JWPR
Journal of World's Poultry Research

J. World's Poult. Res. 5(4): 98-103, December 25, 2015
Research Paper
PII: S2322455X1500014-5

Clinicopathological Manifestations of *Pasteurella Multocida* (Serotypes A: 1, 3 And 4) Infections in Commercial Chickens in Jos, Nigeria

Dashe Yakubu[1*], Raji Moshood[2], Abdu Paul[3], Oladele Blessing[4], Okewole Philip[5], Kumbish Peterside[5], Oluwadare Lola1 and Barde Israel[5]

[1]*National Veterinary Research Institute, Akure, Zonal Office, Hospital Road, Akure, Ondo State, Nigeria.*
[2]*Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Nigeria.*
[3]*Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.*
[4]*Deprtment of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria.*
[5]*Central Diagnostic Department, National Veterinary Research Institute, Vom Jos, Nigeria.*
*Corresponding author`s Email: yakubudashe@yahoo.co.uk

Received: 07 Sep. 2015
Accepted: 20 Oct. 2015

ABSTRACT

This pathogenicity study was conducted to document the clinicopathologic features observed in commercial chickens inoculated with *Pasteurella multocida* serotypes A: 1, 3 and 4. Thirty, eighteen weeks old (adult) commercial chickens were divided into five groups (A, B, C, D and E) of 6 birds each. Chickens in groups A, B and C were inoculated with 0.1 ml of *Pasteurella multocida* serotypes A: 1, 3 and 4 at concentrations of 10^6, 10^7 and 10^8 CFU/chicken respectively using intramuscular route. Group D were inoculated with 0.1 ml fowl cholera vaccine strain of *P. multocida* serotype A: 1 at of concentrations of 10^6, 10^7 and 10^8 CFU/chicken, while group E the uninfected control chickens were given normal saline. All deaths in groups A and B occurred on day 7 and mortality rates were 83.3% for group A and 50% for B. No mortality was recorded in groups C, D (vaccine strain) and E (uninfected control). Gross lesions observed were petechial and ecchymotic haemmorhages on the heart and breast muscles, congestion of the liver and lungs. Histopathological lesions observed were mononuclear cellular infiltration and pulmonary congestion. This study has shown that chickens were susceptible to both *Pasteurella multocida* serotypes A: 1 and 3.

Key words: Chicken, Pathogenic, Jos, Pasteurella, Serotypes

INTRODUCTION

Poultry has been declared the largest group among domesticated livestock in Nigeria with an estimated population of about 140 million (FAO, 2000). Poultry offers the quickest supply of animal protein to man, and provides comparatively greater financial profit than cattle, small ruminants or pigs (Etukudo and Adegboye, 1983; Molokwu et al., 1988). The rural poultry farming contributes 68.5% of the national meat supply; while 11.4% is from commercial chickens and 19.7% from other poultry (Wosu, 1992; Musa et al., 2009).

Despite the unquantifiable economic gains from this industry, it often suffers from disease induced losses due to sustained challenges from environmental factors, viral, bacterial, protozoan, parasitic and fungal infections among others. In Nigeria, a great loss in poultry production is attributed to these agents and it still remains as one of the major constraints hindering the success and growth of the poultry industry, where disease prevention and control measures are rare and high mortality rates are common even in vaccinated flocks in some cases. The epizootic of fowl cholera in the world poses a serious threat to poultry (Pandey, 1992; Ambali et al., 2003). The aetiologic agent of fowl cholera, *Pasteurella multocida* is not host specific and has demonstrated the capacity to infect and cause severe illness and death in livestock. Fowl cholera has often occurred as a sporadic disease in many parts of the world including Nigeria with grave economic consequences on livestock and humans (Ambali, 2003). Fowl cholera is widely identified as one of the major diseases of economic importance wherever intensive poultry production occurs; particularly in the United States of America (Rimler et al., 1998). However, the disease has a significant impact in less intensive systems. For example, fowl cholera was one of the main diseases seen in Mexican village chickens (Gueye, 1999). Fowl cholera was endemic in regions of Asia and Africa where it caused immense economic losses to the poultry industry due to cost of treatment, weight loss and mortality (Townsend et al., 1997). Despite the established role of *P. multocida* as an etiologic agent of fowl cholera in chickens in Jos, Nigeria, there is inadequate information on the clinical, gross pathologic and histopathologic features of the disease caused by *P.*

To cite this paper: Yakubu D, Moshood R, Paul A, Blessing O, Philip O, Peterside K, Lola O and Israel B. 2015. Clinicopathological Manifestations of *Pasteurella Multocida* (Serotypes A: 1, 3 And 4) Infections in Commercial Chickens in Jos, Nigeria. *J. World's Poult. Res.* 5(4): 98-103.
Journal homepage: http://jwpr.science-line.com/

multocida serotypes. This study therefore seeks to document the clinicopathologic features observed in chickens inoculated with *P.multocida* serotypes A: 1, 3 and 4.

MATERIAL AND METHODS

Management and housing of experimental chicken

Thirty, 18 weeks old (adult) commercial chicken were provided by the National Veterinary Research institute, Vom, Nigeria for this experiment. The chickens were divided into five groups (A, B, C, D and E) of 6 chickens each. Groups A, B, C and D were further sub-divided into three sub-groups, with each group consisting of two chickens. One week before the onset of the study, chickens were screened for the presence of *P. multocida* by taking oro-pharyngeal swabs. All chickens were fed with commercial pelleted layer feed and water *ad libitum* before and during the experiment. The house with floor space of the pen was 4×5 m^2 measurement in size was cleaned disinfected prior to the commencement of experiment. Chickens were allowed to acclimatize for two weeks prior to the commencement of the pathogenicity study.

Source of *Pasteurella multocida*

The bacterium *P. multocida* serotypes A: 1, 3 and 4 were recovered by the authors from chickens with clinical cases of fowl cholera in Jos, Nigeria in 2012. The isolates were confirmed by biochemical test, Microbact, Polymerase Chain Reaction and Multiplex PCR. They were sorotyped at United State Department of Agriculture in USA.

Inoculation of Chickens

All groups (A, B, C, D and E) were used for the pathogenicity test. *Pasteurella multocida* serotypes were reconstituted into three different concentrations for groups A, B and C; every chicken in first sub-group of A was administered 0.1 ml intramuscularly of 10^6 CFU (serotype A:1- field strain) contained in Typtose Soya broth, the second sub-group received 10^7 CFU of the same serotypes and the last sub-group received 10^8 CFU of *P. multocida* serotype A: 1, the administration of serotype A: 3 and A: 4 of *P. multocida* at the same doses were replicated for sub-groups B and C respectively. The chickens in group D were inoculated with *P. multocida* serotype A: 1 (fowl cholera vaccine strain) at the same concentrations of 10^6, 10^7 and 10^8 CFU. Chickens in group E (uninfected control) received normal saline at 0.1 ml intramuscularly. Death occurred within the period of twenty one days of the experiment was recorded. Findings from clinical, postmortem examination of carcasses and histopathological features were recorded.

Clinical and pathological examinations

Chickens in all the groups were observed for clinical signs and mortality post inoculation. Postmortem examinations were conducted on chickens that died and tissue section of the spleen, liver, heart and lungs were prepared for histology. The section of grossly affected organs were fixed in 10% buffered formalin; paraffin embedded tissues were sectioned at 5 microns, stained with haematoxylin and eosin (H & E) and mounted on glass slides and examined under light microscope at X200 and X400 respectively.

Statistical Analysis

The entry and sorting of the primary data was performed with Microsoft excel, 2010. Descriptive statistical analysis was conducted using statistical package for social sciences IBM SPSS (version 21.0) (2012) and the results were summarized as percentages in tables.

RESULTS

Ninety two percent of the experimental chickens in groups A and B became sick within 24 h post inoculation. Clinical signs manifested were depression, inappetence, ruffled feathers, dyspnoea, sitting on hock and these signs continued for four days. All deaths in groups A and B occurred on day 7 and mortality rates were 83.3% for group A and 50% for B. No clinical sign or mortality was recorded in groups D (vaccine strain), C and E (uninfected control) (Table 1). Lesions observed at postmortem examinations in chickens inoculated with *P. multocida* serotypes A: 1 and A: 3 were prominent keel with congested heart, liver, kidneys and lungs (Table 2 and Figure 1). Frothy exudates from the lungs, petechial and ecchymotic haemorrhages were seen on the heart, subserosal haemorrhages (Figure 2). Lymphocytic, heterophilic and macrophagic cellular infiltration in the lungs and heart were observed (Figure 3).

DISCUSSION

Pasteurella multocida is one of the bacterial agents responsible for significant economic losses in the poultry industry worldwide. The report of this study indicated that only serotypes A: 1 and A: 3 caused clinical disease in chickens with high morbidity and mortality. Interestingly, these serotypes also demonstrated various degrees of gross and histopathological lesions in the affected chickens. In a similar study conducted in India by Kumar et al. (2004), opined that majority of *P. multocida* belonging to serotypes A: 1, 3 and 4 were associated with fowl cholera in chickens. The findings in this study differ from that of Kumar and others in that serotype A: 4 in this study did not cause mortality in chickens. Similarly, Snipes et al. (1988) also asserted that not all *P. multocida* isolates are pathogenic to chickens. Previous study conducted on fowl cholera by Glisson et al. (2003) indicated that *P. multocida* serotypes vary from one country to the other. Another report of a study from Central Saudi Arabia, by Elfak et al. (2002) reported the involvement of *P. multocida* s erotypes 1, 3, 4 and 5 in an outbreak of fowl cholera in ostriches. The serious implication of these reports is that the production of universal fowl cholera vaccine would be difficult considering the fact that *P. multocida* serotypes vary from one country to the other and immunity is

known to be serotype specific. The congested liver, spleen, lungs as well as petechial and ecchymotic haemorrhages that were observed at postmortem in the experimentally challenged chickens, also confirmed the findings of Visut et al. (2010). The result of this pathogenicity study indicated that all the experimental chickens inoculated with serotypes A: 1 and A: 3 came down with the clinical signs of fowl cholera similar to those reported by Rimler and Glisson (1997). This has buttressed the fact that adult chickens above 16 weeks of age were susceptible to fowl cholera. Host variation in the susceptibility to *P. multocida* serotypes was observed as different clinical signs and gross lesions manifested in the chickens inoculated by the same pathogenic agent. These findings collaborated the findings of Botzler (1991) who also observed that clinical signs of fowl cholera in birds vary depending on various factors such as age, species, dose of strain, route of entry of the bacterium and form of the disease.

Notable histopathological findings in this study indicated a moderate to severe lymphocytic, heterophilic and macrophagic cellular infiltration in lungs and heart. These findings confirmed report of Shilpa and Verma (2006). The findings could possibly signify that the chickens were responding to the inciting bacterial agent, which is a common characteristic in acute phase of fowl cholera. The severe involvement of visceral organs such as congestion noticed in lungs, heart and spleen of chickens clearly demonstrate the septicaemic nature of fowl cholera; this could possibly explain the profound debilitation, massive flock morbidity and mortality observed in chickens suffering from the acute form during outbreaks of fowl cholera.

Table 1. Mortality of chickens after inoculated with P. multocida serotypes A: 1 A: 3 and A: 4

Groups	P. multocida serotypes	Mortality
A	A: 1 (Field Strain)	2 at 10^8 CFU
		2 at 10^7 CFU
		1 at 10^6 CFU
B	A: 3	2 at 10^8 CFU
		1 at 10^7 CFU
		0 at 10^6 CFU
C	A: 4	0 at 10^8 CFU
		0 at 10^7 CFU
		0 at 10^6 CFU
D	A: 1 (Fowl Cholera Vaccine Strain)	0 at 10^8 CFU
		0 at 10^7 CFU
		0 at 10^6 CFU
E	Control (Normal Saline)	0 at 10^6 CFU
		0 at 10^6 CFU
		0 at 10^6 CFU

Table 2. Gross lesions in 18 weeks old chickens inoculated with Pasteurella multocida serotypes A: 1, A: 3 and A: 4

Gross lesions	Group A	Group B	Group C	Group D	Group E
Congested liver	6 (100%)	6 (100%)	-	-	-
Congested heart	6 (100%)	6 (100%)	-	-	-
Congested kidneys	6 (100%)	6 (100%)	-	-	-
Congested lungs	6 (100%)	6 (100%)	-	-	-
Frothy exudates from lung	3 (50%)	0 (0%)	-	-	-
Pet. and Ecc. hae on heart	6 (100%)	6 (100%)	-	-	-
Fatty degeneration	1 (16.7%)	0 (0%)	-	-	-
Egg yolk peritonitis	3 (50%)	3 (50%)	-	-	-
Prominent keel	6 (100%)	6 (100%)	-	-	-

Pet: Petechial, Ecc: Ecchymotic, Hae: Haemorrhages

To cite this paper: Yakubu D, Moshood R, Paul A, Blessing O, Philip O, Peterside K, Lola O and Israel B. 2015. Clinicopathological Manifestations of *Pasteurella Multocida* (Serotypes A: 1, 3 And 4) Infections in Commercial Chickens in Jos, Nigeria. *J. World's Poult. Res.* 5(4): 98-103.
Journal homepage: http://jwpr.science-line.com/

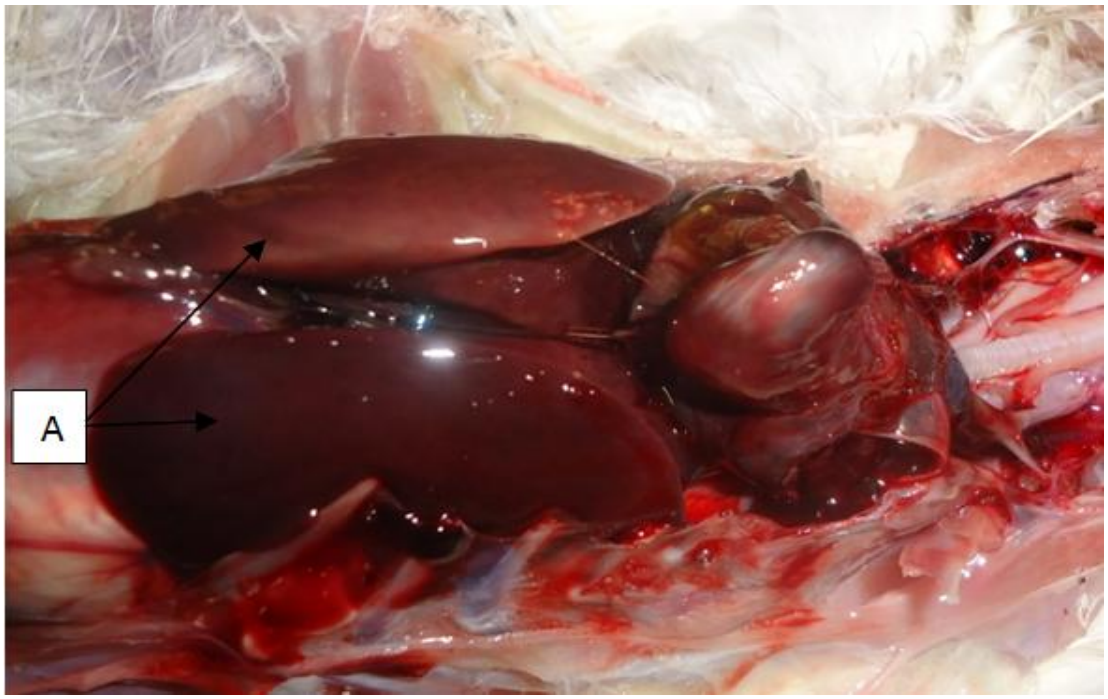


Figure 1. A - Congested liver in a chicken aged 18 weeks inoculated with Pasteurella multocida serotypes A: 3, in Jos, Nigeria

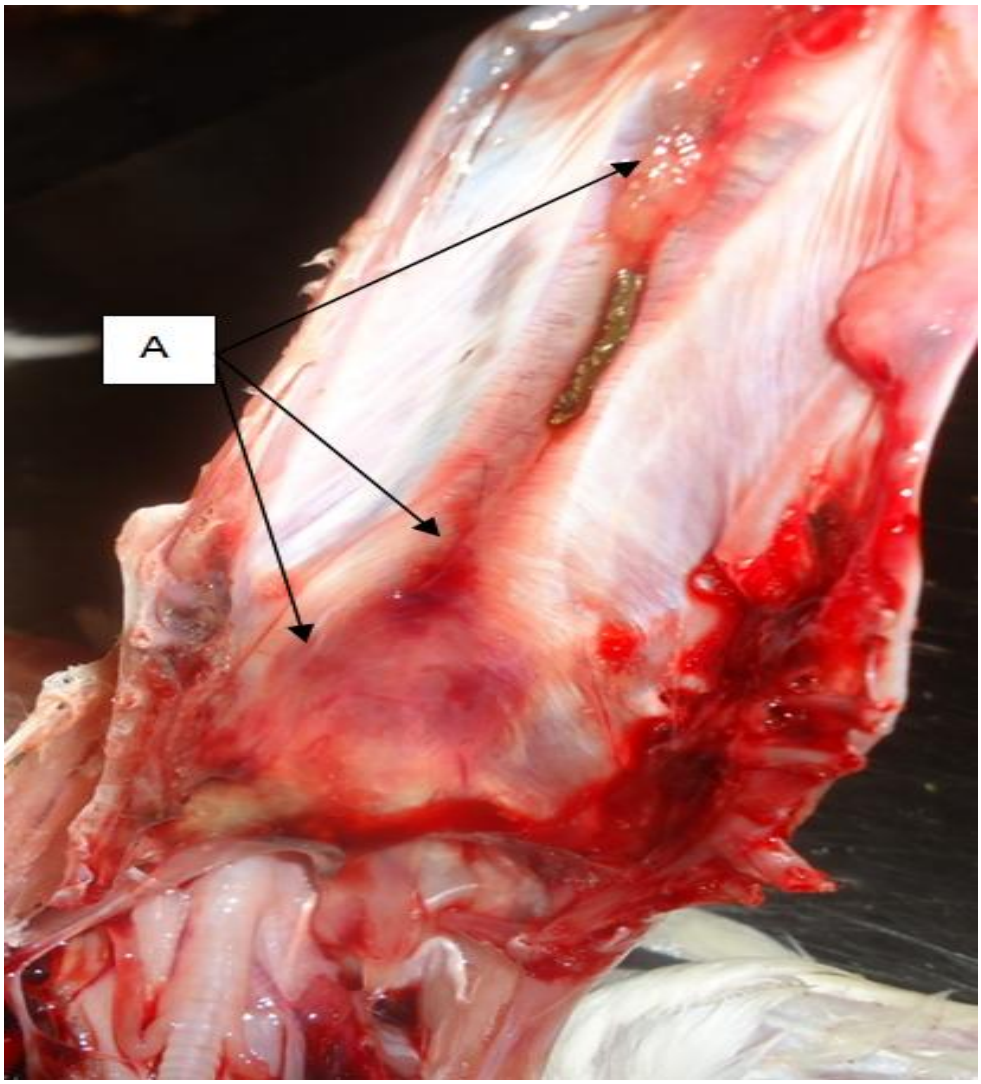
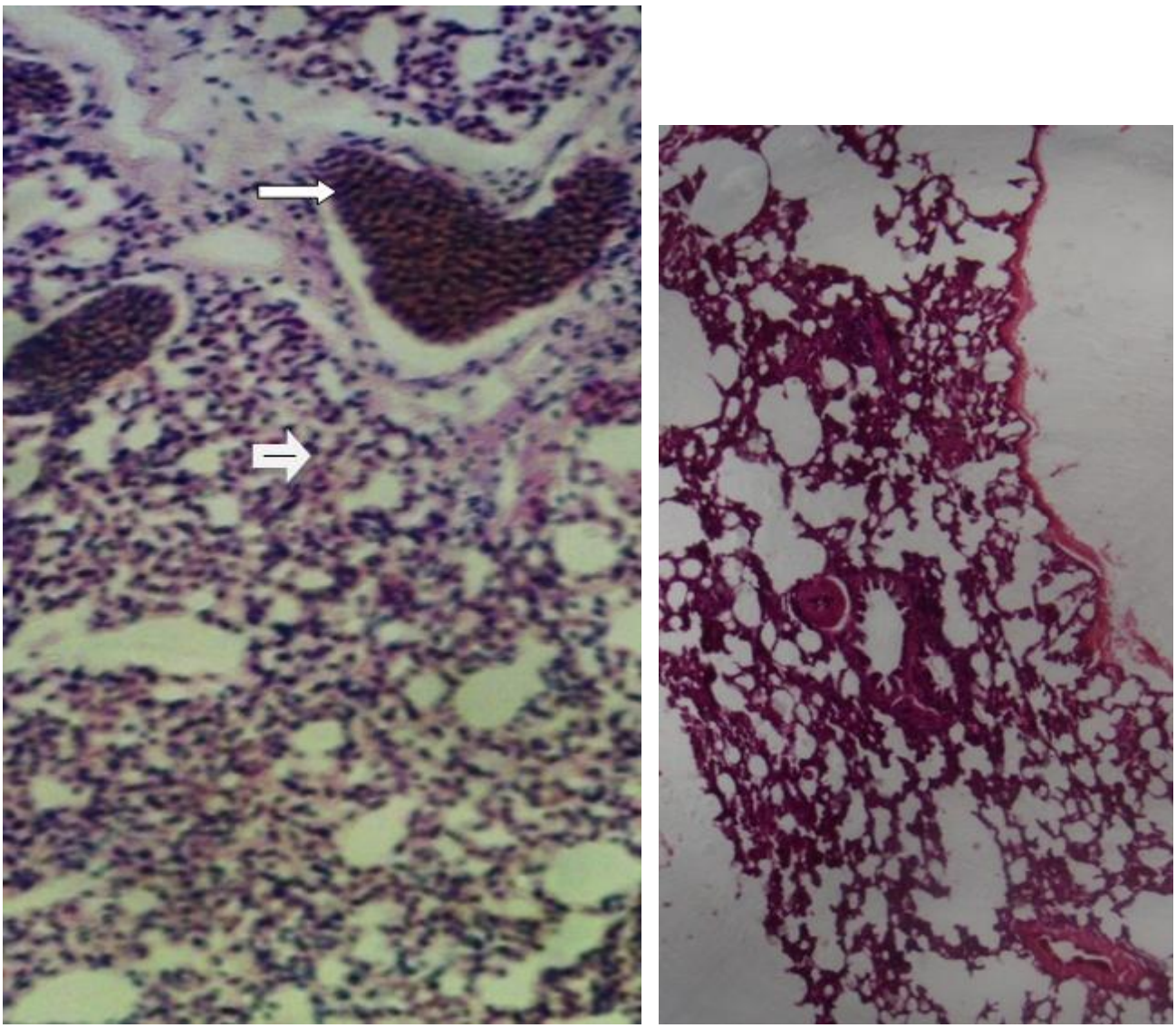


Figure 2. A - Haemorrhages in subserosal layer of sternal bone of a chicken aged 18 weeks inoculated with Pasteurella multocida serotypes A: 1 in Jos, Nigeria.

To cite this paper: Yakubu D, Moshood R, Paul A, Blessing O, Philip O, Peterside K, Lola O and Israel B. 2015. Clinicopathological Manifestations of *Pasteurella Multocida* (Serotypes A: 1, 3 And 4) Infections in Commercial Chickens in Jos, Nigeria. *J. World's Poult. Res.* 5(4): 98-103.
Journal homepage: http://jwpr.science-line.com/



A **B**

Figure 3. A: The lung of a chicken aged 18 weeks infected with *Pasteurella multocida* A:3=10^8. Congested blood vessels (small arrow), areas with obliterated alveoli and infiltrated with inflammatory cells (big arrow) H and E Stain. X 200; **B:** normal lung of a chicken aged 18 weeks at X 400.

CONCLUSSION

The findings of present study have revealed that chickens were highly susceptible to infections with *P. multocida* serotypes A: 1 and 3. These two serotypes of *P. multocida* were found to cause high morbidity, mortality, clinical, gross and histopathologic features in the experimental chickens.

Recommendations

It is therefore recommended that chickens should be vaccinated against fowl cholera with a polyvalent fowl cholera vaccine consisting of *P. multocida* serotypes A: 1 and 3 in order to protect them against *P. multocida* infections.

Acknowledgement

The authors wish to express their profound gratitude to the staff of Central Diagnostic Department, National Veterinary Research Institute, Vom, Nigeria for assisting in carrying out post mortem examination on the carcasses.

REFERENCES

Ambali AG, Abubakar NB and James TE (2003). An assessment of poultry health problems in Maiduguri, Borno State, Nigeria. Tropical Veterinarian, 21: 138-145.

Botzler RG (1991). Epidemiology of avian cholera in wildfowl. Journal of Wildlife Diseases, 27: 367-395.

Elfak MG, Abbas B, Mahmoud MO, Haroun EM. and Abdel-Magied EM (2002). Septicaemic pasteurellosis in ostriches (Stricthio camelus) in Central Saudi Arabia. The Veterinary Journal, 163: 218-221.

Etukudo, BO and Adegboye DS (1983). Studies of chronic respiratory diseases of poultry in Nigeria. Bacterio-logical examination of clinically diagnosed cases. Nigerian Veterinary Journal, 12: 66-69.

Food and Agricultural Organization (2000). Statistic Base, www.fao.org. Food and Agriculture Organization of the United Nations, Rome, Italy. Animal Production and Health Division Publication, No 274415, Rome. FAOSTAT. Accessed 2010 at 3.00 pm.

Glisson JR, Hofacre CL and Christensen JP (2003). Avian pasteurellosis. In: Saif, YM, Barnes H.J, Glison JR., Fadly AM, McDougald LR. And Swayne DE. (Eds), Diseases of Poultry, 11th Ed. Iowa State University Press, Ames, Iowa, USA, Pp. 658-676.

Gueye EF (1999). Ethno-veterinary medicine against poultry disease in African villages. World Poultry Science Journal, 55: 187-198.

Goto Y, Nakura R, Nasu T, Sawada T and Shinjo T (2001). Isolation of P. multocida during an outbreak of infectious septicemia in Japanese quails (Coturnix coturnix japonica). Journal of Veterinary Medical Sciences, 3:1055-1056.

IBM corporated, released (2012). IBM Statistical Package for Social Science (SPSS) statistics for windows, version 21.0. Armonk, New York.

Kumar AA, Shivachandra SB, Biswas A, Singh VP, Vijendra P and Srivastava SK (2004). Prevalent serotypes of Pasteurella multocida isolated from different animals and avian species in India. Veterinary Research Communication, 28: 657-667.

Molokwu JU, Adegboye DS and Emejuawe SO (1988). Bacteria isolates from cases of chronic respiratory disease of chicken in Zaria area of Nigeria. Zariya Veterinarian, 3: 42-44.

Pandey VS. (1992). Epidemiology and economic of village poultry in Africa: An overview. Proceedings of a Workshop on Village Poultry Production in Africa. Rabat city, Morocco, Pp. 124-128.

Rimler RB and Glisson JR (1997). Fowl cholera. In: Calnek, BW., Barnes, H.J., Beard, E.W., McDougald, L.R. and Saif, Y.M. (Eds), Diseases of Poultry, 10th edition, Iowa State University Press, Ames, Iowa, U.S.A, Pp. 143-159.

Rimler RB, Sandhu TS and Glisson JR (1998). In: Swayne, DE., Glisson JR., Jackwood MW. and Reed MW. (Eds.), A laboratory manual for isolation and identification of avian pathogens, 4th Ed. American Association of Avian Pathology, Pennsylvania, USA, Pp 17-28.

Shilpa S and Verma PC (2006). Pathology of Pasteurella multocida infection in chicken. Indian Journal of Animal Research, 40: 15-19.

Snipes KP, Carpenter TE, Corn JL, Kasten RW, Hirsh DC, Hird DW and McCcapes RH (1988). Pasteurella multocida in wild mammals and birds in California: prevalence and virulence for turkeys. Avian Diseases, 32: 9-15.

Townsend KM and Papadimetrious JM (1997). REP-PCR analysis of Pasteurella multocida isolates that cause haemorrhagic septicaemia. Research in Veterinary Science, 63: 151-155.

Visut R, Piyarat C and Niwat C (2010). Comparison of efficacy of enrofloxacin against Escherichia coli or Pasteurella multocida infection in chickens. Thai Journal of Veterinary Medicine, 40: 297-301.

Wosu LO (1992). Strategy for sustained livestock production and development in Nigeria, In: Book of Proceedings of 29th Annual Conference of Nigerian Veterinary Medical Association, Kaduna, Pp. 165-170.