Chukwu, Ogbonna, Muhammad, Olabode, Nwobu, Ogo, Makinde, Elem, and Okewole (2004).
Nig. J. Biotechn. 15 (1) 52 -59

Listeria monocytogenes AND OTHER *Listeria species* IN POULTRY FAECES APPLIED AS MANURE ON FARM LANDS: ENVIRONMENTAL HEALTH AND FOOD SAFETY

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ABSTRACT

Many diseases of poultry, particularly Listeriosis may be disseminated by faecal contamination of the environment or water. An assessment of the prevalence of *L. monocytogenes* and other *Listeria species* in poultry faeces often utilized as manure in soil supplements for maximum crop yield was evaluated. The results of the study showed that of the 480 faecal samples screened, 108 (22.5%) were positive for *listeria monocytogenes* and other *Listeria species*. The local chicken droppings had the highest rate of recovery for number of *Listeria* 36 (7.5%), followed by Spent old layers 27 (5.63%), then turkey 25 (5.2%) and broilers 20 (4.17%). Characterisation of the *Listeria* species showed that *L. monocytogenes* was 64(59.3%), *L. ivanovii*, 20 (18.5%), *L. grayi* 9(8.3%), *L. seeligeri* 4(3.7%), *L. welshimeri* 4(3.7%) and *L. murrayi* 4(3.7%). The public health implication of this pathogen has necessitated a reappraisal of this emerging foodborne pathogen and strategies for its control.

Key words: *Poultry manure, Listeria monocytogenes, Environmental health, food safety*

INTRODUCTION

In recent years, probably due to high cost of inorganic fertilizer, there have been increased interest in the use of low-cost, and relatively high quality livestock waste matters especially poultry droppings (manure) as supplements or replacement for inorganic fertilizer in farms in Nigeria (Jones, 1979). This practice is most common amongst the peasant farmers who are engaged in the cultivation

of fruits, vegetables and other crops. Faecal wastes from domestic animals and poultry are often applied to the soil surface and to a varying extent incorporated into the soil. These faecal wastes applied can also enter water systems by direct contamination of the water or through seepage or surface runoff. Moreover, these faecal wastes harbour a wide variety of pathogenic bacteria like *Listeria monocytogenes* and other pathogens that are of great concern to the public who are usually exposed to these zoonotic pathogens through consumption of faecal contaminated food or water.

It is worthy to note that several works (Jones, 1979; Duarte, *et al*, 2002) on organic matters indicate that when they are not treated before being used as fertilizer, could serve as a major source of pathogenic bacteria into the ground and surface water as well as the food chain. Thus, this practice of using untreated waste matters has continued to pose a challenge to public health officials for the control and prevention of infectious disease pathogens in food and water.

Naturally, ground water has superior quality over surface water with respect to microbial content because of an effective barrier of soil on top of impervious rock strata. Thus, the water is not significantly influenced by climatic, agricultural runoff or storm water migrations as in the case of surface water. Unfortunately, sink holes (bore holes) and caverns can form, through which surface or runoff water can pass, and if contaminated with pathogens, the pathogens will have access to ground water. More so, these have been reported cases that excessive land application of minimally treated animal wastes as (manures) and wastewater can inundate natural soil barriers (Jones, 1979; Jogbloed and Lenis, 1998; Cole, *et al*, 1999). Also excessive distribution of animal wastes from feed lots operations as well as poorly located and contained water plant sludge and garbage waste can contribute to significant pathogen releases in leachiest (Jogbloed and Lenis, 1998). Once the aquifers become contaminated, restoration of ground water purity is very difficult and where possible very slow (Jogbloed and Lenis, 1998; Cole, *et al*, 1999).

The organism *Listeria* is among the infectious organisms which have been known to invade the food chain having been isolated from pasture, soil, water supplies, meat and dairy products among other sources (Weis and Seeliger 1975, Chukwu, 1994, Tauxe, 1997; Loncarevic *et. al.*, 1999,). The genus *Listeria* consists of small, non-spore forming gram-positive rods. Of the several species in this genus, *L. monocytogenes* is the primary and principal pathogenic species for man and animals. Listeriosis, the infectious disease associated with the *Listeria* organism has been recognized in man as a foodborne disease (Schlech *et. al.*, 1983 and Farber and Peterkin, 1991). *L. monocytogenesi* is transmitted to humans by food and waters especially the green food (Lettuce, Cabbage, Sprouts and others) that has been grown using contaminated animal manures used to fertilize them (Schlech, *et al*, 1983; Heseik *et. al.*, 1989; Tauxe, 1997).

The intensification of poultry farm and in particular the disposal or use of their excreta on agricultural lands calls for a reconsideration of the role of this material in the promotion of pathogenic bacteria *L. monocytogenes* and other *Listeria species* as well as other pathogens like *Salmonella species, Yersinia enterocolitica,* in the environment especially in food and water supplies. Applications of contaminated animal waste as manure to fertilize crops have been reported as sources of human *Listeriosis* (Shlech, *et al*, Tauxe, 1997). Also as a potential source of waterborne infections as direct contamination and agriculture runoff can lead to a large numbers of the organism *L. monocytogenes* and other *listeria species* in the environment (Tauxe, 1997; Cole, *et al* 1999).

In view of the increased application of untreated poultry droppings as manure (fertilizers) in the farmlands in Nigeria, the aim of this work was to evaluate the presence of *L. monocytogenes* and other *Listeria* species in chickens and Turkeys excreta from poultry houses whose excreta have been

applied or incorporated into the agricultural farmlands in Bukuru and its environs.

MATERIALS AND METHODS

Collection of Samples: -

In this study, 480 samples of faecal materials were obtained from apparently, healthy Chickens and Turkey with no history of previous Listeric infections diagnosed and treatment effected, but their droppings are being applied as manure and the birds being slaughtered for consumers, were screened for the presence of *L. monocytogenes* and other *listeria species*. The 480 chickens and turkey droppings screened were from poultry houses and farms within the Bukuru axis of Plateau State, Nigeria. One hundred and twenty (120) samples were collected from each Broiler, Spent layers, Local chickens and Turkeys.

Analysis of Samples

Each of the 480 samples was the intestine (5mm long) with its content from the respective chickens and turkeys. About 1gm of each faecal sample was collected aseptically and homogenized in 9mls (10^{-1}) dilution of 0.1% peptone water (1 part to 9mls part of peptone water) and stored at 4^0C for 48hrs. 1ml of each of the diluted samples (10^{-1}) was transferred into 9mls *listeria* enrichment broth-University of Vermount (UVM) with supplement 140 (Oxoid Cm856, SR140) and incubated at 37^0C for 48hrs. Using Centre for Disease Control (CDC) isolation procedures (Doyle and Schoeni, 1986) then processed the broth cultures. These were direct plating and cold enrichment followed by secondary selective enrichment and plating on *listeria* selective medium (Oxford formulation).

Direct Plating Method

0.1ml of each of the homogenized faecal samples in 0.1% peptone water was inoculated onto *listeria* selective medium plate (Oxoid Cm856 SR140) and incubated at 37^0C for up to 48hrs. Typical colonies of *listeria* were examined after 24 and 48hrs of incubation respectively.

Selective Enrichment

1ml each of the homogenized faecal samples in peptone water stored at 4^0C for 48hrs was inoculated into 9mls of UVM selective enrichment broth for a secondary selective enrichment and incubated at 30^0C for 7 days. 1ml of the selective broth culture was inoculated after 24hrs, 48hrs and 7 days onto the *listeria* selective medium plates (Oxford formulation). The plate cultures were incubated at 37^0C for up to 48hrs and examined for typical colonies of *listeria* after 24hrs and 48hrs of incubation.

Selection of Isolates

Three (3) days after periodic subculture of the broth cultures onto *listeria* selective medium plates

(Oxford formulation-Oxoid), Curtis, *et al,* (1989), isolates were collected. The subsequent selection of colonies for morphological and biochemical characteristics were based on the basis of aesculin hydrolysis reaction on *listeria* selective agar medium (Curtis, *et al,* 1989). The *listeria* isolates were kept at 4^0C for further experiments – speciation.

Identification of Isolates

The identification of the isolates was based on gram reaction morphology, tumbling motility at room temperature incubation, and catalase test reaction. Biochemical tests (Sugar fermentation), using 1% solution of lactose, sucrose, xylose, manitol, rahmnose and serotyping were carried out on the isolates according to Weaver (1989) methods.

RESULTS

Out of the 480 samples examined, 108 (22.5%) were positive for *Listeria* organisms (Table I). The distribution of the isolates among the four (4) different types of birds sampled showed that the local chicken had the highest number of *Listera* organisms 36 (7.5%), followed by spent layers 27(5.63%), Turkey 25 (5.21%), and Broilers 20(4.17%) (Table II). On speciation, it was observed that *L. monocytogenes* 64 (59.3%) was predominant in all the birds (Broilers, Spent layers Local chicken and Turkey) sampled. Others include; *L. ivanovii* 20 (18.5%), *L. gayi* 9 (8.3%), *L. seeligeri* 4 (3.7%), *L. welshimeri* 4 (3.7%), *L. murrayi* 4 (3.7%), and *L. innocua* 3 (2.8%) (Table II)

Table I: Prevalence rate of *Listeria* species in the different birds treated

Bird	Number Sampled	Number positive	% Positive
Broilers	120	20	4.17
Spent layers	120	27	5.63
Local Chickens	120	36	7.50
Turkeys	120	25	5.21
Total	480	108	22.5

Table II: The occurrence rate of different *Listeria* species in birds sampled

	% of Tot

Listeria spp	Broilers	Spent layers	Local Chickens	Turkey	Total	Total Isolates
Listeria	9	17	23	15	64	59.3
monocytogenes	4	6	3	7	20	18.5
L. ivanovii	-	1	2	1	4	3.7
L. seeligeri	-	1	2	-	3	2.8
L. innocua	3	-	1	-	4	3.7
L. welshimeri	3	-	4	2	9	8.3
L. grayi	1	2	1	-	4	3.7
L. murrayi						
Total	20	27	36	25	108	22.5

DISCUSSION

L. monocytogenes is the *Listeria* species known to cause listeriosis. The organism can live and survive in the soil, manure piles and grasses (Welshinmeri, 1968, Weis and Seeliger, 1975). Owing to the prevalence of *L. monocytogenes* and other *listeria species* in the poultry droppings examined in this study, its ability to cause disease to humans and animals, knowledge about the bacterium is very important to the Agricultural, Medical, and Veterinary communities in Nigeria (Tables I - II).

Listeriosis associated with poultry manure lies in the difficulty in detection of *L. monocytogenes* and quantitation in complex environmental samples such as manure, compost, soil and foods without pre-enrichment, selective and even post enrichment procedures. Therefore, the application of current standard number methods to a variety of matrices involved in determining the exposure at farm and of the farm-to-table continuum will require adaptation and possibly development of new methods for the detection and qualification of viable *L. monocytogenes* using Polymerase Chain Reaction (PCR). Furthermore, it is no doubt that poultry manure may contain strains of *L. monocytogenes* that establish or spread through the environment in Nigeria unless the manure is disinfected prior to beneficial land use. Although not attempted in this work, treatment of poultry droppings may reduce or eliminate *L. monocytogenes* in poultry droppings before their application on agricultural lands.

Therefore, there is need for effective detective technologies and more concerted efforts to educate the farmers, consumers, industry, government and the general public of the health hazards of applying untreated poultry manures as fertilizers due to the possible presence of pathogenic bacteria *L. monocytogenes* of public health significance.

It has been reported that pathogens spread in the environment due to improper treatment and application of sewage, slaughter offal, sludge, biosolids, slurry, manures and faeces of domesticated and wild animals on land (Fenlon 1985, Wahaab 1995; Bouttfroy, *et al,* 1997; Loncarevic *et. al.* 1999; Duarte, *et al,* 2002). From the results of this investigation, application of such manures and other animal wastes in agricultural farmlands may have adverse effect in the near future in Nigeria. Utilization of contaminated poultry droppings containing *L. monocytogenes* could be an important factor in the occurrence and epidemic of water - and -foodborne listeriosis. Application of contaminated animal manure has been reported as the source of an outbreak of listeriosis (Schlech, *et. al.* 1983). *Listeria* organisms often lead to the contamination of surface waters (Tauxe, 1997). They also colonize birds, rodents and insects. The public health implications of the results obtained in this study cannot be over emphasized. More so, as agronomic areas are more compressed and the proximity of poultry production units to areas used in growing crops, such as fresh produce, without processing the manure may be the potential source for the spread of pathogenic *listeria.* Environmental health implication of this organism could be through the contamination of irrigation water and soils by *L. monocytogenes* and subsequently cross contamination of food crops will be on the increase and possibly ground water contamination in Nigeria. This is because the organism in faecal waste can enter water systems by direct contamination of water or through seepage or surface runoff.

The application of poultry manures to agricultural land without adequate decontamination or elimination of possible pathogens that could contaminate crops and foods will directly increase the risk to human listeriosis or other illnesses via water-or food. The risk of recycling pathogens back to animals on the farm is also there. This tread may be more associated with the local chickens (Tables I and II), which are free rangers as their droppings during the rains may be washed into streams that serve as source of water for human and animal utilization and for irrigation.

In Nigeria, techniques such as composting or deep stacking to reduce or eliminate pathogen level in manure are often not used by producers as this require extra time, attention, special equipment or structures that impose additional costs. However, manures may not be the only on-farm source of *L. monocytogenes.* Other farm sources include farm workers, plant residues and soil *L. monocytogenes* that can be found readily in many soils in association with materials, vegetables and decaying leaves and other plant parts (Weis and Seeliger 1975, Welshimeri 1968, Farber and Peterkin, 1991, Dyer and Stoltenow 2002).

Although many pathogens threaten the safety of the Environment, food and water, *L. monocytogenes* is of particular public health concern. This is in spite of what is known about potential vectors or vehicles of *L. monocytogenes* and its contamination. Up till now, many critical questions remain unanswered. There is a lack of knowledge on the pathogen's ability to survive in manures and on the adequacy of various manure management techniques to reduce or eliminate *L. monocytogenes.*

Therefore, it is critical for human health, animal health and agricultural sustainability reasons that our sources of water and food supplies be protected from contamination by poultry droppings and animal waste. Alternatively, it is our believe that as *L. monocytogenes* which is the principal etiologic agent of *listeriosis* is not spore forming, the danger of disseminating the pathogen in the environment via animal waste can be significantly reduced if stored for some months or pre-treated before spreading on agricultural farmlands in Nigeria.

ACKNOWLEDGMENT

We wish to thank the Director, National Veterinary Research Institute, Vom for permission to publish. Also we are thankful to Dr. E.O. Irokanulo for his positive critics, Mr. Samuel Dimka and Mrs. Amina Danbaki for their secretarial duties.

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