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 (UV) 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*, *Bacillus 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 (Talar 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, motile or slightly curved non-sporing and non-capsule forming small rods or cocccobacilli, 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 species 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, Pondel 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 (Fleming 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* (Pondel 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 prevalence 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 4°C 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 SR1140 (Oxoid CM 856) and incubated at 30°C 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 35°C 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 agglutin 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, mannitol, 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 (19.22%) 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

<table>
<thead>
<tr>
<th>Poultry type</th>
<th>Number Sampled</th>
<th>Number Positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td>30</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>Ducks</td>
<td>40</td>
<td>12</td>
<td>30.0</td>
</tr>
<tr>
<td>Turkeys</td>
<td>30</td>
<td>12</td>
<td>40.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>42</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Discussion

Listeria monocytogenes, the cause of human and animal listeriosis has a temperature range for
growth of 3°C - 45°C with an optimum of 30°C but under certain conditions may survive heating up to
60°C; 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 monocytogenes* and other pathogens in relation to poultry type

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Chickens</th>
<th>Ducks</th>
<th>Turkeys</th>
<th>Total No. of Isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. monocytogenes</em></td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>42 (23.08)</td>
</tr>
<tr>
<td><em>L. grayi</em></td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>10 (5.49)</td>
</tr>
<tr>
<td><em>L. murrayi</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (1.11)</td>
</tr>
<tr>
<td><em>Bacillus spp</em></td>
<td>13</td>
<td>24</td>
<td>16</td>
<td>53 (29.12)</td>
</tr>
<tr>
<td><em>Enterococcus spp</em></td>
<td>10</td>
<td>4</td>
<td>14</td>
<td>28 (15.38)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>9</td>
<td>19</td>
<td>6</td>
<td>33 (18.13)</td>
</tr>
<tr>
<td><em>Streptococcus spp</em></td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>4 (2.19)</td>
</tr>
<tr>
<td><em>Yeast</em></td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>10 (5.49)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>56</td>
<td>74</td>
<td>52</td>
<td>182 (100)</td>
</tr>
</tbody>
</table>

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 illnesses 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 9,195 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.

Pondel 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. monocytogenes* in faecal waste could enter water systems by direct contamination of water or through sewage 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 (Pondel 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


