The Prevalence of Pneumonic Pasteurellosis-Causing Microbes: *Pasteurella multocida* and *Mannheimia haemolytica* in Abattoir Samples in Nigeria.

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Abstract: This study was carried out to investigate the prevalence of pneumonic pasteurellosis-causing microbes: *Pasteurella multocida* and *Mannheimia haemolytica* in cattle. A total of 148 bacterial isolates were identified from 100 samples collected from freshly slaughtered abattoir cattle comprising 20 lung tissue samples and 12 clotted heart blood samples collected from cattle with unhealthy lungs showing signs of congestion, pneumonia, oedema or presence of nodules. Other 41 lung tissue samples and 27 clotted heart blood samples were collected from cattle with apparently healthy lungs. Eighty-three bacterial isolates from lung tissues while 63 were isolated from clotted Heart blood samples. Samples were processed and recovered isolates were identified following standard cultural, morphological and biochemical laboratory techniques. *Pasteurella multocida* was isolated from six unhealthy lung tissue samples (7.22%) and seven clotted heart blood samples (11.11%) of which 4 were from cattle with healthy lungs and 3 from those with unhealthy lungs. *Mannheimia haemolytica* was isolated from one (1.22%) unhealthy lung tissue sample only. Six (6) isolates of *Pasteurella multocida* were found to be motile. Other bacteria isolated in this study include *Proteus mirabilis* (1.20%), Pasteurella *lymphangitidis* (1.20%), *Pasteurella ureae* (1.20%), *Pasteurella pneumotropica* (1.20%), *Pseudomonas aeruginosa* (3.17%), *Klebsiella pneumoniae* (3.61%), *Morganella morganii* (8.43%), *Escherichia coli* (21.69%), *Staphylococcus aureus* (24.00%), *Streptococcus pneumoniae* (26.51%) from lung tissue samples while *Pseudomonas aeruginosa* (1.59%), *Proteus rettgerii* (1.59%), *Pasteurella pneumotropica* (1.59%), *Klebsiella pneumoniae* (1.59%), *Proteus mirabilis* (4.76%), *Morganella morganii* (6.35%), *Shigella dysentariae* (6.35%), *Pasteurella multocida* (11.11%), *Streptoccocus pneumoniae* (15.87%), *Staphylococcus aureus* (22.22%), *Escherichia coli* (26.98%), were isolated from clotted heart blood. The attention of animal handlers and veterinarians is therefore called to the high prevalence of these highly pathogenic organisms in the environment of the abattoir.

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1. Introduction: Bovine respiratory disease (BRD) is among the most important diseases of the cattle industry worldwide, causing great economic loss to farmers and animal owners by reducing average daily gain, feed efficiency, overall performance of beef calves and mortality (Taylor et al 2010a; Hartel et al 2004; Kasimanickam, 2010). Pneumonic pasteurellosis, also known as mannheimiosis, broadly refers to any of the disease conditions caused by bacteria of the genera *Pasteurella or Mannheimia* (Adamu and Ameh 2007). The typical clinical disease is highly infectious, often fatal and with very serious economic impact in animal industry. It is well established that pneumonic pasteurellosis is responsible for the largest cause of mortality in feedlot animals in which the disease accounts for approximately 30% of the total cattle deaths worldwide (Mohamed and Abdulsalem 2008).

It is worth mentioning that *M. haemolytica, P. multocida* and *P. trehalosi (Bibersteinia)* constitute the most important members of the family Pasteurellaceae that pose serious hazards in livestock industry (Babetsa et al 2012).

Pasteurellosis has been reported to be endemic in most countries of Africa, Central and South America, and in some countries in Europe and Asia (Kahrimkhani et al 2011). Prevalence rates have been reported in cattle in Iran (Haji Hajikolaei et al 2008), Scotland (Hotchkiss et al 2010) and in Egypt (Kaoud *et al.,* 2010), also in small ruminants in Ethiopia (Deressa et al 2010) and Jordan (Hawari *et al.,* 2008). Prevalence rate has also been reported in small ruminants in some parts of Nigeria (Tijjani et al 2012; Ugochukwu 2008; Emikpe et al 2013). However there is scarcity of information on the prevalence rate of Pasteurellosis causing microbes, *P. multocida* and *M. haemolytica* in cattle particularly in Ibadan, Nigeria.

The present study was therefore undertaken to determine the frequency of isolation of *P. multocida* and *M. haemolytica* from both unhealthy and apparently healthy cattle slaughtered at the Bodija abattoir, Ibadan, Nigeria.

2 Material and Methods:

Study Location: The study was conducted on cattle slaughtered at Bodija abattoir, Ibadan. Ibadan city is situated at latitude 07°20′ North and longitude 03°50′ East (Niger Delta Working Group, 2015).

Animals for the Study: The study was conducted on domestic cattle (*Bos indicus*) of different breeds including White Fulani, Sokoto Gudali, Adamawa Gudali, Red Bororo and Ndama, slaughtered at the abattoir. These animals were transported from different parts of the country to Ibadan.

Sample Collection: Once the animal was slaughtered and the thoracic cavity opened, the lung was located and examined for the presence or not of any pathologic lesion prior to sample collection. A total of one hundred (100) samples were randomly collected consisting of Twenty (20) lung tissues and twelve (12) samples of clotted heart blood from cattle with unhealthy lungs (showing congestion, pneumonia, oedema or presence of nodules), and forty-one (41) lung tissues and twenty-seven (27) clotted heart blood samples from apparently healthy animals. Samples were collected once in a week, over a period of four months (September to December, 2014). In every case, only one sample was collected per animal. Samples were aseptically collected with scalpel blades into sterile universal bottles; they were tightly closed, then transported to the laboratory for bacteriological examination.

Bacteriological Examination of Samples

Each sample from the lung tissue and clotted heart blood was thoroughly homogenized and were inoculated on both MacConkey agar (Oxoid$^{(R)}$) and 10% Bovine blood agar. The plates were incubated at 37^0C for 24 hours. After incubation, morphological characterisation of each colony was carried out. Subsequently, the colonies were stained using Gram and Giemsa stain technique and examined microscopically. Biochemical tests were performed according to the methods of Carter et al (1991) and Quinn et al (1994) for the following characteristics of each isolate: catalase reaction, oxidase test, urease test, citrate utilization test, indole test, motility test and carbohydrate fermentation tests for the following sugars; sucrose, glucose, salicin, mannitol, lactose and dulcitol. Definitive identification of isolates according to cultural, Gram stain and biochemical reactions was carried out using Flores et al (2009) computer based identification software.

3. Results:

In this study, a higher number (13) of *Pasteurella multocida* organisms were isolated in pure cultures; six from lung tissue samples and seven from clotted heart blood samples, when compared with *Mannheimia haemolytica*, of which only one isolate was obtained from a diseased lung tissue. Three (42.86%) of the *P. multocida* isolates were associated with unhealthy lungs. They were Gram-negative occurring as short rods and coccobacilli, aerobic and facultative anaerobic. All the isolates of *P. multocida* were obtained from blood agar were non-haemolytic, rounded, intermediate in size and sometimes small, grey and glistening, 6 isolates were motile while 7 were non-motile, oxidase, catalase, nitrate and indole positive, and urease negative. They produced acid from: mannitol, sucrose, maltose, xylose, and did not produced acid from raffinose and salicin (Table 1a). They did not grow on MacConkey agar, whereas *M. haemolytica* showed beta haemolysis on blood agar and was also isolated from the MacConkey agar culture of the same sample. Giemsa stained smears from all isolates revealed microscopically bipolar coccobacilli. Result of biochemical characteristics of the isolates are shown on table 1a. An isolated M. haemolytica strain was isolated in pure culture. The colony on 10% bovine blood agar, incubated at 37 ^{o}C for 24 hours, was grey glistening, large, shiny and convex with irregular margins and had brown centre. It had a distinct smell, and clear zones of β – haemolysis beneath. The organism produced pin point pinkish colony on MacConkey agar. It was catalase and oxidase positive, reduced nitrate to nitrite, hydrolysed gelatine, phosphatase positive, it fermented glucose, maltose, mannitol, sorbitol, sucrose and salicin but failed to ferment Lactose. It was negative for arginine, Vogus Proskaur and indole tests. Other bacteria isolated in this study include *Proteus mirabilis* (1.20%), Pasteurella *lymphangitidis* (1.20%), *Pasteurella ureae* (1.20%), *Pasteurella pneumotropica* (1.20%), *Pseudomonas aeruginosa* (3.17%), *Klebsiella pneumoniae* (3.61%), *Morganella morganii* (8.43%), *Escherichia coli* (21.69%), *Staphylococcus aureus* (24.00%), *Streptococcus pneumoniae* (26.51%) from lung tissue samples while *Pseudomonas aeruginosa* (1.59%), *Proteus rettgerii* (1.59%), *Pasteurella pneumotropica* (1.59%), *Klebsiella pneumoniae* (1.59%), *Proteus mirabilis* (4.76%), *Morganella morganii* (6.35%), *Shigella dysentariae* (6.35%), *Pasteurella multocida* (11.11%), *Streptoccocus pneumoniae* (15.87%), *Staphylococcus aureus* (22.22%), *Escherichia coli* (26.98%), were isolated from clotted heart blood.

Table 1a: Result of the Biochemical Test for *P. multocida* and *M. haemolytica* Isolates

Biochemical Test	***Pasteurella Multocida***	***Mannheimia Haemolytica***
Catalase + (%) **– (%)**	**13(100%)** **0**	**1(100%)**
Oxidase + (%) **– (%)**	**13(100%)** **0**	**1(100%)**
Motility + (%) **– (%)**	**6(46.15%)** **7(53.85%)**	**0**
Urease + (%) **– (%)**	**0** **13(100%)**	**1(100%)**
Indole + (%) **– (%)**	**13(100%)** **0**	**0**
Dulcitol + (%) **– (%)**	**0** **13(100%)**	**1(100%)**
Sucrose + (%) **– (%)**	**13(100%)** **0**	**1(100%)**
Glucose + (%) **– (%)**	**13(100%)** **0**	**1(100%)**
Salicin + (%) **– (%)**	**0** **13(100%)**	**1(100%)**
Mannitol + (%) **– (%)**	**13(100%)** **0**	**1(100%)**
Citrate + (%) **– (%)**	**0** **13(100%)**	**1(100%)**
Lactose + (%) **– (%)**	**2(15.39%)** **11(84.61%)**	**0**

Table 1b: LIST OF BACTERIAL ISOLATES FROM LUNG TISSUE SAMPLES

S/No.	Organism	Family	No. from healthy lungs	No. from Unhealthy Lungs	Total	% occurrence of isolates
1.	*Staphylococcus aureus*	Staphylococcaceae	16(80%)	4(20%)	20	24.10%
2.	*Pseudomonas spp.*	Pseudomonadaceae	0	2(100%)	2	3.17%
3.	*Proteus mirabilis*	Enterobacteriaceae	0	1(100%)	1	1.20%
5.	*Pasteurella lymphangitidis*	Pasteurellaceae	0	1(100%)	1	1.20%
6.	*Pasteurella multocida*	Pasteurellaceae	0	6(100%)	6	7.22%
7.	*Pasteurella ureae*	Pasteurellaceae	0	1(100%)	1	1.20%
8.	*Pasteurella pneumotropica*	Pasteurellaceae	0	1(100%)	1	1.20%
9.	*Klebsiella pneumoniae*	Enterobacteriaceae	0	3(100%)	3	3.61%
10.	*Escherichia coli*	Enterobacteriaceae	13(72.22%)	5(27.77%)	18	21.69%
11.	*Morganella morganii*	Enterobacteriaceae	4(57.14%)	3(42.86%)	7	8.43%
12.	*Mannheimia haemolytica*	Pasteurellaceae	0	1(100%)	1	1.20%
13.	*Streptococcus pneumoniae.*	Streptococcaceae	8(36.36%)	14(63.63%)	22	26.51%

Table 1c: List Of Bacterial Isolates From Clotted Heart Blood Samples

S/No.	Organism	Family	No. from Heart with Healthy Lungs	No. from Heart with Unhealthy Lungs	Total	% occurrence of isolates
1	*Staphylococcus aureus*	Staphylococcaceae	3(21.43%)	11(78.57%)	14	22.22%
2	*Pseudomonas spp.*	Pseudomonadaceae	1(100%)	0	1	1.59%
3	*Shigella dysentariae*	Enterobactariaceae	2(50%)	2(50%)	4	6.35%
4	*Proteus mirabilis*	Enterobactariaceae	3(100%)	0	3	4.76%
5	*Proteus rettgerii*	Enterobactariaceae	1(100%)	0	1	1.59%
6	*Pasteurella multocida*	Pasteurellaceae	4(57.14%)	3(42.86%)	7	11.11%
7	*Pasteurella pneumotropica*	Pasteurellaceae	1(100%)	0	1	1.59%
8	*Klebsiella pneumonia*	Enterobactariaceae	0	1(100%)	1	1.59%
9	*Escherichia coli*	Enterobactariaceae	5(29.41%)	12(70.59%)	17	26.98%
10	*Morganella morganii*	Enterobactariaceae	2(50%)	2(50%)	4	6.35%
11	*Streptoccocus pneumoniae*	Streptococcaceae	6(60.00%)	4(40.00%)	10	15.87%

4 Discussion: Several studies have been carried out across the globe on the prevalence of *Pasteurella multocida* and *Mannheimia haemolytica* as the main causative agents of pneumonic pasteurellosis in cattle (Mohammadi et al 2006; Karimkhani et al 2011; Kaoud et al 2010; Haji Hajikolaei et al 2008; Onat et al 2010). Pneumonic pasteurellosis is a disease condition that usually occurs in susceptible hosts subsequent to the dissemination and multiplication of resting pathogens from the upper respiratory tract of convalescent as well as non-clinical carriers, usually facilitated by stressors (Kumar et al 2004).

In this investigation, which was centred on the isolation of *Pasteurella* and *Mannheimia* organisms from bovine lungs and clotted heart blood samples, a relatively high prevalence of *Pasteurella multocida* was observed in diseased lungs while none was isolated from healthy lungs. On the other hand, *Mannheimia haemolytica* organism showed much lower prevalence in the study. These findings are in line with the findings of the study cited by Karimkhani et al (2011) in which 32% prevalence of *P. multocida* organism was isolated in diseased lungs and also to the findings of the study of Araghy (2007) in which no isolate of *P. multocida* and *M. haemolytica* was obtained from apparently healthy cattle. The higher prevalence trend of these pathogens in unhealthy than healthy lung tissues is explainable by the fact that the innate and acquired immunity of the respiratory tracts inhibits the proliferation and dissemination of these pathogens to the lower respiratory tract. However, in conditions of stress, immunosuppression or in disease states the local pulmonary defence mechanisms become impaired leading to increased proliferation and downward spread of the pathogens to the lower respiratory tracts (Ackermann *et al.,* 2010; Mohamed and Abdelsalam, 2008).

The occurrence of *P. multocida* organisms in clotted heart blood has been confirmed by several studies (Naz et al 2012; Buczinski et al2010; Shafarin et al 2007; Al-Zahraa et al 2010) which has been explained to be due to the invasion of the blood stream by the organisms from the primary focus of infection through the lymphatic system to the blood (Al-Zahraa et al 2010). In addition, it has been demonstrated that the lymphoid organs (tonsils and lymph nodes) are the most consistent sites for the persistence of these organisms, particularly *P. multocida* in cattle, from which they can invade the blood stream of even carrier animals (Shafarin et al 2007).

All the isolates of *Pasteurella multocida* in the study were from the non-dulcitol fermenting phenotypes (*P. m. multocida and P. m. septica*). This is in line with the findings of the study of Ekundayo et al (2008) in which 31 isolates from cattle was demonstrated to be non-dulcitol fermenting phenotype. This shows that the non-dulcitol fermenting subspecies of *P. multocida* are the phenotypes occurring mostly in cattle.

Since *Pasteurella multocida* is a well-established non-motile organism (Mohamed and Abdelsalam 2008; Shayegh et al 2009), the observation of motile *P. multocida* isolates in this study is interesting and indicates a possible emergence of a new *P. multocida* phenotype. Further studies however, will be required to verify and ascertain this finding using advanced molecular techniques.

Other bacteria isolated in this study include *Proteus mirabilis*, Pasteurella *lymphangitidis*, *Pasteurella ureae*, *Pasteurella pneumotropica, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Morganella morganii*, *Escherichia coli, Staphylococcus aureus*, *Streptococcus pneumoniae* isolated in this study are in line with the findings of Ahmed et al 2012; Sabiel et al 2012 these researchers

isolated similar bacteria in the lungs of pneumonic sheep in Northern part of Nigeria and in Sudan respectively.

This study reports a high prevalence of *P. multocida* in this environment, a situation that calls for concern from Veterinarians and Farmers as the organism is very pathogenic to both animals and humans and can result in serious economic losses (Dritz et al 1996). It is therefore important that all cattle handlers should ensure the application of adequate preventive measures, including adequate biosecurity measures, proper ventilation of animal house, minimize stress during transport of animals, avoidance of comingling of animals from different sources and proper treatment of animals showing signs of respiratory disease in a herd.

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