Multidrug resistant enterohaemorrhagic Escherichia coli *serogroups in the faeces of hunted Wildlife, Abeokuta, Nigeria*

Olufemi Ernest Ojo[1*], Elizabeth Adesola Amosun[2], Oluwadaisi Oluwaseyi Opebiyi[1], Mufutau Atanda Oyekunle[1], Morenike Atinuke Dipeolu[3] and Ebenezer Babatunde Otesile[4]

[1D]*epartment of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria.*
[2]*Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.*
[3]*Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria.*
[4]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria*

[*]*Corresponding author at: Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria.*
E-mail: ojooe@funaab.edu.ng.

Veterinaria Italiana 2022, **58** (2), 173-179. doi: 10.12834/VetIt.1990.12087.2
Accepted: 14.09.2020 | Available on line: 31.12.2022

Keywords
Antimicrobial resistance, Enterohaemorrhagic *Escherichia coli* (EHEC), Shiga toxins, Hunted wildlife, Zoonosis.

Summary
Wildlife plays significant roles in the dissemination and zoonotic transmission of pathogens. The enterohaemorrhagic *Escherichia coli* (EHEC) are associated with complicated cases of food-borne illnesses. This study investigated the presence of EHEC serogroups (O26, O45, O103, O145, O91, O111, O128, O121 and O157) in wildlife species: cane rats (*Thryonomys swinderianus*), royal antelope (*Neotragus pygmaeus*), African giant rats (*Cricetomys gambianus*) and waterbuck (*Kobus ellipsiprymnus*). EHEC and non-EHEC isolates from these wildlife sources were tested for susceptibility to antimicrobial agents. Overall, 127 (83.0 %) out of 153 samples yielded *E. coli*. Nine (5.9%) samples were positive for EHEC belonging to three serogroups as follows: O26 (n = 2), O111 (n = 2) and O103 (n = 5). The EHEC isolates were from cane rats (n = 6) and royal antelope (n = 3) and possessed virulence-associated genes stx_1 (77.8%), stx_2 (100.0%), *eaeA* (100.0%) and *hlyA* (100.0%). Overall, 127 *E. coli* isolates showed resistance to ampicillin (99.2%), ceftiofur (90.6%), tetracycline (90.0%), cephalexin (87.4%), cefotaxime (50.4%), streptomycin 42.5%, ceftazidime (41.7%), nalidixic acid (37.0%), ciprofloxacin (43.6%), amoxicillin/clavulanic acid (32.3%), gentamicin (27.6%), sulphamethoxazole/trimethoprim (25.2%), norfloxacin (17.3%) and chloramphenicol (11.0%). The role of wildlife in the dissemination and transmission of antimicrobial resistant and zoonotic bacteria should not be neglected for effective preventive and control strategies.

Introduction

Hunted games have served as a source of animal protein for many people of diverse social status and economic backgrounds in Africa for ages (Walz *et al.* 2017, Chausson *et al.* 2019). For many rural households hunted games are the main source of animal protein while for urban elites game meats represent special delicacies (Morsello *et al.* 2015, Chausson *et al.* 2019). In the past decades, rearing of some wild rodents has gained popularity because of the increasing demands for their meat in restaurants and households within major towns and cities (Walz *et al.* 2017). Some people living in peri-urban communities and villages close to towns and cities also engage in hunting of game animals which are brought to urban centres for sales. The processing and marketing of hunted game is a striving business venture in urban centres (Morsello *et al.* 2015, Chausson *et al.* 2019).

In Nigeria, there is no evidence that the activities of hunters and game meat processors are subjected to strict monitoring by government agencies to ensure wholesomeness and safety of the meat. In many parts of West African countries, there are no ante- and post-mortem inspections before selling of game meat to

the public for consumption (Friant *et al.* 2015). Wild animals can serve as reservoirs of pathogens for zoonotic transmission of diseases to humans (Friant *et al.* 2015). Lack of meat inspection increases the risk of game meat serving as vehicle for the transmission food-borne pathogens to consumers and people involved in the processing activities. The likelihood of contamination is particularly high because of the unhygienic conditions under which the game meats are processed and marketed.

The enterohaemorrhagic *Escherichia coli* (EHEC) serogroups are a subset of shiga toxin-producing *E. coli* (STEC) known to cause severe food-borne gastroenteritis characterized by abdominal cramps, vomiting, bloody diarrhea and life-threatening complications such as haemolytic uraemic syndrome (HUS), haemorrhagic colitis and thrombotic thrombocytopaenic purpurae (TTP) (Eichhorn *et al.* 2015, Kanayama *et al.* 2015). While EHEC organisms are associated with fatal infections in humans, they rarely cause clinical disease in the animal hosts (Pruimboom-Brees *et al.* 2000). Ruminants, especially cattle, are the principal reservoirs of EHEC but other farm animals as well as wildlife can harbor EHEC organisms (Stevens *et al.* 2002, Persad and LeJeune 2014). The high pathogenic potentials of EHEC and ease of food contamination have made EHEC a subject of scientific investigations and surveillance programmes in the food industry. In Nigeria, EHEC has been reported in on-farm animals, slaughter-animals and in fresh meat products (Ojo *et al.* 2010). Despite the public health importance of these organisms and the high possibility of contamination during meat processing, there has been no report of EHEC investigations in hunted wildlife being processed as meat for human consumption in Nigeria. Game animals are known to serve as reservoir of zoonotic pathogens including EHEC (Rice *et al.* 2003, Ferens and Hovde 2011, Dias *et al.* 2019).

The present study investigated the presence of EHEC serogroups in the faeces of hunted wildlife that were being processed as meat for human consumption at a game meat processing center in Abeokuta, Nigeria. The antimicrobial susceptibility profile of the EHEC organisms and other *E. coli* isolates from the hunted wildlife was also examined.

Materials and methods

Sample collection

This study was carried out at a game meat processing centre in Abeokuta, the capital city of Ogun State, South-Western Nigeria. A total of 153 faecal samples were collected for microbiological analysis. The samples were collected from fresh carcasses of hunted wildlife that were being processed for human consumption. Four wildlife species, cane rats (*Thryonomys swinderianus*), royal antelope (*Neotragus pygmaeus*), African giant rats (*Cricetomys gambianus*) and waterbuck (*Kobus ellipsiprymnus*) were investigated in this study. The included wildlife species were mammals, the most commonly accepted game meat types. Sample sizes from these species varied according to the available number as at the time of sampling. As much as possible, all freshly looking carcasses of mammals found at the center at the time of visit were included in the sampling. Sampling was between January and May 2013, the period of peak hunting activities in the study area. Moistened sterile swabs were used to scoop faecal materials from the rectum of fresh carcasses without signs of putrefaction. Samples were placed in separate sterile universal bottles, labeled, and transported in icepacks to the laboratory for immediate analysis. The 153 faecal samples examined were from 108 cane rats, 40 royal antelopes, three African giant rats and two waterbucks. All samples were processed within two hours of collection.

Isolation and identification of *Escherichia coli*

Enterohaemorrhagic *E. coli* were isolated and identified in faecal samples as previously described (Ivbade *et al.* 2014). Swab stick containing faecal materials was inoculated directly into nine milliliters of sterile tryptic soy broth (TSB) and incubated at 37 °C for 8 hours for pre-enrichment. Subsequently, one milliliter of the TSB pre-enrichment culture was transferred into 9 ml of modified Tryptic Soy Broth (mTSB) supplemented with novobiocin for selective enrichment at 37 °C for 18 hours. A loopful of the selective enrichment broth culture was inoculated onto MacConkey agar and onto Sorbitol MacConkey agar containing 5-bromo-4-chloro-3-indolyl-β -D-glucuronide (SMAC-BCIG) with cefixime and tellurite supplement. Inoculated agar plates were incubated at 37 °C for 18 to 24 hours. For every sample, five discrete lactose-fermenting (pink) colonies on MacConkey agar were tested for oxidase and catalase production. Similarly, from SMAC-BCIG plates, five colonies of non-sorbitol fermenting bacteria as well as five colonies of sorbitol-fermenting colonies were tested for oxidase and catalase production. All catalase positive and oxidase negative isolates were subjected to biochemical test for the identification of *E. coli* using commercial biochemical tests kits (Oxoid Microbact GNB 24E®) and results interpreted with the aid of accompanying computer software package (Oxoid Microbact® 2000 version 2.03). All isolates identified

as *E. coli* by biochemical tests were preserved on nutrient agar slopes for serological and molecular studies.

Detection of EHEC serogroups

Suspected *E. coli* isolates were screened by serological tests for the identification of common EHEC serogroups. Lactose- and sorbitol- fermenting isolates were transferred from nutrient agar slants onto nutrient agar plates (Petri dishes) and tested for the detection of EHEC serogroups O26, O45, O103, O145, O91, O111, O128 and O121. Serological identification was performed using polyvalent latex agglutination test kit (Dryspot Seroscreen® DR0300, Oxoid, Basingstoke, UK) as well as monovalent latex agglutination test kits from Oxoid, Basingstoke, UK (Dryspot serocheck® O26, O91, O103, O111, O128 and O145) and from Pro-Lab diagnostics, Texas, USA (Prolex™ *E. coli* non-O157 and *E. coli* O91 latex reagent identification kit). Non-sorbitol fermenting isolates were tested for the detection EHEC O157 somatic antigen using a latex agglutination test kit (*E. coli* O157 latex test, Oxoid®).

Detection of virulence genes in EHEC serogroups

Isolates identified as EHEC by serology were investigated for the presence of EHEC-associated virulence genes namely: shiga toxin genes (stx_1, stx_2), intimin gene (*eaeA*) and eneterohaemolysin gene (*hlyA*). Genomic DNA was extracted from overnight tryptic soy broth culture of each isolate by thermolysis as described by Ojo and colleagues (Ojo *et al.* 2016). The target virulence genes were amplified by polymerase chain reaction (PCR) using specific primer sets (Paton and Paton 1998) and previously described amplification conditions (Ojo *et al.* 2010, Ivbade *et al.* 2014). The amplicons were electrophoresed in 1.5% agarose gel, stained with ethidium bromide, and viewed under ultraviolet transilluminator.

Antimicrobial susceptibility testing

One representative isolate from each of 127 samples that yielded *E. coli* was tested for susceptibility to antimicrobial agents using the Kirby Bauer disk diffusion method. A fresh culture of the test isolate was emulsified in normal saline to produce a turbidity corresponding to 0.5 McFarland standard. The bacteria suspension was spread on Mueller Hinton agar (MHA) and antimicrobial disks placed firmly on the agar. The inoculated MHA plates were incubated at 35 ± 2 °C for 18 hours. The diameter of zones of inhibition around each disk was measured and interpreted according to the recommendation of Clinical and Laboratory Standard Institute (CLSI 2013). Isolates were tested for susceptibility to ampicillin (10 µg), amoxycillin/clavulanic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftiofur (30 µg), cephalexin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5µg), nalidixic acid (30 µg), gentamicin (30 µg), norfloxacin (10 µg), streptomycin (10 µg), sulphamethoxazole/trimethoprim 19:1 (25 µg) and tetracycline (30 µg). *Escherichia coli* ATCC 25922 was tested for quality control.

Results

Of the 153 samples examined, 127 (83.0%) yielded *E. coli* isolates. Positive samples included 83 (76.9%) from cane rats, 40 (100.0%) from royal antelopes, two (66.7%) from African giant rats and two (100.0%) from waterbuck (Table I). Overall, EHEC serogroups were detected in nine (5.9%) of 153 samples (Table I). The EHEC isolates belonged to three different serogroups as follows: O26 (n = 2; 1.3%), O111 (n = 2; 1.3%) and O103 (n = 5; 3.3%) (Table II).

The nine EHEC isolates possessed stx_1, stx_2, *eaeA* and *hlyA* virulence genes except for two isolates that lacked the stx_1 (Table III). The EHEC isolates were all (100%) resistant to ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftiofur, nalidixic acid and tetracycline. They showed varying degrees of resistance to ceftazidime (77.8%), ciprofloxacin (77.8%), chloramphenicol (55.6%), gentamicin (55.6%), norfloxacin (55.6%), sulphamethoxazole/

Table I. *Detection of enterohaemorrhagic* Escherichia coli *(EHEC) in the faeces of hunted wildlife in Abeokuta, Nigeria.*

Wildlife species (N)	Number (%) positive for *E. coli*	Number (%) positive for EHEC
Cane rats (108)	83 (76.9)	6 (5.6)
Royal antelope (40)	40 (100)	3 (7.5)
African giant rats (3)	2 (66.7)	0 (0.0)
Waterbuck (2)	2 (100)	0 (0.0)
Total (153)	127 (83.0)	9 (5.9)

Table II. *Enterohaemorrhagic* Escherichia coli *(EHEC) serogroups detected in the faeces of hunted wildlife in Abeokuta, Nigeria.*

Wildlife species	Sample size	Number (%) of EHEC positive samples by serogroups			
		026	0103	0111	Total
Cane rats	108	1 (0.9)	3 (2.8)	2 (2.8)	6 (5.6)
Royal antelopes	40	1 (2.5)	2 (2.5)	0 (0.0)	3 (7.3)
African giant rats	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Waterbucks	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total (A)	153	2 (1.3)	5 (3.3)	2 (1.3)	9 (5.9)

Table III. *Virulence genes and antimicrobial resistance profiles of enterohaemorrhagic* Escherichia coli *(EHEC) serogroups isolated from the faeces of hunted wildlife in Abeokuta, Nigeria.*

Serogroups (Wildlife source)	Virulence genes				Antimicrobial resistance profile
	stx$_1$	*stx*$_2$	*eaeA*	*hlyA*	
O26 (CR)	+	+	+	+	Amp-Amc-Caz-Cef-Cet-Cip-Nal-Str-Tet
O26 (RA)	+	+	+	+	Amp-Amc-Caz-Cef-Cet-Cip-Nal-Str-Tet
O103 (CR)	+	+	+	+	Amp-Amc-Caz-Cef-Cet-Chl-Cip-Gen-Nal-Nor-Str-Stx-Tet
O103 (CR)	+	+	+	+	Amp-Amc-Caz-Cef-Cet-Chl-Cip-Gen-Nal-Nor-Str-Stx-Tet
O103 (CR)	+	+	+	+	Amp-Amc-Caz-Cef-Cet-Chl-Cip-Gen-Nal-Nor-Str-Stx-Tet
O103 (RA)	+	+	+	+	Amp-Amc-Caz-Cef-Cet-Chl-Cip-Gen-Nal-Nor-Str-Stx-Tet
O103 (RA)	-	+	+	+	Amp-Amc-Cef-Cet-Nal-Tet
O111 (CR)	+	+	+	+	Amp-Amc-Caz-Cef-Cet-Chl-Cip-Gen-Nal-Nor-Str-Stx-Tet
O111 (CR)	-	+	+	+	Amp-Amc-Cef-Cet-Nal-Str-Tet

CR = cane rat; RA = royal antelope; + = present; - = absent; Amp = ampicillin; Amc = amoxicillin/clavulanic acid; Caz = ceftazidime; Cef = cefotaxime; Cet = ceftiofur; Chl = chloramphenicol; Cip = ciprofloxacin; Gen = gentamicin; Nal = aalidixic acid; Nor = norfloxacin; Str = streptomycin; Stx = sulphomethoxazole/trimethoprim; Tet = tetracycline.

Table IV. *Antimicrobial susceptibility profile of* Escherichia coli *isolated from hunted wildlife in Abeokuta, Nigeria.*

Antimicrobial agents	Number (%) of isolate with profile		
	Resistance	Intermediate	Susceptible
Ampicillin	126 (99.2)	1 (0.8)	0 (0.0)
Amoxicillin/clavulanic acid	41 (32.3)	72 (56.7)	14 (11.0)
Cefotaxime	64 (50.4)	53 (41.7)	10 (7.9)
Ceftiofur	115 (90.6)	6 (4.7)	6 (4.7)
Ceftazidime	53 (41.7)	39 (30.7)	25 (19.7)
Cephalexin	111 (87.4)	9 (7.1)	7 (5.5)
Chloramphenicol	14 (11.0)	60 (47.2)	53 (41.7)
Gentamicin	35 (27.6)	23 (18.1)	69 (54.3)
Ciprofloxacin	44 (34.6)	47 (37.0)	36 (28.3)
Nalidixic acid	47 (37.0)	75 (59.0)	5 (3.9)
Norfloxacin	22 (17.3)	51 (40.2)	54 (42.5)
Streptomycin	54 (42.5)	51 (40.2)	22 (17.3)
Sulphamethoxazole/trimethoprim	32 (25.2)	41 (32.3)	54 (42.5)
Tetracycline	113 (90.0)	13 (10.2)	1 (0.8)

trimethoprim (66.7%) and streptomycin (88.9%). The two isolates belonging to EHEC serogroup O26 had similar resistance pattern (Amp-Amc-Caz-Cef-Cet-Cip-Nal-Str-Tet) while four isolates from serogroup O103 as well as one isolate from serogroup O111 shared similar resistance pattern (Amp-Amc-Caz-Ce f-Cet-Chl-Cip-Gen-Nal-Nor-Str-Stx-Tet) (Table III). All EHEC isolates displayed multidrug resistance trait with resistance to at least one antimicrobial each from three different classes.

Overall, 127 *E. coli* isolates (EHEC and non-EHEC) showed 99.2% resistance to ampicillin, 90.6% to ceftiofur, 90.0% to tetracycline, 87.4% to cephalexin. 50.4% to cefotaxime, 42.5% to streptomycin, 41.7% to ceftazidime, 37.0% to nalidixic acid, 43.6% to ciprofloxacin, 32.3% to amoxicillin/clavulanic acid, 27.6% to gentamicin, 25.2% to sulphamethoxazole/trimethoprim, 17.3% to norfloxacin and 11.0% to chloramphenicol (Table IV).

Discussion

Enterohaemorrhagic *E. coli* strains are major cause of food-borne infections all over the world. It has been estimated that globally, EHEC organisms cause 2,801,000 acute illnesses, 3,890 cases of HUS and 230 deaths annually (Majowicz *et al.* 2014). Meat contamination especially during processing has been recognized as one of the most significant route of transmission of EHEC from apparently healthy animal reservoirs to humans. Most surveillance programmes focused on the roles of farm animals in the transmission of EHEC with little consideration for the involvement of hunted game animals in EHEC transmission.

In the present study, EHEC serogroups were detected in the faeces of hunted wildlife during processing. The EHEC serogroups were detected in cane rats (rodents) and royal antelope (ungulate) but not in African giant rats and waterbuck. The non-detection of the organisms in African giant rats and waterbuck could be because of the comparatively fewer sample sizes from these species. Earlier studies have shown that wildlife species harboured EHEC serogroups. Enterohaemorrhagic *E. coli* serogroups have been detected in the faeces of wild rodents (Cizek *et al.* 1999, Scaife *et al.* 2006) and ungulates (Dias *et al.* 2019) like the findings in the present study. Only non-O157 EHEC serogroups (O26, O103 and O111) were detected in this study. Although EHEC serogroup O157 is the most commonly implicated in human outbreaks of EHEC foodborne infection, this serogroup was not detected in the present study. A simlar finding was reported by earlier workers where only non-O157 EHEC serogroups were detected in the faeces of wild ungulate (Dias *et al.* 2019). Nevertheless, wildlife has been reported to harbour both O157 and non-O157 EHEC serogroups (Sánchez *et al.* 2010). All the isolates from this study possessed virulence genes especially shiga toxins (*stx*$_1$ and *stx*$_2$) as well as the intimin (*eaeA*) genes which are known to influence the severity of EHEC infections (Ritchie *et al.* 2003).

The detection of EHEC in the faeces of wildlife is of public health and epidemiological significance in the transmission of the organisms to humans and maintenance of their infection cycle in nature. There could be faecal contamination during processing of hunted wildlife for human consumption. This could lead to EHEC infections and associated complications in the consumers. Previous studies have reported contamination of wildlife meat by non-O157 and O157 EHEC serogroups (Magwedere *et al.* 2013, Haindongo *et al.* 2018). Moreover, outbreak of bloody diarrhea and hospitalization due to consumption of wildlife meat contaminated by non-O157 EHEC belonging to serogroups O103 and O145 has been reported (Rounds *et al.* 2012). Wildlife could also serve as sources for dispersal of EHEC organisms for the contamination of vegetables and other crops in the field (Karp *et al.* 2015). This could also lead to human infection following consumption of such contaminated vegetables (Mikhail *et al.* 2018). Likewise, grazing on pasture contaminated by EHEC of wildlife origin could serve as source of livestock infection. This is particularly important in countries of sub-Saharan Africa where majority of cattle population are managed on open field by nomadic herdsmen traversing across wide expanse of forest, which serves as natural habitat to wildlife species.

Antimicrobial resistance in pathogenic and commensal bacteria of human, animal and environmental origins are of significance public health importance with overwhelming socioeconomic implications. *Escherichia coli* is one of the indicator bacteria for monitoring and assessing the status of antimicrobial resistance. In the present study, there were high levels of antimicrobial resistance observed in EHEC and non-EHEC isolates from hunted wildlife. Many of the *E. coli* isolates showed high level of multidrug resistance to at least three different classes of antimicrobial agents. There were varying degrees of resistance to clinically relevant antimicrobial agents such as β-lactams (including third generation cephalosporins such as ceftazidime and cefotaxime), aminoglycosides and fluoro/quinolones. Earlier studies have suggested the important roles of wildlife in the environmental dissemination of antimicrobial-resistant bacteria (Dolejska and Literak 2019). Wildlife have been reported to be carriers of multidrug resistant *E. coli* (Furness *et al.* 2017, Kaspersen *et al.* 2018, Mo *et al.* 2018, Zurfluh *et al.* 2019). There were previous reports on resistance to extended-spectrum cephalosporins and fluoroquinolones in *E. coli* isolates from wild ungulate in Spain (Navarro-Gonzalez *et al.* 2013). Likewise, quinolone resistant *E. coli* isolates have been reported in wildlife from Norway (Kaspersen *et al.* 2018). Multidrug resistant *E. coli* with resistance to ampicillin, tetracycline, fluoroquinolones, trimethoprim and sulphamethoxazole were found in foxes (Mo *et al.* 2019). Similarly, *E. coli* isolates from wildlife in Kenya demonstrated resistance to as many as seven different classes of antimicrobials (Hassell *et al.* 2019). The emergence of antimicrobial resistance is often attributed to exposure to antimicrobial agents. There is no evidence of direct exposure of the hunted wildlife in this study to antimicrobial agents. However, wildlife could be exposed to antimicrobial residues and resistant bacteria that are dispersed in the environment. One important source of wildlife exposure to antimicrobial agent is drinking water from sources that are contaminated due to the discharge of animal manure into waterbodies. In the study area, slaughter-houses and abattoirs discharge effluents directly into streams and rivers (Akanni *et al.* 2019, Elemile *et al.* 2019). Moreover, commercial animal farms especially poultries and piggeries discharge animal wastes into nearby streams and rivers. The discharge of animal wastes into waterbodies leads to contamination of such water sources with antimicrobial residues and antimicrobial resistant bacteria of livestock origins. There is a very high level of dependence on antimicrobial usage in livestock production in Nigeria where there is very poor regulations concerning antimicrobial usage in animal production (Ojo *et al.* 2016, Ojo *et al.* 2017). Such contaminated waterbodies serve as important sources of wildlife exposure to antimicrobial residues and antimicrobial resistant bacteria. Wildlife may also visit livestock farms and pastures where they come in contact with faecal materials from farm animals (Navarro-Gonzalez *et al.* 2013, Hassell *et al.* 2019). Previous studies have reported high incidence of multidrug resistance in *E. coli* strains isolated from farm animals including poultry (Ogunleye *et al.* 2008, Oluduro 2012), ruminants (Amosun *et al.* 2010, Ojo *et al.* 2008) and pigs (Ojo *et al.* 2008) in the study area. Similarly, an earlier study reported that 90% of *E. coli* isolates from bats demonstrated multidrug resistance with 28 different multidrug patterns (Oluduro 2012).

The present study showed that hunted wildlife are potential vehicles for the transmission of EHEC serogroups and other antimicrobial resistant *E. coli* in the study area. It is therefore important to consider the role of wildlife in environmental dissemination and zoonotic transmission of pathogenic and antimicrobial resistant bacteria in the design of preventive and control strategies.

References

Chausson A.M., Rowcliffe J.M., Escouflaire L., Wieland M. & Wright J.H. 2019. Understanding the sociocultural drivers of urban bushmeat consumption for behavior change interventions in Pointe Noire, Republic of Congo. *Hum Ecol*, **47**, 179-191.

Cizek A., Alexa P., Literak I., Hamrík J., Novák P. & Smola J. 1999. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. *Lett Appl Microbiol*, **28**, 435-439.

Dias D., Caetano T., Torres R.T., Fonseca C. & Mendo S. 2019. Shiga toxin-producing *Escherichia coli* in wild ungulates. *Sci Total Environ*, **651** (Pt 1), 203-209.

Dolejska M. & Literak I. 2019. Wildlife is overlooked in the epidemiology of medically important antibiotic-resistant bacteria. *Antimicrob Agents Chemother*, **63** (8), e01167-19.

Eichhorn I., Heidemanns K., Semmler T., Kinnemann B., Mellmann A., Harmsen D., Anjum M.F., Schmidt H., Fruth A., Valentin-Weigand P., Heesemann J., Suerbaum S., Karch H. & Wieler L.H. 2015. Highly virulent non-O157 enterohemorrhagic *Escherichia coli* (EHEC) serotypes reflect similar phylogenetic lineages, providing new insights into the evolution of EHEC. *Appl Environ Microbiol*, **81**, 7041-7047. doi:10.1128/AEM.01921-15.

Ferens W.A. & Hovde C.J. 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog Dis*, **8** (4), 465-487

Friant S., Paige S.B. & Goldberg T.L. 2015. Drivers of bushmeat hunting and perceptions of zoonoses in Nigerian hunting communities. *PLoS Negl Trop Dis*, **9** (5), e0003792.

Furness L.E., Campbell A., Zhang L., Gaze W.H. & McDonald R.A. 2017. Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance. *Environ Res*, **154**, 28-34.

Haindongo N., Nkandi J., Hamatui N., Larai A.A., Hemberger M.Y., Khaiseb S. & Molini U. 2018. The prevalence of non-O157:H7 shiga toxin-producing *Escherichia coli* in Namibian game meat. *Vet Ital*, **54** (3), 185-188.

Hassell J.M., Ward M.J, Muloi D., Bettridge J.M., Robinson T.P., Kariuki S., Ogendo A., Kiiru J., Imboma T., Kang'ethe E.K., Öghren E.M., Williams N.J., Begon M., Woolhouse M.E.J. & Fèvre E.M. 2019. Clinically relevant antimicrobial resistance at the wildlife-livestock-human interface in Nairobi: an epidemiological study. *Lancet Planet Health*, **3** (6), e259-e269. doi: 10.1016/S2542-5196(19)30083-X.

Ivbade A., Ojo O.E. & Dipeolu M.A. 2014. Shiga toxin-producing *Escherichia coli* O157:H7 in milk and milk products in Ogun State, Nigeria. *Vet Ital*, **50** (3), 185-191.

Kanayama A., Yahata Y., Arima Y., Takahashi T., Saitoh T., Kanou K., Kawabata K., Sunagawa T., Matsui T. & Oishi K. 2015. Enterohemorrhagic *Escherichia coli* outbreaks related to childcare facilities in Japan, 2010-2013. *BMC Infect Dis*, **15**, 539.

Karp D.S., Gennet S., Kilonzo C., Partyka M., Chaumont N., Atwill E.R. & Kremen C. 2015. Comanaging fresh produce for nature conservation and food safety. *Proc Natl Acad Sci*, **112** (35), 11126-11131.

Kaspersen H., Urdahl A.N., Simm R., Slettemeås J.S., Lagesen K. & Norström M. 2018. Occurrence of quinolone resistant *E. coli* originating from different animal species in Norway. *Vet Microbiol*, **217**, 25-31.

Magwedere K., Dang H.A., Mills E.W., Cutter C.N., Roberts E.L. & DebRoy C. 2013. Incidence of shiga toxin-producing *Escherichia coli* strains in beef, pork, chicken, deer, boar, bison, and rabbit retail meat. *J Vet Diagn Invest*, **25** (2), 254-258.

Majowicz S.E., Scallan E., Jones-Bitton A., Sargeant J.M., Stapleton J., Angulo F.J., Yeung D.H. & Kirk M.D. 2014. Global incidence of human shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. *Foodborne Pathog Dis*, **11**, 447-455.

Mathusa E.C., Chen Y., Enache E. & Hontz L. 2010. Non-O157 shiga toxin-producing *Escherichia coli* in foods. *J Food Prot*, **73** (9), 1721-1736.

Mikhail A.F.W., Jenkins C., Dallman T.J. Inns T., Douglas A., Martín A.I.C., Fox A., Cleary P., Elson R. & Hawker J. 2018. An outbreak of shiga toxin-producing *Escherichia coli* O157:H7 associated with contaminated salad leaves: epidemiological, genomic and food trace bac investigations. *Epidemiol Infect*, **146**, 187-196.

Mo S.S., Urdahl A.M., Madslien K., Sunde M., Nesse L.L., Slettemeas J.S. & Norström M. 2018. What does the fox say? Monitoring antimicrobial resistance in the environment using wild red foxes as an indicator. *PLoS ONE*, **13** (5), e0198019. doi:/10.1371/journal.pone.0198019.

Morsello C., Yagüe B., Beltreschi L., van Vliet N., Adams C., Schor T., Quiceno-Mesa M.P. & Cruz D. 2015. Cultural attitudes are stronger predictors of bushmeat consumption and preference than economic factors among urban Amazonians from Brazil and Colombia. *Ecol Soc*, **20** (4), 21. doi.org/10.5751/ES-07771-200421.

Navarro-Gonzalez N., Porrero M.C., Mentaberre G., Serrano E., Mateos A., Domínguez L. & Lavín S. 2013. Antimicrobial resistance in indicator *Escherichia coli* isolates from free-ranging livestock and sympatric wild ungulates in a natural environment (Northeastern Spain). *Appl Environ Microbiol*, **79** (19), 6184-6186.

Ojo O.E., Ajuwape A.T.P., Otesile E.B., Owoade A.A., Oyekunle M.A. & Adetosoye A.I. 2010. Potentially zoonotic shiga toxin-producing *Escherichia coli* serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. *Intl J Food Microbiol*, **142**, 214-221.

Ojo O.E., Awoyomi O.J., Fabusoro E. & Dipeolu M.A. 2017. Activities and influence of veterinary drug marketers on antimicrobial usage in livestock production in Oyo and Kaduna States, Nigeria. *J Agric Rur Dev Trop Subtrop*, **118** (2), 207-216.

Ojo O.E., Fabusoro E., Majasan A.A. & Dipeolu M.A. 2016. Antimicrobials in animal production: usage and

practices among livestock farmers in Oyo and Kaduna States of Nigeria. *Trop Anim Hlth Prod*, **48**, 189-197.

Ojo O.E., Schwarz S. & Michael G.B. 2016. Detection and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* from chicken production chains in Nigeria. *Vet Microbiol*, **194**, 62-68.

Paton A.W. & Paton J.C. 1998. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1*, *stx2*, *eaeA*, enterohemorrhagic *Escherichia coli hlyA*, *rfbO111*, and *rfbO157*. *J Clin Microbiol*, **36**, 598-602.

Persad A.K. & LeJeune J.T. 2014. Animal reservoirs of shiga toxin-producing *Escherichia coli*. *Microbiol Spectr*, **2** (4), EHEC-0027-2014.

Pruimboom-Brees I.M., Morgan T.W., Ackermann M.R., Nystrom E.D., Samuel J.E., Cornick N.A. & Moon H.W. 2000. Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. *Proc Natl Acad Sci*, **97** (19), 10325-10329.

Rice D.H., Hancock D.D. & Besser T.E. 2003. Faecal culture of wild animals for *Escherichia coli* O157:H7. *Vet Rec*, **152**, 82-83.

Ritchie J.M., Thorpe C.M., Rogers A.B. & Waldor M.K. 2003. Critical Roles for *stx2*, *eae*, and *tir* in Enterohemorrhagic *Escherichia coli*-induced diarrhea and intestinal inflammation in infant rabbits. *Infect Immun*, **71**, 7129-7139.

Rounds J.M., Rigdon C.E., Muhl L.J., Fordtner M., Danzeisen G.T., Koziol B.S., Taylor C., Shaw B.T., Short G.L. & Smith K.E. 2012. Non-O157 shiga toxin-producing *Escherichia coli* associated with venison. *Emerg Infect Dis*, **18** (2), 279-282.

Sánchez S., Martínez R., García A., Vidal D., Blanco J., Blanco M., Blanco J.E., Mora A., Herrera-León S., Echeita A., Alonso J.M. & Rey J. 2010. Detection and characterization of O157:H7 and non-O157 shiga toxin-producing *Escherichia coli* in wild boars. *Vet Microbiol*, **143**, 420-423.

Scaife H.R., Cowan D., Finney J., Kinghorn-Perry S.E. & Crook B. 2006. Wild rabbits (*Oryctolagus cuniculus*) as potential carriers of verocytotoxin-producing *Escherichia coli*. *Vet Rec*, **159**, 175-178.

Stevens M.P., van Diemen P.M., Dziva F., Jones P.W. & Wallis T.S. 2002. Options for the control of enterohaemorrhagic *Escherichia coli* in ruminants. *Microbiology,* **148**, 3767-3778.

Walz E., Wilson D., Stauffer J.C., Wanduragala D., Stauffer W.M., Travis D.A. & Alpern J.D. 2017. Incentives for bushmeat consumption and importation among West African immigrants, Minnesota, USA. *Emerg Infect Dis*, **23** (12), 2095 - 2097.

Zurfluh K., Albini S., Mattmann P., Kindle P., Nüesch-Inderbinen M., Stephan R. & Vogler B.R. 2019. Antimicrobial resistant and extended-spectrum β-lactamase producing *Escherichia coli* in common wild bird species in Switzerland. *Microbiology open*, **8** (11), e845. https:// doi.org/10.1002/mbo3.845.