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Detection of Verocytotoxigenic *Escherichia coli* O157 Serotype in Dairy Products in Abuja, Nigeria

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Abstract

Ruminants are recognized as healthy carriers of Verocytotoxigenic *Escherichia Coli* (VTEC) organisms and as such most dairy products may provide these bacteria with favourable conditions for their growth. A cross sectional study was conducted to detect the occurrence of VTEC O157 in dairy products in the Federal Capital Territory, Abuja, Nigeria. Raw milk, Nunu and yoghurt were analyzed using standard cultural and biochemical procedures to isolate typical *E. coli.* Isolated *E. coli* samples were sub-cultured into plates of sorbitol MacConkey and Cefixime Tellurite-Sorbitol MacConkey agar. Isolates that are sorbitol negative were further characterized using commercially procured latex agglutination test kits. A total of 367 samples were analyzed out of which 3 tested positive for VTEC O157 (108 of raw milk - 1 (0.93%); 127 of Nunu - 2 (1.57%) and 132 of yoghurt - none). There was no significant association ($p > 0.05$) between season and infection with VTEC O157 in dairy products. Dairy products remained a potential vehicle for VTEC O157 infection.

Keywords

Detection, Prevalence, VTEC O157, Dairy Products

1. Introduction

Escherichia coli O157 is the most common member of a group of pathogenic *E. coli* strains known variously as enterohaemorrhagic, verocytotoxin producing or Shiga-toxin-producing organisms [1] [2]. The first outbreaks caused by *E. coli* O157 occurred in Oregon and Michigan, USA in 1982, when it was isolated from individuals

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who developed bloody diarrhoea and severe abdominal cramps after eating hamburgers in a restaurant chain [3]. Shiga-toxigenenic *Escherichia coli* (STEC) is considered to be most common food-borne zoonotic pathogen causing various disease conditions in both animals and humans [4]. The occurrence of VTEC O157 in dairy farms is highly significant in terms of the potential public health hazards it presents to man via food chain as a result of contamination [5]. Ruminants are considered as important source of VTEC [6] with cattle being regarded as the primary reservoir [7]-[9]. Raw milk may be contaminated with animal faeces and become a good source of infection for human if consume without proper pasteurization [5]. The processing conditions for different milk products are very important from the standpoint of the organism's infection risk. The organism is destroyed in pasteurization process, but insufficient heat treatment of ground meat and rawmilk forms a potential infection risk [10]. Infection with VTEC by human is acquired by the consumption of improperly cooked ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water contaminated with faeces of animals [11]-[13]. VTEC strains are associated with Haemorrhagic colitis (HC) and Haemolyticuraemic syndrome (HUS) in human and also in oedema disease of pigs [12].

In a study in Guwahati city, India, 7 VTEC isolates are detected out of 51 milk samples collected from different unorganized farms [14]. An investigation to determine whether dairy beef cattle raised in Algeria are VTEC carriers show that samples from 61 (30.5%) animals out of the 200 tested are positive [15]. In Northern Italy, it is reported that of the bulk milk samples and milk filters collected in 193 dairy farms, Shiga toxin genes are detected in 30.2% of filters and 12.5% of milk samples [16]. While testing the quality of raw milk intends for direct consumption in Estonia, VTEC genes are detected in 64.3% of the on-line milk filter samples [17]. In Abuja, the Federal Capital of Nigeria and other parts of Nigeria dairy products are commonly consumed without adequate pasteurization and can serve as sources of VTEC infection in the human populace. No recorded work has been done on VTEC O157 in dairy products in FCT, Abuja.

This study is therefore carried out to investigate the occurrence and prevalence of VTEC O157 in dairy products in the Federal Capital Territory, Abuja, Nigeria.

2. Materials and Methods

The research was conducted in the Federal Capital Territory, Abuja, Nigeria located between latitude 8˚ and 9˚25" North of the equator and longitude 6˚45" and 7˚45" East of the Greenwich Meridian [18]. Three area councils (Gwagwalada, Kuje and Municipality) out of 6 area councils were randomly selected by balloting for the study and the work was carried out in the 3 area councils. The study was cross sectional and was carried out between May, 2013 and April, 2014.

A total of 367 samples of dairy products made up of 108 raw milk samples, 127 Nunu (locally processed milk) samples and 132 yoghurt samples were collected and analyzed for VTEC O157. Five milliliters (ml) each of raw milk from farms, Nunu and yoghurt from hawkers were collected in a sterile screw—capped bottles for analysis under aseptic conditions. Of the 367 samples analyzed, 209 were collected during the dry season while 158 were during the wet season. Samples were streaked onto Eosin Methylene Blue (EMB) agar medium and further characterized using biochemical tests. Colonies showing characteristic metallic sheen on EMB agar and confirmed using biochemical tests, viz. indole, methyl red, voges proskaeur and citrate utilization (IMViC) as well as sugar fermentation, urea hydrolysis and production of H_2S were identified as *E. coli*. [19]. *E. coli* isolates ex-EMB were further sub-cultured into plates of sorbitol (MacConkey (SMAC) and cefixime Tellurite-sorbitol MacConkey (CT-SMAC) agar. Isolates that were negative (appearing as colourless or neutral gray with Smokey centre, 1 - 2 mm in diameter) were further characterized using commercially procured latex agglutination test kits from Oxoid Ltd, Hampshire, England. Test results were considered positive for VTEC O157 when agglutination of the latex particles was observed in the test reaction area within 60 seconds. A negative result was obtained if no agglutination was observed in the test reaction area and a smooth blue suspension remains after 60 seconds.

Fisher's exact test was used to determine association between VTEC infection rates with season using statistical packages for social scientist (SPSS) version 20.

3. Results

The *E. coli* isolates ex-EMB exhibited similar IMVic Pattern of + + − − and were negative to both urease and hydrogen sulphide production (**Table 1**). Out of 367 samples collected, 3 (0.82%) yielded VTEC O157. Of the

108 raw milk sample, 1 (0.93%) was positive for VTEC O157, while 2 (1.57%) of the 127 Nunu samples tested positive for VTEC O157. There was no isolate from the yoghurt samples (**Table 2**). Out of the 367 samples collected, 209 were during the dry season while 158 were in the wet season. There was no significant difference ($p > 0.05$) between season and VTEC infection using fisher's exact test (**Table 3**).

Table 1. Biochemical reactions of *E. coli* isolates.

Test carried out	Reaction observed
Indole test	+
Methyl red (MR)	+
Voges Proskauer (VP)	–
Citrate utilization test	–
Urease production	–
H_2S production	–

Key: +: Positive; –: Negative.

Table 2. Prevalence rates of VTEC O157 in specified animal products.

Animal Product	No of Samples	Tested	No Positive	% Positive
Raw milk	108	1	0.93	
Nunu	127	2	1.57	
Yoghurt	132	-	-	
Total	367	3	0.82	

Table 3. Seasonal distribution of VTEC O157 in animal products.

Season	Total Tested	No Positive	% Positive
Dry	209	2	0.96
Wet	158	1	0.63
Total	367	3	0.82

$P > 0.05$.

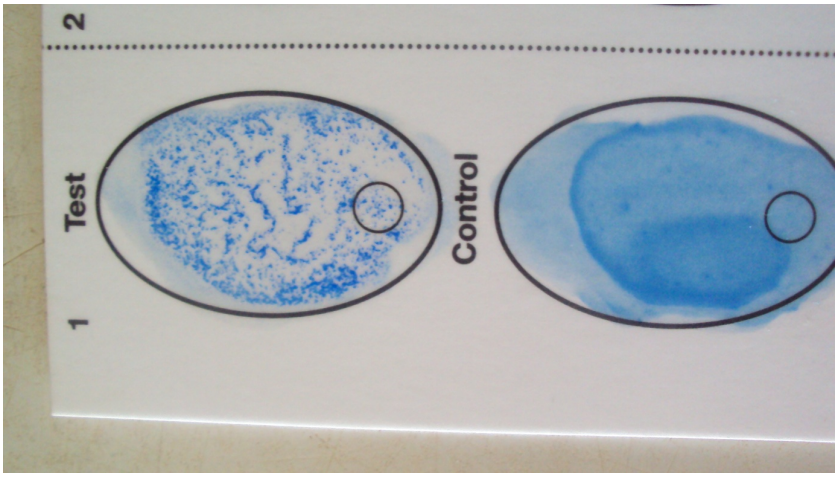

Figure 1. Agglutination reactions of the latex agglutination test kits from Oxoid.

4. Discussion

The confirmation of *E. coli* isolates through biochemical test is in conformation with the IMVic reaction as reported by [19]. The findings in this study using latex agglutination test showed the prevalence of 0.93% for VTEC O157 in raw milk (**Figure 1**). One (0.93%) out of the 108 raw milk samples tested positive. Detection of verocytotoxigenic *E. coli* in raw milk samples of apparently healthy cows observed in this study agreed with other earlier reported findings. VTEC were detected in 2% raw cow's milk [20], of the 30 *E. coli* isolates screened for VTEC, 7 (23.33%) VTEC organisms were isolated [5]. In another study from dairy farms in Trinidad, 8 (0.9%) VTEC O157 out of the 933 *E. coli* isolates tested were isolated [21]. In this study Nunu yielded a prevalence of 2 (1.57%) for O157 VTEC out of the 127 *E. coli* isolates tested and none for the 132 samples from yoghurt. These findings were in agreement with earlier reports such as the observation of VTEC in milk products in Canada [22]; however the findings differ from that in the USA where VTEC was isolated from yoghurt [23]. Other vehicles of dairy product infection implicated are pasteurized milk [24], cream and cheese made from raw milk [25].

The isolation of VTEC O157 in raw milk and Nunu in this study is indicative of cross infection from apparently healthy dairy cows to the dairy products especially as they may not have been properly pasteurized. The yoghurt must have undergone proper pasteurization resulting in zero detection of the VTEC in the yoghurt samples in this study.

The degree of association between season and rate of VTEC 0157 infection in animal products showed no significant association ($p > 0.05$). There was no available literature on previous work on the seasonal variation of VTEC isolation in dairy products.

5. Conclusions

This study observed the presence of VTEC O157 in two of the three dairy products tested (raw milk and Nunu) while there was no VTEC detected in the yoghurt. The presence of VTEC O157 in these dairy products was indicative of an epidemiological causal association to the infection in man as Fulani herdsmen drink raw milk without pasteurization as well as selling the Nunu to the general public for consumption. Consumer awareness programms on food processing, handling and hygiene should be a priority to stakeholders in the food industry. Prevention of cross contamination and temperature control should be the key information.

The limitation of the study was the unavailability of facilities to carry out molecular gene typing and so further analysis using PCR is hereby recommended.

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