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SEROPREVALENCE AND ISOLATION OF CHICKEN INFECTED WITH SALMONELLA HAEMATOLOGICAL AND PATHOGICAL EVALUATION

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ABSTRACT: *The research was conducted to study seroprevalence of bird infected with salmonella and to evaluate the pathology of the affected cockerel chicken in Abia and Umudike both in Aba north and Ikwuano local government area of Abia State, as well as isolate salmonella. This study was carried out from February to June, 2010. Samples used were blood cloaca and liver swabs. Other organs like intestine, ovary, spleen and lungs were also used. The serum plate agglutination method was used. Others are gross, histopathology, morphological, cultural and biochemical tests. The total percentage of seroprevalence was 45.0%. Gross lesion showed congestion, enlargement of the liver and petechial hemorrhages in the intestine. Hematological features showed that there were decreased red blood cells on hemoglobin while the white blood cells increased. And there was no significance difference. (P<0.05). At history, congestion, massive lymphocytes infiltrates in the liver paranchyma. The sum of 54 seropositive salmonella samples from birds were isolated. Further study would also be carried out to investigate the pathogenesis, serotyping and sensitivity test.*

ORIGINAL ARTICLE

Key words: *Isolation and Seroprevalence, chicken, salmonella, heamatology, histology*

INTRODUCTION

Salmonella is one of the major beneficial agents that cause food borne infection in human worldwide (Herickstad et al., 2002). In studies that have examined the prevalence of salmonella on chicken farms, there has also been large interstock variability. The percentage of salmonella positive birds and feaces samples on farms has ranged from 5 – 100% (Carriminana et al., 1997).

Infections with bacteria of the genus salmonella are responsible for a variety of acute and chronic absence of poultry has been reported (Bhattacherjee et al., 1996). With great expansion of poultry rearing and farming, pollurum disease and fowl typhoid have become wide spread problem in (Rahman et al., 1979).

Salmonella is often attributed to the consumption of contaminated food, such as poultry, eggs and fresh produce. Direct contact with infected animals may also serve as a source of salmonella infection as (Tauxe, 1991; Beneson et al., 1995). The shift towards global economy, microbial adaptation, changes in travel and commerce, and lack of knowledge on food safety and handling practices among consumers (Knabel, 1995; Alternese et al., 1997) has contributed to the dynamics of salmonella infection. The study is aimed at evaluating the seroprevalence, heamatological and pathological effect of chicken infected with salmonella and its isolation.

Clinical observation and blood samples were obtained at varying days, after inoculation and red blood cell (RBC), white blood cells (WBC), haemoglobin (HB) and phagocytosis were determined as described Todovora (1987) and Kokosharove (1998).

MATERIAL AND METHODS

This study was conducted in department of veterinary microbiology and parasitology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Samples were obtained from poultry farms located in Aba and Umudike respectively from February to June, 2010.

Sample Collection and Processing

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Samples were collected from cockerel from different farms. The farms had no history of being vaccinated for salmonella. However, they were still screen for salmonella. The samples were collected from birds of varying ages; starter (3 – 6 wks), grower (10 – 16wks), and adult cock (18 – 40wks). Aseptically, 1ml of blood was collected from the jugular vein using 23 gauge needle. The sera were later separated by centrifugation at 1000 rpm for 5 minutes.

Serm Plate Agglutination Test

All isolates were serologically confirmed using commercially available anti-sera Kit (Remel Europe Ltd. UK), specific for all group and type factor antigens. Isolates were sub-cultured on salmonella overnight, serological evaluations were carried out by slide agglutination test. (El-Geridy et al., 1999). The anti sera kit contains salmonella polyvalent O (A-G), salmonella polyvalent O (A-S) and salmonella polyvalent O (A-S) and salmonella polyvalent 09 (A-H). Antigen 0.02ml was pieced on ceramic tiles and 0.02ml of chicken sera was placed on it with the aid of micropipette and mixed properly followed by rocking. Dumping on mixing indicates positive result.

Isolation and Identification of Salmonella

One hundred faecal samples (from the cloaca) were collected using sterile swab sticks. The method of Ewings (1986) was adopted. The culture swab sample was inoculated into selenite -F- and incubated at 37^0C for 24 hours and then plated on salmonella-Shegella agar (SSA) and Triple Sugar iron (TSI) agar to get a pure culture. This was later incubated overnight. The presence of (white or yellow) colony with black centered spot was observed.

Biochemical Test

Besides Gram's stain described by merchant and Packer (1967) other rest carried out were indole test, voges proskuaer (VP) test, methy red test (MR), TSI, agar slant reaction and sugar fermentation test according to (OIE, 2004).

Experimental Chicken

Firstly, the feacal samples of fourteen birds of four weeks old were screened for the presence of salmonella and those found to be negative were grouped into A and B. Group B was infected with salmonella while Group A was the control.

Exactly 0.2ml of 3.3 x 10^8 efu/ml of the salmonella was infected orally to the crop of the chicken with the aid of catheter and monitored for 21 days – (3 weeks). The birds were fed with Animal poultry feed adequately. Blood samples 1ml was collected from the jugular vein using 23 gauge needle at 1, 2, 3, 4, 7, 14 and 21 days after infection (Kokosharov, 1998). The birds were later sacrificed while the control was screened.

Estimation of Haemoglobin (Hb), Red Blood Cell (RBB) White Blood Cell (WBC) of Chicken

HB estimation was determined by adding phosphate buffer saline to the 20mark sahlis apparatus followed by addition of blood sample with pipette. The tube was typed intermittently while distill water was added drop by drop until it assume the consistency recorded. The experiment was repeated thrice. Red blood cell and white blood cell were determined using Neubaur hemocymeter counting chamber (Westhpal et al., 1952). The cover was well fixed to the counting chamber hemocymeter such that there is perfect bridging of the middle line. The suspension was prepared by adding 20ml of chicken of blood with pipette to 4ml of the already prepared red blood cell diluting fluid and 0.4ml white blood cell diluting fluid. A drop of the solution was applied to the tip against the edge of the cover slip. Caution was taken to avoid the fluid from over-flowing into the channels. Focus the objective onto each four corner square and count all the cells contained in them. The WBC and RBC were differently counted and recorded.

Historical examination of infected chickens

The infected birds were sacrificed using surgical blade and forceps and the intestine, liver and lungs tissue were preserved in 10% formalin for histological examination. Each sample was appropriately placed in sample bottle and labeled. The tissues were trimmed, washed, processed in ascending grades of alcohol, cleared in chloroform, embedded in paraffin, sectioned using a microtome and stained with hematoxylin and eosin (Luna, 1968). Photomicrography was taken using photomicrograhic camera (Olympus Pm –C35 model).

Statistical Analysis

All the results were expressed as means of three parallel determinations and the statistical significance was assessed by analysis of variance. The significance was expressed as ($P < 0.05$).

RESULTS AND DISCUSSION

It was found that salmonella increased with the increase of age of birds, table 1. This findings was supported by Sikder et al, (2005) and Young and Teiuquang (2003). A total of 16 isolates (28.5%) was found from 56 seropositive birds and 3 isolates (6.6%) was found from 44 seronegative birds as reflected in Table 2. The isolation rate of seronegative birds was lower than that of seropositve. This finding was also reported Hoque et al, (1996). The birds infected with isolate infection showed that WBC of infected differ significantly from the uninfected

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controls (P<0.05). However, from day 14 to 21 WBC decreased significantly (P<0.05) compared to control. WBC of uninfected was significantly (P<0.05) than that of infected birds on day 1 with mean WBC of 2.18 ±0.06 and 2.22± 0.25 respectively. There was s decrease in the red blood cell from 3.42 ± 0.24 in day 1 to 1.06 ± 0.18 in day 7, as well as heamoglobin from 9.32 ± 0.32 in day 1 to 5.86 ± in day 7. This could be attributed to the infective salmonella which destroys the red blood cell and heamoglobin. The white blood cell increased progressively up to day 7 and the reason is to fight the infective organism, similar report was presented by Kokosharov (1998).

Table 1 - Seroprevalence of chicken infected Salmonella of different ages

Group	No. of Sample Tested	No. of Sample Seropositive	Percentage Seropositive
Starter (3 - wks)	16	2	12.5%
Grower (10 - wks)	34	8	20.6%
Adult (18 - wks)	70	44	62.8%
TOTAL	120	54	45.0%

Table 2 - Relationship between Salmonella Isolate in percentage and Seroprevalence

Agglutination Test	Feacal Swab	Salmonella Isolated
Seropositive	56	16 (28.5%)
Seronegative	44	3 (6.6%)
TOTAL	100	19 (35.1%)

Table 3 - Red and white blood cells of birds orally infected with Salmonella Organism

Parameter	Control	Day after infection						
	0	1	2	3	4	7	14	21
WBC	2.18±0.60	2.22±0.25	2.4±1.0	2.86±1.8	5.42±1.8	5.42±0.10	2.12±0.42	1.86±0.14
RBC	3.64±0.11	3.42±0.24	2.88±.22	2.74±.20	1.66±0.18	1.66±0.18	2.04±1.4	1.66±0.16
HB	9.52±0.42	9.32±0.32	8.98±.54	8.26±.58	6.28±.60	5.86±0.56	5.80±0.54	5.86±0.56
Bandnuclei	2.86	3.16	4.80	4.82	7.12	8.20	9.32	6.82
Lymphocytes	73.10	66.40	58.84	56.40	54.10	56.00	55.00	70.00

Values were calculated from seven individual birds per group and expressed as mean ± SEM. Difference (P<0.05) from control and post infected treated groups. Wbc = White blood cell. Rbc=Red blood cell. Hb=Hemoglobin concentration

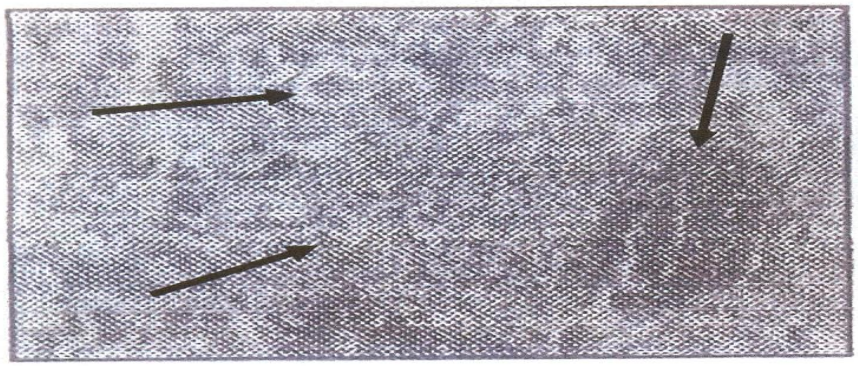

Plate 1. Histopathology of Salmonella sp. infected liver showing congested blood vessels, infiltration of lymphocytes, heterophils around the blood vessel.

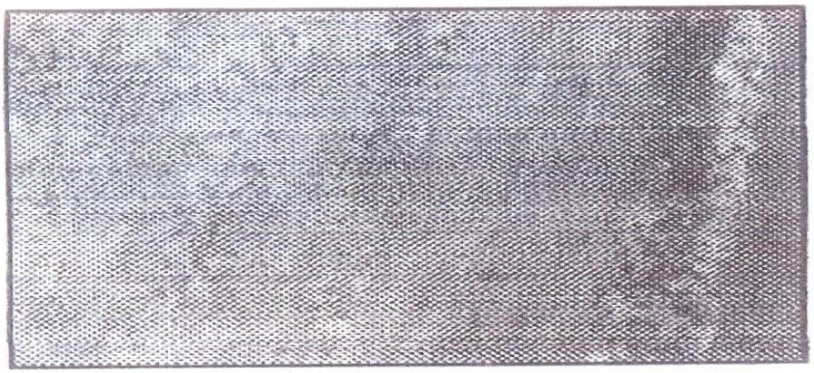

Plate 2: Histopathology of normal liver of a chicken

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Plate 3: The Lesions in the ovary of salmonella infected bird (Pullorum Diesease)

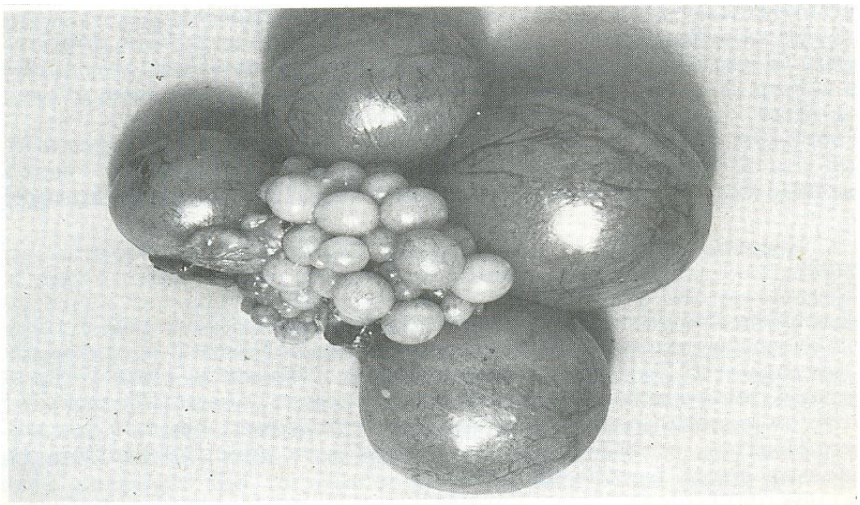

Plate 4. Normal ovary of birds

Similarly, there was a decrease in the lymophocytes from day 1 to 7. The noticeable increase in the white blood cell from day 14 to 21 is suggestive of the inability of the white blood cell to overcome the infective salmonella. The red blood cell, heamoglobin level and hymphocytes of post infection varied. The red blood cell and heamoglobin showed marked progressive decrease as low as 1.66 and 5.86 respectively. The analysis of variance showed no significance difference ($P<0.05$).

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