



Prevalence of bacterial pathogens and serotyping of *E. coli* isolates from diarrhoeic lambs in Sokoto state, Nigeria

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Abstract

A total of 127 fecal samples from diarrhoeic lambs was collected and bacteriologically cultured to determine the prevalence of pathogenic bacteria causing diarrhoea in those lambs. The samples were pre-enriched in peptone water overnight before inoculation on MacConkey agar at 37°C for 24h. The results revealed the presence of *Escherichia coli* 42 (36.84%), *Salmonella sp* 18 (15.79%), *Klebsiella sp* 15 (13.16%), *Arcanobacterium pyogenes* 11 (9.65%) in that order. Other isolates were *Staphylococcus aureus* 10 (8.77%), *Shigella sp* 8 (7.02%), *Proteus vulgaris* 6 (5.26%) and *Streptococcus sp* 4 (3.15%). The most prevalent isolate was serotyped to identify the strains of the isolate involved in diarrhoea. Serotyping of *E. coli* revealed the strains obtained to be O157, O118, O111, O26, and O103, with prevalence rates of 31.0%, 21.4%, 19.0%, 16.7% and 11.9% respectively. This data supports the assertion of *E. coli* as being the most important bacterial pathogen involved in neonatal diarrhoea or colibacillosis and have important public health implication due to the involvement of serotype O157. It was concluded that there is the need for further research to determine the presence of virulent factors of the various serotypes isolated.

Key Words: bacteria, diarrhoea, *E. Coli*, lambs, Prevalence, Serotype

Introduction

Neonatal diarrhoea causes the death of many lambs and is of considerable economic importance to the sheep industry in many countries (Jensen, 1974; Ansari et al., 1978). It is the principal infectious condition affecting young lambs (Snodgrass, 1978) although according to Todd et al., (2008) it is a feature of intensive lambing systems where small paddocks or indoor lambing areas are subjected to build up of disease. Diarrhoea in lambs is a complex, multi-factorial disease involving the animal, the environment, nutrition and infectious agents (Tzipori, 1981; Nasr and Meghawery, 2007; Schoenian, 2006; Shulaw, 2009). The major causes of diarrhoea in lambs during the first month of life are bacteria, viruses, and protozoa (Schoenian, 2006) with lambs that do not receive adequate colostrum being at greatest risk of developing the disease (Shulaw, 2009). However, even animals that received adequate amounts of colostrum may still develop diarrhoea if the colostrum did not contain specific antibodies to these agents (Schoenian, 2006; Anon, 2009).

It is evident that the infectious agents capable of causing diarrhoea in lambs are numerous but the most important bacterial enteropathogens are the

Escherichia coli, *Salmonellae*, *Clostridium perfringens*, *Yersinia spp*, and *Campylobacter sp* (Schoenian 2006). *E. coli* causing scours are however the most important (Schoenian, 2006). *E. coli* infection has been associated with two forms of the disease in lambs: the enteric and septicemic infection (Ansari et al., 1978). *E. coli* is also the most frequently encountered microorganism in the food industry (Arshad et al., 2006). The close contact of young neonatal animals to older lambs and the build up of infectious agents in pens with resultant heavy challenge and exposure is critical to the young lambs coming down with diarrhoea and subsequently death. There is paucity of information on bacterial pathogens commonly associated with lamb diarrhoea particularly in northwestern Nigeria. This study was designed to evaluate the bacterial pathogens associated with lamb diarrhoea and to serotype the most commonly incriminated bacterial pathogen in diarrhoeic lambs in Sokoto state, Nigeria.

Materials and Methods

Ten flocks were randomly selected within Sokoto and environ. The total sheep population in the selected flocks was 684 out of which 246 were lambs. All the sheep were managed under semi - intensive system. Follow - up visits were made to the flocks every fortnight for a period of one year (November, 2006 to

October, 2007) to collect samples from diarrhoeac lambs during the period. Faecal samples were collected from the perineal region of 127 lambs that were diarrhoeac and transported to the laboratory in a cool box. The samples were processed at the microbiology laboratory of the School of Medical Laboratory Sciences, Usmanu Danfodiyo University Teaching Hospital. Samples were directly placed into peptone water and incubated overnight at 37°C as pre-enrichment. A loopful from each of the peptone water was then cultured onto MacConkey agar (MCA) (Oxoid, UK) and Sheep Blood Agar (SBA). All the cultured plates were incubated aerobically and anaerobically at 37°C for 24-48 hours. To obtain pure bacterial culture, suspect colonies were carefully picked and subcultured on blood agar and nutrient agar and incubated for 24h at 37°C. A stock culture from each isolate was then stored on NA further analysis at 4°C.

Isolation and Confirmation of *E. coli*

Preserved isolates were picked using wire loop and directly cultured on Levine eosin methylene blue, EMB (Oxoid, UK) agar. After incubation at 37°C for 24h, dark bluish green colonies with metallic sheen or those possessing dark centres with transparent colourless peripheries were restreaked on EMB or sheep blood agar plates and incubated. 3- 5 isolates with distinct colonial characteristics were obtained in pure culture and identified as *E. coli* based on morphology, Grams stain, oxidase, catalase tests and other biochemical tests as described by Quinn *et al.*, (2002).

Serotyping of *E. coli*

Serotyping of O antigen of *E. coli* was carried out using available O antigen containing *E. coli* antisera. 2ml of commercially prepared polyclonal *E. coli* antisera of serotypes O26, O118, O111, O157 and O103 sourced from Department of Agriculture and Veterinary Sciences, Queens University, Belfast, UK, were used. Each antiserum was diluted using the manufacturer's recommendation. Slide agglutination tests was then used to identify each serotype and positive result was recorded when small minute granules appeared.

Results

Laboratory cultures of bacterial pathogens associated with neonatal lamb diarrhea are presented in Table 1. Out of the 246 lambs, 127 (51.63%) had diarrhea. A total of 114 bacterial organisms were isolated representing eight genera of bacteria. *Escherichia coli* were the most prevalent bacterium isolated numbering 42 (36.84%) and was followed by *Salmonella* sp. as the second most prevalent 18 (15.79%). Other isolates were *Klebsiella* sp. 15 (13.16%), *Arcanobacterium pyogenes* 11 (9.65%), *Staphylococcus aureus* 10 (8.77%) and *Shigella* sp. 8 (7.02%). *Proteus vulgaris* 6 (5.25%) and *Streptococcus* sp. 4 (3.15%) were also isolated. Out of the 42 *Escherichia coli* isolates serotyped, the strains O157 (13); O118 (9); O111 (8); O26 (7) and O103 (5) were identified. The prevalence of *E. coli* serotypes isolated from diarrhoeac lambs in Sokoto metropolis and environs is shown in Table II. The highest serotype was O157 (31.0%) and the lowest was O103 (11.9%).

Table 1: Prevalence of bacteria species isolated from diarrhoeic lambs in Sokoto and environs

Bacteria spp from diarrhoeic lambs	Number isolated	% isolates
<i>Shigella</i> spp	8	7.02
<i>Proteus vulgaris</i>	6	5.26
<i>Escherichia coli</i>	42	36.84
<i>Salmonella</i> spp	18	15.79
<i>Streptococcus</i> spp	4	3.51
<i>Staphylococcus aureus</i>	10	8.77
<i>Arcanobacterium pyogenes</i>	11	9.65
<i>Klebsiella</i> spp	15	13.16
Total	114	100.0

Number of diarrhoeic lambs = 127

Table 2: Characterization of *E. coli* isolated from diarrhoeic lambs

<i>E. coli</i> serotype	Number	% strain
O26	7	(16.7)
O118	9	(21.4)
O111	8	(19.0)
O157	13	(31.0)
O103	5	(11.9)
Total	42	(100.0)

Figures in brackets are percentages of total isolations

Discussion

It is considered that *Escherichia coli* are an important cause of diarrhea in lambs especially at the neonatal stage (Sharif *et al.*, 2005; Hodgson, 1994). In the present study, *E. coli* was the most prevalent isolate with a percentage of 36.84% of all the bacterial isolates. This agrees with the findings of Sharif *et al.*, (2005); Fejes *et al.*, (1990); Hodgson, (1994). Similar results had also been reported in calves by Nahed, (2004); Nasr and Meghawery, (2007) who considered *E. coli* an important cause of neonatal diarrhoea. According to Snodgrass (1978), *E. coli* can be isolated with equal frequency from the faeces of normal non-diarrhoeac lambs. However, the demonstration of enterotoxin and verotoxigenic *E. coli* factors in faeces from lambs with diarrhoea in other studies (Ansari *et al.*, 1978; Sharif *et al.*, 2005) have lend credence to the involvement and significance of *E. coli* as the major culprit in cases of neonatal lamb diarrhoea or colibacillosis.

Another pathogenic bacteria associated with diarrhoea in this study was *Salmonella* spp (not typed) which represents 15.79% of the isolates. This was higher than 5% obtained in camels in Egypt (Ramadan and Sadek, 1971) and 3% in India (Ambwani and Jatkar, 1973). The low isolation rate for *Salmonella* in this study might be because multiple faecal samples are required for higher isolations (Duijkeren *et al.*, 1995). According to Schoenian, (2006) *Salmonella* can cause diarrhoea in older lambs but lambs less than one week old are more likely to die without any clinical signs.

Arcanobacterium pyogenes was ranked fourth with an isolation frequency rate of 9.65%. Other bacteria with

low frequencies in this study were *Staphylococcus aureus* (8.77%), *Shigella* sp (7.02%), *Proteus vulgaris* (5.25%) and *Streptococcus* sp (3.15%). The isolation of *S. aureus* is of significance due to its role in mastitis in ewes as well as contamination of meat and milk products (Quinn and Mackey, 2003; Colville and Berryhill, 2007; Rad and Nazeri, 2008). According to Adeyemi *et al.*, (2003), *S. aureus* is also an agent of severe of diarrhea in humans.

In the present study, *E. coli* serotypes O157, O118, O111, O26 and O103 were obtained. This is in variance with the strains obtained in Jordan (Sharif *et al.*, 2005). In Japan, serotypes O157, O26 and O111 have been reported to be isolated from children with bacterial gastroenteritis (Kodaka *et al.*, 2004). In animals and humans, serotype O157 had attracted increased attention in recent years because of its public health significance (Kodaka *et al.*, 2004; Gyles, 2007). This is the first report on the prevalence of bacterial pathogens and characterization of *E. coli* from diarrhoeac lambs in Sokoto state, northwestern Nigeria.

Conclusion

It was concluded that as in other studies, *E. coli* remained the most important bacterial pathogen involved in neonatal diarrhoea and therefore, further research is required to identify the strains of *E. coli* producing enterotoxins that inhabit the alimentary tract of lambs to cause enteric colibacillosis. In calves, serologic studies suggest that a limited number of strains may be involved in enteric colibacillosis in calves throughout the world. Similar situation probably exist in ovine enteric colibacillosis.

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