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Predictors and risk factors for the intestinal shedding of *Escherichia coli* O157 among working donkeys (*Equus asinus*) in Nigeria

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ABSTRACT

Objectives: *Escherichia coli* are an important group of bacteria in the normal gastrointestinal system but can sometimes cause infections in domestic animals and man. Donkeys are routinely used as multipurpose animal but details of burdens of potentially infectious bacteria associated with it are limited. The prevalence and associations between intestinal shedding of *E. coli* O157 and animal characteristics and management factors were studied among 240 randomly selected working donkeys in north-western Nigeria.

Design: Four local government areas, of Sokoto State in north-western Nigeria were recruited in this study. A multistage randomised cluster design was used to select subjects and donkey owners within selected zones. Confirmation of infection was based on bacterial culture, isolation and biochemical test for *E. coli* O157 from faecal samples.

Results: Of the total bacteria isolated, 203 of the 329 (61.70 per cent) were *E. coli*, 76 of which was *E. coli* serotype O157. A multivariable logistic regression model was used to examine the relation between intestinal shedding of *E. coli* O157 and selected variables. The analysis yielded five potential predictors of shedding: soft faeces in donkeys, Akaza and Fari ecotypes of donkey were positive predictors while maize straw as feed and sampling during the cold dry period were negative predictors.

Conclusions: This study concludes that controlling intestinal shedding of *E. coli* O157 among working donkeys in Nigeria is possible using the identified predictors in planning appropriate interventions to reduced human risk of infection.

INTRODUCTION

Escherichia coli are a group of intestinal microflora in human beings and animals that are usually harmless (Greenland and others 2009). While many of the *E. coli* gut microflora are innocuous, their build-up due to gut stasis, disruptions of the intestinal activities or a sudden change of diet can enhance the accumulation of certain toxins associated with these organisms and cause disease conditions. Some strains of *E. coli* particularly produce powerful toxins that can cause intestinal or extraintestinal diseases (Kaper and others 2004). These strains include the verotoxin-producing *E. coli* (VTEC) also known as shiga toxin-producing *E. coli* (STEC) (Bettelheim and Beutin 2003). The STEC that causes haemorrhagic colitis and haemolytic uraemic syndrome is called enterohemorrhagic *E. coli* (Nataro and Kaper 1998), and it is recognised as an important foodborne pathogen (Gyles 2007). Even healthy animals can harbour human enteric pathogens, many of which have a low infectious dose (Bell and others 1994). Although cattle are the main reservoirs of human pathogenic VTEC, there is evidence that sheep, deer, dogs, poultry and goats can also carry the VTEC strains (Callaway and others 2006, Reinstein and others 2007, Greenland and others 2009). *E. coli* organisms are also classified into serogroups based on the heat-stable 'O' and heat-labile surface 'K' or flagellar 'H' antigens (Vosti and others 1964). The *E. coli* O157:H7 serogroup is the most important cause of severe foodborne illnesses in living organisms and severe infection can result in case fatality of up to 50 per cent. This serogroup among others carries the shigatoxin 1and 2 and the gene responsible for effacement (eae gene) (Gannon and others 1993).

The primary mode of transmission for *E. coli* is the faecal-oral route (contaminated food, milk and water), but there are other possible means of transmission, because animal fur, hair, skin and saliva often harbour faecal material with the infective organism (Keen and Elder 2002, Varma and others 2003, De Schrijver and others 2008).

Donkeys (*Equus africanus asinus*) belong to the family *Equidae*. They are especially useful in arid and semiarid locations in sub-Saharan Africa, because of their hardiness and ability to survive where oxen and most other animals do not thrive. In Nigeria, the population of working donkeys is estimated at over one million; they are classified according to the ecoclimatic conditions and most of them are distributed in the extreme northern region (Blench and others 1992, Pearson and others 1999). They are particularly useful for transport, pulling carts, farm tillage, threshing, fetching/carrying water, milling and other energy-intensive activities, especially in the rural and semiurban locations where road networks are unavailable to motorised vehicles. An adult donkey weighs between 90 kg and 210 kg depending on the breed. Donkeys depend on a low calorie fibre diet (e.g. straw, husks, hay, fresh fodder and dried grasses) for most of their lives (Pearson and others 1999).

Donkeys are known to harbour certain diseases, including trypanosomiasis, babesiosis, African horse sickness, the equine herpes virus, equine influenza, rabies, horse pox, mange and glanders, but their importance in transmitting some zoonotic diseases is not well known. Given the current effort to promote the use of donkeys in drier areas of the world and consequent potential for increased human-animal (donkey) interaction, it is vital to understand donkeys' susceptibility and resistance to disease, and the zoonotic potential of donkey diseases (Pearson and others 1999). Previous study on animal-originated human VTEC O157 infection have shown that only cattle and sheep play more role than donkeys in the epidemiology of the disease (Pritchard and others 2009).

To the authors' knowledge, no prior study has investigated predictors associated with intestinal shedding of *E. coli* O157 among working donkeys in Nigeria and few data exist elsewhere. In addition, there is little information on the diseases affecting donkeys based on data from the veterinary clinics in the country. The objective of this study therefore was to estimate the prevalence of *E. coli* O157 among working donkeys in parts of Nigeria and to identify animal characteristics and husbandry practices that could be potential predictors of intestinal shedding of *E. coli* O157.

MATERIALS AND METHODS

Study area and sampled population

Sokoto is a state in north-western Nigeria. Geographically, the state lies between the longitudes 4°0′E and 6°54′E and latitudes 12°N and 13°58′N. The main occupation of the people in the state is arable farming and rearing livestock. Sokoto State has the second-largest livestock population in Nigeria, with an estimated 3 million cattle, 3 million sheep, 5 million goats, 4600 camels, 52,000 donkeys and hosts of local and exotic poultry species. The State consists of 23 local government areas (LGAs) and is broadly divided into two agricultural zones, namely the northern (comprising of 12 LGAs) and the western (11 LGAs) zones (Fig 1).

This study was conducted between May 2009 and April 2010 in Sokoto State previously described above. To select the study sample, simple balloting was used to pick LGAs in the stratified agricultural zones. Four LGAs were selected from each zone, namely Wurno, Isah, Illela and Tangaza from the northern zone, and Tambawal, Bodinga, Yabo and Sokoto North from the western zone. A multistage randomised cluster design sampling method was then used to select subjects and donkey owners within each of the selected LGAs. Sample collections were done principally to target market days for each of the selected LGAs.

Selection of participants, questionnaire administration, data retrieval and sampling

Four research assistants were recruited and trained in questionnaire administration, retrieval, assessment of scoring criteria and sample collection in a prestudy orientation. Severity of loss of body condition in the donkeys was assessed using the scorecard according to Pearson and Ouassat (2000) as follows: (1) Severe loss of body condition (very thin to less thin); (2) Mild loss of body condition (less than moderate to just moderate); and (3) No loss of body condition (less fat to very fat) and the present health conditions were scored based on the current observations/presenting conditions mentioned by the owners/handlers.

A pilot study was conducted using seven donkeys and their owners/handlers to test and validate the questionnaire and procedures for the data and samples required. For the main study, 30 donkey owners/handlers who had a minimum of one working donkey each were included from each selected LGAs, adding up to a total of 120 (30 owners/handlers×4 LGAs) individuals per zone. Since the two zones were included in the study, a total of 240 closed-ended structured questionnaires were administered to selected donkey owners/handlers on the spot during the visitations in each of the four selected LGAs.

The questionnaire was used to collect data comprising 21 variables with 55 options and it focused on management and husbandry practices relating to the working donkeys. A comprehensive list of variables that have been thought to predict *E. coli* O157 among working donkeys were analysed (Table 1), and the descriptive result on total number of donkeys positive for *E. coli* O157 serotype is shown in Table 1. Since an on-the-spot assessment was conducted, all the 240 donkey owners and handlers contacted responded to the interview (100 per cent return rate) and no respondent had more than 1 donkey. Faecal samples were collected from each donkey at the same time that the questionnaire was being administered, and these samples were taken directly from the rectum of each donkey (n=240), and were transported to the Microbiology Laboratory, Department of Medical Laboratory Sciences, College of Health

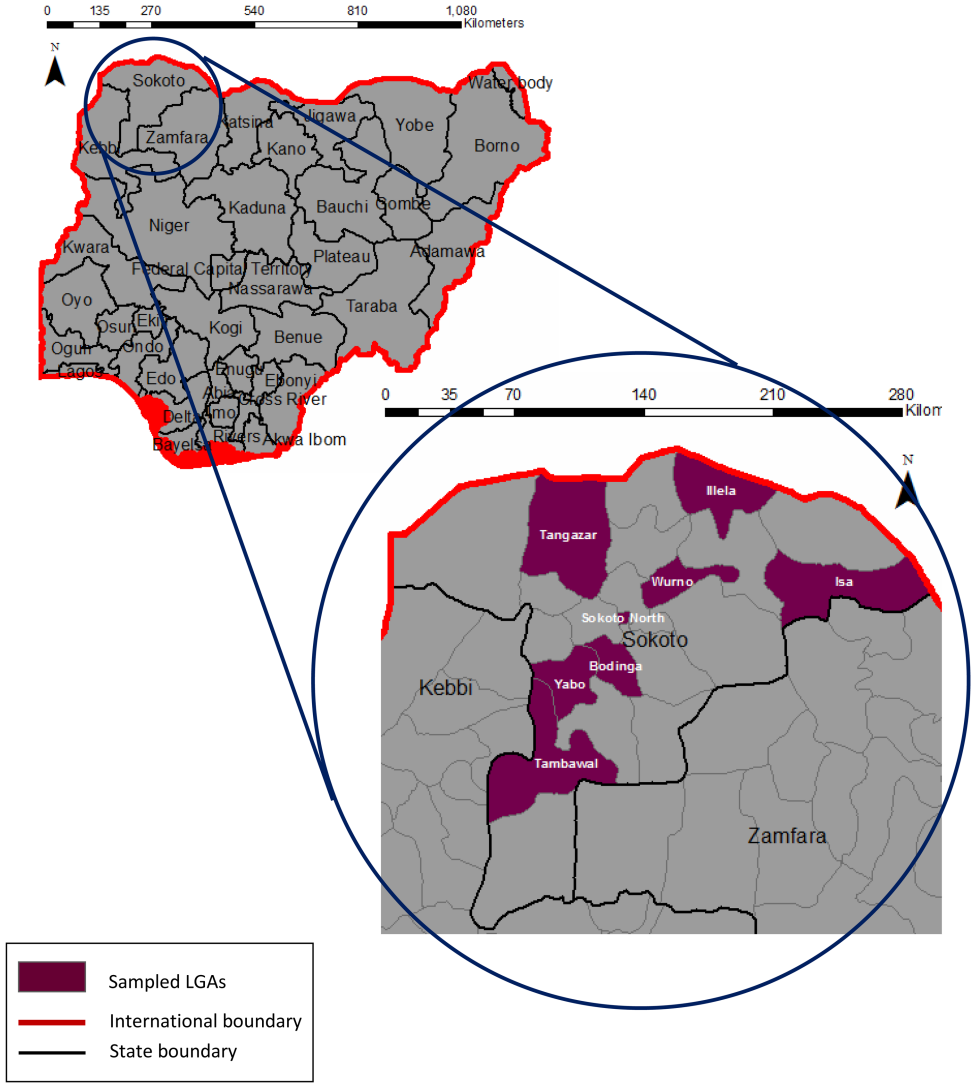

FIG 1: Map of Sokoto State showing boundaries (national and international), agricultural zones and selected (local government areas) LGAs for the study

Sciences, Usmanu Danfodiyo University Sokoto, Nigeria, immediately for processing.

Microbiological and biochemical tests were conducted to characterise the bacterial organisms according to standard techniques (Klein and others 2002, Voetsch and others 2004, Kotgire 2012). Briefly, faeces were observed for consistency, presence of blood, mucous, worms and colour. Cellular exudates were checked for using methylene blue as described by Kotgire (2012). Wet mount were carried out using the hanging drop method. Prepared faecal samples were plated on MacConkey's agar and a selective medium (Sorbitol MacConkey's agar) to identify *E. coli* O157. Further screening for *E. coli* O157 was performing using an antigen-specific latex-agglutination test (Rosario and others 1991). Although few other bacterial organisms were picked alongside the *E. coli* O157, further identification was not carried out on them since they fell outside the objective of this study.

Statistical analysis

Statistical analysis was performed using Stata version 10.0 by StataCorp, Lakeway Drive, College Station, Texas, USA. An initial descriptive analysis of the proportions of faecal samples confirmed positive for *E. coli* O157 in donkeys, stratified by animal and management explanatory variables (Table 1), was conducted in order to identify variables that might be of value for further investigation. Logistic regression analysis was performed with intestinal shedding of *E. coli* O157 (yes=1/no=0) as the observed outcome (dependent variable) based on the microbiological and biochemical test results. Only variables that had unconditional associations with the outcome that were significant using a probability value of $P<0.25$ in the univariable analysis (Table 2) were included for further analysis using a multivariable logistic regression model (Dohoo and others 2009). The correlation among explanatory variables was first checked using a multicollinearity analysis and the observation of

TABLE 1: Donkey ecotypes, sex, definition of potential predictors of intestinal shedding of *Escherichia coli* O157 and prevalence of *Escherichia coli* O157 among working donkeys in Nigeria

		Sex			
	Variable	**Female**	**Male**	**Total number of donkeys**	**Total number of donkeys positive for *E. coli* O157 serotype (+within ecotype %).**
Donkey ecotypes	Aura	32	43	75	22 (29.33%)
	Ehokusu	24	68	92	26 (28.26%)
	Fari	10	2	12	6 (50.00%)
	Jangora	7	19	26	8 (30.77%)
	Akaza	11	14	25	12 (48.00%)
	Duna	3	4	7	1 (14.29%)
	Goho	0	3	3	1 (33.33%)
	Total	87	153	240	76 (31.67%)

Variable	**Description**
Sex	Sex of donkey at the time of sampling (male/female)
Cleaning frequency	The frequency of cleaning the donkey house (daily/any other day)
Purpose for the donkey	Purpose of keeping donkeys (one, two, three or more purposes)
Faecal consistency	Consistency of faecal sample collected during sampling (watery, semisolid or solid)
Duration of diarrhoea	Duration of any previous diarrhoea observed by the owner (days, weeks or months)
Severity of loss of body condition	Severity of loss of body condition observed during sampling (low, mild or severe)
Ecotype*	Ecotype of donkey
Age	Age of donkey based on dentition, in years, at the time of sampling
Feed type†	Feeding of the donkey on single or multiple types of feed in addition to wet grasses (1, 2, 3 or 4 types)
Feeding method	Feeding method applied on the donkey (field grazing, zero grazing/hand-feeding)
Feed supplement	Feed supplement provided in feed for donkey (yes/no)
Present health condition	Health condition of the donkey during sampling (no illness, 1, 2 or more signs of diseases)
Feeding frequency	The frequency of feeding the donkey (any one time, two times daily or three times daily)
Keeping donkey with other animals	Keeping donkey with other animals (don't keep, one animal, more than one animal)

*Ecotype is a subdivision of an ecospecies/species consisting of a population that is adapted to a particular set of environmental conditions
†Feed types include 1: Rice chaff (*Dussa*); 2: Wheat chaff (*Dussa*); 3: Maize straw (*Kara*); and 4: Dry grasses (*Hakki*). These feed types are mixed in certain instances. All local names are italicised

mean variance inflation factors (VIF) (Table 3). A preliminary model was developed using stepwise backward removal of variables. Differences between nested models were assessed using likelihood ratio tests. Variables were retained in the multivariable model if they improved model fit significantly ($P \leq 0.05$), using the Hosmer-Lemeshow goodness-of-fit (GOF) χ^2 test. Results are reported as crude OR (OR_c) in univariable analysis and adjusted OR (OR_a) in multivariable analysis with 95% CIs.

RESULTS

Bacteriological analysis

A total of 235 faecal samples were positive for the presence of one or more bacterial organisms (97.92 per cent) by culture and specifically *E. coli* was identified in 202 samples representing 84.58 per cent of the total faecal samples (240). Only 31.67 per cent (76/240) of the faeces collected from the seven different ecotypes of working donkeys were positive for *E. coli* O157.

Results of univariable logistic regression analysis

No evidence of multicollinearity amongst any of the categorical variables was identified (mean VIF=1.23, Table 3). Of the 48 potential predictor variables tested in a χ^2 univariable analysis, only 11 variables (with bold font P values, Table 2) showed potential association ($P<0.25$) with intestinal shedding of *E coli* O157 and these were considered for inclusion in the multivariable analysis (Table 2).

Using the stepwise backward elimination procedure, only five variables were retained in the final model (Table 3). The Fari (OR_a=3.40, P=0.05, 95% CI 0.98 to 11.78) and Akaza (OR_a=2.77, P=0.02, 95% CI 1.16 to 6.66) ecotypes of donkey were the most susceptible to the risk of intestinal shedding of *E. coli* O157 compared with other ecotypes in this study (Table 3). Similarly, soft faecal consistency is the most important predictor for intestinal shedding of *E. coli* O157 (OR_a=4.16, P=0.002, 95% CI 1.70 to 10.19). Contrastingly, both the feeding of maize straw (OR_a=0.52, P=0.05, 95% CI 0.27 to 0.99) and sampling during the cold dry period (harmattan) in Nigeria (OR_a=0.53, P=0.05, 95% CI 0.28 to 1.00) were negative predictors for intestinal shedding of *E. coli* O157 (Table 3).

The logistic regression χ^2 was 22.75 with 4 degrees of freedom and the Hosmer-Lemeshow GOF χ^2 was 2.59; P=0.63, an indication of the good model fit to the data.

TABLE 2: Results of the univariable logistic regression analysis for associations between the intestinal shedding of *Escherichia coli* O157 and various animal and management factors for 240 samples

Variable	Category	N positive samples	N negative samples	OR_c	95% CI	P value
Sampling season	Cold dry	19	64	1.00	Ref.*	
	Warm wet	36	57	0.47	0.24 to 0.91	**0.24**
	Hot dry	21	43	0.61	0.29 to 1.27	**0.18**
Donkey ecotype	Akaza	12	13	1.00	Reference	
	Aura	22	53	2.21	0.86 to 5.68	**0.09**
	Ehokusu	26	66	2.33	0.92 to 5.85	**0.06**
	Duna	1	6	5.29	0.65 to 138.1	**0.11**
	Goho	1	2	1.81	0.12 to 58.72	0.63
	Fari	6	6	0.93	0.22 to 3.85	0.91
	Jangora	8	18	2.05	0.65 to 6.72	**0.21**
Sex	Female	32	55	1.00	Reference	
	Male	44	109	0.69	0.40 to 1.22	**0.20**
Age	≥10 years	5	16	1.00	Reference	
	1–3 years	23	44	1.66	0.55 to 5.65	0.37
	4–6 years	38	81	1.50	0.52 to 4.87	0.46
	7–9 years	10	23	1.38	0.40 to 5.26	0.60
Keeping of other animals	No	23	32	1.00	Reference	
	Yes	53	132	0.56	0.30 to 1.05	**0.07**
Severity of loss of body condition	Low	10	34	1.00	Reference	
	Mild	39	86	1.53	0.70 to 3.57	0.28
	Severe	27	44	2.07	0.89 to 5.05	**0.09**
Duration of diarrhoea	≤7 days	62	153	1.00	Reference	
	≥7 to ≤28 days	7	7	2.46	0.79 to 7.61	**0.09**
	≥30 days	7	4	4.29	1.20 to 17.24	**0.15**
Faecal consistency	Hard	29	77	1.00	Reference	
	Normal	33	76	1.15	0.64 to 2.09	0.63
	Soft	14	11	3.34	1.35 to 8.43	**0.006**
Purpose of keeping donkey†	One activity	2	6	1.00	Reference	
	Two activities	56	119	1.41	0.29 to 10.42	0.68
	Three activities	17	28	1.80	0.34 to 14.25	0.49
	>three activities	1	11	0.29	0.009 to 4.52	0.31
Present health condition	No illness	9	13	1.00	Reference	
	One sign noticed	50	105	0.69	0.27 to 1.79	0.42
	Two signs noticed	16	44	0.53	0.19 to 1.52	**0.21**
	Multiple signs	0	0	–	–	–
Cleaning frequency	Daily	12	18	1.00	Reference	
	Every other day	64	146	0.66	0.30 to 1.49	0.30
Feed type	Rice chaff	4	7	1.00	Reference	
	Wheat chaff	47	87	0.95	0.26 to 3.86	0.93
	Maize straw	20	60	0.59	0.15 to 2.50	0.42
	Dry grass straw	5	10	0.88	0.16 to 4.93	0.87
Feeding frequency	Thrice daily	35	53	1.00	Reference	
	Once daily	7	20	0.53	0.19 to 1.37	**0.19**
	Twice daily	34	91	0.57	0.32 to 1.02	**0.05**
Feeding method	Field grazing	3	6	1.00	Reference	
	Zero grazing	73	158	0.92	0.22 to 4.63	0.91
Feed supplementation	No	55	127	1.00	Reference	
	Yes	21	37	1.31	0.69 to 2.44	0.39

Crude OR (OR_c) with 95% CIs are reported
*Ref.=reference category to which other categories are compared
†Purposes of keeping donkeys include the following: transport, pulling carts, farm tillage, threshing, fetching/carrying water, milling and other energy-intensive activities

DISCUSSION

E. coli O157 serotypes were shed by a moderately large proportion of the donkeys (31.67 per cent). This organism may serve as a contaminant or it may have direct or indirect zoonotic implications. The authors isolated certain other bacteria from donkey faeces but these were not the main focus of the present study. Derlet and Carlson (2002) previously confirmed that donkeys are

TABLE 3: Final multivariable logistic regression model of predictors and risk factors associated with the intestinal shedding of *Escherichia coli* O157 among working donkeys in Nigeria for 240 faecal samples

Variable	Level	OR_a	95% CI	se	Z score	P value
Severity of loss of body condition	Severe	1.85	0.99 to 3.47	0.59	1.93	0.05
Faecal consistency	Hard	0.20	0.08 to 0.53	0.10	−3.23	0.001
	Normal	0.23	0.09 to 0.60	0.11	−3.01	0.003
Frequency of feeding	Thrice daily	2.30	1.25 to 4.23	0.72	2.68	0.007
Sampling season	Cold dry	0.44	0.23 to 0.85	0.15	−2.45	0.014
Ecotype	Akaza	2.57	1.06 to 6.25	1.17	2.08	0.037

Crude ORs (OR_c) and adjusted ORs (OR_a) with 95% CIs are reported
Hosmer-Lemeshow GOF χ^2=7.27; P=0.51. Mean VIF=1.23
GOF, goodness-of-fit; VIF, variance inflation factors.

reservoirs of certain zoonotic organisms. Since these animals are used for work regularly and the handlers may eat between tasks, sometimes without thorough washing of their hands or observation of strict hygienic measures, they stand the risk of inadvertent exposure to these organisms from donkey. Interestingly, donkey faeces is sometimes used in rural communities to rub/coat the inner walls of mud buildings where human beings live, and this creates a strong potential for contamination and/or infection of those persons who perform this work and people who live in and touch these surfaces (Derlet and Carlson 2002, Pritchard and others 2009). In addition, there is a huge risk of water and environmental contamination since these animals are reared extensively.

Significantly, the predictors that were positively associated with intestinal shedding of *E. coli* O157 included soft faecal consistency, the Fari and Akaza ecotypes of donkey (Tables 3 and 4). Since these Fari and Akaza ecotypes of donkeys seem to have greater susceptibility compared with the other ecotypes, perhaps these ecotypes are not as adapted to the highly fibrous feeds that are fed to these animals in Nigeria or there are intrinsic genetic differences that are yet to be studied. However, there is no proof to support these postulations. It may be possible also that some unknown innate factors predispose these ecotypes to more infection by *E. coli* O157. Since these animals are fed with the largely fibrous diet which is known to increase intestinal transit time in animals with probable increased levels of intestinal *E coli* O157 shedding and associated colienteritis-colisepticaemia, there may be severe diarrhoea (Harvey and others 1973). As such, subclinical diarrhoea may be a consequence of the feed type and *E. coli* O157 build-up in the intestine rather than a direct predictor of the organism. It should be noted, however, that the shedding of *E. coli* in faeces does not always correlate with clinical disease since animals carry this organism regularly.

Finally, sampling during the harmattan-cold dry period (which is usually associated with a surge in animal and human diseases) reduced the risk of shedding of *E. coli* O157 by about half. The reason for this association cannot be immediately established but it is known that during the dry cold harmattan period, free-range donkeys have access to less water, often pass hard dry faeces which are further subjected to the desiccating effects of the harsh environment. It is instructive that faecal sampling for prevalence study in animal within the country may not reveal a true prevalence if collected only during the harmattan period (dry, dusty and cold north-easterly trade wind).

In the course of the interviews, the authors observed that all the donkeys sampled are kept under a traditional (extensive) system of management despite their enormous contributions to the livelihood of the rural dwellers. This husbandry practice is probably not much different from what exists in other parts of Nigeria and

TABLE 4: Correlation matrix for the set of explanatory variables in the analysis of potential predictors of intestinal shedding of *Escherichia coli* O157 among working donkeys in Nigeria

Variable	A	B	C	D	E	F	G	H
A	1							
B	−0.11	1						
C	0.10	0.11	1					
D	0.05	−0.23	−0.13	1				
E	0.02	−0.12	−0.19	0.57	1			
F	0.11	0.04	0.04	−0.07	0.01	1		
G	−0.07	−0.18	0.02	−0.03	0.06	0.13	1	
H	0.03	−0.21	−0.17	0.36	0.35	−0.09	0.24	1

A=Sex, B=Cleaning frequency, C=Feeding frequency, D=Keeping donkey with other animals, E=Feeding with other animals, F=Duration of diarrhoea, G=Severity of loss of body condition, H=History of previous disease

indeed Africa (exposure of these donkeys to inclement weather, diseases and nutritional deficiencies) (Research Inventory Management 1992). The generally held perception that donkeys are very hardy and will withstand adverse conditions, and are resistant to diseases without a drop in outputs may underlie this observed poor management system.

More male donkeys were observed and sampled because they are more frequently owned. The owners' preference for male donkeys is linked to their perception of comparative greater strength as draught animals and probably because female donkeys do not work satisfactorily particularly when they are pregnant (more so, in their last trimester). In this analysis, the authors found out that female donkeys are more likely to shed *E. coli* O157 compared with male donkeys. Keeping of donkeys or with other animals feeding them together does not influence the shedding of *E. coli* O157.

This work has some limitations. While the *E. coli* serotypes were isolated, the isolates were not checked for shigatoxin principles and or the eae gene. The authors are aware that these factors are important in the virulence properties of *E. coli* O157 and its bacteria zoonotic principles. The authors acknowledge that the outcome of this analysis is based on the limited sampled population and small geographical spread. Future studies may benefit from using geographically more diverse samples from different parts of the West African subregion. The test systems may also present with a degree of non-specificities and poor sensitivities. It is in the authors' opinion though that the multiple microbiological tests applied in this study will reduce errors/misclassifications due to this effect. Nevertheless, this study has confirmed the presence of some potential infectious and zoonotic pathogens and contaminants in working donkeys in Nigeria and has associated these pathogens and contaminants with particular predictors thus opening up opportunities for further research in this field.

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Contributors JTJ, AS, FMT and FOF designed the study. JTJ, MBA, MKG and AS collected processed samples. AS and FOF conducted statistical analysis and interpretation of data. FMT, MBA, FOF and JPK coordinated the study. All authors contributed to drafting the manuscript.

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