Full Length Research Paper

Incidence and pathogenicity profile of *Listeria* sp. isolated from food and environmental samples in Nsukka, Nigeria

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Samples of beef, pork, goat meat, poultry, fish and vegetables purchased from a local market located in Nsukka, Enugu State, Nigeria were examined for the presence of *Listeria* species. Surface swab samples taken from butchers' tables as well as soil samples from the field where cows and pigs were kept before slaughter were examined. *Listeria* species were isolated from all samples except the pork and goat meat samples. The highest incidence (100%) was observed in the soil and surface swab samples, respectively, followed by the vegetable samples (85%) and the beef samples (80%). Only *Listeria monocytogenes* and *Listeria ivanovii* were pathogenic to test animals causing death within five days. *L. monocytogenes* induced pathological changes characterized by abscesses in the liver, pericarditis and frank haemorrhage in the kidneys. The most notable change was the accumulation of mononuclear cells in the loose connective tissue surrounding the bile ducts. This study shows that there is a high incidence of listerial organisms in various key elements of the Nigerian environment, including soil and foods of both animal and plant origin, which may pose a health risk for high-risk individuals. The study goes further to establish the major target organs of attack of the pathogenic *Listeria* species.

Key words: *Listeria monocytogenes*, pathogenicity, histopathology.

INTRODUCTION

Listeric infections caused by members of the genus *Listeria* occur worldwide and in a variety of animals including man (Hood, 1993; Low and Donachie, 1997). Seven species of *Listeria* have been identified, but *L. monocytogenes* is the principal pathogen in humans and animals. *Listeria ivanovii* is a pathogen of animals but is occasionally implicated in human disease. The other *Listeria* sp. are generally considered to be non-virulent (Bille and Doyle, 1991; Low and Donachie, 1997).

Listeria monocytogenes is ubiquitous in the environment and has been recovered from dust, soil, water, sewage and decaying vegetation, including animal feed and silage from where the organisms enter the cycle of transmission. Contamination of silage leads to infection of farm animals resulting in possible human infection by way of the food chain (Bockserman, 2000; Ramaswamy et al., 2007; Sleator et al., 2009). Most human infections of *L. monocytogenes* are now considered to be of food-borne origin (Brackett and Beuchat, 1990; NFPA, 1999).

Humans of all ages but especially pregnant women, infants less than four weeks old, the elderly and immuno-compromised individuals are highly susceptible to listeriosis (Ramaswamy et al., 2007). In the USA, listeriosis is responsible for about 400 - 500 deaths each year and an estimated 2500 persons become severely ill within the same period (Stehulak, 1992; DBMD, 2001; FSIS, 2001;

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Ramaswamy et al., 2007).

Although the route of infection is primarily oral, the clinical manifestations of listeriosis are variable between groups and individuals (NFPA, 1999). In the sensitive population, symptoms may be limited to fever, fatigue, nausea, vomiting and diarrhoea, but these may precede a more serious illness such as meningitis and septicemia, which can be fatal (NFPA, 1999; Abdul-Razzak, 2007). In pregnant females, the infection is usually self-limited affecting only the uterine contents and causing miscarriage and stillbirth. The pregnant woman may, however, exhibit flu-like symptoms (NFPA, 1999; FSIS, 2001). In neonates infected *in utero*, the infection causes a condition known as granulomatosis infantiseptium and lesions are common in the liver and placenta but can also be found in the brain, adrenal glands, spleen, kidney, lungs and the gastrointestinal tract (Ramaswamy et al., 2007). In non-pregnant adults and children, listeriosis is principally an infection of immunosuppressed individuals and complications usually involve the central nervous system and the bloodstream, but may include pneumonia and endocarditis (NFPA, 1999; Sleator et al., 2009).

The incidence of listeriosis in the Nigerian environment is not well documented. This study was undertaken to evaluate the incidence of *Listeria* species in some food and environmental samples and to establish target organs affected by the pathogenic species.

MATERIALS AND METHODS

Sample Collection

Meat samples (beef, pork, goat, chicken, turkey, and fish) were purchased randomly from butchers in the local market. The samples were pre-cut and packaged by the butchers. The meat samples consisted of offal and beef while the fish samples included both fresh and dried varieties. Surface swab samples were also taken from the butchers' tables. Soil samples were collected randomly from the field where cattle and pigs were kept and fed before slaughter. The vegetables (cabbage, lettuce, carrots, green peas) were purchased from different vendors in the local market. All samples were stored at 4°C for not more than 24 h before analysis.

Enrichment

Pre-enrichment and enrichment were carried out according to the method of the United States Food and Drug Administration and Center for Food Safety and Applied Nutrition (Hitchins, 2001).

For each sample, a 25.0 g amount was added to 225 ml of the listerial enrichment broth, (University of Vermont broth) (UVM), without antibiotics. The meat and vegetable samples were prepared by the rinsing method of Rorvik et al. (1995). The UVM broth used in rinsing the samples were subsequently plated out on PALCAM agar plates while the remaining broth was incubated for enrichment. Enrichment was carried out by initial incubation at 30°C for 4 h in 500-ml Erlenmeyer flask, after which antibiotics (20 mg Nalidixic acid and 12 mg Acriflavine hydrochloride) were added before further incubation for 44 h at the same temperature.

For collection of surface samples, the swabs for each sample

were first soaked in 5 ml of enrichment broth before use. The sample-loaded swabs were then placed in 30 ml enrichment broth, and the culture subjected to enrichment as earlier described.

Isolation and Identification of *Listeria* species

After 24 and 48 h enrichment, respectively, broth cultures were streaked onto PALCAM agar plates and incubated at 35°C for 24 h. Thereafter, plates were examined for grey-green colonies with black background, typical for *Listeria*. Enumeration was done after 48 h. Total viable counts were taken from plates containing 30 - 300 colonies, from samples plated without enrichment.

Selected representative colonies were further identified to species level based on haemolytic patterns and biochemical analysis, as described by Bille and Doyle (1991). Haemolytic activity was tested on *L. monocytogenes* blood agar (LMBA) containing sheep blood. Test isolates were streaked onto the LMBA plates and incubated at 37°C for 24 - 48 h. Haemolytic *Listeria* species grow as small light-coloured colonies surrounded by narrow zones of beta haemolysis. Only *L. monocytogenes, L. ivanovii* and *Listeria seeligeri* show haemolysis, with *L. ivanovii* having wider zones of haemolysis than the other two. Other *Listeria* species show no haemolysis. Biochemical tests used included; catalase, coagulase, nitrate-reduction, growth in 10% sodium chloride, sugar fermentation and *CAMP* tests. Typical colonies of different species were transferred to trypticase soy agar (TSA) and purified by repeated subculturing. Colonies from the TSA plates were preserved on TSA agar slants and used for further tests.

Mouse pathogenicity test

Listerial cultures were grown for 24 h at 35°C in trypticase soy broth plus yeast extract (TSBYE). The cells were then centrifuged at 3,000 rpm for 30 min, after which the pellets were re-suspended in 1 ml of 0.1% peptone water. Female albino mice weighing 16 - 18 g were then inoculated intraperitoneally with 0.1 ml of the listerial suspension. Groups of five mice each were used per isolate and the animals were given access to food and water *ad libitum* and were observed for death over a period of five days.

Histopathology

To determine the target organ(s) of attack by the pathogen, ten-fold serial dilutions of *L. monocytogenes* suspension were prepared. One tenth of a milliliter of each of the different dilutions (ranging from 1:10-7:10) was inoculated intraperitoneally to the experimental animals. Different dilutions were used initially to determine which dilution would produce the desired pathological effects (animals showing signs of illness for four to five days before death). The 4:10 dilution gave the best results and was used for subsequent tests.

After death of the animals, different organs were examined histologically for pathological manifestations. Organs examined included the liver, kidneys, brain, lungs, stomach, intestines and spleen. The organs were excised immediately after death of the animal, cut with a sharp scalpel blade into small pieces and fixed by immersing the cut pieces in modified Bouin's fluid made up of picric acid (300 ml), formalin (100 ml), and 1% tricarboxylic acid (20 ml) according to the methods of Harris et al. (1973). Fixation was for 24 h after which the fixed tissues were dehydrated in a series of ascending ethanol concentrations (70, 80, 90, 95%, and absolute) and then embedded in paraffin. Sections were thereafter cut with a microtome at 4 μm thickness, dewaxed in xylene and hydrated in a series of descending ethanol concentrations (absolute ethanol, 95,

Table 1. Incidence of *Listeria* species in various food and environmental samples.

Sample	Number of Samples Examined	Number of Positive Samples (%)
Beef	20	16 (80)
Fish	15	6 (40)
Poultry	10	7 (70)
Vegetables	20	17 (85)
Pork and Goat	7	0 (0)
Soil	5	5 (100)
Surface swabs	5	5 (100)
Total	82	56 (68)

Table 2. Average count of *Listeria* species from positive samples.

Sample	Range of Counts (log$_{10}$ CFU/g)	Number of Samples in Range
Beef	Negative [a]	4
	Not detected [b]	3
	3.90 - 4.90	2
	4.91 - 5.90	11
Fish	Negative [a]	9
	Not detected [b]	5
	3.90 - 4.90	1
Goat and Pork	Negative [a]	3
Poultry	Negative [a]	3
	Not detected [b]	2
	5.00 - 5.25	2
	5.26 - 5.50	3
Vegetables	Negative [a]	3
	Not detected [b]	6
	3.90 - 4.90	2
	4.91 - 5.90	8
	5.91 - 6.90	1
Soil	4.90 - 5.90	1
	5.91 - 6.90	4
Surface swabs	3.90 - 4.90	1
	4.91 - 5.90	4

[a] No *Listeria* species isolated; [b] *Listeria* species isolated after enrichment.

90, 80 and 70%). Cut tissues were stained with haematoxylin and eosin before microscopic examination.

RESULTS

Incidence of *Listeria* species in meat and environmental samples

The incidence of *Listeria* in the examined samples is shown in Table 1. *Listeria* species were found in 68% of the samples. The highest positive results were obtained from surface swabs and soil samples (100%), followed by vegetable samples (85%) and beef samples (80%). No *Listeria* could be isolated from the pork and goat meat samples. Table 2 shows the ranges of listerial counts (log$_{10}$ CFU/g) and the number of samples within each range. The highest counts were associated with the soil and the surface swab samples. Among the food samples, the vegetable samples had the highest incidence followed by the beef samples. The fish samples had the least counts
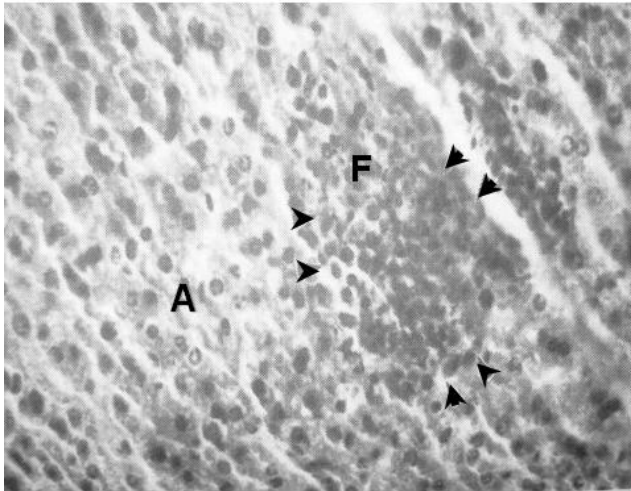
Figure 1. Liver section showing loss of normal architecture. Note the necrotic focus (F) characterized by necrosis of hepatocytes and dense infiltration of mononuclear cells (arrowheads) (x 400).
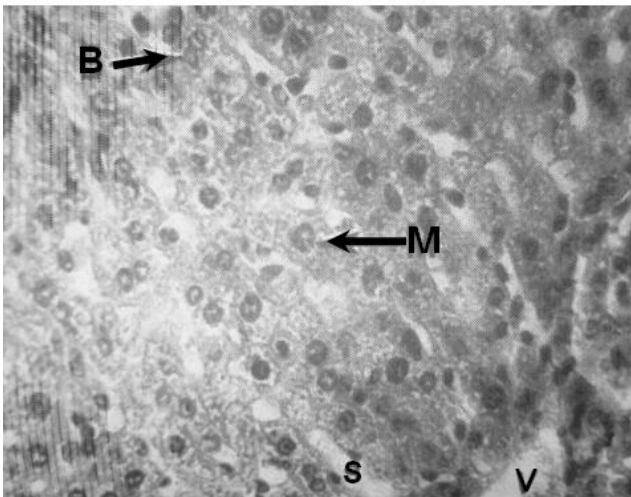

Figure 2. Section of mouse liver showing normal architecture of the hepatocytes. Note the mononucleated (M) and binucleated (B) hepatocytes, sinusoid (S) and the central vein (V) (x540).

and the positive samples were only obtained following 48 h enrichment.

Speciation of listerial isolates

Representatives of all *Listeria* species were encountered. Of the 26 isolates examined, five (19.2%), seven (26.9%), and two (7.7%) were *L. monocytogenes, Listeria murrayi/ grayi* and *L. seeligeri*, respectively, while four (15.4%)
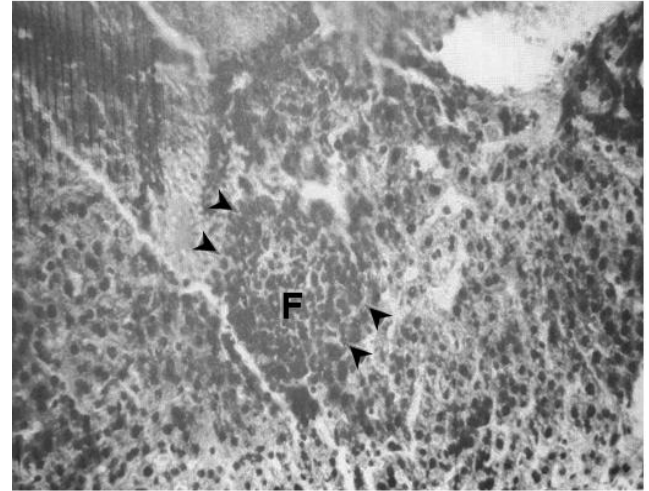
Figure 3. A large necrotic focus marked by dense infiltration of mononuclear cells (x 380).

each belonged to *L. ivanovii, Listeria innocula,* and *Listeria welshimeri.*

Mouse pathogenicity test

Of all the haemolytic species, only isolates of *L. monocytogenes* and *L. ivanovii* caused death of test animals.

Histopathological examination

Histopathological changes were observed in the liver, heart and the kidneys. The liver was the most affected showing overall mild degeneration of the hepatocytes (Figure 1) as compared to the normal architecture of the hepatocytes (Figure 2). There was also the presence of scattered foci characterized by necrosis of the hepatocytes, dense infiltration of mononuclear cells (Figure 3) and degrees of accumulation of mononuclear cells in the loose connective tissue surrounding the bile ducts (Figures 4 and 5).
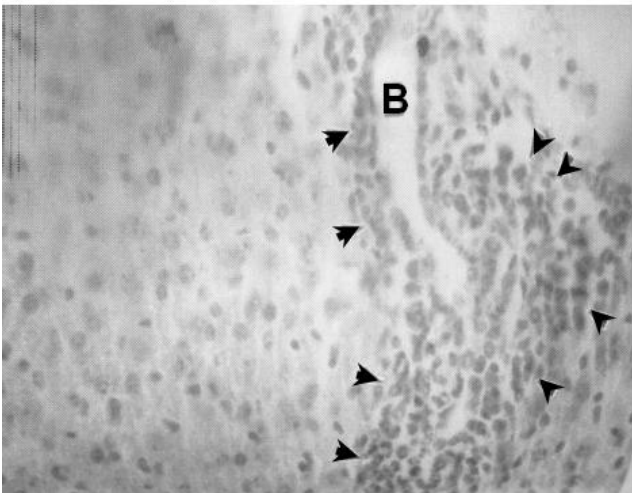
The heart showed mild pericarditis in the region of the coronary groove, within which the adipose connective tissue was infiltrated by mononuclear cells (Figure 6). The effects on the kidneys were characterized by frank hemorrhage and occasional mononuclear infiltration of the cortical interstitium (Figure 7).

DISCUSSION

Reports from developed countries, like the United States of America, have implicated contaminated foods as a major vehicle of transmission for listeriosis (Schuchat et
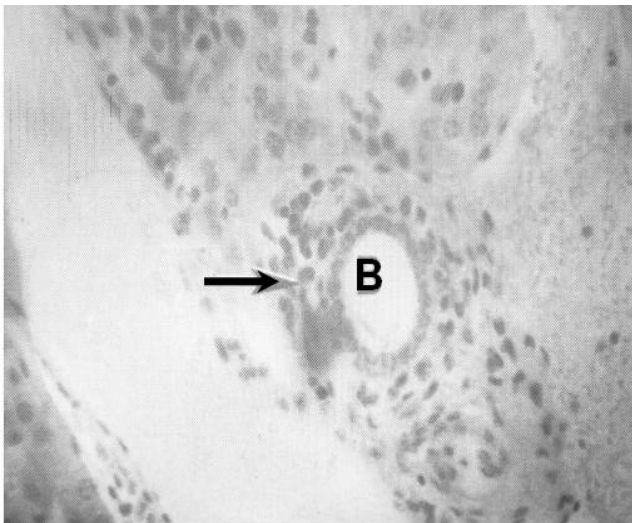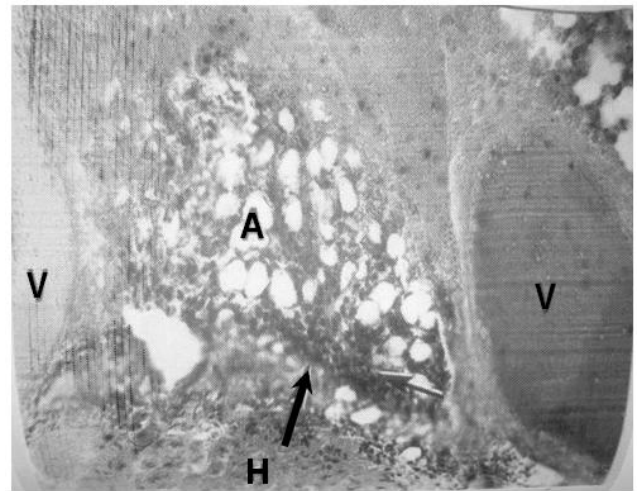
Figure 4. Section of mouse liver showing mild infiltration of mononuclear cells in the connective tissue surrounding a bile duct (B) (x 400).

Figure 6. Section of the coronary groove of the heart showing mild peridarditis characterized by the mononuclear infiltration of the adipose connective tissue (arrows). V, coronary blood vessels; A, adipose cells; H, cardiac muscle (x 400).
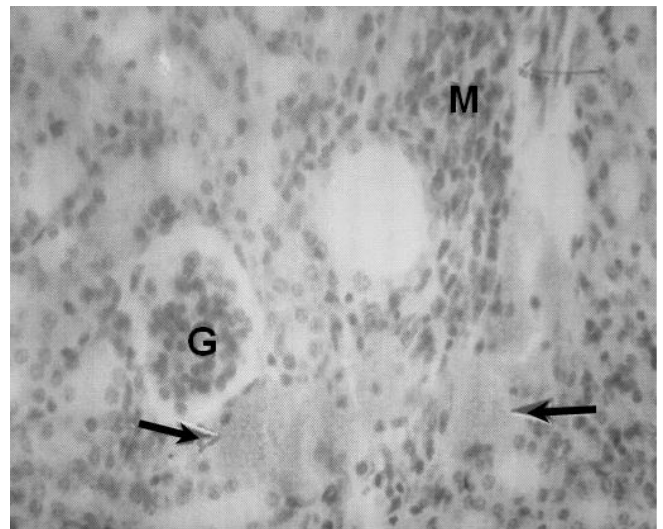

Figure 5. Severe infiltration of the periductal connective tissue by mononuclear cells. B indicates the bile duct while the arrowheads mark the extent of the infiltration (x 400).

Figure 7. A section of the cortex of the kidney showing frank haemorrhage (arrows) and accumulation of mononuclear cells (M) in the interstitium. G indicates the glomerulus of the renal corpuscle (x 400).

al., 1992; WHO, 2000). *L. monocytogenes*, the species predominantly responsible for listeriosis, is found in soil and water from where vegetables get contaminated. Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin such as meats and dairy products (Schlech et al., 1983; Schuchat et al., 1992; Hood, 1993; Bockserman, 2000; DBMD, 2001). Our findings in this study indicate a high incidence of *Listeria* in beef (80%) but not pork and goat meat samples. Although, the beef samples were made up of offal (while the goat and pork meat samples consisted of

limbs of the animals), these offal are usually sold in contact with the other parts of the animal, thus, facilitating cross-contamination.

Surface swab samples taken from the butchers' tables in the abattoirs equally had a very high incidence (100%) of *Listeria* species. This is indicative of poor practice of hygiene by the butchers. Although, meat is thoroughly cooked before consumption, the chances of cross-contamination of ready-to-eat (RTE) food products in the

refrigerator or kitchen, still exists. Considering the ability of *Listeria* species to grow at refrigeration temperature, their presence on any food is totally undesirable and should be controlled if not eliminated. Other workers in different countries (Sheridan et al., 1994; Choi et al., 2001; Miettinen et al., 2001; Hassan et al., 2001) have reported an incidence of between 62 and 85% of *Listeria* species in various foods which is comparable with the results of this study.

Contrary to our observations in this study, there are reports of isolation of *Listeria* species from pork (Soriano et al., 2001; Choi et al., 2001; Kanuganti et al., 2002). Poultry samples have also been shown to have a high incidence (70%) of *Listeria* species, even when purchased in a frozen state, while sporadic cases of listeriosis have been known to result from the consumption of under-cooked chicken (Greenwood et al., 1991; Morris and Ribeiro, 1991; Tan et al., 2007).

The fish samples examined in this study had a rather low incidence (40%). This could be due to low level of contamination of the river or seawater from which the fishes were caught. A number of earlier workers reported the absence of *Listeria* from tropical fish and attributed this to environmental factors (Manoj et al., 1991; Karunasagar et al., 1992; Kamat and Nair, 1994). Jeyasekaram et al. (1996) however, attributed it to inadequate isolation procedures. In a study conducted in Sokoto, Nigeria, Salihu et al. (2008) reported an incidence of 25% in smoked fish. Like in the case of poultry products, viable *Listeria* species were isolated in this study from frozen fish samples, thus further underscoring the fact that both frozen foods and non-frozen foods marketed by commercial outlets may represent a health hazard.

Among the food samples examined, the highest listerial incidence (85%) was encountered in the vegetable samples. This finding represents a major threat to human safety, as some of these vegetables receive no further heat treatment prior to consumption. It is on record that the first documented outbreak of listeriosis involved cabbage, as the vehicle of transmission (Schlech et al., 1983). Other vegetables from which Listariae have been isolated include: Sprouts, lettuce, celery, cauliflower, and broccoli (Thunberg et al., 2001). Contamination of vege-tables with *Listeria* species is thought to occur on the farm mainly through soil and animal manure, although, contaminated wash water may also play a role.

The high incidence (100%) observed with the soil samples from fields where cows and pigs were kept prior to slaughter, is suggestive of possible fecal carriage of *Listeria* species by these animals. The findings here are similar to those of MacGowan et al. (1994) and tend to agree with the suggestion of Bockserman (2000) that the usual habitat of *L. monocytogenes* is the intestinal tract of mammals and birds from where the organism enters the soil via animal droppings.

The pathogenicity profile of *L. monocytogenes* isolated in this study was evaluated by intraperitoneal injection of mice with cultures of the organism. Histopathological examination of various organs showed that the organism induced various pathological changes in different organs. By far the most notable change was the accumulation of mononuclear cells in the loose connective tissue sur-rounding the bile ducts. This effect suggests a possible breach in the integrity of the bile duct epithelium leading to leakage of an irritant, like bile, into the peritubular connective tissues. It would therefore appear that the liver is a major target organ of the pathogen; this is in accord with the reports of Miller and Burns (1970) and Al Dajani and Khatib (1983). Kennedy and Miller (1992) also showed coagulative necrosis and infiltration by macro-phages and neutrophils as were observed in this study.

The foregoing account, suggests high incidence of listerial organisms in various key elements of our environment, and that the pathogenic species (for mice) consist of *L. monocytogenes* and *L. ivanovii* with the liver, heart and kidney as the major target organs.

ACKNOWLEDGMENTS

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