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 diarrhoeac 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 0157, 0111, 026, and 0103, with prevalence rates of 31.0%, 21.4%, 6.11%, and 0.11% 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, multifactorial 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 perfringes, 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 diarrhoeac 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...
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 environ 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

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

Number of diarrhoeic lambs = 127

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

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

Figures in brackets are percentages of total isolations.
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.

**References**


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