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REGULAR ARTICLE

OCCURRENCE OF *SALMONELLA, VIBRO AND E. COLI* IN EDIBLE LAND SNAIL IN NIGER DELTA, NIGERIA

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ABSTRACT

We determined the presence of foodborne pathogens from proximal gut of edible land snail (*Archachatina marginata*) sampled from Itam, Akpan Andem, Afaha and Ikpa markets in Uyo metropolis during the dry season. Fresh snail samples were collected from open market tables presented for sale were screened in the laboratory for microbial load. The total bacteria, *Salmonella, Vibrio* and *Escherichia coli* pathogens were measured. The results showed ($p<0.05$) pathogens in snail meat were found to be above $10^2 cfu^{-g}$ recommended microbiological limits. The foodborne pathogenic bacteria rating of sampled markets was Itam < Akpan Andem < Afaha < Ikpa. Edible snail can be a bioindicator and vector of foodborne pathogens. It is critical that producers, retailers, processors and consumers take responsibility to prevent contamination, cross-contamination, mishandling, as well as proper holding, storage and cooking of snail meat to eradicate foodborne pathogenic incidence.

Keywords: Edible mollusc, consumer health, food pathogens, meat contamination, zoonoses

INTRODUCTION

Snail meat is a delicacy in diets of people in southern Nigeria **(Ebenso and Ebenso, 2011).** Snails are prone to environmental contaminations and pollutants **(Ebenso and Ologhobo, 2009).** Molluscs continually ingest bacteria from the soil and their environment **(Walker *et al.,* 1999).** Fresh mollusc is a highly perishable product and spoilage develops aerobically, especially as it concerns improper handling after harvest, processing and storage.

Molluscs have been reported to have been implicated as vehicles for human infections caused by *E. coli*. The *E. coli* have been reported to have long-term survival in manure, soil and pasture **(Fenlon *et al.,* 2000).** Laboratory research demonstrates the potential of invertebrates to act as vectors in the transmission of *E. coli* **(Graczyk *et al.,* 2001).** A mortality of 3 to 5% has been reported on infections caused by *E. coli* **(Thorpe, 2004).**

In accordance to the European Union provisions, *Salmonella* must be absent from 25g of examined samples of food in order for them to be destined for human consumption **(Giaccone *et al.,* 2012).** Today in Nigeria, persistent fever could be linked to typhoid fever. Starting from *Salmonella(enterica)* ser. typhi the reason for contamination could be related to errors and omissions in handling food and the way of harvesting mollusc from uncontrolled areas **(Popovic, 2010).** Sources of food contamination can be numerous, as the *Salmonella* pathogen can be present in the intestine of livestock, without causing any infection to the animals ("healthy carrier" condition) **(Giaccone *et al.,* 2012).** Besides in animals and animal products, *Salmonella* can adhere well to the work surfaces, and from there spread to other foodstuff by cross-contaminations **(Moretro *et al.,* 2011).** When chronic complications from salmonellosis such as ocular and urinary disorders set in, they are hard to treat even with common antibiotics **(Castillo *et al.,* 2011).**

According to the **WHO, (2009)** the estimates for the direct cost from foodborne illness (especially diarrhea, caused by *Salmonella* and *E. coli*) are equated at US $3 billion or 17-25% of the total costs from all illness in Nigeria. Foodborne infection is endemic in Nigeria. The Federal Ministry of Health reported 90,000 cases of food poisoning in 2007, which is certainly a gross understatement. The WHO estimates 200,000 deaths from foodborne pathogens (especially *E. coli* and *Salmonella*) in Nigeria **(WHO, 2009).** The 1997 Local Government Health Systems profile for Nigeria on reported leading causes of deaths in different geo-political zones showed that foodborne pathogenic illnesses (like diarrhoea) cases accounted for 25% of mortality followed by malaria (21%) and accidents (19%) **(FAO/WHO, 2002).** In Akwa Ibom State of Nigeria, bloody diarrhoea accounted for 31% of all cases of foodborne diseases in humans **(Akinjogunla *et al.,* 2009).**

Nonetheless **Collins, (1997)** reported that, food safety and public health officials attribute a rise in incidence of foodborne illness to changes in demographics and consumer life styles that affect the way food is prepared and stored. According to **USDA, (1997)** educating people about steps they must take to prevent and control foodborne illness is a virtal link in the food preparation chain.

The objective of this study was to identify and enumerate foodborne pathogens isolated from proximal gut of edible land snail *Archachatina marginata* sold at markets in Uyo metropolis and to highlight the impact of potential foodborne contamination, as a public health challenge.

MATERIAL AND METHODS

Sample Collection

A total of 96 snail (*A. marginata*) of fresh weight of 100 ± 5.00g were sampled from open market tables, with 24 snails from each of four locations at Itam, Akpan Andem, Afaha and Ikpa markets respectively, in Uyo metropolis, of Akwa Ibom State in the Niger Delta region of Nigeria within latitude $4^{o}31'$N and $4^{o}45'$N and longitude $7^{o}31'$E and $45^{o}5'$E (Figure 1), with mean temperature of 30^{o}C and rainfall of 2000 – 3000 mm per annum **(Udosen, 2000)** during the dry season months of October and November.

Fresh snail samples for microbiological analyses were collected in sterile isotherm container and transported to the laboratory. The snails were extensively washed with water and rinsed with normal saline to remove all surface contaminants. The edible parts of snails were dissected to remove intestinal extracts from the proximal gut, for subsequent homogenization and serial dilutions.

Identification and Enumeration of Bacteria

One gram of sample was diluted serially in ten fold dilution blanks and properly mixed with sterile glass rod. The 0.1 ml of diluted sample was pipetted into sterile plate and molten sterile agar medium (45°C) was poured. The media used were plate count agar (PCA, Biotech), nutrient agar (NA Biotech), xylose lysine desoxycholate agar (XLD, Biotech) and DeMan Rogosa Sharpe agar (MRS, Biotech). The plates were rotated gently to disperse inoculum in medium and allowed to solidify. This was done in triplicates and plates were incubated at 37°C.

Colonies that developed on the plates were grouped on the bases of their cultural characteristics. Pure cultures of all bacterial isolates were obtained by repeated streaking on

NA, PCA and MRS plates. Morphological characteristics of each isolate were examined after Gram-staining, spore stain, and motility under the light microscope (X1000) using oil immersion objectives. For the purpose of identification the following biochemical tests were performed on the isolates: gelatin hydrolysis, catalase, indole, nitrate reduction, Voges Proskauer, methyl red and sugar utilization (glucose, lactose, galactose, maltose, mannitol, sorbose,cellobiose, arabinose, raffinose sorbitol, fructose, xylose and sucrose).

The identification of the isolates was done by comparing the cultural, morphological and biochemical characteristics of the cultures with the characteristics of known taxa using the Bergey's manual of determinative bacteriology **(Holt *et al.*, 1994; Cheesbrough, 2006; Oyeleke and Manga 2008)** and Cowan and Steel's manual for the identification of medical bacteria **(Barrow and Feltham, 1992).**

Statistical Analysis

Data obtained were subjected to one way analysis of variance (ANOVA), using statistical analysis system (SAS) package and means separated using least significant difference (LSD) **(SAS, 1992).**

RESULTS AND DISCUSSION

In the present study, the total *Vibrio* count ($p<0.05$) range from 2.00 – 9.66 x $10^4 cfu^{-g}$, these values were higher than $10^3 cfu^{-g}$ limits by **HPA, (2009); ICMSF, (1986)** respectively. Results confirm strong evident which may occur due to poor sanitary conditions and cross-contamination. These indicate that consumption of snails from Uyo markets would be potentially injurious to health and/or unfit for human consumption. Snails were sampled during the dry season, whereby temperature could be over 30^oC at the markets. According to **Kaysner and DePaola, (2000); CDC, (2007)** detection of *Vibrio spp* is more likely in mollusc harvested in summer than in winter. **Su and Liu, (2007)** concluded that *Vibrio* can multiply rapidly in oysters upon exposure to elevated temperatures. In consuming snail meat contaminated by *Vibrio*, **Lhafi and Kuhne, (2007)** reported that the significance of public health is dependent on the health status of the consumer as well as on the concentration and on the virulence of the pathogen. In their study **Eduok *et al.*, (2010)** reported inferior values for *Vibrio spp* isolated from fresh mangrove oyster *Crassostrea tulipa* from Douglas creek of Niger Delta, with foodborne infection becoming worrisome because of the unhygienic environment, handling of the harvested biota and

mild heat. The **FDA, (2005)** stated that the total and pathogenic *Vibrio* grows and survives equally during post-harvest handling and processing.

In Table 1, *Salmonella* count ($p<0.05$) ranged from 8.67–18.33 x 10^4cfu^{-g} with reference to **ICMSF, (1986)** the recommended microbial count for *Salmonella* in fresh and frozen fish, crustaceans and oysters (mollusc) is 10^2cfu^{-g}. While discussing the infective dose issue, **Giaccone *et al*., (2012)** suggested that, generally it is accepted that *Salmonella* becomes truly dangerous for humans when it reaches in a food a change of at least 10^4cfu^{-g}. According to **Lindhardth *et al.,* (2009)** it is important to note that the foods contaminated by *Salmonella* do not usually show any modification in their sensory characteristics, even though the pathogens within have reached very high levels, concretely harmful to human health. The present study may serve as a surveillance testing of few samples of snail meat from open market environment, but the risk for foodborne illness is increasing.

The *E. coli* count ($p<0.05$) ranged from 0.00 – 9.33 x 10^4cfu^{-g} (Table 1). These values were above 10^2cfu^{-g} limits of **HPA, (2009).** According to HPA template, cause of contamination may be poor hygiene due to undercooking, or cross contamination from raw food especially meat or food contact surfaces, as well as poor temperature and time control. According to **Olowe *et al.,* (2008)** *E. coli* can induce grastroentritis. The occurrence of *E. coli* in the samples indicated recent fecal pollution of human origin **(Duffour *et al.,* 1985).** Molluscs could be potential carried of *E. coli* **(Sproston *et al.,* 2006).** It has been reported that gastropods find mammalians feces (manure) an attractive food source **(Speiser, 2001),** which together with the regular ingestion of contaminated soil, demonstrates the potential to internal pathogen carriage.

The pathogenic load ratings of the four markets are Itam<Akpan Andem<Afaha<Ikpa markets. The microorganisms isolated in this study have health implications to man **(Omenewa *et al.,* 2011).**

According to **Popovic *et al.,* (2010)** the seafood with the largest number of unsatisfactory rates of indicators and pathogens are molluscs. The **FDA, (2011)** reported that application of heat is one of the simplest and most effective methods of eliminating pathogens from food, recommending that heat application of 90^{o}C for 1.5min in the center of the molluscs and 100^{o}C for 4 mins for shellfish are accepted as safe processes before consumption. **Oraei *et al.,* (2011)** affirmed that the irradiation (up to 7.00 kiloGray) of fisheries product, is a physical treatment involving direct exposure to electron of electromagnetic rays for their longtime preservation and improvement of quality and safety. **Norhana *et al.,* (2010)** stated that refrigeration (below 4^{o}C) and freezing are well known techniques for extending the shelf-life of food products.

Table1 Bacterial counts isolated from proximal gut of *A. marginata* sold at Uyo Markets

Paramater (x10^4 cfu^{-g})	Itam	Andem	Afaha	Ikpa	SEM
Total bacteria count	9.00^c	17.00^b	9.33^c	24.33^a	1.25
Salmonella sp	8.67^c	11.33^b	18.33^a	12.33^b	2.74
Vibrio sp	2.00^c	8.00^b	9.00^a	9.66^a	1.51
Escherichia coli	0.00^c	4.33^b	3.00^b	9.33^a	1.25

Figure 1a **Figure 1b**

Figure 1 Map of Nigeria showing Akwa Ibom State (Fig 1a) and Map of Akwa Ibom State showing market (sampling) locations in Uyo (Fig 1b).

CONCLUSION

The edible snail can serve as a vector for spread of foodborne illnesses. In this study samples recorded pathogens above (10^2cfu^{-g}) standard microbiological limits in food. The *A. marginata* edible land snail can be used as a bioindicator for microbiological and food safety assessment. Epidemiological data of this study indicates that safety in foods is critical to public health and safety of consumers.

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