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SEROPREVALENCE OF FAECAL SHEDDING OF *Escherichia Coli* O157:H7 FROM EXOTIC DAIRY CATTLE IN NORTH-WESTERN NIGERIA

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SUMMARY

One thousand eight hundred feacal samples were collected from six diary herds in Kaduna and Sokoto States for the isolation and serological confirmation of enterohaemorrhagic *E. coli* O157:H7. The overall sero-prevalence rate was 0.9%. This rate was not dependent on season, breed, management and water quality; but was significantly associated statistically with the sex, diarrhea status and age of the animals. The prevalence of 0.9% in the exotic dairy cattle is low. A very large scale surveillance of livestock is needed to have a clearer picture of the occurrence and distribution of the enteropathogen.

KEY WORDS: *E. coli* 0157:H7, Enterohaemorrhagic, Seroprevalence, Cattle

INTRODUCTION

Escherichia coli O157:H7 was identified in 1982 as an important food-borne human pathogen causing haemorrhagic colitis (HC), and haemolytic uraemic syndrome (HUS) (Riley, 1983). The pathogen has since then been the subject of intense inquiry during the past decade (Doyle, 1991; Griffin and Tauxe, 1991 and Padbye and Doyle, 1992). The increased frequency of reporting has continued into the new millennium (Zhao *et al.*, 2001; Wang *et al.*, 2002; Smith *et al.*, 2003; Cagney *et al.*, 2004; Bidet *et al.*, 2005; Islam *et al.*, 2005 and Keen *et al.*, 2006).

Dairy cattle, especially young animals have been implicated as the major reservoir host of *E. coli* O157:H7 (Montenegro *et al.*, 1990; Doyle, 1991; Griffin and Tauxe, 1991 and Whipp *et al.*, 1994). The dairy and fast food industries are often incriminated as the sources of the pathogen in epidemics and sporadic outbreaks (Cagney *et al.*, 2004 and Mora *et al.*, 2005).

These industries are fast growing in Nigeria, particularly in the northern part that harbours the larger part of the Nigerian cattle population (Bourn *et al.*, 1994). The aim of this study therefore was to ascertain the prevalence rate of *E. coli* O157:H7 from exotic cattle in the north-western part of Nigeria.

MATERIALS AND METHODS

Sampling Sites

Using the multistage (cluster) sampling method (Snedecor and Cochran, 1976), Kaduna and Sokoto states were selected by a simple random sampling from seven states in the geo-political zone namely: Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto and Zamfara. Four of the six dairy farms in Kaduna State and two of the four dairy farms in Sokoto State were selected in the second stage of the multistage sampling method by a second simple random sampling. The selected dairy farms in Kaduna State were: Dairy unit farm, National Animal Production Research Institute (NAPRI) Shika; Jamil farms, Kaduna-Jos road; Niyya farms, Kaduna Abuja road and Rio Hondo farms, Kaduna. In Sokoto State, SMTA and Yahaya Abdulkareem farms, Sokoto were selected in the second simple random sampling of the four dairy farms in Sokoto State.

Collection of Faecal Samples from Cattle

Samples of 10 to 50g of faeces were collected from the rectum of each animal by retrieval on rectal palpation with a clean gloved hand, following adequate restraint of the animals (Chapman *et al.*, 1993).Each sample was labelled and a questionnaire was filled for each animal obtaining information on the animal's ear-tag number and/or nickname, age, breed, sex, location of the farm (name of the farm), management type, presence of diarrhoea, nature of diarrhoea, duration of diarrhoea, number of animals in the herd, number currently with diarrhoea, history of diarrhoea in the herd, source(s) of water available to the animals, other conditions observed on the animal and history of antimicrobial use on each animal. Samples were held in plastic bags which were knotted and kept in a cool box containing ice blocks and taken to the laboratory for bacteriological culture and isolation (Chapman *et al.*, 1993).

Isolation of *E. coli* 0157:H7 from faecal samples

E. coli 0157:H7 was isolated as described by Chapman *et al* (1993), with some minor modifications. Briefly, about 1g of faeces was enriched in 9ml of modified tryptone soya broth (MTSB) supplemented with novobiocin (Oxoid Ltd, Hampshire England). This enrichment phase was carried out on the farm soon after sample collection. On arrival at the laboratory the enriched samples were incubated at 37°C for 24hours, 50 µl of which was cultured on Sorbitol MacConkey agar supplemented with cefixime (CR-SMAC) (Oxoid Ltd, Hampshire England) and incubated at 37°C for 24 hours. The first 193 faecal samples were inoculated (without the enrichment phase) onto Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. Colonies showing a greenish metallic sheen on EMB were selected and screened biochemically (Coghlan *et al.*, 1975). Isolates that were biochemically *E. coli* were then plated on CR-SMAC agar. Colourless colonies on CR-SMAC were selected and stored on nutrient agar (NA) slants at 4°C until required. All colonies that showed colourless appearance on CR-SMAC agar were screened biochemically using procedures described in detail by Coghlan *et al* (1975). Each isolate was tested for citrate utilization, urease production, H_2S production in triple sugar iron (TSI) agar and sulphide indole motility (SIM) medium, motility and indole production using SIM medium, fermentation of glucose, sucrose and /or lactose in TSI medium, methyl red (MR) and Voges Proskauer (VP) reactions using MRVP medium, ability to produce an acid or a non-acid reaction from fermentation of lactose, arabinose maltose, sorbitol and manitol, and gas from glucose.

Serological Confirmation of *E. coli* 0157:H7

Serological testing was carried out using CR-SMAC negative isolates that had been biochemically tested. The Remel Wellcolex *E. coli* 0157:H7 kit (Remel Europe Ltd. Dartford Kent, U.K) was used. Wellcolex *E. coli* 0157:H7 is a rapid latex agglutination test for the presumptive identification of *E. coli* 0157:H7 isolates on laboratory media. The test contains two test reagents. The somatic (0157) antigen test reagent consists of red latex particles coated with antibodies specific for *E. coli* (O) antigen. When a drop of the reagent is mixed on a card with a suspension of *E. coli* 0157 organisms, rapid agglutination occurs through the interaction of specific IgG and 0157 lipopolysaccharide antigen. Similarly the H7 test reagent consists of blue latex coated with antibodies specific for the flagellum, (H7) antigen. This method has been widely used for the confirmation of *E. coli* O157:H7 from cattle (Chapman *et al.*, 1993 and Smith *et al.*, 2003). The instructions of the manufacturer concerning choice of media and laboratory practice were followed to the letter. The performance of the test and control latex reagents was confirmed using fresh overnight cultures of a reference strain EHEC EDC 933.

Statistical Analyses

The data generated from the microbiological and serological screening was sent into Microsoft Excel 2003 (Microsoft Corp., Redmond, WA, USA) and descriptive analysis was performed. The files were imported into the statistical package for social sciences (SPSS) for Windows 11.0 (Standard Version SPSS Inc., Chicago, IL, USA). Chi-square and Fisher's Exact Tests were used to test for significance ($p<0.05$) (Snedecor and Cochran, 1976).

RESULTS

Seroprevalence of *E. coli* 0157:H7

The seroprevalence of *E. coli* 0157:H7 was 0.9% (17/1800) (Table I). This rate was not dependent on season (Pearson à^2, P>0.05), breed (Fishers Exact Test, Exact significance (2-sided) =0.053), management (Fishers Exact Test, Exact significance (2-sided) =0.002) and water quality (Pearson à^2, P>0.05). However, the seroprevalence of *E. coli* 0157:H7 in cattle was significantly associated statistically with sex (Pearson à^2, P<0.05) (Table II), and diarrhea status (Fishers exact test, exact significance (2-sided) =1.000) (Table III) and age (Fishers Exact Test, Exact Sig. (2-sided) =0.0445).

TABLE I: Seroprevalence of *E. coli* O157:H7

Number of samples collected	Number Positive by REMEL Welcolex *E. coli* Test [1]	Seroprevalence
1800	17	0.9%

[1] **Latex agglutination test kit**

TABLE II: Distribution of *E. coli* O157:H7 by diarrhea status

	E. coli O157:H7		
Diarrhea Status	**Positive**	**Negative**	**Total**
Present	15	1543	1558
Absent	2	240	242
Total	17	1783	1800

TABLE III: Distribution of *E. coli* O157:H7 by Sex

	E. coli O157:H7		
Sex	**Positive**	**Negative**	**Total**
Female	12	1114	1126
Male	5	669	674
Total	17	1783	1800

DISCUSSION

The prevalence of *E. coli* O157:H7 in the US and Europe has been reported to range from less than 1 to 5% (Armstrong *et al.*, 1996, Mora *et al.*, 2005, Vali *et al.*, 2005). The result of our study was consistent with observations in other countries revealing a prevalence of 0.9%. This falls in the lower category of the range from these countries and could be due to better technologies that are used to detect the organism in Europe, US and other advanced countries. Jih *et al* (2005) and Sharma (2006), published-microarray analysis and real time reverse transcription multiplex PCR respectively and this greatly improve detection of the organism. The use of these technologies for detection of *E. coli* might explain the higher prevalence reported in these countries.

The present study revealed that of the 17 isolates, 6 were from yearling heifers (35%), 4

from weaned calves (24%) and 2 from pre-weaned calves (12%). Only 5 isolates (29%) were from cattle from 2 or more years in age. Studies of the pathogen in cattle have revealed that calves and heifers have higher prevalence rates of faecal shedding (Orskov *et al.,* 1987 and Wilson *et al.,* 1991, 1993). Similar results have been observed by others in studies involving cattle (Chapman *et al.,* 1993 and Wilson *et al.,* 1991, 1993). The peak time of infection in cattle ranges from 3-18 months of age supporting the consistency between our findings and other workers that calves and heifers shed the organism more frequently than adult cattle (Anon., 2007).

The prevalence was not dependent on season of the year, breed, management and water quality. Changes in management practices on dairy farms such as early calf weaning, grain feeding and zero-grazing of the cattle have been proposed to have created the environment for faecal shedding of *E. coli* O157:H7 (Garber *et al.*, 1995). These practices were not observed on the farms sampled and could account for our findings.

Enterohaemorrhagic *E. coli* O157:H7 has emerged in exotic the cattle population in north-western Nigeria. There is a need for extensive surveillance in indigenous breeds of cattle used for dairying, domestic animals and food to ascertain the associated risks to humans.

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