

## PRELIMINARY STUDIES ON THE PREVALENCE OF *Listeria monocytogenes* IN *Clarias gariepinus* and *Oreochromis niloticus* IN IBADAN, OYO STATE

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### ABSTRACT

*Prevalence of Listeria species in Clarias gariepinus and Oreochromis niloticus were examined. Sixty (60) of fishes were randomly selected from commercial outlet in Ibadan. Sex, Total Length, and Weight were determined. Simple percentages were used to analysis the data. The results show that 12 out of 60 (20%) were positive for Listeria species. Male has highest number of infection (18.91%) fishes with average length of 20-30cm had 41.17%, 351-400g had highest number of infection (28.57% ). C. gariepinus (26.67%) shows the higher presence of Listeria species than O. niloticus. There is no correlation between infection and species, sex or length. The result shows high presence of Listeria species and further suggested that there is high risk of contacting listeriosis though the listeriosis outbreak has not been recorded in the study area.*

**Key words:** *Listeria species*, Prevalence, Infection

### INTRODUCTION

Fish is considered as one of the safest and highly nutritional source of protein. The need for fish to be readily available in the market cannot be overemphasized, as the gap between its demand and supply is very huge. Despite a noticeable decrease in capture production, aquaculture is assuming a profitable venture economically (Adeshina & Umma, 2012). Fish consumption per capita of 12.0kg is considered globally adequate for normal and healthy growth (Ogbonnaya & Ibrahim 2009). It can be observed that this has not been met in developing countries like Nigeria. The quality of fish and its products must be standard and safe for consumption if fish must remain in the market. Fish can harbour some pathogens/diseases which can be transmitted to person(s) who have contact or eat the infected fish. It has been recorded that Nigeria is the largest consumers of fish throughout the world (1.5million tonnes/year) (Imam and Dewu, 2010), yet, Nigeria is deficit in fish requirement as the country imports over 900,000 metric tonnes of fish with domestic production lingering behind at 450,000 metric tonnes/year (Biu and Akorede, 2013).

There are several pathogens associated with fish but the case of *Listeria monocytogenes* drawn some attentions. *Listeria* spp. are considered ubiquitous organisms and causes serious illness in man and fish called listeriosis (Autio, *et al.*, 1990). The organism is found in the soil, vegetation, plants, mud, water, sludge, animals and birds. *L. monocytogenes* is a harmful bacterium that can be found in a variety of foods. In pregnant women, *L. monocytogenes* caused illness can result in miscarriage, fetal death, or severe illness or death of a newborn infant. *L.*

*monocytogenes* and other listeria spp. have been isolated from fishery products on a regular basis in tropical and temperate regions 1980s. *L. monocytogenes* is considered as psychrotrophic pathogen and may thus be less common in tropical water (Siavash, *et al.*, 2011). Ben Embarek (1974) reviewed the incidence *Listeria* in seafood worldwide and found that the prevalence of *L. monocytogenes* varies from 4 to 12% in temperate areas and 12 to 17% in tropical areas (Adesiyun, 1993). Ecological niche of *L. monocytogenes* is very difficult to define because it can survive in many environmental conditions (Mittinen, 2006). It has unique abilities to grow at 4<sup>0</sup>c (refrigerated temperatures). It has been isolated from mud, plants, soil, water, vegetation, and in the internal organs of both man and animals (Mietinnen 2006; Chukwu 2007). Chukwu, (2007) found *L. monocytogenes* in sheep faeces, rabbit faeces and cow dung had about 78 (41.27%) of total sample.

Chukwu (2007) investigated 900 samples of plant, soils, animal wastes and farm debris and reported that *L. monocytogenes* occurred 78 times (8.6%) which is the highest. In fish species, roe and equipments ranges from slightly above 4% to as high as 88% in fin fishes and as also significant from 0 to 51% from shelf fish (Walker *et al.*, 1990; Swaminathan and Feng 1994; Rorvik *et al.*, 1995; Jinneman *et al.*, 1999; Rocourt *et al.*, 2001; Zhu *et al.*, 2005; Mietinnen, 2006; Salihu *et al.*, 2008; Amusan, 2012). The economic and social impact of *L. monocytogenes* on animals and the Nigerian population are largely unknown. Each year USA estimates about 1500-2500 cases of *L. monocytogenes* infection with 350-500 deaths. More so, old adults, pregnant women and immunodeficient people are more prone to infections (Dowe, *et al.*, 1997). Reports of *Listeria* infections in Africa are scanty and particularly in Nigeria, despite many pathonogonomic signs and symptoms suggestive of listeriosis (Bayles *et al.*, 1996). Significant human demographics, behaviour and land use changes have been witnessed in Nigeria within the last decade. These changes have influenced the lifestyle of the citizenry contributing to new disease emergence by changing transmission dynamics to bring animals (including fish) and people into closer and more frequent contact with pathogens (Chukwu, 2007). Such changes have led to increases in human agricultural and aquacultural activities in both residential areas and their environments (Botzler *et al.*, 1974). The animal wastes of commercial farms of this area are used directly as bio-organic fertilizer of some farm (Dauphin *et al.*, 2001) which could serve as source of contamination.

Fish is recommended to old people and pregnant women by medical practioners because of high protein contents but very unfortunately the people prone to listorios are these categories (Bayles *et al.*, 1996; Anon, 1999), hence, the need for the study of fish listeriosis. There are still a paucity of knowledge on the distribution of listeria species couple with unreported cases are presently lacking. The study investigated the prevalence of *L. monocytogenes* in fish in Ibadan, Nigeria.

## **MATERIALS AND METHODS**

This study was conducted in Ibadan, Oyo State. It lies within the forestry zone of Western Nigeria. Ibadan is located on Latitude 7° 25' North and Longitude 3° 5' East approximately 145 kilometers north of Lagos (OYSG, 2015).

## Prevalence of *Listeria monocytogenes*

### Sample collection

*C. gariepinus* and *O. niloticus* were used for the study because of its popularity, culture and consumption rate. Thirty (30) *C. gariepinus* and thirty (30) Tilapia (*Oreochromis niloticus*) were purchased live from local fish sellers within Ibadan metropolis between September and December 2013. Twenty fishes per location (Orita Agbowo, Iwo road, and Eleyele markets) were collected three times. The fish samples were transported in bowl (plastic) containing clean water from a well to the Pathology and Parasitological laboratory of Veterinary Hospital, Mokola Ibadan where they were examined for listeriosis. The fishes were separated based on their sexes using genital observation. Total length and Weight of the fish were recorded. The samples were stored at 4°C for not more than 24 h before analysis.

### Isolation and Identification of *Listeria* species

Samples were analysed for *Listeria* spp. Using the enrichment, selection and isolation protocol recommended by the US Food and drug Administration (Lovett, 1988). For each sample, 5g of intestine and 5g of gills were removed aseptically and placed separately in 20ml of listeria enrichment broth (LEB), Merck Germany) containing potassium thiocyanate 937.5g/L, Merck). The compound was homogenized and incubated at 35°C. On day 1, 2, and 3 the culture were plated onto listeria selective agar (Oxford formulation, Himedia, India) and incubated at 37°C for 2 days (48h). The plates were observed for listeria colonies (greenish-yellow colonies) and at three colonies were subjected to sub-culture (Brain heart infusion Agar (BHIA, Hispanblab) and incubated at 37°C for 48h. The colonies were characterized by Gram staining, motility, catalase and oxidase test and acid production from xylose.

### Statistical analysis

The data were subjected to descriptive statistics and correlation using Statistical Package for Social Sciences (SPSS) Version 20.

## RESULTS AND DISCUSSION

The result shows that 12 out of 60 (20%) were positive for *Listeria* species (Table 1) which is high enough to cause damage, loss and infection. Table 2 shows highest number of infected species based on sex was high in male (18.91%) but the percentage of infection was high in female (21.74). Table 3 shows the occurrence of *Listeria* species based on Total length, where fishes with 20-30cm has highest infection (41.17%) while fishes with 30-40cm has lowest (9.09%) and there is negative correlation ( $r = -0.774$ ) between the percentage of infection and infection intensity in reference to length. Table 4 shows the prevalence based on body weight. It was observed that fishes with 351-400 have highest number of infection with 28.57% and there is positive correlation ( $r = 0.892$ ) between the percentage of infection and infection intensity in reference to bodyweight. *C. gariepinus* shows the highest presence of *Listeria* species with over 26.67% (*L. monocytogenes* = 16.67%; *L. innocua* = 6.67%; and *L. grayii* = 3.33%) while *O. niloticus* shows total of 13.33% presence of *Listeria* species (*L. monocytogenes* = 10.00%; *L. innocua* = 3.33%; and *L. grayii* = 0.00%). This result in agreement with the work of Siavash, *et al.*, (2011) who recorded 18%, Salihu, *et al.*, (2008) and Mittenin, (2007). The dominant two species (*L. monocytogenes* and *L. innocua*) shares the same or similar ecological niche so the similarity in their occurrence is in order.

**Table 1: Occurrence of *Listeria* species in Fish Samples by Species**

| Species              | No. of samples | Number(%)               |                   |                 |
|----------------------|----------------|-------------------------|-------------------|-----------------|
|                      |                | <i>L. monocytogenes</i> | <i>L. innocua</i> | <i>L. grayi</i> |
| <i>C. gariepinus</i> | 30             | 5(16.67)                | 2(6.67)           | 1(3.33)         |
| <i>O. niloticus</i>  | 30             | 3(10.00)                | 1(3.33)           | 0(0.00)         |
| Total                | 60             | 8(13.33)                | 3(5.00)           | 1(1.67)         |

**Table 2: Occurrence of *Listeria* species in Fish Samples by Sex**

| Sex     | No. of samples | No. of infected (%) | Intensity of Infection |
|---------|----------------|---------------------|------------------------|
| Male    | 37             | 7(18.91)            | 0.19                   |
| Females | 23             | 5(21.74)            | 0.20                   |
| Total   | 60             | 12(20.00)           | 0.20                   |

**Table 3: Occurrence of *Listeria* species in Fish Samples by Total Length**

| Total Length (cm) | No. of samples | No. infected (%) | Intensity of infection | Correlation between % and Intensity of infection |
|-------------------|----------------|------------------|------------------------|--------------------------------------------------|
| 10-20             | 21             | 3(14.29)         | 0.14                   | -0.774                                           |
| 21-30             | 17             | 7(41.17)         | 0.41                   |                                                  |
| 30-40             | 22             | 2(9.09)          | 0.09                   |                                                  |
| Total             | 60             | 12(20.00)        | 0.20                   |                                                  |

\* Correlation is significant at the 0.05 level (1-tailed), \*\* Correlation is significant at the 0.01 level (1-tailed)

## Prevalence of *Listeria monocytogenes*

**Table 4: Occurrence of *Listeria* species in Fish Samples by Weight**

| Body weight (g) | No. of samples | No. infected(%) | Intensity of infection | Correlation between % and Intensity of infection |
|-----------------|----------------|-----------------|------------------------|--------------------------------------------------|
| 100-150         | 0              | 0(0.00)         | 0.00                   | 0.892*                                           |
| 151-200         | 9              | 1(11.11)        | 0.11                   |                                                  |
| 250-300         | 13             | 3(23.07)        | 0.23                   |                                                  |
| 301-350         | 0              | 0(0.00)         | 0.00                   |                                                  |
| 351-400         | 28             | 8(28.57)        | 0.24                   |                                                  |
| Total           | 60             | 12(20.00)       | 0.20                   |                                                  |

\* Correlation is significant at the 0.05 level (1-tailed) , \*\* Correlation is significant at the 0.01 level (1-tailed)

## CONCLUSION AND RECOMMENDATIONS

Isolation of *L. monocytogenes* in fish (*Clarias* and *Tilapia*) in Ibadan in this study indicates that there is high presence of *Listeria* species and this further suggested that there is high risk of contacting listeriosis in the study area. Although the case of listeriosis in Ibadan is yet to be formally reported, but the study opined that it infection might went unreported, hence consumers should sensitized on its prevalence in the area. Further studies should be conducted to ascertain its health and economic implication as well as knowledge and attitude of the populace.

## REFERENCES

- Adeshina, I. and Umma, S.B. (2012): Effect of different smoking periods on nutritional value of *Heterotis niloticus*, *Int. J. of Sci. and Dev. St.* 7(7):117-124.
- Adesiyun, A.A. (1993): Prevalence of *Listeria* spp. *Campylobacter* spp. *Salmonella* spp. *Yersinia* spp. and toxigenic *escherichia coli* on meat and seafood in trinidad *Int. j. F. Microbiol.* 10:395-403.
- Amusan, EE (2012): Detection of *Listeria monocytogenes* in frozen fish in Lagos, Nigeria, *Proceedings of the 27<sup>th</sup> Annual Conference and Biennial General Meeting of the Fisheries Society of Nigeria* held at Banguet Hall Government House/Faculty of Law, Niger-Delta University, Bayelsa State, 25<sup>th</sup>-30<sup>th</sup> November, 307-310.
- Anon, B. (1999). Opinion of scientific committee on veterinary measures relating to public health on *Listeria monocytogene*. *European Commission*. Health and Consumer Protection Directorate- General Manual, 12-16.

- Autio, T., Hielm, S. Miettinen, M., Sjoberg, A.M., Aarnisalo, K., Bjorkroth, J., Mattila-Sandholm, T. & Korkeala, H. (1999). Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. *Appl. Environ. Microbiol.* 65:150-55.
- Bayles, D.O., Annous, B.A. & Wilkinson, B.J. (1996). Cold stress proteins induced in *Listeria monocytogenes* in response to temperature downshock and growth at low temperatures. *Appl. Environ. Microbiol.*, 62:1116–1119.
- Ben-Embarek, P.K. (1994). Presence, detection and growth of *Listeria monocytogenes* in seafoods: A review. *Int. J. Food Microbiol.* 23:17-34.
- Biu, A.A. and Akorede, G.J. (2013). Prevalence of Endoparasites of *Clarias gariepinus* (Burchell 1822) in Maiduguri, Nigeria *N. j. of Fisheries & Aq.* 1(1);1-6.
- Botzler, R.G., Cowan, A.B. and Wetzler, T.F. (1974): Survival of *Listeria monocytogenes* in soil and water. *J. Wildl. Dis.* 10, 204-212.
- Chukwu, O.O.C. (2007): Studies of *Listeria monocytogenes* (Bacterium) in some selected Local Government Areas of Plateau State, Nigeria, *Ph.D Thesis* submitted to Department of Botany, University of Jos, 256.
- Dauphin, G., Ragimbeau, C. & Malle, P. (2001). Use of PFGE typing for tracing contamination with *Listeria monocytogenes* in three cold-smoked salmon processing plants. *Int. J. Food Microbiol.* 64:51-61.
- Dowe, M.J., Jackson, E.D., Mori, J.G. & Bell, C.R. (1997). *Listeria monocytogenes* survival in soil and incidence in agricultural soils. *J. Food Prot.* 60, 1201-1207.
- Imam, T.S. and Dewu, R.A. (2010). Survey of Piscine ecto and intestinal parasites of *Clarias* spp. Soldat Galadima Road Fish Market, Kano Metropolis, Nigeria *Bio. Res. Comm.* 2294:209-214.
- inneman, KC; Wekell, MM & Eklund, MW (1999): *Incidence and behavior of Listeria monocytogenes in fish and seafood products*. In: Ryser, E.T. and Marth, E.H. (eds.), *Listeria, listeriosis and food safety*, 2 ed. Marcel Dekker, New York, USA p. 601-630.
- Lovett, J. (1988). Isolation and Enumeration of *Listeria monocytogenes*. *Food Technol.*, 42:172-175.
- Miettinen, H (2006): *Listeria monocytogenes* if Fish Farming and Processing, *PhD Thesis*, University of Helsinki, Finland, 77.
- Miettinen, H. (2007). *Listeria monocytogenes* in fish farming and processing. Academic dissertation To be presented with the permission of the Faculty of Veterinary Medicine, University of Helsinki, for public examination in Auditorium P3, Porthania, Yliopistonkatu.
- Ogbonaya, O.P. & Ibrahim, I.A. (2009): Food production in Nigeria, A report submitted to the Ministry of Agriculture, Nigeria. 234p.

## **Prevalence of *Listeria monocytogenes***

Oyo State Government (OYSG) (2015): Official Website of Oyo State Government. Available on <http://www.oyostate.gov.ng/ministries-departments-and-agencies/local-government-and-chieftaincy-matters/local-government-chairmen/> accessed on 1/6/2015 9:59pm.

Rocourt, J; Hogue, A; Toyofuku, H; Jacquet, C & Schlundt, J (2001): *Listeria* and listeriosis: risk assessment as a new tool to unravel a multifaceted problem A., *J. Infect. Control.* 29:225-227.

Rørvik, LM; Caugant, DA & Yndestad, M (1995): Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. *Int. J. Food Microbiol.* 25, 19-27.

Salihu, M. D., Junaidu, A. U., Manga, S.B., Gulumbe, M.L., Magaji, A.A., Ahmed, A., Adamu, A.Y., Shittu, A. & Balarabe, I. (2008). Occurrence of *Listeria monocytogenes* in smoked fish in Sokoto, Nigeria *African Journal of Biotechnology* 7(17):3082-3084.

Salihu, MD; Junaidu, AU; Manga, SB; Gulumbe, ML; Magaji, AA; Ahmed, A; Adamu, AY; Shittu, A & Balarabe I (2008): Occurrence of *Listeria monocytogenes* in smoked fish in Sokoto, Nigeria, *Journal of Biotechnology* 7 (17):3082-3084.

Siavash, M., Ali, F. & Samira, E. (2011). Incidence of *Listeria* species in Farmed Tropical Fish in khuzestan, Iran *W. J. of F. and M. Sci.* 3(3):206-209).

Swaminathan, B & Feng, P (1994): Rapid detection of Foodborne Pathogenic Bacteria. *Annu. Rev. Microbiol.* 48:404 – 426.

Walker, SJ; Archer, P & Banks, JG (1990): Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. Appl. Bacteriol.* 68, 157-162.

Zhu, M; Du, M; Cordray, J & Ahn, DU (2005): Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Compr Rev Food Sci Food Saf* 4:34–42.