The Occurrence of *Listeria monocytogenes* in Faeces of Domesticated Poultry

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Abstract

A study designed to evaluate the occurrence of Listeria monocytogenes (the cause of human and animal listeriosis) in faeces of domesticated poultry in Jos, Plateau State, Nigeria was undertaken. A total of 100 fresh faecal samples were obtained from a variety of poultry (40 ducks, 30 each of chickens and turkeys) and screened for Listeria monocytogenes using the University of Vermont (UVM) Listeria enrichment broth and Listeria selective media agar base (Oxford formulation). The results obtained showed that of the 100 samples screened, 42 (42%) were positive for Listeria monocytogenes. Chickens accounted for the highest percentage 18(60%) of birds positive for Listeria monocytogenes followed by Turkeys 12 (40%) and the ducks 12 (30%). Other bacteria isolated included: Listeria grayi, Listeria murrayi, Baccillus spp, Enterococcus spp, Staphylococcus aureus, Streptococcus spp, and Yeast. The public health implications of this pathogen detected in poultry faeces are discussed in relation to the use and methods of disposal of poultry faeces.

Introduction

In the last two to three decades, poultry keeping in Nigeria has become common practice. Many individuals keep poultry either on a large or small scale within their homes. These birds are kept for their egg production or for meat, while the droppings are used as supplements or replacement for inorganic fertilizer in farms in Nigeria (Jones, 1979; Chukwu *et al*, 2004a)

The faecal material which may harbour a variety of pathogenic bacteria of great public health significance may be washed by rain water and may enter water systems either by direct contamination or seepage or surface runoff (Talaro and Talaro, 1996, Chukwu *et al;* 2004a)

The intensification of poultry farming and in particular the disposal or use of their excreta on agricultural land calls for a reconsideration of the role of this material in the promotion of pathogenic bacteria. Application of contaminated animal waste as manure to fertilize crops has been reported as a source of human listeriosis (Schlech *et al,* 1983; Tauxe, 1997).

Listeriosis is a zoonotic disease caused by an emerging food borne pathogen called *Listeria monocytogenes* (Talaro and Talaro, 1996). It may show different symptoms, which include abortion in animals, septicaemia, encephalitis, meningitis, endocarditis, personality change and other disorders in man and animals.

The genus *Listeria* consists of gram-positive, straight or slightly curved non-sporing and non-capsule forming small rods or coccobacilli, 2 to 3 μm by 0.5 μm in size (Duguid *et al*, 1985). Of the several species in this genus, *L. monocytogenes* is the principal pathogenic specie for man and animals. Listeriosis has been recognised as a very important emerging infectious foodborne disease (Schlech *et al.* 1983; Faber and Peterkin, 1991., Talaro and Talaro 1996., Prescott *et al.*, 2002, Adak *et al.*, 2005). *L.monocytogenes* is transmitted to humans through ingestion of contaminated food and water. Also, vegetables may get infected when grown on soils contaminated with this bacterium (Schlech *et al.*, 1983., Tauxe, 1997, Pondei and Ogbonna, 2004). The first documented outbreak of food borne listeriosis occurred in Nova Scotia, Canada in 1981 (Schlech *et al.,* 1983). Since then several other cases have been reported: Massachusetts in 1983, Los Angeles and Orange county, California in 1985, England and Wales between 1996 and 2000 (Flemming *et al.,* 1985; James *et al.,* 1985; Adak *et al.*, 2005).

In Nigeria, several cases of *L. monocytogenes* have been diagnosed in man and animals (Oni *et al.,* 1989, Chukwu *et al.,* 1997). Reported cases include: genital infection (urethritis) of a dairy animal attendant associated with *L. monocytogenes* in Vom, Nigeria (Chukwu *et al,* 1997); an outbreak in a cattle ranch in Jos, Plateau State leading to the death of 20 animals within about 10 minutes (Chukwu *et al.*, 2004b); and the first case of natural infection by *L. monocytogenes* in dog, leading to canine Listeriosis manifesting as a neurologic disorder mimicking rabies infection (Chukwu *et al.*, 2004c). *L. monocytogenes* has also been isolated from some vegetables commonly grown in Jos, Nigeria and the type of manure applied on the different farms affected the occurrence of *L. monocytogenes* (Pondei and Ogbonna, 2004).

The intensification of poultry farming calls for intensive research to ensure that the faecal wastes generated by poultry birds are friendly to the environment and to man. This research was carried out to evaluate the presence of *Listeria monocytogenes* in faeces of poultry kept in homes and to recommend public environmental health safety practices which may check the contamination of human habitats and homes by pathogenic microorganisms seen in poultry faeces.

Materials and Methods

Collection of samples: One hundred (100) samples of fresh faecal material were obtained from apparently healthy chickens, turkeys and ducks

which have neither been diagnosed nor treated for listeriosis and are kept in domestic homes as free rangers or in poultry houses in Jos metropolis, Plateau State, Nigeria.

The 100 poultry screened consisted of 30 chickens, 40 ducks and 30 turkeys. The samples were collected immediately after they were dropped; using a clean spatula they were placed inside separate sterile polythene bags and labelled. The samples were kept at refrigerator temperature until they reached the laboratory for analysis the next day, as suggested by Pagotto *et al.*,(2002).

Analysis of samples: Analysis of the faecal samples was done in four phases – Pre-enrichment, selective enrichment, selective plating and identification as described below.

Pre-enrichment: One gram (1g) of each of the 100 faecal samples was weighed out aseptically and homogenized in 9ml of 0.1% peptone water; (1 part to 9 parts peptone water) as suggested by Pagotto *et al.*, (2002). The homogenized faecal material in peptone water was stored at 4ºC for 48 hours.

Selective enrichment: One millilitre of each of the homogenized samples was transferred into 9ml of University of Vermont *Listeria* enrichment broth (UVM) with supplement SR140 (Oxoid CM 856) and incubated at 30ºC for 72 hours as recommended by Curtis *et al.*, (1989).

Selective Plating: Plating was done using the procedures of the Centre for Disease Control and as elaborated by Doyle and Schoeni (1986). Briefly, using a sterile wire loop, the broth cultures were inoculated onto *Listeria* selective medium agar base plates (Oxford formulation) with supplements Oxoid CM 856 & SR140, then incubated at 35ºC for 48 hours under anaerobic conditions. Typical colonies of *L. monocytogenes* were examined after 48 hours incubation as recommended by Curtis *et al.*, (1989).

Identification: Three days after periodic subculture of the broth cultures onto listeria selective medium plates (Oxford formulation - Oxoid), resultant isolates were collected. The subsequent selection of colonies for morphological and biochemical characterization was based on the black zones around colonies of *L. monocytogenes* due to formation of black iron phenolic compounds derived from the aglucon content of the selective medium (Curtis *et al.*, 1989).

The identification of the isolates was based on a battery of morphological, physiological and biochemical tests: Gram reaction, morphology, tumbling motility at room temperature incubation, and catalase test reaction. Biochemical tests (sugar fermentation) were conducted using 1% solution of each sugar - Lactose, xylose, manitol, and rhamnose as described by Chukwu *et al.*, (2004c).

Other bacteria, which thrived in the listeria selective medium were identified based on Cowan and Steel's manual for identification of medical bacteria (Barrow and Feltham, 1993).

Results

Of the 100 samples examined, 42 (42%) were positive for *Listeria monocytogenes* (Table I). The distribution of the isolates among the three different poultry types sampled showed the chickens having the highest percentage of *Listeria monocytogenes*, followed by the turkeys while the least was the ducks. Of the 30 chickens sampled, 18 (60%) were positive for *L. monocytogenes*. Among the turkeys, 12 (40%) of the 30 sampled were positive for *L. monocytogenes* while 12 (30%) of the ducks were positive out of 40 sampled (Table I).

Other *Listeria* organisms identified are *Listeria grayi* (10%) and *Listeria murrayi* (2%). Of the 30 chickens sampled, 2 (6.67%) were positive for *L. grayi* and no *L. murrayi* was recovered. Of the 40 ducks sampled, 6 (15%) were positive for *L.grayi* and 2 (5%) were positive for *L. murrayi*. Of the 30 turkeys sampled, 2 (6.67%) were positive for *L. grayi* and no *L. murrayi* was recovered (Table 2).

Other bacterial species isolated and identified are also shown in Table 2. Overall, a total 182 isolates were recovered (54(29.68%) *Listeria species* and 128(70.31%) other bacteria). Of the 54(29.68%) *Listeria* organisms recovered, 42 (77.78%) were *L. monocytogenes*, 10 (18.52%) were *L. grayi* and 2(3.70%) were *L. murrayi*. Of the 128 (70.31%) other bacteria recovered, *Bacillus* spp accounted for 53(41.40%), *Staphylococcus aureus* - 33(25.78%), *Enterococcus* spp - 28 (21.88%), yeast - 10 (7.81%) and *Streptococcus* spp 4 (3.13%) respectively.

Table I: Occurrence of *Listeria monocytogenes* in the different poultry types treated

Poultry type	Number Sampled	Number Positive	% positive
Chickens	30	18	60.0
Ducks	40	12	30.0
Turkeys	30	12	40.0
Total	**100**	**42**	**42.0**

Discussion

Listeria monocytogenes, the cause of human and animal listeriosis has a temperature range for growth of 3ºC - 45ºC with an optimum of 30ºC but under certain conditions may survive heating up to 60ºC; for this reason, the organism can survive at the temperature of most soils. *L. monocytogenes* was isolated from 42% of the poultry faeces screened. These faeces were obviously soil bound in the form that they were collected. The prevalence rate is higher than the 22.5% documented by Chukwu *et al* (2004a) for *Listeria species* among broilers, spent layers, local chickens and turkeys. This difference may be due to differences in sample size and poultry type screened. Overall, the chickens had the highest incidence (20%) compared to the 12% each in ducks and turkeys. Chukwu *et al* (2004a) also recorded higher rates in local chickens (7.5%) compared to 4.17% in broilers, 5.6% in spent layers and 5.21% in turkeys.

Adak et al, (2005) reported that in England and Wales, chicken consumption accounted for more disease, death and healthcare usage than any

Table 2: Frequency of occurrence of *Listeria species* and other pathogens in relation to poultry type

Pathogen	Chickens	Ducks	Turkeys	Total No. of isolates (%)
L. monocytogenes	18	12	12	42 (23.08)
L. grayi	2	6	2	10 (5.49)
L. murrayi	-	2	-	2 (1.11)
				54 (29.68)
Bacillus spp	13	24	16	53 (29.12)
Enterococcus spp	10	4	14	28 (15.38)
Staphyloccocus aureus	9	18	6	33 (18.13)
Streptococcus spp	2	2	-	4 (2.19)
Yeast	2	6	2	10 (5.49)
				128 (70.31)
Total	**56**	**74**	**52**	**182 (100)**

other food type between 1996 and 2000; a finding corresponds with the findings in this study where 60% of chickens screened were positive for *L. monocytogenes*, this rate is higher than that obtained in other poultry types. Many other bacterial species were also isolated from these chickens.

In England and Wales, *L. monocytogenes* and *Escherichia coli O157:H7* together accounted for 15% of all deaths due to food borne diseases between 1996 and 2000. *L. monocytogenes* alone accounted for 221 cases of diseases and 78 deaths which showed that 35.3% of individuals that came down with listeriosis died of the disease, this death rate is very high especially when compared to some other pathogenic bacteria like *Staphylococcus aureus* and *Bacillus species* where 9,196 and 10,717 cases respectively were reported but no death resulted in either (Adak *et al*, 2005).

In Nigeria, reported deaths due to *L. monocytogenes* is rare but unreported deaths may be high since there are increasing reports of the presence of this pathogen in our environment.

Pondei and Ogbonna (2004) in a survey of some vegetables commonly grown in Jos, found *L. monocytogenes* in 7.5% of the vegetables screened. Those vegetables grown on soils where cow dung was applied as manure showed occurrence of *L. monocytogenes*, whereas vegetables grown on soils where poultry droppings were used as manure did not harbour *L. monocytogenes*. The public health implications of the results obtained in this study could be the possible contamination of irrigation-water and soils by *L. monocytogenes* and subsequently cross contamination of food crops which has been on the increase.

Consumption of such food product is a very important source of human listeriosis. Chukwu *et al* (2004a) reported that *L. mon*ocytogenes in faecal waste could enter water systems by direct contamination of water or through seepage or surface runoff.

It has also been reported that pathogens spread in the environment due to improper treatment and application of sewage, slaughter offal, sludge, biosolids, slurry and manures of faecal origin (Loncarevic *et al*, 1999; Duarte *et al*, 2002).

It is therefore critical for environmental safety and agricultural sustainability that our sources of water, food and our environment be protected from contamination by pathogens in poultry faeces. To achieve this, the public need to employ certain safety practices to reduce contamination of the environment by these faecal pathogens. Such practices include- restriction by fencing the area for scavenging poultry, treating or disinfecting poultry faeces before they are disposed of or applied on farmland.

Treatment of poultry faeces may be achieved by the use of heat. Storing of the faecal material for some months to dry may significantly reduce *L. monocytogenes* since the organism is not spore forming.

There is ignorance on the existence of *L. monocytogenes* by public health workers and its isolation and diagnostic procedures are poor in Nigeria (Pondei and Ogbonna, 2004) and this should be corrected. It is important that public health workers in Nigeria do not overlook listeriosis as an emerging food borne illness. Diagnosis of human listeriosis needs to be routinely carried out in diagnostic laboratories in Nigeria, especially when its symptoms are noticed. This would help to prevent, control and treat cases of the disease.

References

Adak G.K., Meakins S.M., Yip H., Lopman B.A., O'Brien S.J. (2005). Disease Risks from Foods, England and Wales, 1996-2000; *Emerging Infectious Diseases. 11 (3): 365-372.*

Barrow G.I; Feltham R.K.A (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria.* Cambridge University Press, Cambridge, Pp 1-40.

Chukwu O.O.C, Ogbonna C.I.C., Goebel W. Bubart A, Nwobu G.O., Chima J.C. (1997). *Genital infection of a dairy animal attendant associated with Listeria monocytogenes* in Vom, Nigeria. *Journal of Medical Laboratory Sciences.* 6: 12-14.

Chukwu O.O.C. Ogbonna C.I.C., Muhammad M.J. Olabode O.A., Nwobu O.G, Ogo I.N. Makinde A.A. Elem H.E, Okewole P.A, (2004a) *Listeria monocytogenes* and other *Listeria spp* in poultry faeces applied as manure on farmlands; Environmental health and food safety. *Nigerian Journal of Biotechnology 15(1).* 52-59.

Chukwu O.O.C, Nwankpa N.D, Muhammad M., Ogbonna C.I.C., Chukwuedo A.A., Olabode O.A., Nwosu C.I., Okewole P.A. (2004b). Listeriosis in a pregnant bitch–A case report. *Proceedings of the 41st Congress of Nigeria Veterinary Medical Association,* 30-31.

Chukwu O.O.C, Muhammad M.J, Ogunjumo S.O. Ngulukun S.S, Ogbonna C.I.C, Nwankpa N.D., Molokwu U.J. (2004c). Sporadic bovine listeriosis in Jos, Nigeria. *Proceedings from the 41st Congress of Nigerian Veterinary medical association,* 50-51.

Curtis G.D.W, Mitchell M.G., King A.F and Griffin E.J. (1989). Differential medium for the isolation of *Listeria monocytogenes. Letters in Applied Microbiology 8*: 95-98.

Doyle P.M, and Schoeni L.R. (1986). Selective enrichment procedure for the isolation of *Listeria monocytogenes* from faecal and Biological specimens. *Applied and Environmental Microbiology 51*, 1127-1129.

Duarte L.E, Guera M.M, and Bernardo M.F. (2002). *Salmonella* and *Listeria* species carriage by gulls (Lairds). *Revista Portuguese Ciencias Verterinarias* 97 (544): 181-187.

Duguid, D. Marmion P, Swain H.A. (1985). *Mackie and McCartney Medical Microbiology Vol.1*: *Microbial infections*, Thirteenth edition. Churchill Livingstone Publishers. Pp.43-284.

Faber M.J. and Peterkin I.P. (1991). *Listeria monocytogenes*_a food borne pathogen. *Microbiology Rev. 55*, 476-511.

Fleming D.W. , Cochi S.L. McDonalds K.I. Brondum J., Hayes P.S, Pukaytis B.D. Holmes M.B. Auduries A, Broome C.V. and Reingol A.L. (1985). Pasteurised milk as a vehicle of infection in an outbreak of listeriosis. *New England Journal of Medicine 312*; 404-407.

James S.M. Fannim S.L., Agree B.A., Hall B, Parker E. Voot J, Run G, William J, Lieb L, Salinen C, Pandegrase T, Werner S.B. Chin J (1985). Listeriosis Out break association with Mexican style cheese California. *Morbidity and Mortality Weekly Report/CDC 34*: 347-359.

Jones, W. (1979). Health Hazards associated with the handling of animal wastes. *Veterinary Records* 106: 4-7.

Loncarevic S, Arturson K, and Johansson I, (1999). A case of canine cutaneous listeriosis. *Veterinary Dermatology 10 (1)*: pp.69-71.

Oni O.O. Adesiyum A.A. Adeleke O.J. Saidu A.N.S, (1989). Prevalence and characteristic of some *Listeria monocytogenes* isolated from cattle and milk in Kaduna State, Nigeria. *Israeli Journal of Medicine 45*: 12-17.

Pagotto F, Daley E, and Faber J.M (2002). Enumeration of *Listeria monocytogenes* in food. *The compendium of Analytic methods, Vol.3*, http://www.hc-sc.gc.ca/foodailment .

Pondei J.O, Ogbonna, C.I.C. (2004). Incidence of *Listeria monocytogenes* in some vegetables grown in Jos, Nigeria. *Nigerian Journal of Experimental and Applied Biology 5(2):* 161-164.

Prescott L.M, Harley J.P. Klein D.A, (2002) *Microbiology* 5th Edition. McGraw Hill Higher Education. USA. Pp.421-990.

Schlech W.F., Lavingne M.P. Burtalussi A.R. Allen C.A, Haldene V.E; Wort J.A, Hightower W.A., Johnson E.S. King H.S., Nicholas S.E. and Broom V.C. (1983). Epidemic Listeriosis evidence of transmission of food. *New England Journal of Medicine 308*: 203-206.

Talaro K. and Talaro A. (1996). _*Foundations in Microbiology* 2nd edition. WCB McGraw Hill U.S.A.; Pp.90-670.

Tauxe R.V. (1997). Emerging food borne diseases an evolving public health challenge. *Emerging Food Borne Disease (3):* 425-434.