African Journal of Cellular Pathology 9:66-72 (2017)
The Official Journal of the Society for Cellular Pathology Scientists of Nigeria
ISSN 2449 - 0776
www.ajcpath.com

SOME PLASMA BIOCHEMICAL CHANGES IN LAYERS EXPERIMENTALLY INFECTED WITH SALMONELLA GALLINARUM

Chiroma Mohammed Adam[1], Adamu Sani[2], Gadzama Joseph John[1], Esievo King Akpofure Nelson[2], Abdulsalam Hassan[1], Balami Arhyel Gana[3], Enam Samson James[2,] Muhammad Ya'u[4] , Atata Abdulhamid Jamila[5]

1. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria.
2. Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Zaria, Nigeria.
3. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria.
4. Department of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia, Jigawa State
5. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin, Kwara State.

Corresponding Author: Chiroma M.A
Email: drmohammedchiroma78@gmail.com

Abstract

Aim: The study was conducted to investigate some plasma biochemical changes in layers experimentally infected with Salmonella Gallinarum

Methods: A total of 20 eighteen-week- old ISA Brown layers were used in the experiment. The birds were randomly divided into two groups; infected and control, of 10 birds each. Each bird in the infected group was orally administered 0.5 ml of the inoculum containing $9x10^8$CFU/ml. Similarly, birds in the control group were each administered 0.5 ml normal saline only. All the experimental birds were closely monitored for clinical signs of fowl typhoid. Blood samples were collected from each group at day zero (Day 0), 2, 4, 7, 14, 21, 28, 35 and 42, post-infection and used for determination of plasma biochemical parameters.

Results: By day seven post infection, all birds in the infected group showed clinical signs typical of fowl typhoid; weakness, ruffled feathers, huddling together, somnolence, greenish-yellow diarrhea, weight loss, drop in egg production, decrease in feed and water consumption and mortality rate (50%). There were, however, marked increase in the plasma activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and the level of urea and significant hypoprotinemia.

Conclusion: The experimental Salmonella Gallinarum infection induced alteration in the liver and kidney functions.

Key Words: Salmonella Gallinarum, Typhoid, ISA brown layers

INTRODUCTION

Fowl typhoid caused by Salmonella Gallinarum is recognized worldwide as a disease of social and economic significance (Shivaprasad, 1997). In Africa, it has been reported in many countries including Tanzania, Uganda (Okoj, 1993), Senegal (Arbelot et al., 1997), Nigeria (Sa'idu et al., 1994) and Morocco (Bouzoubaa et al., 1987). It is a septicaemic disease that affects primarily chicken and turkey, although natural infections in many other avian species have been reported (Wray et al., 1996; Shivaprasad,1997). Although Salmonella Gallinarum infection is frequently

considered a problem of adult and grower chicken, chicks are often affected. The outbreak of fowl typhoid in young chicks may be associated with vaccination against fowl typhoid practiced by most breeders which leads to vertical transmission of the disease (Jordan and pattison, 1992; Roa, 2000). Efforts at controlling fowl typhoid through the application of a co-ordinated policy of hygienic measures, together with serological testing and slaughter of positive reactors, have led to the seemingly eradication of Salmonella gallinarum in many developing countries (Barrow, 1999). However, fowl typhoid remains a leading disease of the poultry industry in many areas of the world (Okwori et al., 2013). Acute form of the disease manifests as respiratory distress and depression with a characteristic clinical sign of greenish- yellow diarrhea, there may be enlarged and congested liver, spleen and kidney. Liver may have white foci of 2-4mm in diameter (Beyaz et al., 2010). In acute to subacute cases, there is multifocal necrosis of hepatocytes with accumulation of fibrin and infiltration of heterophils mixed with a few lymphocytes and plasma cells can be seen in the liver (Kokosharov et al., 1997; Hossain et al., 2006). In sub-acute outbreaks, sporadic mortality over a long period is experienced while in chronic cases, especially in cases where there are large nodules in the heart, the liver will have congestion with interstitial fibrosis. The spleen may have severe congestion or fibrin deposits and severe hyperplasia (Chishti et al., 1985). The transmission of Salmonella Gallinarum can be through fecal droppings of infected birds, bird carcasses and laid eggs.The infection could be introduced by importation of live infected chickens and hatched eggs. Mechanical spread may be by humans, wild birds, mammals, flies, ticks, feedsacks, etc (Steigh and Duguid, 1989). For the past few decades, poultry production has become increasingly organized, specialized and integrated into an industry of major national and international importance (Mai et al., 2004; Khan et al., 2007). As a result, poultry diseases are every poultry farmer's nightmares. The economic losses attributed to these infections are enormous and in most cases unquantifiable. In Nigeria, early detection of the disease in any locality can help reduce/eliminate the losses that may occur in the event of the disease outbreak (Okwori et al., 2013). This study evaluated the plasma biochemical changes in layers experimentally infected with Salmonella Gallinarum in Zaria, Kaduna State, Nigeria.

MATERIALS AND METHODS

Area of Study

The study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah Zone of Nigeria, between latitude 7^0 and 11^0N, and longitude 7^0 and 44^0E; the average rainfall of this zone ranges from 1,000 to 1,250 mm, and the average temperature ranges from 17^0C to 33^0C (Sa'idu et al., 1994).

Experimental Birds

A total of twenty 18-week old ISA Brown layers were purchased from kujama farm in Kaduna. These birds were duly vaccinated against endemic infectious diseases except fowl typhoid. On arrival, they were housed and managed intensively in washed, cleansed and disinfected poultry research pens of veterinary teaching hospital Ahmadu Bello University, Zaria. From the day of arrival and throughout the experiment, the birds were fed on standard commercial layer mash (Hybrid Feed®) and water was provided ad libitum. The birds were acclimatized for a period of four weeks to get used to all the handling conditions.

Source of bacterial organism

Salmonella Gallinarum was obtained from the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Sub-culture of organisms

The bacterium from the previously prepared slant was reactivated by sub-culturing on MacConkey agar (MCA). The resulting colonies were then examined for their characteristic features, color and morphology and tested for the gram stain reaction (Gram negative). Mcfaland turbidity standards were made in the laboratory by preparing a 1% solution of anhydrous Barium Chloride and 1% solution of sulfuric acid and they were mixed to obtain a barium precipitate. The volumes of the two reagents were adjusted to prepare standards of different turbidities that represent different concentrations of bacterium. The standards were used to visually compare the turbidity of a suspension of bacteria.

Pre-infection bacteriological monitoring of experimental birds

During the period of acclimatization, all birds were checked to ensure they were free from Salmonella spp. Individual cloacal swabs were collected and then immersed in buffered peptone water, and then followed by plating them in MacConkey agar (MCA) and blood agar (BA).

Both cloacal swab and plates were incubated in a bacteriological oven at 37^0C for 24 hours according to the standard laboratory methods (Wigley et al., 2001; Parmer and Davies, 2007).

Challenge of the birds with Salmonella Gallinarum

At 22 weeks old, the chickens were allocated into two groups at random (infected and control) of 10 birds each. Few colonies were scooped from the cultured plate and inoculated into a sterile test tube, each containing 20 ml of 0.5% normal saline, until the turbidity was equivalent to 9 x 10^8 CFU/ML. At 26 weeks old, after reaching there peak point of lay, each of the birds in the infected group was challenged by oral administration of 0.5 ml inoculum containing $9x10^8$CFU/ML of Salmonella Gallinarum, while the birds in control group which were uninfected with the bacterium, but given distilled water only.

Clinical Observation

Following inoculation of the birds with the Salmonella Gallinarum, the infected group was observed daily for clinical signs of fowl typhoid and findings were recorded.

Determination of Plasma Biochemical Parameters

Blood samples of 2.5 ml each was collected from the infected and control groups via wing vein, using 25 gauge needle and syringe on days 0, 2, 4, 7, 14, 21, 28, 35, and then 42 post infection. The blood was dispensed into (EDTA) as anticoagulant and used for plasma biochemical evaluations of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), level of plasma urea concentration and plasma total protein.

Bacteriological Isolation

At post-mortem, tissues from the ovary, liver, kidney and spleen were aseptically taken for isolation of Salmonella Gallinarum using standard laboratory methods (Wigley et al., 2001; Parmer and Davies, 2007).

Statistical Analysis

Data obtained were subjected to statistical analysis including the calculation of the mean and standard error of the mean. Data between groups were evaluated by student t-test and values of $P<0.05$ were considered significant using Graph Pad Prism Version 5.00 for Windows, GraphPad Software, San Diego California USA.

RESULTS

Clinical Manifestations of Fowl Typhoid in the infected Commercial Layers

All the infected group showed clinical signs of fowl typhoid starting at day 7 post-infection, which include: depression and huddling, ruffled feathers, somnolence, greenish-yellow diarrhea, loss of weight, a decrease in feed and water consumption, decreased egg production and sudden death, while the control Group showed no sign of any disease. There was mortality in the infected group, with mortality rates of 50% among experimentally infected layers while no abnormal signs or gross lesions were observed in normal control layers during the experimental periods.

Bacterial recovery from infected birds

Salmonella Gallinarum organisms were isolated from the liver, spleen, kidney and ovary of the infected layers beginning from day 9 post-infection and throughout the experimental period. Biochemical test revealed indole negative, urea negative, catalase and citrate positive and it produces hydrogen sulphide (H_2S) in triple sugar iron agar TSI.

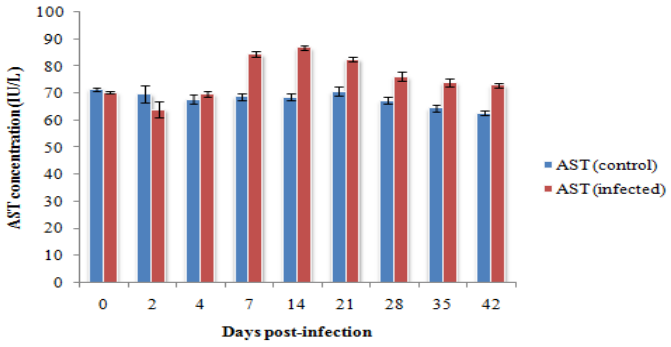

Figure 1: Mean (±SEM) plasma aspartate aminotransferase activity in *Salmonella Gallinarum* experimentally-infected and control layers.

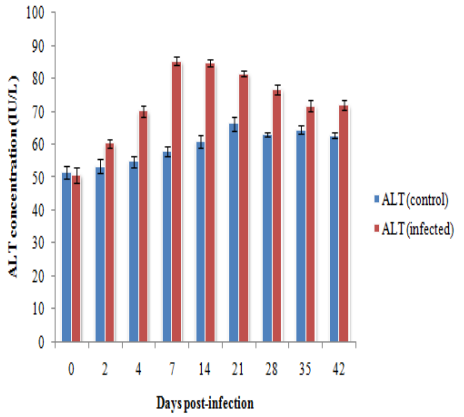

Figure 2: Mean (±SEM) plasma alanine aminotransferase activity in *Salmonella Gallinarum* experimentally-infected and control layers.

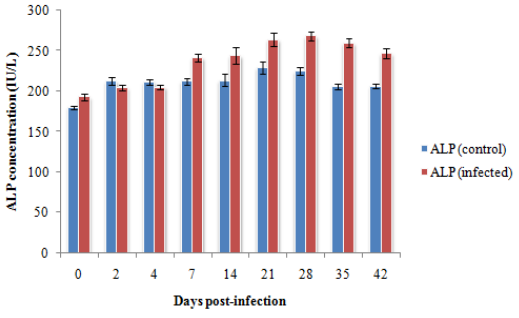

Figure 3: Mean (±SEM) plasma alkaline phosphatase (ALP) activity in *Salmonella Gallinarum* experimentally-infected and control layers.

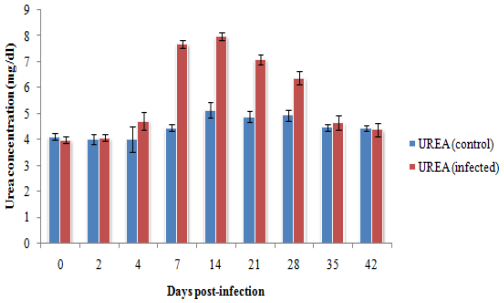

Figure 4: Mean (±SEM) plasma urea concentration in *Salmonella Gallinarum* experimentally-infected and control layers.

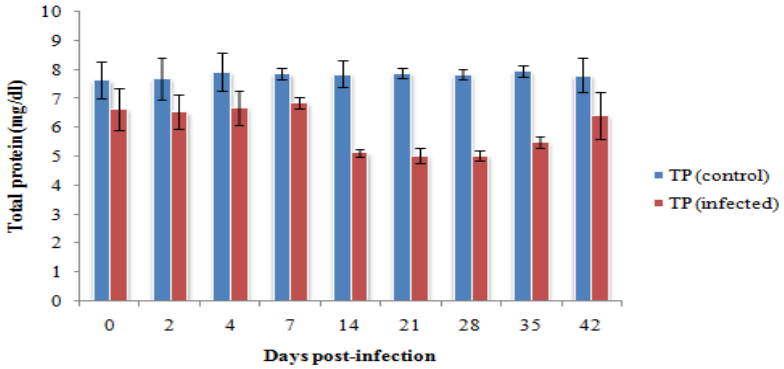

Figure 5: Mean (±SEM) plasma total protein in *Salmonella Gallinarum* experimentally-infected and control layers.

DISCUSSION

The clinical signs observed in the Salmonella gallinarum-infected layers in this study, which included depression, ruffled feathers, huddling, loss of body weight, drop in egg production, somnolence and greenish-yellow diarrhoea were consistent with findings in previous reports (Shivaprasad, 2000; Freitas Neto et al., 2007; Ezema et al., 2009; Garcia et al., 2010), except for the incubation period, which was 3 days as reported by Garcia et al. (2010) as opposed to 7 days in this study. The difference in the incubation period could be due to; infective dose of the bacteria, the pathogenicity of the organism, virulence and the host's capacity of building an adequate immune response to fight the pathogenic agent (Lahiri et al., 2010). The 50% mortality in the layers recorded in this study, which started 9 days pi was in the range (10-100%) reported, previously (Shivaprasad, 1996;

Uzzau et al., 2000; Oliveira et al., 2005; Paiva et al., 2009), in chickens. The significant increase ($P<0.05$) suggested hepatic dysfunction as AST, ALT and ALP are good indicators of hepatocellular damage (Hegab et al 2004., Ahmed et al., 2014). Elevated urea concentrations was also observed on day 4 post infection, and which was significantly higher ($P<0.05$) on day 7, 14, 21 and 28 post infection. And these increase may be attributed to kidney dysfunction caused by Salmonella infection (Ahmed et al., 2014). A slight increase in mean plasma total protein was initially observed on days 4 and 7 post infection, even though was not significant ($P>0.05$) and this slight increase may be due to dehydration or volume contraction secondary to fluid loss and this is similar to the one reported by Hegab (2004) who reported hyperalbumenaemia in Salmonella gallinarum infected broiler chickens. Thereafter, a significant decrease ($P<0.05$) in mean plasma total protein level was observed starting from day 14 up to day 35 post infection and this decrease may be caused by protein loss associated with renal dysfunction, starvation and hepatic dysfunction leading to hypoaproteinaemia (Meyer et al., 1995; Kokosharov et al., 2006). In conclusion, experimental Salmonella Gallinarum infection in layers induced alteration in liver and kidney functions. The observed hypoproteinamia is considered as one of the diagnostic tools for acute fowl typhoid disease.

Acknowledgments

We thank Samson James Enam, Mohammed Yusuf from the Department of Veterinary Pathology and Habib Paul Mamman and Hajiya Salamatu, from the Department of Veterinary Microbiology Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for their immense laboratory support.

References

Ahmed MA, Youssef FM, AbdelRahman AG, (2014). Studies on the effect of thyme oil on Salmonella bacteria in broiler chicks. Assiut Veterinary Medical Journal, 60(140): 82-95.

Arbelot B, Dayon, JF, Mamis D, Gneye JC, Tall F, Samb H. (1997). Sero-survey of Dominant avian disease in Senagal; Mycoplasmosis, Fowl Typhoid and Pullorum disease, Newcastle, Infectious Bursal and Infections Bronchitis disease. Revue d' Elevage et de Medicine veterinarier des Pays tropicaus, 50, 197-203.

Barrow PA, Lowell MA, Murphy CK, Page K (1999). Salmonella infection in a commercial line of ducks;Experimental studies in virulence, intestinal colonization and immune protection. Epidemiology of Infection,123:12-132

Beyaz L, Atasever A, Aydin F, Gumusoy KS, Abay S (2010). Pathological and clinical findings and tissue distribution of Salmonella gallinarum infection in turkey poults. Turkish Journal of Veterinary and Animal Sciences,34: 101-110

Bouzoubaa KKV, Nagarya JA, Newman, Pomeraj BS (1987). Use of membrane proteins from Salmonella gallinarum for prevention of fowl typhoid infection in chickens. Avian Dis. 31: 699-704

Cheesbrough, M. (2000). District Laboratory practice in Tropical Countries part 2 (pp. 132 142)

Chishti, M.A., Khan, M.Z. and Siddique, M. (1985). Incidence of salmonellosis in chicken in and around Faisalabad (Pakistan). Pakistan Veterinary Journal, 5: 79- 82

Ezema, W.S., Onuoha, E. and Chah, K.F. (2009). Observations on an outbreak of fowl typhoid in commercial laying birds in Udi, South Eastern Nigeria. Comparative Clinical Pathology, 18(4): 395-398

Feldmann, B.V., J.G. Zinki and N.C. Jain, (2000). Shalm's Veterinary Hematology. 5th ed. Lea and Fibiger, Philadelphia, USA.

Freitas-Neto OC, Arroyave W, Alessi AC, Fagliari JJ, Berchieri Jr. A (2007). Infection of commercial laying hens with Salmonella gallinarum: Clinical, anatomopathological and haematological studies. Brazilian Journal of Poultry Science,9(2): 133-141

Garcia KO, Santana AM, Freitas Neto OC, Berchieri Jr. A, Fagliari JJ (2010). Experimental infection of commercial layers using a Salmonella enteric serovar Gallinarum strain: blood serum component and histopathological changes. Brazilian Journal of Veterinary Pathology, 3(2): 111-117

Hegab, AHH., (2004). Clinicopathological studies on salmonellosis in chicken. MVSc Thesis, Fac. Vet. Med., Suez Canal Univ., Egypt.

Hossain MS, Chowdhury EH, Islam MM, Haider MG, Hossain MM (2006). Avian Salmonella infection: isolation and identification of organisms and histopathological study. Bangladesh Journal of Veterinary Medicine,4: 7-12

Jordan FTW, Pattison M. (1992). Poultry Disease 4th Edition. W.B. Sauder Company Ltd London pp 169-171.

Khan, M.A., Hussain, I., Siddique, M., Rahman, S.U. & Arshad, M. (2007). Adaptation of a local wild Infectious bursal disease virus on chicken embryo fibroblast cell culture. International Journal of Agriculture and Biology, 9(6): 925-927.

Kokosharov, T., Hristov, H, Belchev, L (1997). Clinical, bacteriological and pathological studies on experimental fowl typhoid. Indian Veterinary Journal, 74: 547-549

Kokosharov, T., (2006). Changes in the protein profile in birds with experimental acute fowl typhoid. Bulgarian Journal of Veterinary Medicine, 9(3): 189-192.

Lahiri A, Iyer N, Das P, Chakravortty D. (2010). Visiting the cell biology of Salmonella infection. Microbes and infection, 12(11): 809-818.

Mai HM, Ogunshola OD, Obasi OL (2004). Serological survey of Newcastle disease and infectious bursal disease in local ducks and local guinea fowl in Jos, Plateau state, Nigeria. Revue Eleve Medical Veterinari pavstrop Tropica Pathologie Infectieuse Communication 57 (1-2):41-44.

Meyer, DJ., coles, EH., rich, LJ (1995). Medicina de Laboratório Veterinária. São Paulo: Interpretação e Diagnóstico. Roca, 307.

Okoj, L. (1993). Diseases as important factors affecting increased poultry production in Uganda. Der Tropenlandwin, Zeitschrift in dentropen and Subtropen Jahrgag, 94, S37-S44.

Okwori, AEJ, Ogbe, R. J., Chollom, S. C., Agada, GOA, Ujah A, Okwori E, Adeyanju ON, Echeonwu GON (2013). Isolation of Salmonella Gallinarum From Poultry Droppings in Jos Metropolis, Plateau State, Nigeria. IOSR Journal of Agriculture and Veterinary Science (IOSR-JASR). Vol 5 (2): 14-44.

Oliveira, G.H., Jr. A. Berchieri and A.C. Fernandes,(2005). Experimental infection of laying hens with Salmonella enterica serovar gallinarum. Brazilian Journal of Microbiology, 36(1): 51-56.

Paiva, J.B. de, R.A.C. Penha Filho, Y.M.S. Argüello, M.D. da Silva, Y. Gardin, F. Resende, Jr. A. Berchieri and L. Sesti, (2009). Efficacy of several Salmonella vaccination programs against experimental challenge with Salmonella gallinarum in commercial brown layer and broiler breeder hens. Brazilian Journal of Poultry Science, 11(1): 65-72.

Parmer, D. and Davies, R. (2007). Fowl typhoid in small backyard laying flock. The Veterinary Record, 160:348

Prasanna, K. and Paliwal, O. P. (2002). Experimental Fowl Typhoid and Pullorum Disease in Chickens: Clinical and Patho-morphological Studies. Indian Journal of Veterinary Pathology, 26:528-531

Roa G (2000). A Comprehensive Textbook on Poultry Pathology. Medical publisher ltd pp,7-10.

Sa'idu, L., Abdu., P.A. Umoh, J.U and Abdulahi, U.S (1994). Disease of Nigerian indigenous chickens. Bulletin of Animals Health Production in Africa, 42, 19- 23.

Shivaprasad, H.L. (1996). Pullorum Disease and Fowl Typhoid. Calneck BIN (ed). Disease of poultry Tenth Edition. Pp82-96. Iowa State University Press.

Shivaprasad HL (1997). Pullorum disease and fowl typhoid. In B.W. Calnek., H.J. Barnes., C.W. Beard, L.R. McDougald & Y.M. Saif (Eds.), Disease of Poultry 10th edition (pp. 82–96). Ames, IA: Iowa State University Press.

Shivaprasad, H. L. (2000). Fowl typhoid and Pullorum disease. Review Science, 19 (2):405-424.

Steigh JD, Duguid JP. (1989). Salmonella**.** In: 13th ed. Collee JG, Duguid JP, Fraser AG, Marmion BP editor. Practical Medical Microbiology. Volume 2:New York: Churchill Livingstone p. 456–479.

Uzzau, S., Brown, D.J., Wallis, T., Rubino S., Leori, G., Bernard, S., Casadesus, J., Platt, D.J. and Olsen, J.E. (2000). Host adapted Serotypes of Salmonella enteric. Epidemology of infection, 125: 229-255.

Wigley, P., Berchieri Jr. A., Page, K.L., Smith, A.L. and Barrow, P.A. (2001). Salmonella enterica serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. Infection and Immunity, 69 (12): 7873-7879

Wray, C., Davies, R.H and Corkish, J.D (1996). Enterobacteriaceae. In F.T.W. Jordan and M. Pattison (Eds). Poultry Diseases 4th edition (pp 9-43) London; Saunders company Ltd.