academicJournals

Vol. 9(13), pp. 938-944, 1 April, 2015
DOI: 10.5897/AJMR2013.6278
Article Number: CE3D3C552154
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation of *Escherichia coli* from cattle and lechwe antelopes at the livestock/wildlife interface area of the Kafue flats in Zambia

Charles Mubita[1], Bernard Mudenda Hang'ombe[1]*, John Bwalya Muma[1], Musso Munyeme[1], Evans Mulenga[1], Manda Chitambo[1], Hideto Fukushi[2], Jun Yasuda[3], Hiroshi Isogai[4] and Emiko Isogai[5]

[1]School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia.
[2]Department of Applied Veterinary Sciences, United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan.
[3]Veterinary Teaching Hospital, Faculty of Agriculture, Iwate University, Morioka, Japan.
[4]Institute of Animal Experimentation, Sapporo Medical University, Sapporo, Japan.
[5]Laboratory of Animal Microbiology, Department of Microbial Biotechnology, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.

Received 15 August, 2013; Accepted 2 June, 2014

This study was conducted at the livestock/wildlife interface areas of national parks and traditional cattle grazing areas. The main aim of the study was to establish the occurrence of *Escherichia coli* serogroups isolated from Kafue lechwe antelopes (*Kobus leche kafuensis*) and pastoral cattle. A total of 593 faecal samples from lechwe (232) and pastoral cattle (361) were conveniently picked from the grazing pastures in the interface areas and cultured for *E. coli*. From each faecal sample, two or three presumptive *E. coli* isolates were picked to constitute 1,283 isolates with 574 from lechwe and 708 from cattle. Some of these isolates were found to be similar to Shiga toxin-producing *E. coli* O157 on CHROMagar and sorbitol MacConkey agar. Only 18 *E. coli* isolates from Kafue lechwe antelopes were grouped into eight serogroups while 32 from pastoral cattle were grouped into 16 serogroups. The most prevalent type-able serogroups from lechwe antelopes and pastoral cattle were O125 (5.8%) and O29 (4.2%), respectively. On further analysis by fermentation of various sugars, some isolates showed a similar pattern suggesting that the strains were indistinguishable.

Key words: *Escherichia coli*, diarrheagenic, Shiga toxin, *Kobus leche kafuensis*, pastoral cattle.

INTRODUCTION

The genus *Escherichia coli* comprise several serotypes among which are the most important bacterial public health hazards. The serotypes are known to cause diverse intestinal and extra intestinal diseases by means

*Corresponding author. E-mail: mudenda68@yahoo.com. Tel: +260 977 3262 88. Fax: +260 211 293727.

of different mechanism that affect a wide range of cellular processes (Kaper et al., 2004). The serotypes that are associated with disease of humans are classified under six recognized categories; Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC) and Enteroaggregative *E. coli* (EAEC) (Garcia and Bouguenec, 1998; Nataro and Kaper, 1998; Presterl et al., 2003). Each category has distinct virulence factors, serogroups, epidemiological features and produces characteristic clinical symptoms (Levine, 1987). Diarrheagenic outbreaks are mainly because of consumption of contaminated food of animal origin and water. Meat from food animals (wild and domestic) has been identified as an occasional vehicle of enteric infections (Keene et al., 1997). Various studies have shown meat from deer and cattle as being responsible for some reported cases (Rabatsky-Her et al., 1987; Hornitzky et al., 2005; Fairbrother and Nadeau, 2006).

In Zambia, meat from wildlife is a major source of animal protein, with the Kafue lechwe (*Kobus leche kafuensis*) antelope being one of the most sought after game animal for consumptive utilization (Stafford, 1991; Siamudaala et al., 2003). It is for this reason that it becomes imperative to know the microbiological safety of meat and meat products derived from Kafue lechwe antelopes given the less than optimal handling and hygienic standards during evisceration and general processing. Other investigators suggest that poorly processed wildlife meat could serve as a source of human infections (Rabatsky-Her et al., 1987; Keene et al., 1997). Therefore, this study was aimed at elucidating evidence that Kafue lechwe antelopes which are in close contact with pastoral cattle in the livestock/wildlife interface areas may be asymptomatic carriers of diarrheagenic *E. coli* (DEC) serogroups. Human infections can occur from the ingestion of contaminated food of animal origin (Angulo et al., 2000). For instance, ETEC serotype 078 is known to cause severe diarrhea outbreaks (Ryder et al., 1976; Germani et al., 1985) while EPEC serogroup O125 is also known to cause a profuse watery diarrheal disease in developing countries (Regua et al., 1990). EPEC-like organisms have also been isolated from animals such as rabbits, pigs and dogs (Zhu et al., 1994; Nataro et al., 1998) and therefore knowledge on the occurrence of pathogenic *E. coli* in animal species meant for food is important.

MATERIALS AND METHODS

Study area

The study was conducted in the livestock/wildlife interface areas of Lochinvar (410 km^2) and Blue-Lagoon (420 km^2) National Parks on the Kafue Flats in Zambia that provide a unique interaction between livestock and wildlife as over 300,000 herds of cattle are moved from the upland areas to the wetlands during the drier months of the year. The Blue Lagoon and Lochnivar National Parks provide a rich lucurstrine habitat for the Kafue lechwe antelopes. GMAs are interface areas where interaction between wildlife and humans is facilitated through transhuman livestock herding activities.

Sample collection

Samples were collected from Kafue lechwe antelope and cattle that shared grazing pasture on the Kafue flats. Faecal samples from lechwe antelopes were collected in two categories. The first category, involved 2 g faecal contents from the rectum of the 77 hunter harvested animals from Lochinvar (n = 52) and Blue-lagoon (n = 25). The second category involved 155 faecal samples of about 5 g each from freshly voided grazing lechwe antelopes in Lochinvar (n = 110) and Blue-lagoon (n = 45). The collected samples were placed in a 150 x 100 mm polythene self adhesive bag and then stored at 4°C till laboratory analysis. Hunter harvested lechwe antelopes faecal samples were from animals that were cropped on special permission from Zambia Wildlife Authority (ZAWA). The sample size was pre-determined as authorized by the ZAWA research quota system for scientific research. In the case of pastoral cattle, a total of 361 freshly voided faecal samples were picked from the grazing pastoral cattle in Lochinvar (n = 261) and Blue-lagoon (n = 100).

Isolation and identification of *Escherichia coli*

All faecal samples were processed according to the method described by Seker and Yardimci (2008) with slight modification. One gram of faeces was transferred into tubes containing 9 ml peptone water and homogenized by vortexing for 5 min. The vortexed mixture was incubated at 37°C for four hours and then a loopful of the mixture was sub-cultured on Desoxycholate Hydrogen Sulfide Lactose agar (DHL) (Nissui Pharmaceutical Co., Tokyo, Japan). About four suspicious *E. coli* colonies from each sample were transferred to Eosine Methylene Blue agar (EMB) (Eiken Chemical Co., Tokyo, Japan) and then incubated at 37°C for 24 h as described by Balows et al. (1991). Suspected isolates on EMB were confirmed as *E. coli* according to previous workers (Carter and John, 1990; Barrow and Feltham, 1993; Quinn et al., 2002). Screening for the presence of Shiga toxin (*Stx*) producing *E. coli* serogroups was done using Sorbitol MacConkey agar (SMAC) (March and Ratnam, 1986; Boyce et al., 1995) and CHROMagar O157 (Bettelheim, 1998; Hirvonen et al., 2012). The *E. coli* isolates were serogrouped with commercially available *E. coli* antisera test kit (Denka Seiken Co. Tokyo, Japan) for specific O antigens. Following serogrouping, 12 isolates (5 belonging to O27, 2 isolates from O78 and 5 isolates from O125 serogroup) were selected randomly for fermentative reaction tests to a range of 18 sugars as described by O'Sullivan et al. (2006).

Statistical analysis

The database was established in Excel Spread sheets and for statistical analysis it was transferred to STATA SE/11 for windows statistical package (Stata Corp. College Station, Texas. USA). A p-value of <0.005 was considered indicative of a statistically significant difference.

RESULTS

A total of 1,283 *E. coli* presumptive isolates from 593 faecal samples comprising lechwe (n = 232) and pastoral cattle (n = 361) were picked for analysis. In lechwe, 575

Table 1. Distribution of *E. coli* isolates from Kafue lechwe and cattle by selective media and serogrouping

Serogroup	Lechwe isolates (*n* = 104)		Cattle isolates (*n* = 144)	
	Non-sorbitol fermenting isolates on SMAC	Mauve coloured isolates on CHROMagar O157	Non-sorbitol fermenting isolates on SMAC	Mauve coloured isolates on CHROMagar O157
O8	0	0	1	2
O8/O27	0	0	0	1
O8/O27/O125	0	0	1	0
O8/O27/O55/O125	0	0	2	0
O8/O27/O55/O125/O169	0	0	3	0
O15	2	0	0	0
O27	2	4	3	1
O27/O115	0	0	1	0
O27/O55/O125	0	0	1	0
O29	0	0	2	4
O55	0	0	1	0
O55/O125/O169	0	0	2	0
O78	0	1	0	1
O8/O125	1	0	0	0
O29/O143	0	1	0	0
O55/O125	0	2	0	0
O125	6	0	4	0
O125/O169	0	0	2	0
O159	0	0	0	1
None-typeable	69	27	45	84
Total (%)	80 (76.9)	35 (33.6)	68 (47.2)	94 (65.2)

E. coli isolates were recovered and identified with 104 (18.1%) being non-sorbitol-fermenting (NSF) isolates on SMAC and appeared mauve coloured on CHROMagar O157 (Table 1). Eighty (76.9%) isolates out of 104 presumptive STEC O157 isolates from Kafue lechwe antelopes were NSF, while 35 (33.7%) were mauve coloured. Of these isolates, 11 exhibited both characteristics. All suspected STEC isolates exhibited a typical green metallic sheen on EMB agar. Out of 104 *E. coli* isolates, 79 (76%) were from Lochinvar NP, while 25 (24%) were from Blue-lagoon NP (Table 2). All the lechwe isolates from Blue-lagoon examined were NSF while only 69.6% (55/79) of *E. coli* isolates from Lochinvar were NSF. Thirty-four of 35 (97.1%) mauve coloured isolates were from Lochinvar while only one mauve coloured isolate was from Blue-lagoon. Of the 35 mauve coloured isolates, 18 (51.4%) were from the faecal samples picked from the pastures while 17 (48.6%) were coming from the rectal samples. The proportion of mauve coloured isolates from Lochinvar (43%) was higher than those from Blue-lagoon. Based on the serogrouping results, the isolates were classified into 8 O serogroups (Table 1). Serogrouping of 104 isolates revealed that 18 (17.3%) isolates were typeable while the rest were non-typeable. All isolates belonging to serogroups O15 and O125 were NSF isolates on SMAC, whereas the isolates belonging to serogroup O78 and the majority of the strains belonging to serogroup O27 (P=0.004) were mauve coloured on CHROMagar O157. There was a significant association (P = 0.004) between serogroups and the choice of the selective media used. All isolates belonging to serogroups O15 and O125 were NSF isolates on SMAC, whereas the isolates belonging to serotype O78 and the majority of the strains belonging to serotype O27 (P=0.004) were mauve coloured on CHROMagar O157. The most frequently isolated DEC serogroups were EPEC O125 (5.8%) followed by ETEC serogroup O27 (4.8%) (Table 3).

In pastoral cattle, *E. coli* isolates were cultured and selected from 361 cattle faecal samples. Altogether 708 isolates were isolated from these samples, out of which, 144 (20.3%) were either NSF on SMAC and appeared mauve coloured on CHROMagar O157 (Table 1). Seventy-one (19.7%) faecal samples out of 361 samples were found to harbour the suspected STEC O157 isolates. The area distribution of the 71 faecal samples were 52 (73.2%) from Lochinvar and 19 (26.8%) from Blue-lagoon. Of the 144 suspected STEC O157 isolates, 110 (76.4%) were from Lochinvar, while 34 (23.6%) were from Blue-lagoon NP (Table 2). Of 144 isolates, 68 (47.2%) were NSF while 94 (65.3%) were mauve coloured on CHROMagar 0157 with eighteen isolates exhibiting both characteristics. Seventy-five out of 94 (79.8%) mauve coloured isolates were from Lochinvar,

Table 2. Distribution of *E. coli* serogroups from Kafue lechwe and pastoral cattle by area of sampling.

Serogroup	Pastoral cattle (*n* = 144)		Kafue Lechwe (n = 104)	
	Lochnivar	Blue Lagoon	Lochnivar	Blue Lagoon
O15	0	0	1	1
O125	4	0	3	3
O125/O169	1	1	0	0
O159	0	1	0	0
O27	1	3	5	0
O27/O115	0	1	0	0
O27/O55/O125	1	0	0	0
O8/O125	0	0	0	1
O8/O27	0	1	0	0
O29	0	6	0	0
O55	0	1	0	0
O55/O125/O169	0	2	0	0
O55/O125	0	0	2	0
O78	1	0	1	0
O8	1	1	0	0
O8/O27/O125	1	0	0	0
O8/O27/O55/O125	2	0	0	0
O8/O27/O55/O125/O169	3	0	0	0
O29/O143	0	0	1	0
Non-typeable	95	17	66	20
Total	110	34	79	25

Table 3. Occurrence estimates of *Escherichia coli* serovars from Kafue lechwe and pastoral cattle.

Serogroup	Category	Frequency of isolation	
		Pastoral cattle (n = 144)	Kafue lechwe (n = 104)
O15	ETEC	0	2 (1.9)
O125	EPEC	4 (2.8)	6 (5.8)
O125/O169	EPEC/ETEC	2 (1.4)	0
O159	ETEC	1 (0.7)	0
O27	ETEC	4 (2.8)	5 (4.8)
O27/O115	ETEC	1 (0.7)	0
O27/O55/O125	ETEC/EPEC	1 (0.7)	0
O8/O125	ETEC/EPEC	0	1 (0.96)
O8/O27	ETEC	1 (0.7)	0
O29	EIEC	6 (4.2)	0
O55	EPEC	1 (0.7)	0
O55/O125/O169	EPEC/ETEC	2 (1.4)	0
O55/O125	EPEC	0	2 (1.9)
O78	ETEC	1 (0.7)	1 (0.96)
O8	ETEC	2 (1.4)	0
O29/O143	EIEC	0	1 (0.96)
O8/027/O125	ETEC/EPEC	1 (0.7)	0
08/027/055/0125	ETEC/EPEC	2 (1.4)	0
08/027/055/0125/0169	ETEC/EPEC	3 (2.1)	0
Non-typeable	-	112 (77.8)	86 (82.7)
Total		144	104

Table 4. Comparison of fermentative reaction results of selected *E. coli* biovars from Kafue lechwe and pastoral cattle (*n* = 12).

Test	*E. coli* serogroup tested											
	O27					O78		0125				
	Lechwe			Cattle		Lechwe	Cattle	Lechwe			Cattle	
	1	2	3	4	5	6	7	8	9	10	11	12
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-
Dextran	-	-	-	-	-	-	-	-	-	-	-	-
Dulcitol	-	+	+	-	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	+	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+	+	+
Meliobiose	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+
Resorcinol	-	-	-	-	-	-	-	-	-	-	-	-
Ribitol	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	+	+	+	+	+	+	-	-	+	-	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	-	+	+	+	+	+	-	-	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	+	+	+	+	+	+
Barbitol	-	-	-	-	-	-	-	-	-	-	-	-

+ = Positive reaction, - = negative reaction.

while 19 (20.2%) were from Blue-lagoon. Thirty-two (22.2 %) of these isolates were grouped into 16 O serogroups (Table 1). Most of the defined serogroups were from Lochinvar (Table 2). The most frequent serogroup was the non-typeable *E. coli* which accounted for 66% (95/144) and 11.8% (17/144) of the isolates from Lochinvar and Blue-lagoon, respectively (Table 2).

The metabolic fingerprint of *E. coli* serogroups from cattle and lechwe was determined by biochemical profile. Twelve *Escherichia coli* isolates belonging to serogroups O27 (5), O78 (2) and O125 (5) respectively were randomly selected from lechwe antelopes and pastoral cattle. These isolates were subjected to fermentation of various carbohydrates (Table 4). The results of sugar utilization from various source suggested that isolates belonging to serogroup O27 were of three distinctive fermentative groups with one group comprising isolates numbers 1 and 4, while the second group were isolate number 3 and 5 from lechwe antelopes and pastoral cattle, respectively. The third group is of isolate number 2. The O78 serogroup had 2 distinct fermentative groups while in the O125 serogroup, 3 distinct groups were recorded (Table 4).

DISCUSSION

This study has shown that Kafue lechwe antelopes and domestic cattle on the Kafue Flats are potential carriers of food-borne pathogens. *E. coli* is generally used as an indicator organism because of the ease of isolation (Anonymous, 2003). In this study, we used the culture method followed by serogrouping and biochemical typing to confirm the presence of *E. coli*. According to our observations, the use of SMAC agar was specific in the proportion of recovery of *E. coli* serogroup O125 than CHROMagar O157. The use of SMAC agar and CHROMagar O157 to screen for presumptive STEC O157 strains was cost effective in the sense that they helped to reduce the material costs and the number of *E. coli* isolates needed to be confirmed by serogrouping. Though it is most likely that some unusual sorbitol positive *E. coli* O157 strains could have been missed which could not be differentiated from normal intestinal flora as observed by Ammon et al. (1999).

Kafue lechwe antelopes are carriers of at least seven diarrheagenic *E. coli* serogroups which include enterotoxigenic *E. coli* (O15, O27 and O78), enteropathogenic *E. coli* (O55 and O125) and enteroinvasive *E. coli* (O29/O143). It is worth noting that the ETEC serogroup O27 and O78 were most predominant in Lochinvar, while EPEC serogroup O125 and ETEC serogroup O15 were evenly distributed between Lochinvar and Blue-lagoon, suggesting that the Kafue lechwe antelopes may be the probable reservoir host. The present study attempted for the first time to classify *E. coli* from faecal material of Kafue lechwe and

pastoral cattle into O serogroups. The most commonly association was between serogroups O27, O78 and O125 from Kafue lechwe and cattle, respectively or vice versa. The isolation of pathogenic *E. coli* serogroups in the present study is in agreement with reports that wild animals may be involved in the epidemiology of zoonoses and may serve as reservoirs (Kruse et al., 2004). We reported O15, O27, O78 and O125 serogroups from Kafue lechwe, which could be potential sources of human gastroenteritis. Elsewhere, studies focusing on the Shiga toxin – producing *E. coli* isolates from wildlife meat as a threat to public health have been conducted (Miko et al., 2009).

There were more *E. coli* serogroups from faeces of pastoral cattle than Kafue lechwe antelopes, as pastoral cattle tend to graze varied epidemiologically in different areas because of their transhumant nature and are thus exposed to pasture with different microbiological profiles. Our finding may be in agreement with the existing knowledge which has always implicated cattle as a major reservoir of DEC (Trevena et al., 1999; Bach et al., 2002; Cookson et al., 2006). The presence of typeable DEC serogroups from pastoral cattle was higher in Blue-lagoon than Lochinvar. This could be attributed to the fact that Blue-lagoon is more transhumance in nature than Lochinvar, because pastoralist villages are much closer to the livestock/wildlife interface area. Furthermore, *E. coli* serogroups O29 and O125 could be ecologically adapted to respective area animal sanctuaries. Collectively, these results strongly intimate that the eventual predominance of ETEC O27 and EPEC O125 on the pasture may have important public health implications in the study area. Interestingly, we also noted that ETEC serogroup O15 and EIEC serogroup O29 are only confined to faecal samples from Kafue lechwe antelope and pastoral cattle, respectively; despite the latter being the most frequently isolated DEC serogroup from the pasture. We are tempted to assume that serogroup O29 was perhaps host specific though we would like to suggest that more research be conducted to substantiate these findings. A heightened interaction between wild animals and cattle is likely to lead to bacteria interspecies transfer (Gilbreath et al., 2009).

In this study, serogrouping and biochemical profiling of the bacterial isolates provided information on the relatedness between *E. coli* serogroups from Kafue lechwe and pastoral cattle. This study has shown that ETEC (O27 and O78) and EPEC (O125) are common serogroups haboured by Kafue lechwe antelopes and pastoral cattle. This is in agreement with the report by Rice et al. (1995) that wild animals and cattle sharing common areas would likely experience interspecies transfer. Therefore, current findings in our study may confirm that bacterial strains from Kafue lechwe antelopes are somewhat related to some strains from pastoral cattle posing a risk to the pastoral communities living in these areas.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Our study was supported in part by the following research grants: No. 15255021 from the International Scientific Research Program, Ministry of Education, Science and Culture, Japan and The University of Zambia Research grant, Zambia.

REFERENCES

Ammon AL, Petersen R, Karch H (1999). A large outbreak of hemolytic uremic syndrome caused by anusual sorbitol-fermenting strain of *Escherichia coli* O157:H-. J. Infect. Dis. 179:1274-1277.

Angulo F, Johnson K, Tauxe R, Cohen M (2000). Significance and sources of antimicrobial-resistant non-typhoidal *Salmonella* infections in the United States. Microbial. Drug Res. 6: 77-83.

Anonymous (2003). Feacal coliform an indicator organism. New Hampshire Department of Environmental Services. WD-WEB-18. www.des.nh.gov.

Bach SJ, McAllister TA, Veira DM, Gannon VPJ, Holley RA (2002). Transmission and control of *Escherichia coli* O157:H7. Can. J. Anim. Sci. 82:475-490.

Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ (1991). Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D. C.

Barrow IG, Feltham RKA (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria. Third Edition. Cambridge University Press.

Bettelheim AK (1998). Reliability of chromagar O157 for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroup. J. Appl. Microbiol. 85: 425-428.

Boyce TG, Pemberton AG, Wells JG, Griffin PM (1995). Screening for *Escherichia coli* O157:H7 – a nationwide survey of clinical laboratories. J. Clin. Microbiol. 33: 3275-3277.

Carter GR, John RC (1990). Diagnostic Procedures in Veterinary Bacteriology and Mycology. Fifth Edition. Academic Press, Inc. 107-128.

Cookson AL, Taylor SC, Attwood GT (2006). The prevalence of Shiga toxin-producing *Escherichia coli* in cattle and sheep in the lower North Island, New Zealand. N. Z. Vet. J. 54: 28-33.

Fairbrother JM, Nadeau E (2006). *Escherichia coli*: on farm contamination of animals. Rev. Sci. Tech. Off. Int. Epiz. 25: 555-569.

Garcia MI, Le Bouguenec C (1998). Enteric infections due to *Escherichia coli*. Clin. Microbiol. Infec. 4: 38-43.

Germani Y, Chaplain JC, Pochemaliky G, Degenne P, Brethes B (1985). Epidemiologic study of *Escherichia coli* isolated from infantile diarrhea cases on the Wallis and Futuna Islands (French Overseas Territory). Bull. Soc. Pathol. Exot. Filia. 78: 141-149.

Gilbreath JJ, Shields MS, Smith LR, Farrel LD, Sheridan PP, Spiegel KM (2009). Shiga toxin producing *Escherichia coli* and the genes encoding them, in faecal samples from native Idaho ungulates. Appl. Environ. Microbiol. 75: 862-865.

Hirvonen JJ, Siitonen A, Kaukoranta SS (2012). Usability and performance of CHROMagar STEC medium in detection of Shiga Toxin producing *Escherichia coli* strains. J. Clin. Microbiol. 50: 3688-3690.

Hornitzky MA, Mercieca K, Bettelheim KA, Djordjevic SP (2005). Bovine feces from animals with gastrointestinal infection are a source of serologically diverse atypical enteropathogenic *Escherichia coli* and shiga toxin-producing *E. coli* strains that commonly possess intimin. Appl. Environ. Microbiol. 71: 3405-3412.

Kaper JB, Nataro JP, Mobley HL (2004). Pathogenic *Escherichia coli*.

Nat. Rev. Microbiol. 2:123-140.
Keene WE, Sazie E, Kok J, Rice DH, Hancock DD, Balan VK, Zhao T, Doyle MP (1997). An Outbreak of *Escherichia coli* O157:H7 Infections Traced to Jerky made from Deer Meat. J. Am. Med. Assoc. 277:1229-1231.
Kruse H, Kirkemo AM, Handeland K (2004). Wildlife as source of Zoonotic Infections. Emerg. Infect. Dis. 10: 2067-2072.
Levine MM (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohaemorrhagic and enteroadherent. J. Infect. Dis. 155: 377-389. March SB, Ratnam S (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. 23:869-872.
Miko A, Pries K, Haby S, Steege K, Albrecht N, Krause G, Beutin L (2009). Assessment of Shiga toxin-producing *Escherichia coli* isolates from wildlife meat as potential pathogens for humans. Appl. Environ. Microbiol. 75:6462-6470.
Nataro JP, Kaper JB (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11:142-201.
O'Sullivan J, Bolton DJ, Duffy G, Baylis C, Tozzoli R, Wasteson Y, Lofdahl S (2006). Methods for detection and molecular characterization of pathogenic *Escherichia coli*. Co-ordination Action Food-CT-20006-036256. Pathogenic *Escherichia coli* Network. www.antimicrobialresistance.dk/data/ima.
Presterl E, Zwick HR, Reichmann S, Aichelburg A, Winkler S, Kremsner GP, Graninger W (2003). Frequency and Virulence properties of diarrheagenic *Escherichia coli* in children with diarrhea in Gabon. Am. J. Med. Hyg. 69:406-410.
Quinn PJ, Carter ME, Markey B, Carter GR (2002). Clinical Veterinary Microbiology (First Edition). Mosby 209-247.
Rabatsky-Her T, Dingman D, Marcus R, Howard R, Kinney A, Mshar P (1987). Deer meat as the source for sporadic cases of *Escherichia coli* O157:H7 infection, Connecticut. Lancet 2: 276-280.
Regua AH, Bravo VLR, Leal MC, Lobo Leite MEL (1990). Epidemiology survey of the enteropathogenic *Escherichia coli* isolated from children with diarrhoea. J. Trop. Pediatr. 36:176-179.
Rice DH, Hancock DD, Besser TE (1995). Verotoxigenic *E. coli* O157 colonization of wild deer and range cattle. Vet. Rec. 137: 524.
Ryder WR, Wachsmuth KI, Buxton A, Evans G D, Dupont LH, Mason E, Barrett FF (1976). Infantile diarrhea produced by heat-stable enterotoxigenic *Escherichia coli*. N. Engl. J. Med. 295: 849-853.
Seker E, Yardimci H (2008). First isolation of *Escherichia coli* O157:H7 from faecal and milk specimens from Anatolian water buffaloes (*Bubalus bubalus*) in Turkey. J. S. Afr. Vet. Assoc. 79(4):167-170.
Siamudaala VM, Muma JB, Munag'andu HM, Mulumba M (2003). Conservation and development interventions: at the wildlife/wildlife interface: implication for wildlife, livestock and human health: 14th to 15th August 2003. Durban, South Africa; Veterinary challenges regarding the utilisation of the Kafue lechwe (Kobus leche kafuensis) in Zambia. 75-80.
Stafford KJ (1991). A review of diseases of parasites of the Kafue lechwe (*Kobus leche Kafuensis*). J. Wildl. Dis. 27: 661-667.
Trevena WB, Willshaw GA, Cheasty T, Domingue G, Wray C (1999). Transmission of Vero Cytotoxin Producing *Escherichia coli* O157 Infection from Farm Animals to Humans in Cornwall and West Devon. Commun. Dis. Public Health 2:263-268.
Zhu C, Harel J, Jacques M, Desautels C, Donnenberg MS, Beaudry M, Fairbrother JM (1994). Virulence properties and attaching-effacing activity of *Escherichia coli* O45 from swine post weaning diarrhea. Infect. Immun. 62:4153-4159.