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Isolation and characterisation of *Listeria* species from ruminants in Maiduguri north–eastern Nigeria

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A cross sectional study was carried out to determine the prevalence of *Listeria* species in ruminants in Maiduguri. Three hundred faecal samples were randomly collected from ruminants at the Maiduguri central abattoir from January – March, 2011. One hundred faecal samples each were collected from cattle, sheep and goat at ante mortem by balloting comprising of fifty samples each from male and female animals. Forty (13%) of the three faecal samples were identified as positive *Listeria* species. Out of the forty positive samples, 15(37.5%) were from cattle, 16(40%) from sheep and 9(22.5%) were from goats, the difference was not statistically significant (P > 0.05). The sex distribution of the animals positive for *Listeria* species showed that 21(7%) of the positive samples were from males and 19 (6%) were from females. The sex specific prevalence in the animal species sampled was not statistically significant (P > 0.05). Biochemical characterisation of the *Listeria* isolates showed *Listeria monocytogenes* 4(10%), *Listeria innocua* 17 (42.5%), *Listeria ivanovii* 12 (30%), *Listeria seeligeri*4(10%), and *Listeria welshimeri* 3(7.5%). This study affirms the isolation of *Listeria* species in the faeces of ruminants brought for slaughter at the abattoir which could serve as a source of contamination of meat meant for human consumption.

Key words: Listeria species, ruminants, north-eastern Nigeria.

INTRODUCTION

The genus *Listeria* represents a group of closely related, Gram-positive, facultative anaerobic, non-spore-forming, rod-shaped bacteria 0.5 µm in width and 1–1.5 µm in length, and with a low G+C content. There are a total of 7 species of *Listeria* as *Listeria monocytogenes, Listeria innocua, Listeria welshimeri, Listeria seeligeri, Listeria ivanovii, Listeria murray*iand *Listeria grayi* (Gebretsadik et al., 2011). *L. monocytogenes* and *L. ivanovii* are pathogenic (Liu et al*.,* 2006). While *L. monocytogenes* infects both man and animals, *L. ivanovii* is principally an animal pathogen that rarely occurs in man (Low and Donachie, 1997). Sporadic human infections due to *L. seeligeri* and *L. innocua* have also been reported (Perrin et al*.,* 2003). The organism is ubiquitous in nature often found in animal products such as raw milk and raw meat due to unsanitary practices during milking and slaughtering (Schuchat et al*.,* 1991). Prevalence of *Listeria* species from milk, meat, vegetables, faeces and environmental samples have been reported by several authors (Ikeh et al., 2010; Atil et al*.,* 2011; Yakubu et al., 2012; Abay et al., 2012; Brian et al*.,* 2012). The presence of *Listeria* specie in faeces was associated with the prevalence of these

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bacteria in feed (Buncic, 1991; Sanaa et al., 1993). *L. innocua*exists in the environment and in animal intestines quite commonly as such it was reported to be more commonly found in food than other *Listeria* species (Erol and Sireli, 1999). Previous studies reported that *Listeria* specie was found between 0.5 and 67% of the isolation rate in cattle and sheep feces (Skovgaard and Mogen, 1988; Husu, 1990; Vilar et al., 2007). Listeriosis is of major veterinary importance in cattle, sheep, and goats (Low and Donache, 1997), not only due to significant economical losses in livestock production through morbidity and high mortality but also with regard to food safety and public health representing a possible link between the environment and human infection. There are paucity of information on the prevalence of *Listeria* species in ruminants in Maiduguri. The present study therefore aimed at characterisation and determination of the prevalence of *Listeria* species in ruminants (cattle, sheep and goats) in Maiduguri, north-eastern Nigeria.

MATERIALS AND METHODS

Study area

The study area (Maiduguri) is located in the arid zone of Borno State with an area of about 69,436 km^2 and lies within latitude 10-13°N and longitude 12-15°E. It lies within the savannah with low records of rainfall. The area falls in the tropical continental north with dry season of between 4 to 8 months, starting from October to May followed by a short rainy season from late June to early October. The state is located within the North Eastern corner of Nigeria and has boundaries with Chad to the North East, Cameroon to the East and Adamawa State to the South West. According to the 2005 census, the population is estimated to be 4,558,668 and ranked 12th in the country.

Sample collection

A total of three hundred (300) fecal samples were aseptically collected from Maiduguri central abattoir between January -March, 2011 from apparently healthy goats, sheep and cattle. Equal numbers of samples were collected from the animals, cattle (100), goat (100) and sheep (100) comprising fifty (50) samples each from male and female animals. The fecal samples were collected per rectum after proper restraint and put into a sterile sample bottle and transported to the Faculty of Veterinary Medicine Microbiology Laboratory in an ice pack for bacteriological analysis.

Bacteria isolation

Aseptically, 10 g of each sample was added to 90 ml *Listeria* enrichment broth (LEB) (Oxoid®) containing selective *Listeria* enrichment supplement in a bottle; this was homogenized for 2 min at room temperature and incubated at 37°C for 24-48 h. After incubation, a loopful of growth from LEB was streaked onto the surface of *Listeria* selective agar (Oxoid ®) and PALCAM agar. The plates were incubated at 37°C for 24 to 48 h. Typical *Listeria* colonies appeared greyish-black with a black zone in surrounding medium of both plates. This black colour was due to the utilization of esculin in the media. Presumptive colonies of *Listeria* species on both plates having a black colouration on PALCAM and LSA were streaked on nutrient agar slants incubated at 37°C for 24 h and stored at 4°C.

Presumptive colonies were subsequently subjected to Gram staining and further biochemical characterisation. Biochemical characterization was conducted using beta haemolytic reactions, catalase, oxidase, urease and acid production from (glucose, manitol, galactose, xylose and rhamnose) in order to differentiate the various *Listeria* species according to the methods of OIE (2008) manual.

Statistical analysis

The data collected was subjected to Fisher's Exact Chi-square test and odds ratio using Graph PadInstat statistical package to determine if there is significant association between sex and isolation of *Listeria* species in ruminants in the study area. A P value less than 0.05 was considered statistically significant. The prevalence rate and the odds ratio (OR) were calculated using (2x2) contingency table to test for association between isolation of *Listeria* species in the faeces and sex as well as animal species (cattle, sheep, goats).

RESULTS

A total of three hundred (300) faecal samples comprising of 100 samples each from cattle, sheep and goats were collected at the Maiduguri central abattoir and analysed for the presence of *Listeria* species. Forty (13%) out of the three hundred samples were presumptively identified as positive for *Listeria* species, while the remaining 260 (87%) samples were found to be negative for *Listeria* species. Out of the 40 positive samples, 21 (7%) were male (OR = 0.576 – 2.186) and 19 (6%) were female (OR = 0.457 – 1.735). There was no significant statistical association (P > 0.05) between male and female positive animals as regards to isolation of *Listeria* species (Table 1).

Table 2 shows the sex specific prevalence of *Listeria* species in cattle, sheep and goats in Maiduguri. *Listeria* species were isolated in fifteen (15%) out of the one hundred faecal samples collected from cattle, this consist of 7% males (OR = 0.284 – 2.568) and 8% females (OR = 0.389 – 3.516) which was not statistically significant (P > 0.05). Out of the hundred faecal samples collected from sheep,16% were positive for *Listeria* species comprising of 8% male and 8% female with the same (OR = 0.343 – 2.914) having no statistical difference (P > 0.05) between both sexes. A total of 9% out of the hundred faecal samples examined from goats were positive for *Listeria* species, out of which 6% were males (OR = 0.503 – 9.017) and 3% were females (OR = 0.110 – 1.987). There was also no statistically significant association (P > 0.05) between the sex of the animals and the isolation *Listeria* species.

The source specific prevalence of *Listeria* species in ruminants is shown in Table 3. Out of the fifteen cattle from which *Listeria* species were isolated, six each were *L. ivanovii* and *L. innocua* and one each were *L. monocytogenes*, *L. seelighreii* and *L. welshimeri*. The isolation rate of *Listeria* species in sheep was sixteen, these comprises 8 positive samples for *L. innocua*, 4 for *L. ivanovii* and 2 each for *L. monocytogenes* and *L. seeligheri*. *Listeria* species were isolated in 9 goats in the present study. These consist of 3 positive samples for *L. innocua*, 2 each for *L. ivanovii* and *L. welshimeri* and 1 positive sample each for *L. monocytogenes* and *L. seeligheri*.

Table 1. Sex specific prevalence of *Listeria* species in faeces of ruminants sampled in Maiduguri central abattoir (January – March 2011).

Sex	Positive	Negative	Total	X2	Odds ratio (OR)	95% CI on OR	
						Lower	Higher
Male	21(7%)	129(44%)	150(50%)	0.115*	0.122	0.576	2.186
Female	19(6%)	131(43%)	150(50%)		0.891	0.457	1.735
Total	40(13%)	260(87%)	300(100%)				

*The difference in the sex prevalence was not statistically significant (P > 0.05).

Table 2. Sex specific prevalence of *Listeria* species in faeces of cattle, sheep and goats in Maiduguri central abattoir (January – March 2011).

Animal	Sex	Positive	Negative	Total	X^2	Odds ratio (OR)	95% CI on OR	
							Lower	Upper
	Male	7	43	50		0.855	0.284	2.568
Cattle	Female	8	42	50	0.079*	1.170	0.389	3.516
	Total	15	85	100				
	Male	8	42	50		1.00	0.343	2.914
Sheep	Female	8	42	50	0.00*	1.00	0.343	2.914
	Total	16	84	100				
	Male	6	44	50		2.136	0.503	9.017
Goats	Female	3	47	50	1.099*	0.468	0.110	1.987
	Total	9	91	100				

*****The sex specific prevalence among the animal species was not statistically significant (P>0.05).

Table 3. Source specicific prevalence of *Listeria* species in faeces of cattle, sheep and goats in Maiduguri central abattoir (January – March 2011).

Animal specie	Total no. of isolates	*Listeria* species				
		L. monocytogenes	*L. ivanovii*	*L. seeligeri*	*L. innocua*	*L. welshimeri*
Cattle	15	1	6	1	6	1
Sheep	16	2	4	2	8	-
Goat	9	1	2	1	3	2
Total	40	4	12	4	17	3

DISCUSSION

An isolation rate of 13% *Listeria* species in the faeces of ruminants have been reported in the present study which is similar to the reports of Abay et al*.* (2012) who also reported the isolation rate of 22 and 10% of *Listeria* species in the faeces of cattle and sheep, respectively. The variation in the isolation rate could be due to the number of animals sampled and the difference in geographical locations or the sampling technique employed. In the present study, phenotypic methods of characterisation were employed using 300 animals comprising 100 each of cattle, sheep and goats. Other workers in different countries (Choi et al*.* 2001) in Korea, Miettinen et al. (2001) in Finland and Hassan et al*.* (2001) in Malaysia) have reported an incidence of between 62 and 85% of *Listeria* species in various foods. The findings here are similar to those of MacGowan et al*.* (1994) that the usual habitat of *Listeria species* is the intestinal tract of mammals and birds from where the organism enters the soil via animal droppings. From the results of the present study, more male animals (21 (7%)) were positive for *Listeria* species than female animals (19 (6%)). The difference in the sex specific prevalence was not statistically significant (P > 0.05). In cattle, more female cattle (8(8%)) are affected than male cattle (7(7%)) but the diffe-

rence was not significant statistically (P > 0.05). There was no difference in the number of male and female sheep that were positive for *Listeria i*n the present study. In the goats sampled in the present study, more male goats (6(6%)) were positive for *Listeria* species than females (3(3%)) but the difference was not statistically significant (P > 0.05).

The findings in the present study has affirmed the isolation of *Listeria* species in ruminants which may serve as reservoir for human pathogenic strains and therefore its impact on food safety cannot be over-emphasised (Borucki et al*.,* 2004; Nightingale et al*.,* 2004; Okwumabua et al*.,* 2006). Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin such as meats and dairy products (Schuchat et al*.,* 1992; Hood, 1993; Bockserman, 2000). Although the portal of entry of *L. ivanovii* has not been fully established, *L. ivanovii* infection in ruminants is associated with eating spoiled silage or hay, as happens with *L. monocytogenes*, suggesting foodborne origin (Gaya et al*.,* 1996).

Ruminant farm animals play a key role in the persistence of *Listeria* spp. in the rural environment via a continuous faecal-oral cycle (Vazquez-Boland et al., 2001). In the present study, the majority of *Listeria* species isolated were *L. innocua* as it has been reported to be present in much larger numbers in feed than other species, and therefore has a higher chance of being detected in animal faeces. It has been reported that *L. innocua* isolated from beef minced meat and other *L. innocua* isolated from cattle faeces have 99% similarity (Abay et al*.,* 2012). Hence *Listeria* isolated in this study (faeces) could be considered as a potential risk for meat contamination that could play a role in the epidemiology of listeriosis.

*L. ivanovii*is considered to be mildly pathogenic and seems to affect almost exclusively ruminants, resulting in abortion, still-births, and neonatal septicemia, but not central nervous system infection (Low and Donachie, 1997; McLauchlin and Jones, 1999; V'azquez-Boland et al*.,* 2001). The association between the isolated pathogens and silage consumed by the ruminants could not be ascertained because no sample was collected from the silage and the type of the feed consumed by these ruminant were not collected for microbiological analysis, though it has been reported that investigation of an epidemiological link between silage feeding and listeriosis in ruminants gave inconsistent results. Whilst some studies could isolate matching *Listeria* strains in brains of affected animals and silage samples, others yielded unrelated strains (Mohammed et al*.,* 2009). Previous study reported a higher prevalence of the bacterium in samples collected from the immediate cattle environment (feed bunks, water through and beddings) and in cattle feces than in silage challenging the view that silage is the only source of Listeriosis (Mohammed et al*.,* 2009).

Conclusion and recommendations

The study established the findings that *Listeria* species are found in the faeces of ruminants (13%) in the study area. *Listeria* species were more commonly isolated in cattle (15%) and sheep (16%) than in goats (9%). *L. innocua*17 (42.5%) and *L. ivanovii*12 (30%) were the *Listeria* species more commonly isolated in the faeces of ruminants than the other species. We therefore recommend the use of good hygienic practices and standard procedures in the abattoir to minimise the level of contamination of meat.

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