starling (n = 124), wild turkey (n = 83), coyote (n = 7), rodent feces (n = 300), and fish (n = 4).

 Table 1. Antimicrobial susceptibility profiles of the 213 non-O157 *E. coli* isolates recovered from small Indian mongooses in Grenada

Antimicrobial (Disc conc. ^a (µg))	Resistant	Intermediate	Susceptible
		# (%)**	-
Ampicillin (10)	17 (8)	11 (5.2)	185 (86.9)
Amoxicillin-clavulanic Acid (20, 10)	1 (0.5)	3 (1.4)	209 (98.1)
Cefotaxime (30)	0	0	213 (100)
Ceftazidime (30)	0	0	213 (100)
Ciprofloxacin (5)	3 (1.4)	0	210 (98.6)
Enrofloxacin (5)	5 (2.3)	5 (2.3)	203 (95.3)
Gentamicin (10)	1 (0.5)	0	212 (99.5)
Imipenem (10)	0	0	213 (100)
Nalidixic acid (30)	7 (3.3)	3 (1.4)	203 (95.3)
Streptomycin (10)	81 (38)	29 (13.6)	103 (48.4)
Tetracycline (30)	77 (36)	9 (4.2)	127 (59.6)
Trimethoprim-sulfamethoxazole (1.25, 23.75)	12 (5.6)	0	201 (94.4)

**#: number, % (percentage): values are rounded up and down to one decimal place; ^aResistant, intermediate or susceptible according to CLSI guideline for all drugs

In Trinidad and Tobago, no *E. coli* O157:H7 was found in any of the fecal samples: free-ranging wildlife (n = 271), captive wildlife (n = 175), wild bird (n = 293), and zoo animals (n = 373) examined in the survey of wildlife with only a single mongoose (*H. auropunctatus*) seen among the 373 zoo animals tested [38]. In 2014, a study conducted in Grenada on both domestic and wild green iguanas (*Iguana iguana*) revealed the absence of *E. coli* O157:H7 in fecal samples from the tested iguanas [36].

Studies have shown that pathogenic non-O157 E. coli serotypes such as the O26, O103, O145, 0172, 0174, 0113 and 0111, which are nonsorbitol fermenters that give a negative reaction to E. coli O157:H7 latex kits have been associated with infections in humans [50,51]. In this current study, 8 (3.8%) out of the 213 E. coli isolates were non-sorbitol fermenters that gave a negative reaction to the two E. coli O157:H7 latex kits. This study targeted only the E. coli O157:H7 serotypes which are typically nonsorbitol fermenters that give a positive reaction to the E. coli O157:H7 latex kits. In comparison with other studies, the survey carried out on wildlife in Trinidad and Tobago, showed the presence of non-sorbitol fermenting E. coli that were not agglutinated by O157 antiserum from: freeranging mammals (<1%), captive wild animals (2%) and animals in a zoo (0.5%) [38]. Sylvester et al. [36] reported a 29% (12 out of 42) prevalence rate of non-O157 E. coli serotypes in green iguanas in Grenada that were non-sorbitol fermenters that gave a negative reaction to the E. coli O157:H7 latex kits. This indicates that both wild and domestic animals in Grenada and other parts of the world may be potential reservoirs for the non-O157 group. Further studies that targets the non-O157 group is required to determine whether the 8 (3.8%) non-O157 E. coli isolates recovered in this study are among the pathogenic non-O157 group. This information will enable us to determine whether mongooses in Grenada are reservoirs for the pathogenic non-O157:H7 E. coli groups and may possibly be a source of environmental contamination.

The antibiotics susceptibility assays of this present study revealed low resistance rate ranging from 0.5% to 8% of all the 213 *E. coli* isolates to seven out of the 12 antibiotics tested (Table 1). This is somewhat similar to the findings of Sylvester et al. [36] who tested non-O157:H7 *E. coli* isolates from green iguanas to similar types of antibiotics. Their results revealed

low resistance rates to ampicillin (7%), nalidixic acid (2%), and trimethoprim-sulfamethoxazole (2%) which are similar to the resistance rates to ampicillin (8%), nalidixic acid (3.3%), and trimethoprim-sulfamethoxazole (5.6%) observed in this current study. However, resistance rate of 12% to amoxicillin-clavulanic acid observed by these authors [36] was higher than the 0.5% resistance rate to amoxicillin-clavulanic acid observed in the current study. The authors [36] also observed a resistance rate of 2% to cefotaxime which is contrary to the zero resistance to cefotaxime observed in our study. A study on young healthy pigs in Grenada by Amadi et al. [52], revealed a low resistance rates to ampicillin (3%), amoxicillin-clavulanic acid (1%), trimethoprim-sulfamethoxazole (3%), and a zero resistance rate to cefotaxime, which closely corresponds to the findings of this current study.

The low resistance rates to ciprofloxacin (1.4%). enrofloxacin (2.3%), and gentamicin (0.5%) observed in this current study is contrary to the zero resistance rates to the same antibiotics reported by Sylvester et al. [36] and Amadi et al. [52]. In this current study, we observed a zero resistance rate to ceftazidime and imipenem which concurred to findings of these authors [36,52]. The resistance to streptomycin (38%) and tetracycline (36%) were the highest resistance rates observed in this present study. This is in contrast to the resistance rates to streptomycin (12%) and tetracycline (2%) reported by Sylvester et al. [36]. On the other hand, the high resistance rate to tetracycline (36%) observed in this present study somewhat concurred to the higher resistance rates of 100% and 96% to tetracycline observed in pigs in Grenada by Sabarinath et al. [53] and Amadi et al. [52], respectively. It is possible that the mongoose E. coli in our study acquired resistance to streptomycin and tetracycline via transmissible plasmids from human and animal strains because of the closeness of the habitats of mongooses to human settlements and livestock farms.

Some of the non-O157:H7 *E. coli* isolates recovered in this present study showed a moderate to high intermediate resistance rates (1.4 to 13.6%) to some of the tested antibiotics with the highest rates being to streptomycin (13.6%). This is similar to the 14% intermediate resistance rates to streptomycin observed in green iguanas [36]. Fifty seven (27%) out of the 213 non-O157:H7 *E. coli* isolates recovered in this present study were resistant to two or more

antibiotics. This is of public health concern since these multiple antibiotic resistant organisms can be transmitted from animals to humans.

5. CONCLUSION

This current study showed that presently, mongooses in Grenada are not reservoirs for the *E. coli* O157:H7 serotype. It also indicates that the tested mongooses are presently not major reservoirs for multiple resistant *E. coli* strains. Among the 213 non-O157:H7 *E. coli* isolates, the resistance rate to drugs other than streptomycin and tetracycline was very low.

ACKNOWLEDGEMENTS

The authors are thankful to the Board of Trustees of St. George's University, the Chancellor Dr. C. Modica for providing funds for the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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