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Short Communication

Occurrence of pathogenic *yersinia* species in locally fermented cow milk (nono) in jos, nigeria.

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Abstract

Yersiniosis has been reported globally due to contamination of milk and other related products. 'Nono' is the Fulani word for the locally fermented milk product (from cow) produced by Fulani cattle rearers and popularly consumed in Northern Nigeria. This study was aimed at investigating locally fermented cow milk (nono) on sale to the public in Jos metropolis for the presence of pathogenic *Yersinia* species. A total of 300 milk (nono) samples were collected and examined using the cold enrichment technique and *Yersinia* selective culture media. Confirmatory biochemical tests were carried out using the API 20E strip test. Three (3) samples of *Yersinia* species were isolated indicating a 1% occurrence rate. Only *Yersinia enterocolitica* was implicated in this study. Serotyping revealed that all strains were of serotype 0:9 which is one of the two most common serotypes representing the most virulent worldwide causes of yersiniosis. The results of this study indicate a low occurrence rate of pathogenic *Yersinia* species in locally fermented cow milk (nono) hawked in Jos. In a developing country like Nigeria where hygiene standards are to an average extent unchecked, and animal disease not properly controlled, milk still remains a vehicle for a variety of human disease.

Keywords: *Yersinia enterocolitica,* nono, Jos.

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Introduction

Members of the genus *Yersinia* belong to the Kingdom Animalia; Class Schizomycetes; Order *Eubacteriales*; Family *Enterobacteriaceae* (Smith *et al.*, 1978). There are eleven (11) species but only three members of the genus are pathogenic for humans and animals: *Yersinia pestis* is the causal agent of bubonic and pneumonic plague; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are both enteropathogenic bacteria which cause the disease known as yersiniosis in man. They have an extensive animal reservoir and may cause extensive epizootic outbreaks with various patterns of disease/yersiniosis (Davis *et al*, 1980). The mode of transmission is faecal-oral by contact with infected persons or animals or by eating and drinking faecally contaminated food and water. Nosocomial transmission by infected blood products have been reported (Health Canada, 2001). Infections and subsequent diseases due to these organisms can quickly lead to death if not promptly treated.

'Nono' is the Fulani word for the locally fermented milk product (from cow) produced by Fulani cattle rearers and popularly consumed in Northern Nigeria. It forms (part of) the staple diet of a great majority of the local populace (Shehu, 1995). It is taken as a refreshing drink (plain) or with fura/dembu (cooked mashed millet balls) as a cereal drink. All milk and milk products have the potential to transmit pathogenic organisms to humans. People who prefer, for whatever reasons to drink fresh (raw) milk face the greatest risk of contact with these pathogens.

All over the world, cases of disease outbreaks have been reported due to contamination of milk and other related products by *Yersinia* species. In Montreal, Canada, 58 school children after a field trip developed illness from drinking raw milk in 1975. *Yersinia enterocolitica* serovar 0:5,27 was implicated. In 1976, there was a chocolate milk outbreak in Oneida, New York, involving children. This was the first food-borne outbreak reported in the United States with *Yersinia pseudotuberculosis* (Jani, 2003).

Later in the same year in New York, 217 school children reportedly became ill after drinking pasteurized chocomilk (syrup added after pasteurization). Thirty six of them were hospitalized, 16 of whom underwent appendectomy. Causal organism was found to be *Yersinia enterocolitica* serovar 0:9. In the 1980s, outbreaks involving *Yersinia pseudotuberculosis* in Finland and Japan constituted most of the sporadic cases reported in literature (Jani, 2005). A study of the microbiological composition of raw milk from 27 selected farms in the Camembert region of Normandy revealed that 36% of the samples tested positive for *Yersinia enterocolitica* (Desmasures *et al*, 1997). In Nigeria, diarrhoea and gastroenteritis due to *Yersinia enterocolitica* have been reported (Agbonlahor *et al*, 1983; Onyemelukwe, 1993; Omoigberale and Abiodun, 2002; Okwori *et al,* 2007). *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* have also been detected in 'nono' in Bauchi Metropolis of North Eastern Nigeria (Okwori *et al,* 2008).

This study was aimed at investigating locally fermented cow milk (nono) on sale to the public in Jos metropolis, Nigeria for the presence of pathogenic *Yersinia* species.

Materials and Methods

Sample Collection: A total of three hundred (300) 'nono' samples were randomly collected from 'nono' hawkers within Jos Metropolis, Nigeria. Six (6) sampling points were identified namely: Dilimi, Farin gada, Katako, Gada Biu, Massalacin-Juma'a and Terminus markets. 20mls of 'nono' samples were collected into sterile McCartney bottles and transported to the laboratory in ice-cold boxes.

Bacteriological Isolation: The cold enrichment method as adapted from Weagant *et al* (1982) was employed: A 1:10 dilution of each 'nono' sample was carried out using phosphate buffered saline (PBS). The mixture was then homogenized and incubated at 4^0C for twenty one (21) days. Subculturing was subsequently done from inoculated PBS unto MacConkey and Deoxycholate agar respectively. At the end of day one (1), only small (1-2 mm diameter) flat, colourless or pale pink colonies were selected. Pure and discrete colonies were obtained by repeated subcultures of these unto fresh MacConkey agar. These selected colonies were further inoculated unto Cefsulodin-Irgasan-Novobiocin (CIN) agar and incubated for one (1) day.

Presumptive Identification of Bacterial Isolates: Typical small colonies (1-2mm in diameter) having deep red centers with sharp borders surrounded by clear colourless zones (red "bull's eyes" colonies) with entire edges were selected as presumptive *Yersinia.* They were subjected to and subsequently identified by the biochemical tests/reactions as obtained using the API 20E system following the manufacturer's instructions.

Results

Table 1 gives the distribution of *Yersinia enterocolitica* in milk (nono) samples from different sampling points in Jos Metropolis.
Table 2 gives the frequency of *Yersinia* species isolates.
Table 3 gives the serotype of *Yersinia enterocolitica* isolates.
Plate 1 shows *Yersinia enterocolitica* as seen on the API 20E strip.
Plate 2 shows the API 20E strip scoring chart for *Yersinia enterocolitica.*

Table 1: Distribution of *Yersinia enterocolitica* in milk samples collected from different sampling points in Jos Metropolis.

Sampling points	Number of Milk samples	Number of isolates of *Yersinia enterocolitica* (%)
Dilimi	70	1 (0.33)
Farin gada	40	1 (0.33)
Katako	65	-
Gada Biu	55	1 (0.33)
Massalacin-Juma'a	40	-
Terminus Market	30	-
TOTAL	300	3 (1)

Table 2: Frequency of *Yersinia* species isolates.

Yersinia species	Number of Milk samples	Frequency (%)
Yersinia enterocolitica	300	3 (1%)
Yersinia pseudotuberculosis	300	0

Table 3: The serotype of *Yersinia enterocolitica* isolates

Serotype	Number of isolates serotyped	Number of reactive isolates (%)
0:3	3	-
0:6	3	-
0:8	3	-
0:9	3	3 (100%)

Plate 1: *Yersinia enterocolitica* as seen on the API 20E strip

KEY:

ONPG - Beta galactosidase (-ve)
ADH - Arginine dihydrolase (-ve)
LDC - Lysine decarboxylase (-ve)
ODC - Ornithine decarboxylase (+ve)
CIT - Citrate (-ve)
H_2S - Hydrogen Sulphide (-ve)
URE - Urease (+ve)
TDA - Tryphan deaminase (-ve)
IND - Indole (+ve)

VP - Voges – Proskaeur (-ve)
GEL - Gelatin (-ve)
GLU - Glucose (+ve)
MAN - Mannitol (+ve)
INO - Inositol (-ve)
SOR - Sorbitol (+ve)
RHA - Rhamnose (-ve)
SAC - Salicin (+ve)
MEL - Melibiose (-ve)
AMY - Amylose (+ve)
ARA - Arabinose (+ve)

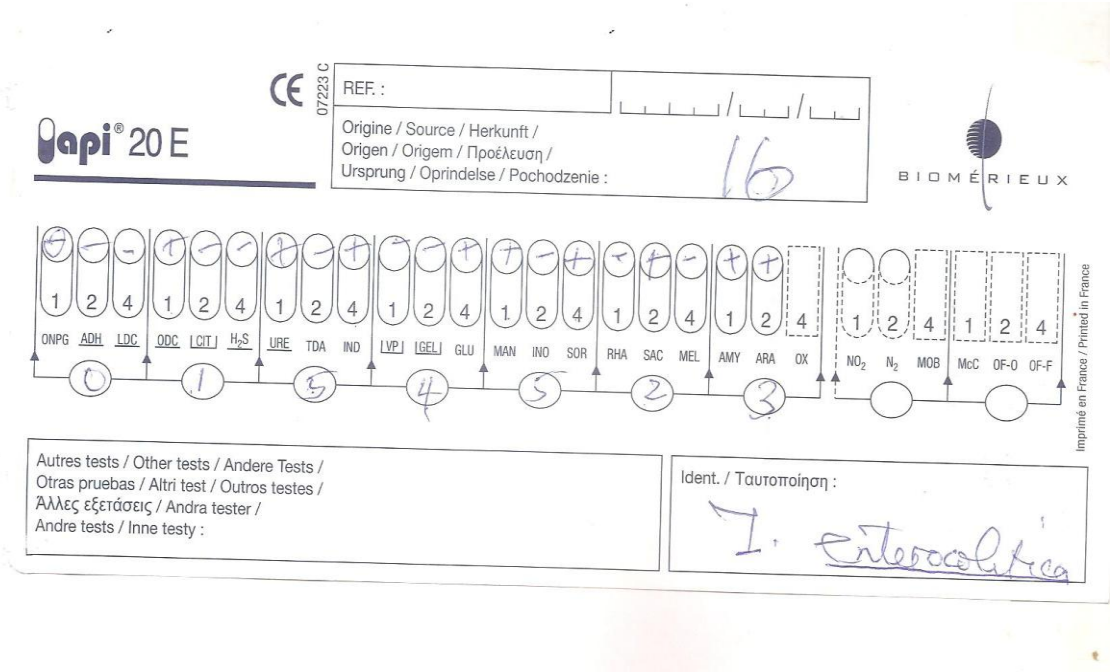

Plate 2: The API 20E strip scoring chart for *Yersinia enterocolitica*

Discussion and Conclusion

This study shows a low prevalence rate (1%) of pathogenic *Yersinia* in locally fermented cow milk (nono) hawked in Jos Metropolis. Only *Yersinia enterocolitica* was implicated in this study. *Yersinia enterocolitica* has been isolated from animals (Okwori *et al,* 2005), raw food materials, environment (Fredriksson-Ahomaa and Korkeala, 2003), water and human beings (Kapperud, 1977; Agbonlahor *et al,* 1983; Okwori *et al,* 2007). In man, *Yersinia enterocolitica* produces a severe form of gastroenteritis called yersiniosis (Health Canada, 2001) which is typically manifested by acute watery diarrhoea and also characterized by fever, abdominal pain and cramps and sometimes vomiting. The serotype 0:9 is one of the two most common serotypes which represent the most virulent worldwide causes of human yersiniosis (Bronfin, 2005).

The 1% prevalence rate recorded in this study is of clinical significance because *Yersinia enterocolitica* is capable of multiplying rapidly in milk (especially at refrigerated temperatures). In developing countries where hygiene is poor and animal disease uncontrolled, milk remains the vehicle for a variety of human disease (Gill, 1996). Here in Nigeria, 'nono' is produced under poor sanitary conditions by Fulani local herdsmen not only of unknown but rather questionable health status (Shehu, 1995). The unhygienic processing and handling techniques usually associated with milking may have been possible sources of contamination. Shehu, 1995 carried out a study which reported that the palms and sputum of (female) 'nono' vendors harbored disease-causing pathogens. Since 'nono' is an uncontrolled fermentation product, fermentation failures resulting

in multiplication of microbes during processing presents yet another possible source of contamination. The health significance of either fermentation failure or post processing contamination is enormous. A number of hitherto non-pathogenic microorganisms are known to become pathogenic under various processing or conditions (Solytys, 1963; Ndukwe *et al,* 1992). The need for 'nono' sellers to ensure proper sanitary, conditions in handling milk (nono) is critical to the production of a wholesome product (nono) for consumption by the general public.

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