THE PREVALENCE OF PNEUMONIC PASTEURELLOSIS-CAUSING MICROBES: *PASTEURELLA MULTOCIDA* AND *MANNHEIMIA HAEMOLYTICA* IN ABATTOIR SAMPLES IN NIGERIA.

Uchechukwu Chukwudi Obiekwe[+1] Olalekan Taiwo Lasisi[*] Elizabeth Adesola Amosun[^]

[+1]Department of Veterinary Medicine, University of Nigeria, Nsukka.
uche4zion2009@gmail.com+2348069300845
[^]Department of Veterinary Microbiology, University of Ibadan, Ibadan Nigeria
[*]Department of Veterinary Medicine, University of Ibadan, Ibadan Nigeria.

Abstract
This study was done to investigate the prevalence of pneumonic pasteurellosis-causing microbes: *Pasteurella multocida* and *Mannheimia haemolytica* in cattle. A total of 143 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, 41 lung tissue samples and 27 clotted heart blood samples from cattle with apparently healthy lungs. Samples were processed and recovered isolates were identified following standard cultural, morphological and biochemical laboratory techniques. 83 bacteria isolates were obtained from lung tissues while 63 were isolated from clotted heart blood samples. *Pasteurella multocida* was isolated from 6(7.22%) unhealthy lung tissue samples and 7(11.11%) clotted heart blood samples (4 from cattle with healthy lungs and 3 from those with unhealthy lungs). *Mannheimia haemolytica* was isolated from 1 (1.22%) unhealthy lung tissue sample only. Six isolates of *P. multocida* were found to be motile. Other bacteria isolated in this study include *Proteus mirabilis*, *Pasteurella lymphangitidis*, *Pasteurella ureae*, *Pasteurella pneumotropica*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Morganella morganii*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia*, from lung tissue samples while *Pseudomonas aeruginosa*, *Proteus rettgerii*, *Pasteurella pneumotropica*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Morganella morganii*, *Shigella dysentariae*, *Pasteurella multocida*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, 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.
Keywords: Prevalence, Pneumonic Pasteurellosis, Abattoir Samples, Microbes, Ibadan-Nigeria

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 weight gain, feed efficiency and overall performance of beef calves and mortality (Taylor *et al.*, 2010). Pneumonic pasteurellosis, also known as mannheimiosis, broadly refers to any of the disease conditions caused by bacteria of the genera *Pasteurella* or *Mannheimia*. The typical clinical disease is highly infectious, often fatal and with very serious economic impact in the animal industry. It is worth mentioning that *M. haemolytica*, *P. multocida* and *Bibersteinia trehalosi* 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 small ruminants in Ethiopia, Jordan and some parts of Nigeria (Ugochukwu, 2008; Emikpe *et al.*, 2013). However, there is still 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.

Materials and Methods

A total number of 100 cattle of different breeds slaughtered at the abattoir were sampled, these animals were transported from different parts of the country to Ibadan. At the opening of the thoracic cavity, following slaughter, the lungs were examined for the presence or not of any pathologic lesion prior to sample collection. A total of 100 samples were randomly collected consisting of 20 lung tissues and 12 samples of clotted heart blood from cattle with unhealthy lungs (showing congestion, pneumonia, oedema or presence of nodules), and 41 lung tissues and 27 clotted heart blood samples from apparently healthy animals. Samples were collected once in a week, over a period of 4 months. Each sample was aseptically collected from a different animal.

Samples were placed in sterile bottles and transported to the laboratory in an ice box. Samples were homogenized and inoculated on both MacConkey agar (Ozoid®) and 10% Bovine blood agar. The plates were incubated at 37^0C for 24 hours. Definitive identification of isolates according to cultural, Gram stain, and biochemical reactions (including sugar fermentation tests) were carried out using Flores *et al.* (2009) computer based identification software.

Results and Discussion

Table 1 shows list of bacteria organisms isolated from lung tissue samples, 12 different bacterial genera representing 5 different families were isolated. *Streptococcus pneumoniae* was mostly isolated, followed by *Staphylococcus aureus* and *Escherichia coli.*

Table 1: 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%
4.	*Pasteurella lymphangitidis*	Pasteurellaceae	0	1(100%)	1	1.20%
5.	*Pasteurella multocida*	Pasteurellaceae	0	6(100%)	6	7.22%
6.	*Pasteurella ureae*	Pasteurellaceae	0	1(100%)	1	1.20%
7.	*Pasteurella pneumotropica*	Pasteurellaceae	0	1(100%)	1	1.20%
8.	*Klebsiella pneumonia*	Enterobacteriaceae	0	3(100%)	3	3.61%
9.	*Escherichia coli*	Enterobacteriaceae	13(72.22%)	5(27.77%)	18	21.69%
10	*Morganella morganii*	Enterobacteriaceae	4(57.14%)	3(42.86%)	7	8.43%

11	*Mannheimia haemolytica*	Pasteurellaceae	0	1(100%)	1	1.20%
12	*Streptococcus pneumoniae*	Streptococcaceae	8(36.36%)	14(63.63)	22	26.51%

Table 2 shows list of bacterial organisms isolated from clotted heart blood samples, 11 different bacterial genera from 5 different families were equally isolated. *Escherichia coli* was mostly isolated, followed by *Staphylococcus aureus* and *Streptococcus pneumoniae*. This shows the uniquitous nature of these organisms in the environment.

Table 2: 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.	*Streptococcus pneumonia*	Streptococcaceae	6(60%)	4(40%)	10	15.87%

In this study, a higher prevalence of *P. multocida* organism was observed, 6 (7.22%) from lung tissue samples and 7 (11.11%) from clotted heart blood samples when compared with *M. haemolytica* of which only 1 (1.20%) isolate was obtained from an unhealthy lung and none (0%) from clotted heart blood samples. Out of the 13 *P. multocida* isolates, 9 (69.23%) were associated with unhealthy lungs. These findings are in line with the findings of the study of Karimkhani *et al.* (2011) who observed 32% prevalence of *P. multocida* organism isolated in diseased lungs and also the findings of Araghy (2007) in which no isolate of *P. multocida* and *M. haemolytica* was obtained from apparently healthy cattle. The higher prevalence of these pathogens in unhealthy than healthy lung tissues is explainable by the fact that in conditions of stress, immunosuppression or disease states, the local pulmonary defense mechanisms become impaired leading to increased proliferation and downward spread of the pathogens from the upper respiratory tract to the lower respiratory tracts (Ackermann *et al.,* 2010).

The occurrence of *P. multocida* organisms in clotted heart blood is as a result of the invasion of the blood stream by the organisms from the primary focus of infection through the lymphatic system to the blood (Shafarin *et al.,* 2007; Al-Zahraa *et al.,* 2010). Moreso, it has been shown that 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 carrier animals (Shafarin *et al.,* 2007).

All isolates of *P. multocida* obtained from blood agar were non-haemolytic, 6 isolates were motile while 7 were non-motile, oxidase, catalase, nitrate, and indole positive, urease negative. Since *Pasteurella multocida* is a well-established non-motile organism (Shayegh *et al.,* 2009), the observation of motile *P. multocida* isolates in this study 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.

The isolation of several other microbes from bovine lungs in this study are in line with the findings of Ahmed *et al.* (2012) who isolated similar bacteria organisms in the lungs of pneumonic sheep in Northern part of Nigeria and shows that cattle in this environment harbour potentially pathogenic organisms in their lower respiratory tract.

Conclusion and Recommendation

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 highly pathogenic to animals and zoonotic to humans and can result in serious economic losses. It is therefore, important that all cattle handlers should ensure the application of adequate preventive measures, including adequate biosecurity measures, proper ventilation of animal houses, 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.

References

Ackermann, M.R., Derscheid, R. and Roth, J.A. (2010). Innate Immunology of Bovine Respiratory Disease. *Vet. Clin. North Am Food Anim. Pract. 26(2): 215 – 228.*

Ahmed, A., Egwu, G.O., Garba, H.S., Magaji, A.A. and Tambuwal, F.M. (2012). Bacteriological and Histopathologic studies in Pneumonic Lambs in Sokoto, North Western Nigeria. *Bull. Anim. Hlth. Prod. Afr., 60, 39-44.*

Al-Zahraa, A., El-Din, K., Mohamed, A.M., Walaa, H.G. and Ghada, S.L. (2010). Prevalence of Microbial Pathogens in Blood Cultures: An Etiological and Histopathological Study. *Journal of Taibah University for Science;* 3: 23–32.

Araghy, S.A. (2007). Etiological Study of Calf Pneumonia by Analysis of Bronchoalveolar Fluid. Ph. D. Thesis Veterinary Faculty, University of Tehran.

Babetsa, M., Sandalakis, V., Vougidou, C., Zdragas, A., Sivropoulou, A., Psaroulaki, A. and Ekateriniadou, L.V. (2012). Tetracycline resistance genes in *Pasteurella multocida* Isolates from Bovine, Ovine, Caprine and Swine Pneumonic Lungs Originated from Different Greek Prefectures. *African Journal of Microbiology Research, 6* (17): 3917-3923.

Emikpe, B.O., Jarikre, T.A. and Eyarefe, O.D. (2013). Retrospective Study of Disease Incidence and Type of Pneumonia in Nigerian Small Ruminants in Ibadan, Nigeria. *Afr. J. Biomed. Res., 16: 107 – 113.*

Flores, O., Belanche, L.A. and Blanch, A.R. (2009). New Multiplatform Computer Program for Numerical Identification of Micro-organisms. *J. Clin. Microbiol 47: 4133 – 4135.*

Karimkhani, H., Zahraiesalehi, T., Sadeghizali, M.H., Karimkhani, M. and Lameyi, R. (2011).Isolation of *Pasteurella multocida* from cows and Buffaloes in Urmia's Slaughter House. *Archives of Razi Institute, 66 (1): 37-41*

Shafarin, M.S., Zamri-Saad, M., Jamil, S.M., Siti-Khairani, B. and Saharee, A.A. (2007). Experimental Transmission of *Pasteurella multocida* 6:B in Goats. *J Vet Med A Physiol Pathol Clin Med*; 54(3):136-9

Shayegh, J., Sharaf, D., Mikaili, P. and Namvar, H. (2009). Pheno- and Genotyping of *Pasteurella multocida* Isolated from Goat in Iran. *African Journal of Biotechnology; 8(16): 3707 – 3710.*

Taylor, J.D., Fulton, R.W., Lehenbauer, T.W., Step, D.L. and Confer, A.W. (2010). The Epidemiology of Bovine Respiratory Disease: What is the Evidence for Predisposing Factors. *Can. Vet. J. 51(10): 1095 – 1102.*

Ugochukwu, E.I. (2008). Isolation and Characterization of *Pasteurella multocida* from Caprine Pneumonic Lungs. *Animal Research International 5(2): 880 – 882*