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Susceptibility and Pathological Changes in Chickens Experimentally Infected with *Salmonella Enterica* Serovar Zega

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Abstract:

Salmonella enterica serovar Zega was first isolated in dead duckling in 1952 and in dead chickns in 2016. Since then there has not been any documented effort to study the pathogenicity of this bacterium in chickens. Three groups of six weeks old cockerels were inoculated with the bacterium intraperitoneally (IP), intranasally (IN) and orally (OR). The fourth group was the uninoculated control (CT).Clinical signs were mainly somnolence, anorexia and droopy wings. There was no mortality. The total morbidity was 32, 40 and 24 % in the IN, IP and OR groups respectively. The gross lesions were mainly enlargement of the liver, spleen and kidney. There was atrophy of the bursa and spleen in the IP chickens. There were degeneration, necrosis and haemorrhages in the liver generally. The IP chickens showed depletion of the lymphocytes in the bursa and spleen. The bacterium was re-isolated from the liver, intestines, spleen and heart. The above observations showed that the organism could be moderately pathogenic in young cockerels.

Keywords:

Salmonella Enteric Serovar Zega, Chickens, Pathological Changes, Clinical Signs

1. Introduction

In developing countries especially in Africa, modern poultry farming is a major source of livelihood and a source of animal protein supply in the urban settings [1]. Poultry occupies a prominent position in the provision of animal protein and this

accounts for about 25% of local meat production in Nigeria [2]. [3] identified disease prevalence as one of the major factors that caused low performance in poultry production. *Salmonella* infection is a serious medical and veterinary problem worldwide and causes great concern in the food industry [4]. The infection in poultry is important both as a cause of clinical disease in poultry and as a source of food-borne contamination in humans [5]. Host-adapted salmonellae (*S. pullorum* and *S. gallinarum*) are responsible for severe systemic diseases that have become relatively rare in countries with testing and eradication programmes [6] and [7]. These infections are however common in developing countries [8,9,10] and have been reported in Nigeria [11], [12], and [13], Tanzania [14], Uganda [15], Zambia [16], Libya [17] and Senegal [18] among others.

The genus *Salmonella* is a typical member of the Family *Enterobacteriaceae* and consists of gram-negative, non-spore forming bacilli. The bacteria in this genus contain three different types of antigens: the agglutinating properties of the somatic "O", flagella "H" and capsular "Vi" antigens, which are used to differentiate among more than 2500 serologically distinct types of *Salmonella* [19]. The genus consists of only two species: *Salmonella bongori* and *Salmonella enterica*, with the latter being divided into six sub species: I-*entericae*, II-*salamae*, III-*arizonae*, IV-*diarizonae*, V-*houtennae* and VI-*indica*. Within *Salmonella enterica* subspecies I (*Salmonella enterica* subspecies *entericae*), the most common 'O' antigen serogroups are A, B, C1, C2, D and E. Strains within these sero-groups cause approximately 99% of *Salmonella* infections in human and warm blooded animals [20].

The pathogenicity of *Salmonella* depends on the invasive properties and the ability of the bacteria to survive and multiply within the cells, particularly macrophages [21]. The main site of multiplication of the bacterium is the digestive tract which may result in widespread contamination of the environment due to bacteria excretion through faeces [22].

Salmonella enterica serovar Zega was first isolated from dead ducklings in the Belgian Congo (now Democratic Republic of Congo) in 1952 [23]. It was recently isolated from cases of mortality among commercial layer chickens in south western Nigeria [24]. The bacterium therefore appears to be a significant pathogen for chickens. The aim of this project therefore was to study the susceptibility, clinical signs and pathological changes associated with *Salmonella enteric* serovar Zega infection in young chickens.

2. Materials and Methods

2.1. Experimental Chickens

A total of 100, five-week old Yarkon White cockerels obtained from the Poultry Unit of National Veterinary Research Institute, Vom, were used for the experiment. These birds were housed in deep litter system in a concrete pen and commercial starter diet was provided for the chicks throughout the experiment. The chicks were not vaccinated against any disease and were kept in isolation at the departmental experimental poultry facility. Feed and water was provided ad-libitum. Brooding heat was generated by kerosene stove and electric bulbs during the first four weeks of age.

2.2. Bacteriological Monitoring Before Challenge

Before challenge, cloacal swabs were collected from the chickens on days 2 and 5 post arrival. This was done by pre-enrichment of the swab samples in buffered peptone water, followed by plating on MacConkey agar (MCA) using standard laboratory methods [25] and [26]. The chickens were found to be free from Salmonella infection.

2.3. Bacterial Isolate used in the Study

Stock culture of Salmonella enterica serovar Zega originally isolated from commercial layers in south western Nigeria, identified at the Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, South Dakota, USA [24] and [27] and maintained at the Bacterial Research Department, National Veterinary Research Institute, Vom, Nigeria was used in the study.

2.4. Culture and Determination of the Titer of the Bacterial Inoculum

The lyophilized bacterium from the culture bank was reactivated by culturing in peptone water, incubated overnight at 370C and sub-cultured on MacConkey agar (MCA). The resulting colonies were examined for their colony characteristics (colour and morphology) and tested for gram-reaction (Gram-negative). The bacterium was passaged twice in chickens before the final culturing. Five colonies were scooped and inoculated into 10 ml of phosphate buffered saline (PBS) and this was incubated for 24 hours at 37 °C after which a ten-fold dilution was carried out in test tubes. The colony counts from the test tubes were determined. To obtain the number of organisms that was inoculated into the birds, the number of organisms was multiplied by volume by the dilution factor (CFU = No. of colony × Volume × Reciprocal of Dilution factor) [28]. Salmonella zega inoculum (in PBS) containing 1 x 108 cfu/ml was used for the experimental challenge

2.5. Experimental Challenge

The 100 chickens were randomly assigned to 4 groups of 25 birds per group at six weeks of age. Each chicken in the three groups received 0.5 ml of PBS containing 1 X 108cfu/ml of Salmonella enterica serovar Zega as follows:

Group A chickens were each inoculated with Salmonella zega suspension intra-nasally. This was achieved by dropping 0.25ml of the inoculum into each nostril (IN).

Group B chickens were each inoculated with 0.5 ml of the Salmonella zega suspension intra-peritonealy (IP).

Group C chickens were each inoculated with 0.5 ml of the Salmonella zega suspension orally (OR).

Group D was the uninoculated control (CT).

2.6. Examinations for Clinical Signs and Lesions

The birds were observed twice daily for clinical signs from days 0 to 21 post challenge (PC). On days 3, 5, 7, 10 and 13 PC, three birds from each group were euthanised and necropsied. Gross lesions were noted. Samples of the liver, spleen, heart, intestine and bursa of Fabricius were fixed in 10 % formal saline, dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax.

Sections were cut, stained with haematoxylin and eosin (H&E) and studied under the light microscope.

2.7. Bacterial re- isolation

Samples of the liver, spleen, heart and intestine from each group were aseptically collected. They were subsequently inoculated on MacConkey agar for Salmonella isolation and identification using standard laboratory methods [25] and [26].

3. Results and Discussion

3.1. Results

3.1.1. Clinical signs

The onset of clinical signs in the infected birds was rapid and varied depending on the route of administration. On day 1 PC (18hrs), two chickens (8%) from the IP group showed somnolence, loss of appetite, hurdling together and ruffled feathers. By day 2 PC, a total of four chickens (16%) in this group had these signs which had progressed to include droopy wings. Also on day 2 PC, one chicken (4%) in the IN group showed signs of somnolence and ruffled feathers. These same signs were observed in four chickens in the OR group on day 3 PC. Unilateral ocular discharge was further observed in two of these birds. On day 4 PC, the conjunctiva was congested in one chicken in the OR group, while three birds showed the same sign in the IP group. On day 5 PC, three chickens in the IP group showed conjunctivitis. Misshapen/dry and pale comb was observed in four birds on day 7 PC in the IP group. From day 11 PC, no signs were seen in all the groups. Generally, 32%, 40% and 24% of the infected chickens showed clinical signs in the IN, IP, and OR routes respectively without any mortality. No clinical sign was observed in the CT group.

3.1.2. Lesions

The gross lesion distribution and persistence in all the infected groups is recorded in Table 1. Congestion was the primary observation on day 3 PC. The liver, kidneys and spleen were enlarged and congested at day 5 PC. Peritonitis was observed in all infected groups on days 5, 7 and 10 PC. Atrophy of the bursa of Fabricius and spleen was observed in the IP birds on days 5 to 13 PC (Figure 1, Figure 2).

Sections of the liver in birds in the IP, IN and OR groups showed congestion, degeneration, necrosis and mononuclear cellular infiltration on days 3 to 5 PC. In the spleen, mild depletion of lymphocytes was observed at days 5, 7, and 10 PC in all infected groups. Kidney showed degeneration and necrosis of the tubular epithelium in all the infected groups. Sections of the heart in all the infected groups from day 3 PC onwards showed necrosis of the myocardium with lymphocytic infiltration (Figure 3). In the bursa of Fabricius, there were necrosis and depletion of lymphocytes in the IP and OR groups (Figure 4)). Erosion of the epithelial lining of the villi and mononuclear cellular infiltration were observed in the intestines of all the infected groups from day 3 PC. The CT chickens had no lesion.

3.1.3. Re-isolation of Salmonella Enterica Serovar Zega

Salmonella enterica serovar Zega was re-isolated from the organs (liver, spleen, heart and intestines) on days 3, 5, 7 and 10 PC (Table 2).

Table 1. *Gross lesions distribution and persistence in infected chickens.*

Post Mortem, Days	Intra-peritoneum (IP)	Intra-Nasal (IN)	Oral (OR)
DAY 3 Post challenge	Congestion of Liver 2/3* Intestinal congestion 2/3 Enlargement of kidney 1/3	Congestion of Liver 1/3 Intestinal congestion 2/3 Congestion of spleen 1/3	Intestinal congestion 2/3
Day 5 PC	-Congested Breast Muscle 1/3 -Peritonitis 3/3 -Cloudy mesentery 2/3 -Enlarged Liver 2/3 -Haemorrhagic Liver 2/3 -Congested Liver 1/3 -Enlargement of the spleen 2/3 Intestinal congestion 2/3 -Atrophy of bursa of fabricious 3/3	-Peritonitis 1/3 -Enlargement of spleen 1/3 -Intestinal congestion 1/3	-darkening of the peritoneum 2/3 Peritonitis 3/3
Day 7 PC	-Mild peritonitis 2/3 -Haemorrhagic Liver 1/3 -Atrophy of the Bursa of Fabricious 3/3	-Mild peritonitis1/3 -Necrotic spleen1/3 -Congestion of Liver1/3	-Mild peritonitis 1/3
Day 10 PC Day 13 PC	-no lesions seen -no lesions seen	-mild peritonitis 2/3 -liver congestion 1/3 -no lesions seen	-mild peritonitis 1/3 -no lesions seen

* = $\frac{\textit{no. with lesions}}{\textit{no. examined}}$

Table 2. *Salmonella enterica serovar Zega re-isolation in organs on days 3, 5, 7 and 10 PC.*

Days PC	Organs	CT group	IN group	IP group	OR group
Day 3	Liver	Negative	Positive	Positive	Positive
Day 3	Heart	Negative	Negative	Positive	Negative
Day 3	Spleen	Negative	Positive	Positive	Positive
Day 3	Intestine	Negative	Negative	Positive	Positive
Day 5	Liver	Negative	Positive	Positive	Positive
Day 5	Heart	Negative	Negative	Positive	Negative
Day 5	Spleen	Negative	Positive	Positive	Positive
Day 5	Intestine	Negative	Negative	Positive	Positive
Day 7	Liver	Negative	Positive	Positive	Positive
Day 7	Heart	Negative	Negative	Positive	Positive
Day 7	Spleen	Negative	Positive	Positive	Positive
Day 7	Intestine	Negative	Positive	Positive	Positive
Day 10	Liver	Negative	Positive	Positive	Positive
Day 10	Heart	Negative	Positive	Positive	Positive
Day 10	Spleen	Negative	Positive	Positive	Positive
Day 10	Intestine	Negative	Positive	Positive	Positive
Organs positive post- infection (%)		0%	68.75%	100%	87.5%

CT-Control IN-Intranasal IP-Intraperitoneal OR-Oral

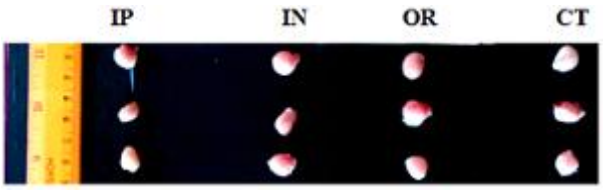

Figure 1. *Moderate atrophy of the bursa in the IP chickens on day 10 PC.*

IP-Intraperitoneal route of inoculation

IN-Intranasal route

OP- Oral route

CT-Control

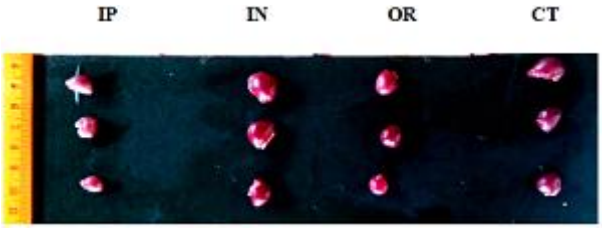

Figure 2. *Moderate atrophy of the spleen in the IP chickens on day 13 PC.*

IP- Intraperitoneal route of inoculation

IN- Intranasal route

OR- Oral route

CT- Control

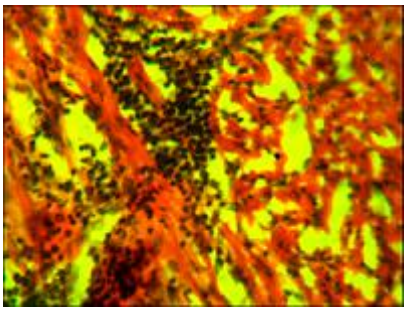

Figure 3. *Necrosis of the myocardium with infiltration of lymphocytes in IP chickens on day.*

5 PC. H & E x 400

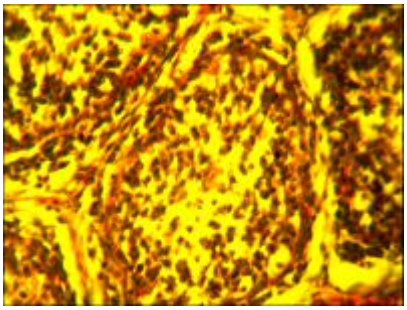

Figure 4. *Necrosis and depletion of lymphocytes in the bursa of IP chickens on day 3 PC.*

H & E x 400

3.2. Discussion

The above observations show that Salmonella enterica serovar Zega can be moderately pathogenic in young cockerels. To the best of our knowledge this is the first attempt to study the pathogenicity of this organism in chickens. More work will be required to determine the pathogenicity of the organism in other types of chickens, the effect of age on the susceptibility and any possible public health dangers posed by the organism. The clinical signs observed in this study have been described for Salmonellosis by [8], [29] and [30]. The enlargement of the liver, spleen and kidney

have also been observed by [13], [31] and [32] in Salmonella gallinarum and Salmonella pullorum infections. But the atrophy of the bursa and spleen and the depletion of the lymphocytes in the two organs observed in this study do not appear to have been described for Salmonellosis. This is a very important observation because of possible suppression of the immune response of the infected chickens. The histopathological changes observed in liver have been reported in Salmonellosis by [33], [34], [35] and [32]. The highest rate of bacterial re-isolation was from the intestine and liver in this study. These results are in agreement with those of [36] and [37]. [38] reported that caeca, caecal tonsil and caecal contents were the sites more likely to offer maximum recovery of the Salmonella organisms. The table on gross lesions distribution and persistence showed that the IP route produced more severe lesions than the other routes even though it is not a natural route of infection.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical Approval

Ethical approval for this project was given by the University Committee on Medical and Scientific Research Ethics, University of Nigeria, Nsukka.

Author Contributions

Conceptualization: E.P.E.; K.J.; C.K.; Methodology: E.P.E.; K.J.; C.K.; Investigation: E.P.E.; K.J.; C.K.; Resources: O.I.; E.D.C.; A.J.S.; C.T.P.; M.B.; F.I.O.; O.J.N.; Data Curation: A.J.S.; C.T.P.; M.B.; F.I.O.; Writing – original draft preparation: E.P.E.; K.J.; C.K.; Writing – review and editing: E.P.E.; O.J.A.; C.K.; O.O.D.; Supervision: K.J.; C.K.; Project administration: D.E.; S.N.M.; A.Y.; J.E.O.

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References

[1] Cardinale, E.; Tall, F.; Gueye, E.F.; Cisse, M.; Salvat, G. Risk factors for Salmonella enterica subspecie enterica infection in Senegalese broiler-chicken flocks. *Preventive Veterinary Medicine,* 2004, 63, 151-161.

[2] Agbaje, M.; Davies, R.; Oyekunle, M.A.; Ojo, O.E.; Fasina, F.O.; Akinduti, P.A. Observation on the occurrence and transmission pattern of S.G in commercial poultry farm in Ogun state, southwestern Nigeria. *Africa Journal of Microbiology Research,* 2010, 4, 776-800.

[3] Nebiyu, Y.; Brhan, T.; Kelay, B. Characterization of Village Chicken Production Perfomance under Scavenging Systems in Halaba District of Southern Ethiopia. *Ethiopia Veterinary Journal,* 2013, 17, 69-80.

[4] Begum, F.; Adachi, Y.; Kham, H.S.R. Immunodominant surface protein of some Salmonella serovars. *Bangl. Journal of Veterinary Medicine,* 2008, 6, 145-151.

[5] Waltman, W.D.; Gast, R.K.; Mallinson, E.T. Salmonellosis. In A Laboratory Manual for The isolation and Identification of Avian Pathogens. Swayne, D.E.; Glisson, J.R.; Jackwood, M.W.; Pearson, J.E. Reed, W.M. (Eds) American Association of Avian Pathologists 4th edition. University of Pennsylvania, USA, 1998; pp. 4-13.

[6] Pomeroy, B.S.; Nagaraja, K.V. Fowl typhoid. In: Diseases of Poultry, 9th ed. Calnek, B.W., Barnes, H.J., Beard, C.W., Reid, W.M and Yoder, H.W Jr., eds. Iowa State University Press, Ames, Iowa 1991; pp. 73-86.

[7] Snoeyenbos, G.H. Pullorum disease. In: Diseases of Poultry, 9th ed. Calnek, B.W.; Barnes, H.J.; Beard, C.W.; Reid, W.M.; Yoder, H.W Jr., eds. Iowa State University Press, Ames, Iowa, 1991; pp. 73-86.

[8] Shivaprasad, H.L. Fowl typhoid and pullorum disease. *Revue Scientifique et Technique,* 2000, 19, 405-424.

[9] Orji, M.U.; Onuigbo, H.C.; Mbata, T.I. Isolation of Salmonella from poultry droppings and other environmental sources in Awka, Nigeria. *International Journal of Infectious Diseases,* 2005, 9, 86-89.

[10] Prakash, B.; Krishnappa, G.; Muniyappa, L.; Kumar, B.S. Epidemiological characterization of avian Salmonella enterica serovar infections in India. *International Journal of Poultry Science,* 2005, 4, 388-395.

[11] Oboegbulem, S.I.; Orajaka, L.J.E.; Okoye, J.O.A.; Erojikwe, E.E. Fowl typhoid outbreak in university laying flocks, A case report. *Nigerian Veterinary Journal,* 1980, 9, 24-26.

[12] Saidu, L.; Abdu, R.A.; Umoh J.U.; Abdullahi, U.S. Diseases of indigenous chickens. *Bulletin of Animal Health Production in Africa,* 1994, 42, 19-23.

[13] Ezema, W.S.; Onuoha, E.; Chah, K.F. Observations on an outbreak of fowl typhoid in commercial laying birds in Udi, South-Eastern Nigeria. *Comparative Clinical Pathology,* 2008, 18, 395-398.

[14] Mtie, B.J.; Msami, H.M. Reflection on current animal health status in Tanzania. *Tanzania Veterinary Journal,* 1991, 16, 37-49.

[15] Ojok, L. Diseases as important factor affecting increased poultry production in Uganda. *Tropical Landwirt,* 1993, 94, 37-44.

[16] Sharma, R.N.; Pandey, G.S.; Hhan, A.Q. Salmonella isolation from poultry in the Republic of Zambia. *Bulletin of Animal Health and Production in Africa,* 1991, 39, 173-175.

[17] Hamid, M.A.; Sharma, R.N. Epidemiology of fowl typhoid in and around Tripoil (Libya). *Bulletin of Animal Health and Production in Africa,* 1990, 38, 315-317.

[18] Arbelot, B.; Dayon, J.F.; Mamis, D.; Gueye, J.C.; Tall, F.; Samb, H. Sero survey of dominant avian diseases in Senegal: Mycoplasimosis, fowl typhoid and polloruim disease, Newcastle disease, infectious bursal disease and infectious bronchitis disease. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux,* 1997, 50, 197-203.

[19] Popoff, M.Y.; Bockemuhl, J.; Gheesling, L.L.. Antigenic Formulas of the Salmonella Serovars, 8th revision. WHO Collaborating Centre for Reference and

Research on Salmonella. Supplement 2001 (No: 45) to the Kauffmann-White Scheme. *Research in Microbiology,* 2003, 154, 173-174.

[20] Uzzau, S.; Brown, D.J.; Wallis, T.; Rubino, S.; Leor,i G.; Bernard, S.; Casadeus, J.; Platt, D.J.; Olsen, J.E. Host adapted serotypes of Salmonella enterica. *Epidemiology and Infection,* 2000, 125, 229-255.

[21] Humbert, F.; Salvat, G. The risk of transmission of Salmonella in poultry farming. Detection and prevention in Europe. *Review Science and Technology,* 1997, 16, 83-90.

[22] Berchieri, Jr A.; Murphy, C.K.; Marston, K.; Barrow, P.A.. Observations on the persistence of vertical transmission of 7 Salmonella enterica serovars Pullorum and Gallinarum in chickens: effect of bacteria and high genetic background. *Avian Pathology,* 2001, 30, 221-231

[23] Fain, A.; Kauffmann, F.; Schoetter, M. A new Salmonella type (S. zega) from the Belgian Congo. *Acta Pathology and Microbiology, Scand*, 1952, 31, 325.

[24] Useh, N.M.; Ngbede, E.O.; Akange, N.; Thomas, M.; Foley, A.; Keena, M.C.; Nelson, E.; Christopher-Hennings, J.; Tomita, M.; Suzuki, H.; Scaria, J. Draft genome sequences of 37 Salmonella enteric strains isolated from poultry sources in Nigeria. *Genome Announcement,* 2016, 4, 00315-16.

[25] Wigley, P.; Berchieri, Jr A.; Page, K.L.; Smith, S.L.; Barrow, P.A. Salmonella enterica serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. *Infectious Immunology,* 2001, 69, 7873-7879.

[26] Parma, D.; Davies, R. FT in a small backyard laying flock. *Veterinary Research*, 2007, 160-348.

[27] Mshelbwala, F.M.; Ibrahim, N.D.G.; Saidu, S.N.; Azeez, A.A.; Akinduti, A.A.; Kwanashie, C.N.; Fakilahyel, Kadiri, A.K.; Muhammed, M.; Fagbamila, I.O.; Luka, P.D. Motile Salmonella serotypes causing high mortality in poultry farms in three South-Western States of Nigeria. *Veterinary Record Open,* 2017, 4, 000247.

[28] Miles, A .A.; Misra, S S. *Journal of Hygiene (London),* 1938, 38, 732

[29] Freitas, Neto, O.C.; Arroyave, W.; Alessi, A.C.; Fagliari, J.J.; Berchieri, A. Infection of commercial laying hens with Salmonella gallinarum: Clinical, anatomopathological and haematological studies. *Brazilian Journal of Poultry Science,* 2007, 9,133-141.

[30] Barde, I.J.; Bale, J.O.O.; Oladele, S.B.; Fatihu, M.Y.; Kumbish, P.R.; Chukwu, D.I.; Rimfa, A.G.; Akanbi, B.O.; Moses, G.D.; Ahmed, J.S.; Okewole, P.A. Gross and histo-pathological changes in Japanese Quail (Coturnix Coturnix Japonica) experimentally infected with Salmonella enterica serovar Gallinarum. *Animal and Veterinary Sciences,* 2015, 3, 84-88.

[31]. Barde, I.J.; Bale, J.O.O.; Oladele, S.B.; Fatihu, M.Y.; Kumbish, P.R.; Rimfa, A.G.; Ahmed, J.S.; Okewole, P.A. Serum biochemical changes in Japanese quail (Coturnix Coturnix Japonica) experimentally infected with Salmonella enterica Serovar Gallinarum. *African Journal of Cellular Pathology,* 2014, 4, 42-47.

[32] Dutta, P.; Borah, M.K.; Gangil, R.; Singathia, R. Gross/Histological impact of Salmonella gallinarum isolated from layer chickens in Jaipur and their

antibiogram assay. *International Journal of Advanced Veterinary Science and Technology,* 2015, 4, 153-159.

[33] Wong, R.A.; Tellez, G.I.; Valladares, J.; Hargis, B.M. Pathogenicity of Salmonella gallinarum on an experimental infection of one-day-old broiler chicks. *Poultry Science,* 1997, 75, 44.

[34] Beyaz, L.; Atasever, A.; Aydin, F.; Gumusoy, K.S.; Abay, S. Pathological and clinical findings and tissue distribution of Salmonella gallinarum infection in turkey poults. *Turkish Journal of Veterinary and Animal Sciences,* 2010, 34, 101-110.

[35] Deshmukh, S.; Asrami, R.K.; Ledoux, D.R.; Rottinghaus, G.E.; Bermudez, A.J.; Gupta, V.K. Pathologic changes in extrahepatic organs and agglutinin response to Salmonella gallinarum infection in Japanese Quail fed Fusarium Verticilloides culture material containing known levels of Fusarium B1. *Avian Diseases,* 2007, 51, 705-712.

[36] Gast, R.K.; Beard, C.W. Serological detection of experimental Salmonella enteritidis infections in laying hens. *Avian Diseases,* 1989, 34, 721-728.

[37] Zaida, H.H.M.A. Isolation and Identification of Salmonella species from Chickens in Khartoum State. A thesis submitted to Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, 2003.

[38] Calnek, B.W.; Barnes, H.J.; Beard, C.W.; McDoougald, L.R.; Saif, Y.M. Diseases of Poultry. 10th Ed. Iowa State University Press, America USA, 1997.