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PATHOGENIC POTENTIAL OF *LISTERIA MONOCYTOGENES* ISOLATED FROM CATTLE FAECES IN ADO-EKITI

*Oyinloye^{1,2}, M. A., David¹, O. M and Famurewa¹, O.

¹Ekiti State University^{1,2}, Ado-Ekiti, Ekiti State; ²Afe Babalola University, Ado-Ekiti, Ekiti State

*correspondence: oyinloyema@abuad.edu.ng, +234 705 0455 585

ABSTRACT

Listeria monocytogenes is an opportunistic food-borne pathogen causing listeriosis especially among immune-compromised persons. Its high rate of morbidity and mortality has classed the organism among the top watch list in foods. It is known to produce several virulence factors which aid its survival in harsh conditions and its dissemination within host cells. The pathogenicity of *L. monocytogenes*, isolated from cattle faeces in Ado-Ekiti, was determined in Wister albino rats for two weeks and the relative virulence was calculated. Rats were challenged with isolates producing listeriolysin O and phospholipase orally, intraperitoneally and subcutaneously. Biochemical parameters and haematoxylin and eosin (H and E) stained sections of selected organs were examined for significant changes ($p < .05$) and histopathological effects post-experiment. Relative virulence was recorded at 0% with rats showing no signs of infection or death. However, significant changes in total protein, lipid profile and some selected antioxidant enzymes, as well as cytological changes in the examined H and E sections of organs showed that an infection had occurred. Bacteria may have however been eradicated by the immune-competent rats. This study therefore concludes that isolates may be pathogenic especially for persons tagged 'high risk' due to low immunity.

Keywords: *L. monocytogenes*, listeriosis, pathogenicity, histopathology, cattle faeces

POTENTIEL PATHOGÈNE DE *LISTERIA MONOCYTOGENES* ISOLÉES DE FÈCES DE BOVINS DANS ADO-EKITI-

*Oyinloye^{1,2}, M. R., David¹, O. M. et Famurewa¹, O.

¹L'état d'Ekiti, Université de l'état d'Ekiti, Ado-Ekiti ; ²Afe Babalola University, Ado-Ekiti, l'état d'Ekiti

*Correspondance : oyinloyema@abuad.edu.ng, 2347050455585

ABSTRACT

Listeria monocytogenes est un agent pathogène d'origine alimentaire opportuniste causant la listériose en particulier chez les personnes immunodéprimés. Le taux élevé de morbidité et de mortalité a classé l'organisme parmi les top liste de surveillance dans les aliments. Il est connu pour produire plusieurs facteurs de virulence dont l'aide sa survie dans des conditions difficiles et sa diffusion dans les cellules hôtes. La pathogénicité de *L. monocytogenes*, isolées de fèces de bovins à Ado-Ekiti, était déterminé à Wister albino rats pendant deux semaines et la virulence relative a été calculée. Les rats ont été remis en question avec les isolats produisant la listériolysine O et la phospholipase, oralement et par voie sous-cutanée. Les paramètres biochimiques et l'hématoxyline-éosine (H et F) les articles tachés de certains organes ont été examinés pour des changements significatifs ($p < .05$) et des effets histopathologiques post-expérience. Virulence relative a été enregistré à 0 % avec les rats ne montrant aucun signe d'infection ou de la mort. Cependant, des changements importants dans les protéines totales, le profil lipidique et certains enzymes antioxydantes, ainsi que des changements cytologiques dans les sections H et E d'organes ont montré qu'une infection s'est produite. Des bactéries peuvent cependant avoir été éliminé par le système immunitaire des rats-compétents. Cette étude conclut donc que les isolats peuvent être pathogènes en particulier pour les personnes avec le tag 'risque' en raison d'une faible immunité.

Mots-clés: *L. monocytogenes*, listériose, pathogénicité, histopathologie, bovins faeces

INTRODUCTION

One of the earliest recorded large outbreak of listeriosis was traced to locally made coleslaw (contaminated cabbage via manure) and the strain isolated was the same as the outbreak/epidemic strain, *L. monocytogenes* serotype 4b (1). Fleming *et al.*

(2) reported an outbreak of listeriosis which occurred in Massachusetts in 1983. This outbreak involved non pregnant adults who had immunosuppressive condition.

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At the period of the outbreak, there were simultaneous diagnostic cases of listeriosis in fetuses and neonates with fatality rate of 29% in both adults and neonatal groups. Post pasteurisation contamination of milk was concluded as the likely explanation for the outbreak. In the United Kingdom, McLaunchlin *et al.* (3) reported an unusual strain, *L. monocytogenes* serotype 1/2a, in an outbreak in Carlisle, East Columbia, with 11 cases of listeriosis. Similarly, another listeriosis outbreak implicated raw vegetables in Boston (4). Linnan *et al.* (5) reported one of the largest outbreaks of listeriosis in the USA. In this outbreak, majority of the cases were in pregnant Hispanic mothers and their neonates. Out of 93, 81(87%) cases were pregnant-associated resulting to 29 foetal or neonatal deaths. In recent reports, the Centres for Disease Control and Prevention (CDC) have recorded outbreaks in multiple States of the USA. In 2011, cantaloupes contaminated with the bacteria *L. monocytogenes* caused the deadliest food-borne disease outbreak in the United States in nearly 90 years. A total of one hundred and forty seven illnesses were recorded across 28 States with thirty three deaths and a miscarriage (6). The year 2012 also witnessed another multistate outbreak of listeriosis involving 14 States, linked with an imported cheese, recording twenty two illnesses, twenty hospitalizations and four deaths (7). Another cheese-infected outbreak involving 5 States recorded six illnesses, six hospitalizations, one death and a miscarriage (8). In 2014, eight cases of listeriosis linked with a contaminated dairy product across 2 States were reported. Five of the cases involved two mothers and their newborns, and a third newborn, leading to seven hospitalizations and one death (9). Very recently, the health implication of *L. monocytogenes* presence in milk was reported by CDC in a multistate infection of listeriosis outbreak in old people (73 to 81 years). The outbreak resulted in 50 % mortality of persons infected (10).

Listeria monocytogenes is a remarkable bacterium that has evolved over a long period to acquire a diverse collection of molecules, each with unique properties and functions, and each contributing to the success of *L. monocytogenes* as an intracellular pathogen (11). Upon ingestion by the host via contaminated food, *L. monocytogenes* withstands exposure to host proteolytic enzymes, the acidic stomach environment (pH 2.0), bile salts and non-specific inflammatory attacks,

largely through the actions of several stress-response genes (*opuCA*, *lmo1421* and *bsh*) and related proteins (11). Having survived this initial stage, *L. monocytogenes* adheres to and is internalized by host cells with the assistance of a family of surface proteins called internalins (12). The most notable internalins are internalin A (InlA) and internalin B (InlB). Following its uptake by host cells, *L. monocytogenes* is primarily located in single-membraned vacuoles. Two virulence associated molecules are responsible for lysis of the primary single-membraned vacuoles and subsequent escape by *L. monocytogenes*: listeriolysin O (LLO) and phosphatidylinositol-phospholipase C (PI-PLC) (13, 14). After lysis of the primary single-membraned vacuoles, *L. monocytogenes* is released to the cytosol, where it undergoes intracellular growth and multiplication. The intracellular mobility and cell-to-cell spread of *L. monocytogenes* require another surface protein, ActA (a 67 kDa protein encoded by *actA*), which is cotranscribed with PC-PLC and mediates the formation of polarized actin tails that propel the bacteria toward the cytoplasmic membrane. At the membrane, bacteria become enveloped in filopodium-like structures that are recognized and engulfed by adjacent cells, resulting in the formation of secondary double-membraned vacuoles. A successful lysis of the secondary double-membraned vacuoles signals the beginning of a new infection cycle, which is dependent on PC-PLC upon activation by *Mpl* (a 60 kDa metalloprotease encoded by *mpl*) (14).

The primary manifestations of listeriosis in humans include septicaemia, meningitis (or meningoencephalitis) and encephalitis, usually preceded by influenza-like symptoms including fever. Gastroenteric manifestations with fever also occur. Although the morbidity of listeriosis is relatively low, the mortality can reach values around 30% (15). These rates exceed those from other bacteria such as *Salmonella* and *Clostridium*, making listeriosis a leading cause of mortality among food-related infections (15). In pregnant women, infection may result in abortion, stillbirth or premature birth (16, 17), while infection is characterised by septicaemia and meningitis among adults (18). Listeriosis induced meningitis is often exacerbated by encephalitis which is very unusual for a bacterial infection (18). Listeriosis affecting the central nervous system accounts for around 55-75% of cases whereas septicaemia accounts for 15-20% with non-typical infections making up the remainder (14). Despite reports from different countries around the world of the involvement of this pathogen in different food-borne outbreaks, little is reported of its medical importance in Nigeria. Njoku-Obi and Njoku-Obi (19) reported the first evidence of *Listeria* through serological evidence among blood donors, staff and

students of the Lagos University Teaching Hospital. The study reported the presence of *L. monocytogenes* by somatic agglutination and complement fixing antibodies. The first case of isolation of the organism in Nigeria from an adult female patient was reported by Eyo *et al.* (20). The first neonatal case was reported in a neonate showing signs of listerial meningitis, haven been infected from the mother. Both mother and child were effectively treated with ampicillin (21). A 26.32% mortality rate from 19 patients presenting with meningitis and meningoencephalitis due to *L. monocytogenes* in a one-year prospective study was reported in another study (22). Results from other reports are as follow: in 420 faecal samples from patients presenting with gastro-enteritis, none yielded a positive result to *L. monocytogenes*; 0.4% positive samples from 1097 cerebrospinal fluid (23); 5 positive cases of *L. monocytogenes* out of 66 septicaemic neonates (24); from 33 septicaemic neonates screened, none was positive to *L. monocytogenes* (25); a microbiological screening of 162 donated blood samples for transfusion showed none positive to *L. monocytogenes* (26); and in a three-year retrospective study of 1500 paediatric patients, none was positive to listerial infection (27). Several reports have been made on the occurrence and spread of *L. monocytogenes* in Nigeria in food, food-drinks, and environmental samples (28). This study therefore aimed at evaluating the pathogenic potential of *L. monocytogenes* isolates from cattle faeces in Wister albino rats, challenged through the oral, peritoneal and subcutaneous routes.

METHODS

A total of thirty (30) faecal samples were gotten from the intestines of different slaughtered cows into sterile universal bottles and were transported on ice-packs within 2hrs to Afe Babalola University Microbiology laboratory for bacteriological analysis.

The method of Food and Drug Administration (FDA) bacteriological and analytical method (BAM) described by Hitchins (29) was used using a combination of selective enrichment broth (University of Vermont Medium Modified *Listeria* Enrichment Broth, UVM-MLEB; Alpha Biosciences, USA) and selective Brilliance *Listeria* Agar Base (BLAB; Oxoid, Basingstoke, Hampshire, UK). Cattle faeces were collected from the intestine after slaughter. One gram (1g) of sample was homogenised in 9 ml of Tryptone Soya broth. A volume of 1 ml of this homogenate was then inoculated into 9 ml of UVM-MLEB (Alpha Biosciences, USA), to which selective supplement (Oxoid, Basingstoke, Hampshire, UK) was added after 4 hours of incubation at 37°C, and subsequently incubated at 37°C for 44 hours. The enriched culture was streaked at 24 and 48 hours on Brilliance *Listeria* agar with differential and selective supplements

(Oxoid, Basingstoke, Hampshire, UK). After incubation at 37°C, blue-green colonies on the medium were presumed to be *Listeria* species. These isolates were subjected to Oxoid Biochemical Identification (OBIS-mono kit) reagents to differentiate between *L. monocytogenes* and non-*monocytogenes* species; confirmed *L. monocytogenes* were further subjected to biochemical characterization (Gram stain, catalase, oxidase, sugar fermentation, and motility at 25°C).

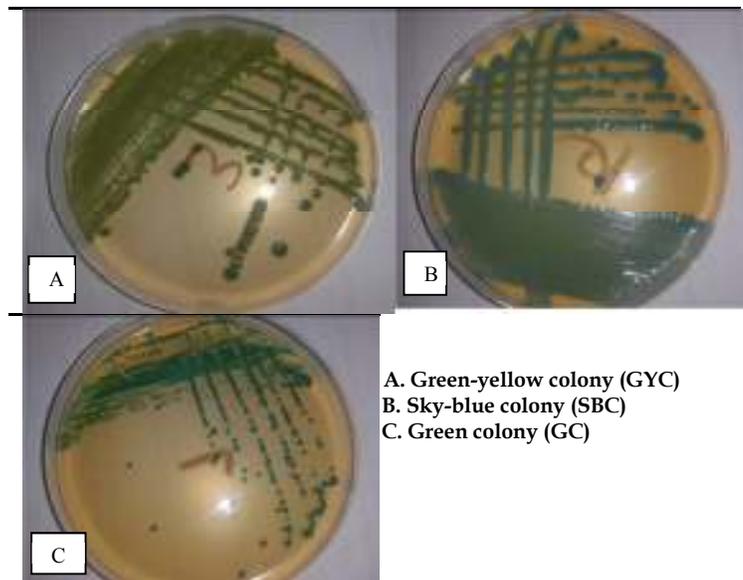
Histopathological study: Isolates were examined for the production of listeriolysin O and phospholipase using 7% sheep blood agar and differential supplement (Oxoid, Basingstoke, Hampshire, UK) respectively. Positive isolate for virulence factors were used to challenge Wister albino rats. National guidelines on the use of animals in experimental research was followed and approved by the University's Research Ethical Board. Inoculum density was determined using the method described by Stelma *et al.* (30). Four groups of five rats each were challenged orally, intraperitoneally and subcutaneously, while the fourth group served as the control and were challenged with sterile normal saline, and were observed for two weeks for signs of infection. Serum and organs (liver, spleen and kidney) were collected post-experiment; blood and faeces were cultured for re-isolation of bacteria; biochemical parameters were determined using commercial diagnostic kit (Crumlin, Co. Antrim, UK); and haematoxylin and eosin stained sections of organs were examined.

RESULT

A total of 176 bacteria were isolated from the samples having different colonial colours on the selective agar. However, blue-green colonies (Plate 1), *Listeria* species were recorded at 87 (49.43%) as green-yellow (GYC, 30/30), sky-blue (SBC, 30/30) and green colonies (GC, 27/30). *Listeria monocytogenes* was identified by OBIS-mono kit at 27 (90%), which were the green colonies (GC) with halo on the chromogenic selective agar and biochemical reactions also identified the 27 isolates as *L. monocytogenes* (Table 1). All isolates of *L. monocytogenes* demonstrated the production of listeriolysin O and phospholipase (Plate 2), known virulence factors. The pathogenic prowess of the isolate recorded a relative virulence of 0% and the bacteria were not re-isolated among all inoculated groups. However, different biochemical parameters revealed varying levels of infection signs. A significant elevated level ($p < .05$) of total protein seen among the intraperitoneal and subcutaneous groups revealed inflammation or infection among the groups

and the liver showed more signs of infection by *L. monocytogenes* than spleen and kidney (Table 2). Changes in the lipid profiles had no significant relationship to signs of infection (Table 3). Also an

elevated level of alanine aminotransferase (ALT) observed among oral and subcutaneous groups indicated acute hepatocellular damage (Table 4).



A. Green-yellow colony (GYC)
B. Sky-blue colony (SBC)
C. Green colony (GC)

PLATE 1: APPEARANCE OF *LISTERIA* SPP. ON BRILLIANCE *LISTERIA* AGAR

TABLE 1: BIOCHEMICAL CHARACTERISTICS OF GC ISOLATES OF *LISTERIA* SPECIES

Biochemical tests	GC	n = 27(%)
Gram reaction	+	27 (100)
DALase production	-	27 (100)
Catalase production	+	27 (100)
Oxidase production	-	27 (100)
Xylose fermentation	-	27 (100)
Mannitol fermentation	-	27 (100)
Lactose fermentation	+	21 (77.78)
Galactose fermentation	+	22 (81.48)
Motility at 25°C	+	27 (100)

Key: GC - green colony; +: positive; -: negative; V: variable; DALase: D-alanyl aminopeptidase

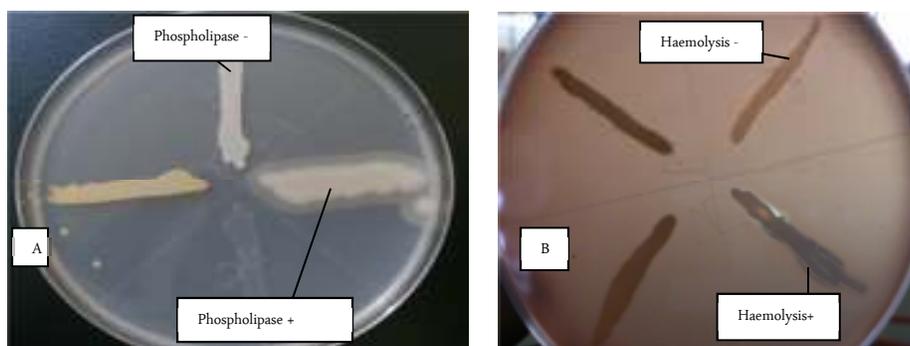


PLATE 2: PRODUCTION OF SOME VIRULENCE FACTORS BY *L. MONOCYTOGENES*
A. showing the production of phospholipase (white halo around colony); B. β -haemolysis (clear area around the edge of colony)

TABLE 2: EFFECTS OF *L. MONOCYTOGENES* INFECTION ON TOTAL PROTEIN (MG/DL) IN SELECTED ORGANS OF RATS

Treatment	Organs		
	Spleen	Kidney	Liver
Control	2.96 ± 0.228 ^a	2.28 ± 0.234 ^a	2.21 ± 0.154 ^a
Oral	2.30 ± 0.595 ^a	2.28 ± 0.312 ^a	1.94 ± 0.334 ^a
Intraperitoneal	2.65 ± 0.420 ^a	2.36 ± 0.228 ^a	3.47 ± 0.453 ^b
Subcutaneous	3.06 ± 0.591 ^a	2.36 ± 0.133 ^a	2.53 ± 0.147 ^{a, b}

Key: Values are Mean ± SEM in mg/dL, values of the same alphabet superscripts have no significant difference ($P < .05$), while values with different alphabet superscripts have significant differences ($P < .05$) by column.

TABLE 3: EFFECTS OF *L. MONOCYTOGENES* INFECTION ON LIPID PROFILE (MG/DL) IN SERUM OF RATS

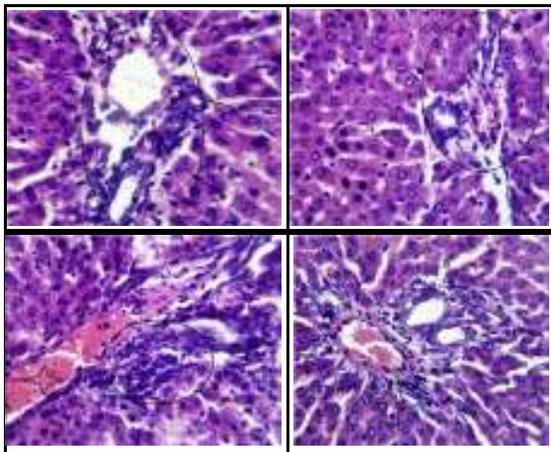
Treatments	TC	TGC	HDL	LDL
Control	229.30 ± 26.08 ^{a, b}	188.25 ± 14.20 ^b	19.55 ± 1.90 ^a	172.10 ± 24.92 ^a
Oral	354.63 ± 29.41 ^b	115.76 ± 19.05 ^a	17.93 ± 0.57 ^a	313.55 ± 36.98 ^b
Intraperitoneal	211.38 ± 43.16 ^a	126.57 ± 7.74 ^a	20.52 ± 0.90 ^a	165.55 ± 45.27 ^a
Subcutaneous	252.46 ± 28.71 ^{a, b}	183.05 ± 17.16 ^b	19.14 ± 1.86 ^a	196.71 ± 24.69 ^a

Key: Values are Mean ± SEM, values of the same alphabet superscripts have no significant difference, while different superscripts represent values with significant differences ($P < .05$) by column. TC-total cholesterol, TGC-triglyceride, HDL-high density lipoprotein, LDL- intermediate density lipoprotein, LDL-low density lipoprotein

TABLE 4: EFFECTS OF *L. MONOCYTOGENES* INFECTION ON SOME SELECTED ANTIOXIDANT ENZYMES IN ORGANS OF RATS

Organ	Enzyme	Treatment			
		Control	Oral	Intraperitoneal	Subcutaneous
Liver	AST	9.17 ± 4.15 ^a	7.57 ± 2.43 ^b	6.69 ± 1.60 ^b	4.37 ± 0.87 ^c
	ALT	4.11 ± 0.97 ^a	5.82 ± 1.56 ^a	4.07 ± 1.45 ^a	6.98 ± 1.04 ^a
	CAT	283.90 ± 1.06 ^a	338.72 ± 4.46 ^b	328.04 ± 1.37 ^b	316.97 ± 1.93 ^b
	SOD	20.00 ± 3.79 ^a	30.00 ± 1.85 ^b	35.00 ± 2.25 ^b	55.00 ± 0.95 ^c
Spleen	AST	22.12 ± 14.62 ^a	14.27 ± 10.25 ^b	5.67 ± 2.05 ^c	8.00 ± 2.64 ^c
	ALT	4.51 ± 1.43 ^a	4.95 ± 0.61 ^a	4.22 ± 1.37 ^a	5.53 ± 1.55 ^b
	CAT	216.45 ± 1.66 ^a	242.76 ± 1.08 ^b	233.74 ± 0.69 ^a	257.31 ± 0.76 ^b
	SOD	50.00 ± 12.91 ^a	50.00 ± 12.91 ^a	50.00 ± 10.00 ^a	49.98 ± 11.06 ^a

Key: Values are Mean ± SEM in mg/dL, values of the same alphabet superscripts have no significant difference ($P < .05$), while values with different alphabet superscripts have significant differences across row. ALT - alanine aminotransferase, AST - aspartate aminotransferase, CAT - Catalase, SOD - superoxide dismutase



A	B
C	D

PLATE 3: HAEMATOXYLIN AND EOSIN STAINED SECTION OF LIVER (MAG. X 400)

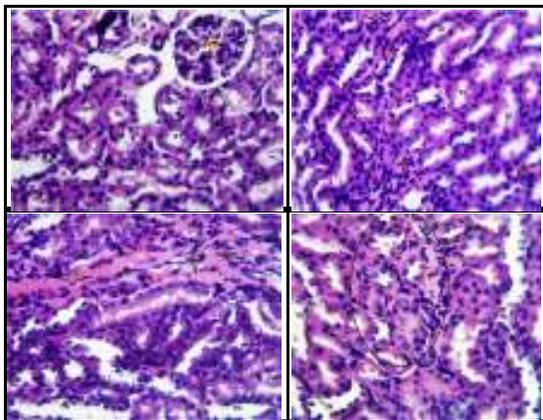
Key: A- Control; B- Oral; C- Intraperitoneal; D- Subcutaneous
A - section of the hepatic tissue composed of hepatocytes (H) disposed in sheet and separated by the sinusoids (S). The portal triads (Circle) composed of the bile duct and vessels. Section is free from collections and inflammatory cells.
B - Section of the hepatic tissue composed of hepatocytes (H) disposed in sheet and separated by the sinusoids (S). The portal triads (Circle) composed of the bile duct and vessels. Section with mild collections and inflammatory cells.
C - Section of the hepatic tissue with marked periportal infiltration with inflammatory cell (Circle), there is marked congestion of vessels (Star). The hepatocytes appear unremarkable.
D - Photomicrograph shows mild periportal infiltration and vascular congestion (Arrow). The sinusoidal spaces appeared mildly dilated (DS). The hepatocytes appear unremarkable.

Significant increases ($p < .05$) were observed in the levels of superoxide dismutase (SOD) and catalase (CAT) among all experimental groups (Table 4). Significant biochemical changes among the tested rats revealed that infection signs were more consistent in Intraperitoneal and Subcutaneous groups than in Oral group.

The haematoxylin and eosin (H and E) stained sections of liver, kidney and spleen showed signs of infection among the experimental groups. Bacteria cells were however not seen in the stained sections. Stained sections of the liver revealed mild collections and inflammatory cells in the portal triad of both oral and subcutaneous group. Section showed infiltration of inflammatory cells in the intraperitoneal group, and hepatocytes appear abnormal (Plate 3). While the renal tubules appear

normal with the oral group, mild to heavy tubular necrosis was revealed with stained sections from subcutaneous to Intraperitoneal group respectively (Plate 4). The stained section of the spleen also showed pockets of congestion and most especially marked proliferation of the white pulp which contains the lymphocytes, and the proliferation of lymphocytes among subcutaneous group all indicated infection (Plate 5). In all stained sections, marked signs of infection were more prominent in intraperitoneal group than others.

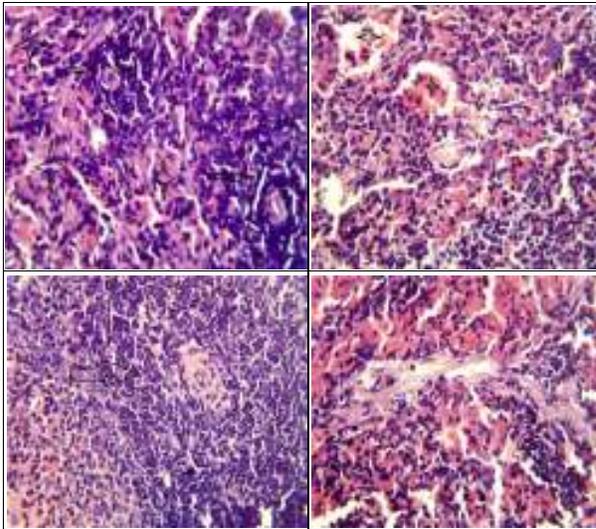
Both the biochemical and histopathological parameters indicated that an infection had occurred among the rats but none died during the experimental period.



A	B
C	D

Key: A- Control; B- Oral; C- Intraperitoneal; D- Subcutaneous
A - Photomicrograph shows section of the renal tissue composed of the renal corpuscles (Arrow) composed of the glomerulus (G), the bowman's space (BS) is well defined. The renal tubules (RT) are lined by regular epithelium. Section is free from collection and inflammatory cells.
B - Photomicrograph shows marked interstitial collection (Star). The renal tubules appear normal.
C - Photomicrograph shows marked interstitial collection (Star). The renal tubules showed degenerative changes (tubular necrosis).
D - Section shows marked interstitial congestion and mild inflammatory infiltrates. The renal tubules show mild degenerative changes (TN).

PLATE 4: HAEMATOXYLIN AND EOSIN STAINED SECTION OF KIDNEY (MAG. X 400)



A	B
C	D

Key: A- Control; B- Oral; C- Intraperitoneal; D- Subcutaneous

A - White pulp (Arrow) and red pulp (Star). The blood vessels (Arrow head).

B - Section shows pockets of congestion (Star)

C - Section shows marked proliferation of the white pulp. (Arrow)

D - Arrow shows cluster of lymphocytes.

PLATE 5: HAEMATOXYLIN AND EOSIN STAINED SECTION OF SPLEEN (MAG. X 400)

DISCUSSION

In the isolation and identification of *L. monocytogenes*, time and accuracy are important factors to consider as the rate of mortality due to the organism is relatively high (31, 32, 33, 34). This study employed the use of a chromogenic medium, Brilliance *Listeria* Agar Base (formerly Oxoid Chromogenic *Listeria* Agar, OCLA), which have been reported reliable in the differentiation of *L. monocytogenes* from other species. The sensitivity and specificity of Brilliance *Listeria* agar in this study was at 100 % and 100 % respectively. This report agrees with the study of Park *et al*, (35) at a sensitivity and specificity rate of 92.0 % and 96.5 % respectively. The medium ranked second highest at 98.7 % (second to lecithin and levofloxacin medium (LLM) at 98.8 %) overall for its confirmation rate of *Listeria* species. Other chromogenic media reported alike are BBL CHROMagar *Listeria*, Agar *Listeria* according to Ottaviani and Agosti (ALOA), Rapid'L-mono agar, and CHROMagar *Listeria*. Chromogenic media have been proven efficient in the isolation and differentiation of *Listeria* species (33).

The cycle of relationship among faeces, water and soil in relations to humans makes the samples possible sources of *L. monocytogenes* infection.

Locally, cattle dung is employed in land fertilization for the cultivation of crops meant for human consumption. *Listeria monocytogenes* was isolated from 90 % of cattle faeces in this study, and occurred at 9.7 % in the total population of *L. monocytogenes* from all samples. This result agrees with report of Umeh and Okpokwasili (36) that screened faecal droppings of domesticated animals and found the highest occurrence of *L. monocytogenes* in cattle faeces. Animal droppings have been widely implicated around the world in the occurrence of the organism (37, 38, 39, 40, 41, 42). Most animals have been identified as healthy carriers of *L. monocytogenes* as signs of infection are not noticed on them. These animals shed the organisms in faeces and thus contaminate their products and the environment (39, 43). The report of this study thus discouraged the use of animal dung in farm land fertilization as this can lead to contamination in animals during grazing, water bodies by surface run-offs, and in man through consumption of farm products from such farms.

The pathogenicity of *L. monocytogenes* was studied by using the mouse virulence assay method (44). The relative virulence was at 0 %, with no dead rat at the end of the experiment, and *L. monocytogenes* was not recovered from the stool and the blood. However, biochemical markers and histopathological results showed signs of infection. Isolates used in the pathogenicity tests expressed virulence factors such as listeriolysin, phospholipase and biofilm production (45). Elevated levels of total protein and alanine aminotransferase (ALT) are suggestive of acute hepatocellular damage, while low alkaline phosphatase (ALP) could be due to low protein deficiency among the orally-challenged group. Significant increased levels of superoxide dismutase (SOD) are suggestive of the presence of phagocytes which are known to produce superoxide in great quantities as a weapon against foreign particles such as bacteria. This increase is directly proportional to the significant increase of catalase, as both enzymes are important in neutralizing the damaging effects of superoxide and hydrogen peroxide to water and oxygen (46, 47, 48). *Listeria monocytogenes* strains have been classified as virulent and avirulent. Strains of lineage III have been identified to be avirulent or weakly virulent due to production of lower levels of virulence factor, thus, these strains do not cause death of infected animals (44, 49). The putative gene, *lmo2821 (inl)*, has been identified as a means of differentiating virulent from avirulent strains of *L. monocytogenes*. Thus, despite the production of listeriolysin, phospholipase and biofilm, strains may still be avirulent in mouse virulence assay (49).

Conclusion: The rats used in the experiment were immuno-competent, thus, may have successfully eradicated the bacteria as *L. monocytogenes* is an opportunistic pathogen causing diseases in immuno-compromised hosts. Thus, the isolates used in this study can be said to be virulent considering its effects in biochemical and histopathological changes in immuno-competent rats.

Conflict of Interest: Authors declare that there is no conflict of interest in this study.

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Authorship: FO designed and supervised the study, OMA carried out the study and wrote the manuscript, while DOM proofread the manuscript and carried out the data analysis.

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