

Full Length Research Paper

Efficacy of two probiotics in the control of *Escherichia coli* O157:H7 in experimentally infected lambs

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This study was designed to determine the efficacy of probiotics mixture in the control of Shiga-toxin *Escherichia coli* (STEC) O157:H7 in experimentally infected lambs. Fifteen Yankassa breed of lambs aged between 3-4 weeks old were used. The lambs were divided into three groups of five lambs each (n=5). Group A: Neither probiotics nor STEC O157:H7 were administered (control), Group B: lambs were administered viable STEC O157:H7 cells at 6×10^8 CFU/ml together with daily administration of probiotics mixture at 4.5×10^8 CFU/ml, Group C: lambs were administered only viable STEC O157:H7 cells at 6×10^8 CFU/ml without probiotics. Faecal samples from all the experimental lambs were screened for the presence of STEC O157:H7 before the commencement of this study using Tryptone soy broth (TSB) as an enrichment media and Cefixime-tellurite sorbitol MacConkey agar (CT-SMAC) as a selective media. Following oral inoculation of the lambs with STEC cells, faecal samples were collected once weekly for six weeks, for STEC O157:H7 isolation and enumeration. STEC O157:H7 was confirmed by its reaction with O157 and H7 anti-sera (Wellcomex^r). STEC O157:H7 was not shed by lambs in group A (control). However, Group B lambs administered a mixture of probiotics shed significantly lower ($P < 0.05$) counts of STEC cells in the six weeks post-infection than Group C lambs that received only STEC cells without probiotics. In conclusion, the use of probiotics mixture significantly ($P < 0.05$) reduced the faecal shedding of STEC O157:H7. It was therefore recommended that probiotics should be administered to lambs to help control these pathogens.

Key words: Shiga-toxin producing *Escherichia coli* O157:H7, probiotics mixture, cefixime-tellurite Sorbitol MacConkey Agar, tryptone soy broth, anti-sera.

INTRODUCTION

Shiga-toxin-producing *Escherichia coli* O157:H7 (STEC) is a food borne pathogen primarily transmitted to humans

through the consumption of contaminated water or food (Caprioli et al., 2005). STEC represent the only

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pathogenic group of *E. coli* that has a definite zoonotic origin, although not all the STEC strains have been demonstrated to cause disease in humans. Six non-O157 groups have been identified by CDC (2008) to be responsible for over 70% of non-O157 STEC-associated illness (Bosilevac and Koochmarai, 2011). The predominant STEC serotype associated with outbreaks and sporadic cases of serious STEC illnesses is STEC O157 (Karmali et al., 2010).

Ruminants such as cattle, sheep and goats are regarded as the main animal reservoirs of STEC (Nataro et al., 2011). Adult ruminants can be asymptomatic carriers of these pathogens, but it has been reported to cause mild to severe diarrhoea in lambs, calves and pigs (Cray and Moon, 1995). Similarly, Dean-Nystrom et al. (1997) also reported cases of mucoid diarrhoea and mortality in neonatal lambs and calves. Infected ruminants can also serve as source of infection to other susceptible animals such as pigs and rabbits. STEC O157:H7 also causes a wide range of human diseases, including mild to severe diarrhoea, haemorrhagic colitis (HC) and life threatening haemolytic uremic syndrome (HUS) (Caprioli et al., 2005). Both the European Food Safety Authority (EFSA) and the U.S. Department of Agriculture (USDA) have issued recommendations for laboratory testing for these pathogens (Eblen, 2010),

Lema et al. (2001) and Everlon et al. (2013) indicated that some strategies may be used in the rumen to decrease the number of viable STEC cells. One of such strategies is the use of probiotics supplemented in the ration. Probiotics are live organisms with the capacity to benefit the gastrointestinal tract microflora by promoting health or preventing diseases in the host (Papadimitriou et al., 2007; Hill et al., 2014). Several mechanisms have been proposed to explain the beneficial effects of probiotics. Among them are the production of organic acids by bacterial probiotics which can help decrease the gut pH, create more favourable ecological conditions for the resident microbiota and decrease the risk of pathogen colonization (Servin, 2004).

There is paucity of information on the use of probiotics in the control of STEC in Nigeria, Hence, this study will help explore alternative treatment regimen through the use of probiotics to improve small ruminant production in Nigeria.

MATERIALS AND METHODS

The study was carried out at the Livestock Investigation Division of the National Veterinary Research Institute, Vom, plateau State, Nigeria. The State is located in the North Central Geopolitical zone of Nigeria and has a land area of 26,899 sq km (NPC, 2006).

Experimental animals

Fifteen (15) Yankassa breed of lambs, aged between 3 and 4 weeks were used for this research. The lambs were sourced from the Small Ruminant Section of the Livestock Investigation Division

of the National Veterinary Research Institute, Vom, Plateau State.

The lambs were tagged and allocated into pens. They were confined to their dams to enable them suckle. The dams were fed on concentrate and hay. Water was provided *ad libitum*

Escherichia coli strain used in the study

Shiga-toxin producing (STEC) *E. coli* O157:H7 strain, which is under the entero-haemorrhagic serogroup of *E. coli* was sourced from the National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Cultures for use as inocula were produced in 10 ml of 0.9% saline solution after an overnight incubation on Sorbitol MacConkey agar (SMAC) supplemented with cefixime tellurite (Oxoid, UK) at 37°C for 24 h. Cell numbers were determined spectrophotometrically using the McFarland standard and were adjusted to contain 6×10^8 CFU/ml.

Probiotics used in the study

Sky-flo® Probiotics containing *Lactobacillus acidophilus* and Sanolife® PRO-F probiotics containing a balanced mixture of *Bacillus pumilus*, *Bacillus licheniformis* and *Bacillus subtilis* were used for this research. It was manufactured by Inve Aquaculture, Belgium.

Preparation of the daily doses of probiotics mixture

To prepare the daily doses of the probiotic strain used, individual tube containing the lyophilized bacteria, were inoculated into 9 ml of *Lactobacillus* selective broth de Mann, Ragosa and Sharpe (MRS) (Oxoid, UK) for selective enrichment and incubated at 37°C for 24 h aerobically. After the period of incubation, a loop full of the positive broth was then streaked on MRS agar (Oxoid, UK) plates and incubated at 37°C for 24 h aerobically. Colonies that grew were re-suspended in 10 ml of saline to generate a suspension containing *Lactobacillus acidophilus* 4.5×10^8 CFU/ml, *Bacillus subtilis* 4.5×10^8 CFU/ml, *Bacillus licheniformis* and *Bacillus pumilus* 4.5×10^8 CFU/ml using the McFarland standard.

Experimental design

Animal groupings

Lambs were divided into three (3) groups (A, B and C) of five (5) lambs each and confined to separate pens. The animals were all tagged for the purpose of identification.

Pre-experimental management of animals

The lambs were allowed to acclimatize to the environment for two weeks prior to the commencement of this study, during which the dams were de-wormed with albendazole (10 mg/kg) *per os* and administered ivermectin (0.2 mg/kg) sub-cutaneously to control ectoparasites. The lambs were weaned before the commencement of the study at four weeks of age, and fed concentrate and water during the study period.

Ethics

Ethical clearance was obtained from the Ahmadu Bello University Zaria Committee on Animal Use and Care, with approval number:

ABUCAUC/2016/Vet.Medicine/003.

Pre-infection data

Pre infection data collected from the lambs included: Faecal samples collected directly from the rectum of all the lambs into sterile polythene bags and immediately taken to the laboratory to screen for the presence of *E. coli* O157:H7 as described by Chapman et al. (1994). Briefly, 1 g of the faecal sample from each lamb was added to 10 ml of TSB and incubated at 37°C for 24 h aerobically for enrichment. Following incubation, a loop full of the overnight broth was streaked on CT-SMAC to selectively isolate *E. coli* O157:H7 colonies.

Animal groupings and treatment regimen

At the end of the two weeks acclimatization period and after the screening of all the lambs for the presence of *E. coli* O157:H7, they were subsequently subjected to different treatment regimens as follows: Group A: neither probiotic non *E. coli* (STEC O157:H7) was administered, as they served as control; Group B: Lambs were administered viable STEC O157:H7 (6×10^8 CFU/ml) together with a mixture of probiotics (*L. acidophilus*, *B. pumilus*, *B. subtilis* and *B. licheniformis*) at 4.5×10^8 CFU/ml daily throughout the research period. Both were administered in a 1 ml normal saline, orally through the use of sterile syringe directly into the mouth; Group C: Lambs were administered 1 ml inoculum containing viable STEC O157:H7 (6×10^8 CFU/ml) without probiotics, through the use of sterile syringe directly into the mouth.

Faecal sample collection, detection and enumeration of *E. coli* (STEC) O157:H7 post-infection

Faecal samples were collected from all the lambs on day three following the inoculation of *E. coli* O157:H7 to confirm the presence of the pathogen in the faeces. Subsequently, faecal samples were collected once weekly, directly from the rectum of each lamb every morning using sterile swab sticks, for a period of 6 weeks for detection and enumeration of *E. coli* O157:H7 colonies as described by Chapman et al. (1994). Briefly, 1 g of faeces from each lamb was aseptically added into 10 ml of normal saline and homogenized by shaking vigorously. Subsequently, 1 ml of the homogenate was then added to 9 ml of normal saline and a hundred (100) fold serial dilution was made after which 0.1 ml from each dilution tubes was spread plated onto CT-SMAC to selectively isolate *E. coli* O157:H7 colonies for enumeration. Creamy white colonies that grew were regarded as presumptive isolates due to the inability of *E. coli* O157:H7 to ferment Sorbitol and was further confirmed serologically using *E. coli* O157:H7 Monoclonal Antisera (Wellcolex *E. coli* O157:H7 kit, Oxoid). Presumptive *E. coli* colonies were also further verified by conventional biochemical methods as described by Harrigan (1998).

Presumptive colonies of *E. coli* O157:H7, were further confirmed by serotyping using commercial latex kit for *E. coli* O157:H7 (Wellcolex *E. coli* O157:H7 kit) which is a rapid latex agglutination test for confirming non-sorbitol fermenting colonies as possessing the O157 somatic antigen and H7 flagellar antigen.

Data analysis

The data obtained were expressed as mean \pm standard error of mean (SEM) and presented in graphs. One way ANOVA with Tukey's post hoc test using SPSS version 20 for windows was used to determine significant difference in the shedding of STEC

O157:H7 among the groups. Values of $P < 0.05$ were considered significant at 95% confidence interval.

RESULTS

Mean number of STEC O157:H7 isolates recovered

No STEC O157:H7 isolate was recovered from the faeces of all the lambs in group A throughout the six weeks of the study (Figure 1). However, the mean STEC O157:H7 isolates recovered from group B lambs increased significantly ($P < 0.05$) from zero pre-infection to 2×10^4 CFU/g at the first week post infection and then decreased significantly ($P > 0.05$) to 1.1×10^4 CFU/g during the second week, and progressively decreased to 8×10^3 CFU/g in the third week, 6×10^3 CFU/g in the fourth week, 4×10^3 CFU/g in the fifth week and 2×10^3 CFU/g in the sixth week post-infection (Figure 1). Group C lambs had a significant increase ($P < 0.05$) in the mean STEC O157:H7 isolates over the six weeks post-infection: from no isolate pre-infection to 3.08×10^5 CFU/g in the first week post infection then decreased progressively to 2.3×10^4 CFU/g in the sixth week (Figure 1).

There were higher number of STEC O157:H7 isolates recovered from group C lambs that were challenged with viable STEC O157:H7 cells without the administration of a mixture of probiotics, than the group B lambs that received the viable STEC O157:H7 cells together with a mixture of probiotics over the six weeks period.

DISCUSSION

STECs are zoonotic pathogens that can cause food borne diseases in humans, ranging from diarrhoea to haemolytic colitis (HC) and the deadly haemolytic uremic syndrome (HUS) (WHO, 1998). There are several studies showing the prevalence of STEC in ruminants in different parts of the world. Therefore, the control of STEC is of great public health significance (Everlon et al., 2013; Martins et al., 2015). Several reports exist on the efficacy of probiotics in the control of STEC O157 (Avila et al., 2000; Lema et al., 2001; Everlon et al., 2016).

In this study, there were no clinical signs observed from all the lambs in the experimental groups throughout the six weeks period of research. This could be because ruminant such as cattle, sheep and goat are regarded as the main animal reservoirs and are usually asymptomatic carriers of these pathogens (La Ragione et al., 2009). It could also be due to the fact that the dose of the STEC O157:H7 inoculum used for the challenged was not high enough to produce clinical infection in the lambs. This finding agrees with the report by Lema et al. (2001), Avila et al. (2000) and Everlon et al., (2013) who also reported absence of clinical signs of STEC O157:H7 in experimentally infected ruminants. However, six weeks

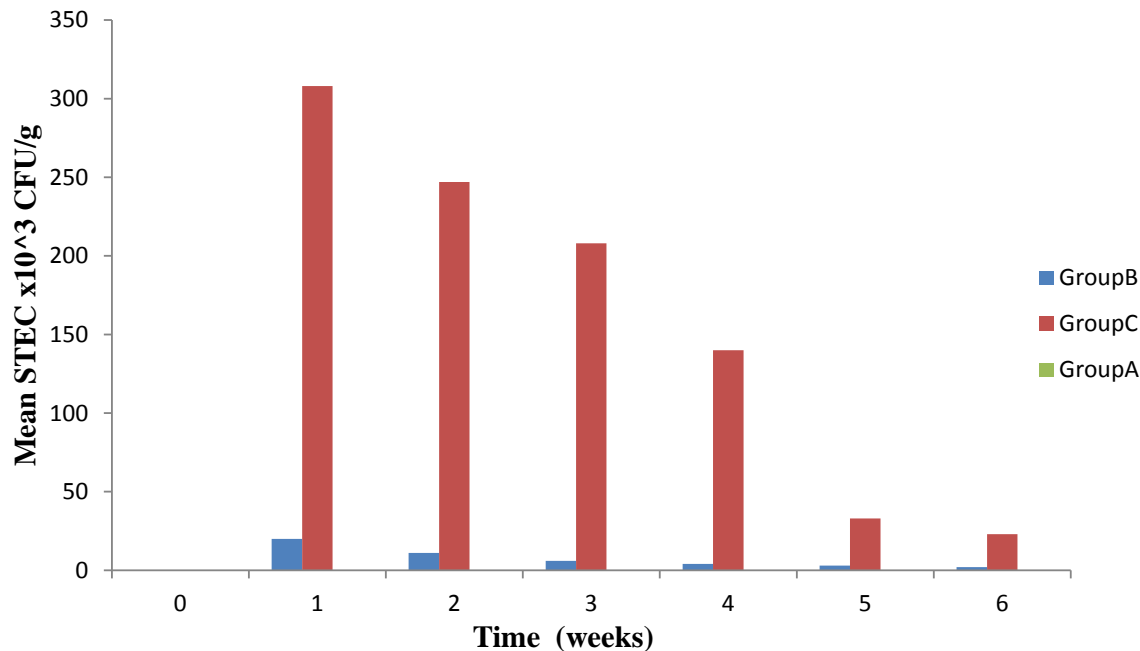


Figure 1. Mean *E. coli* (STEC) O157:H7 isolates recovered from the three experimental groups of lambs six weeks post-infection. Group A: Control; Group B: Lambs inoculated with STEC cells together with probiotics administered daily; Group C: lambs inoculated with STEC cells only without probiotics administration 0 week represents pre-infection period. 1 to 6 weeks represents post-infection period.

post-infection, it was observed that a lower count of *E. coli* (STEC) O157:H7 was recovered from lambs in Group B that received *E. coli* O157:H7 inoculum together with the mixture of probiotics strains in comparison with Group C lambs that received only *E. coli* (STEC) O157:H7 inoculum without probiotics strains. The fewer counts of STEC O157:H7 recovered from the lambs in Group B could be as a result of the effect of probiotic strains administered. The mixture of probiotic strains perhaps, produced inhibitory substances which inhibited the adhesion and proliferation of the STEC cell, thereby leading to the death of these cells. Furthermore, the absence of a well-developed normal flora at this age of the lambs may also have facilitated the establishment of the probiotics strains in the gut and consequently, the establishment of the STEC O157:H7 was hampered. While the higher number of STEC strains recovered from group C lambs, could also be as a result of the absence of a well-developed normal flora in the young lambs; thereby, facilitating the adhesion and proliferation of the STEC O157:H7 cells in this group of lambs. It could also be as a result of the absence of any intervention (probiotics), so, the STEC O157:H7 cells did not suffer any competition with other micro-organism.

This finding agrees with the report of Lema et al. (2001) who showed a protective effect of probiotics on STEC O157:H7 in lambs. Also, work done by Everlon et al. (2013) showed that probiotics was more efficient in reducing the faecal shedding of STEC cells in sheep that

were younger than 45 days as compared to sheep that were older than 45 days. Similarly, Stanford et al. (2014) also reported a reduction of STEC O157:H7 in finishing feedlot cattle fed a mixture of probiotics.

The findings suggest the efficacy of probiotic mixture in the control of STEC O157:H7 in lambs through the reduction of faecal shedding of these pathogens.

Conclusion

Administration of a mixture of probiotics strains (*L. acidophilus*, *B. pumilus*, *B. subtilis* and *B. licheniformis*) at 4.5×10^8 CFU/ml was effective in the control of *E. coli* O157:H7 (STEC) in experimentally infected lambs. It was therefore recommended that probiotic mixture should be added to feed or water of lambs to help control these pathogens.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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