Pathogenicity of *Salmonella* Paratyphi A in Pullets

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**Keywords**

Chicken – *Salmonella* Paratyphi A – Pathogenicity – Nigeria.

**Summary**

The pathogenicity of *Salmonella* Paratyphi A isolated at Debiwise Poultry Farm during a fulminating outbreak was tested. Twenty 16-week-old pullets were inoculated orally with 0.5 ml of $1.3 \times 10^8$ CFU/bird *Salmonella* Paratyphi A, while 20 others of the same age served as uninfected control. By the fourth day postinoculation (p.i.) dullness, ruffled and unkempt feathers, somnolence, yellowish-green diarrhea, decreased water and feed consumption were observed in the infected birds; mortality was 95% by day 16 p.i. Remarkable pathological lesions were recorded between days 7 and 14 p.i. The liver was moderately enlarged, with multiple necrotic foci, and dark brown to brown coloration. The kidneys were swollen, with widespread focal pale necrotic areas, while the spleen was slightly enlarged. Histopathologically, the proventriculus showed focal glandular necrosis, mononuclear cell infiltration and moderate perivascular leucocytic infiltration. There was mucosal hemorrhage, matting of intestinal villi with reduction of villous height and presence of epithelial debris in the lumen with increased leucocytic infiltration in the lamina propriae. The liver showed moderate, diffuse congestion of the sinusoid and central veins, as well as multiple foci of necrosis of hepatocytes, mononuclear infiltration and perivascular cellular infiltration. There was diffuse lymphoid depletion in the nodule and around the splenic arterioles as well as throughout the parenchyma. The kidneys were congested with tubular epithelial necrosis characterized by karyorrhexis of the nuclei. The organism was recovered from the liver, spleen, heart, heart blood and bone marrow of infected birds. No clinical sign or gross lesion or pathogen was observed in the negative control.

**INTRODUCTION**

Domestic poultry constitute the largest single reservoir of *Salmonella* organisms found in nature (12, 13). Salmonellosis is of major economic importance in terms of losses in farm animals and the disease is endemic in commercial poultry flocks in Nigeria (17, 24). Both morbidity and mortality in salmonellosis are highly variable in chickens and are influenced by age, strain susceptibility, nutrition, flock management, and characteristics of exposure (22). In Nigeria, salmonellosis due to *Salmonella* Gallinarum, Pullorum and Typhimurium have been reported (2, 18, 19). *Salmonella enterica* serotypes Typhi and Paratyphi A, B, C are responsible for typhoid fever and paratyphoid enteric fever, respectively (15, 23). A large outbreak of *Salmonella enterica* serotype Paratyphi B infection caused by goats’ milk cheese was reported in France (7).

**MATERIALS AND METHODS**

**Experimental chickens**

The S and D Aderupoko Farm®, Abeokuta hatchery division, donated 150 day-old chicks (Yaffa® pullets) out of which 40 pullets were randomly selected for this investigation. The chicks were raised under strict hygienic conditions for 16 weeks at the Teaching and Research Farm University of Ibadan in Nigeria. They were fed *ad libitum* with chicks’ mash, without antibiotics, containing 17% crude protein, 2531 metabolizable energy kcal/kg from a day old to the eighth week of age. The growers’ mash, containing 18% crude protein, 2448 metabolizable energy kcal/kg, was fed from the ninth week until the end of the investigation. Clean water was provided in troughs *ad libitum*. The chicks were routinely


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Salmonella organism

In July 2002 there was a suspected salmonellosis outbreak with high morbidity and mortality in a commercial (Debiwise Poultry) farm near the border of Ibadan Oyo State. Postmortem specimens were taken from the liver, spleen, heart and heart blood of dead chickens for bacteriological investigation (4). The isolates on MacConkey agar were non-lactose fermenting, yellowish glistening colonies and were found to be Gram negative, motile, short rod, oxidase negative, urease negative, citrate negative, indole negative, salcinic negative, sucrose negative, glucose positive, sorbitol positive, dulcitol positive, and H₂S negative (4, 9). A pure colony of Salmonella isolate was emulsified with normal saline and tested by the slide agglutination method and was positive for both polyvalent agglutinating antisera O and H (Laboratory Diagnostic Products, UK), and was identified as a Salmonella species.

The serological identification was done by inactivating the Salmonella isolate as described by Bernard et al. (5). The isolate was inoculated into sterile trypticase soy broth (TSB), and incubated at 37°C overnight. One milliliter of 0.1% formalin was added to 1.0 ml broth culture of Salmonella isolate. The mixture was then diluted by another 4.0 ml sterile TSB and incubated at 37°C overnight. The inactivated Salmonella isolate was inoculated onto blood agar and MacConkey agar and incubated at 37°C for 48 h. Sera from two weaner rabbits were collected before they were inoculated orally with 1.0 ml of the inactivated Salmonella isolate. The pre-inoculation sera were screened using febrile antigen kit controls were used for the slide agglutination test. Two weeks after, the sera of the two rabbits were screened for Salmonella antibodies by the slide agglutination test using the above febrile antigen kit. The antibody titers to the two antigens (Salmonella Paratyphi AH and Salmonella Paratyphi AO) that were positive by the slide agglutination test were determined by using the serological diagnosis by Widal reaction previously described by Cruickshank et al. (6) after a slight modification. Briefly, two sets of serial dilutions of each serum sample from the rabbits were constituted in sterile tubes with normal saline (0.85% NaCl), starting from 1:20 to 1:2560, while the ninth tube contained only saline. A volume of 0.5 ml of each of the two slide agglutination test-positive antigens was added to each of the two sets of serially diluted serum from the rabbits. The tubes were incubated at 37°C for 2 h followed by incubation at room temperature for 30 min and examined for agglutination for (H) Salmonella Paratyphi AH, while for (O) Salmonella Paratyphi AO the tubes were incubated at 37°C for 4 h and kept at 4°C overnight before reading.

Pathogenicity test

Twenty of the forty randomly selected sixteen-week-old pullets that tested negative for Salmonella were housed in one cage designated “experimental birds”, while twenty others (control) were housed in another cage. The weighing balance was used to take the grammetric data of the birds before they were housed. The exercise was repeated on days 7, 14 and 21 postinoculation (p.i.) as a means of evaluating the subclinical disease. A viable count was effected 24 hours after inoculation of broth culture of Salmonella Paratyphi at 37°C using the plate count, a method by Miles et al. (16) to determine the concentration of the infective dose. Each experimental bird was fed orally 0.5 ml of the 8 h broth containing 1.3 x 10⁸ CFU/ml Salmonella Paratyphi A, while the control birds were fed orally with 0.5 ml of sterile TSB each. The two groups were subsequently given growers’ rations and water without antibiotics. The birds were observed daily for clinical signs and dead birds were taken for postmortem examination. The only surviving bird was euthanized with chloroform on day 21 p.i. (13).

Bacteriology and histopathology

Specimens were taken aseptically from the liver, small intestines, spleen, heart and heart blood, and bone marrow of infected birds for bacteriological and histopathological examinations. The bacteriological specimens were stored in the deep freezer at –20°C, in the Department of Veterinary Microbiology and Parasitology at the University of Ibadan, until they were examined by standard methods (1, 4, 9). Histopathological specimens were fixed in 10% formal saline and processed routinely for histopathological examination (21).

RESULTS

The cultured formalin-inactivated broth culture of the Salmonella isolate showed no growth on blood agar and MacConkey agar, respectively, after 48 h incubation. Also the sera from the weaner rabbits were negative when tested with Salmonella Typhi O, Salmonella Typhi H, Salmonella Paratyphi AO, Salmonella Paratyphi AH, Salmonella Paratyphi BO, Salmonella Paratyphi BH, Salmonella Paratyphi CO and Salmonella Paratyphi CH antigens. However, two weeks after the oral inoculation of the rabbits with formalin-inactivated broth culture of the Salmonella isolate, the sera from the inoculated rabbits reacted with only Salmonella Paratyphi AO and Salmonella Paratyphi AH in the slide agglutination test. The results of the tube agglutination test are presented in Table I. The suspected isolate was identified morphologically, biochemically and serologically as Salmonella Paratyphi A.

The experimental birds showed slight inactivity on day 2 p.i. By day 4 p.i. dullness, ruffled and unkempt feather, somnolence, yellowish green diarrhea, decreased water and feed consumption were observed in the infected birds, and the severity increased until day 16 p.i. Mortality in the infected group was 95% by day 16 p.i. The initial mean body weights of infected and control birds were 0.76 ± 0.20 and 0.78 ± 0.21 kg, respectively. However, the weighing scheduled to be repeated on days 7, 14 and 21 p.i. could not be performed because of the rapid course of the disease.

Table 1

Results by the tube agglutination test

<table>
<thead>
<tr>
<th>Salmonella Paratyphi AO</th>
<th>Salmonella Paratyphi AH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody titer</td>
<td>Antibody titer</td>
</tr>
<tr>
<td>Rabbit A</td>
<td>1:320</td>
</tr>
<tr>
<td>Rabbit B</td>
<td>1:160</td>
</tr>
</tbody>
</table>
Remarkable pathological lesions were recorded between days 7 and 14 p.i. The liver was moderately enlarged, with multiple necrotic foci of about 1 mm in diameter, with dark brown to bronze coloration (Figure 1). The kidney was swollen, with widespread focal pale necrotic areas, while the spleen was slightly enlarged. Histopathologically, the proventriculus showed focal glandular necrosis (Figure 2) with mononuclear cell infiltration and moderate perivascular leucocytic infiltration. There was a mucosal hemorrhage and matting of intestinal villi (Figure 3), with reduction of villous height and presence of epithelial debris in the lumen with increased leucocytic infiltration in the lamina propriae. The liver showed moderate, diffuse congestion of the sinusoids and central veins, as well as multiple foci of necrosis of hepatocytes, and mononuclear infiltration and perivascular cellular infiltration. There was diffuse lymphoid depletion in the nodule and around the splenic arterioles (Figure 4) as well as throughout the parenchyma. The kidneys were congested with tubular epithelial necrosis characterized by karyorrhexis of the nuclei (Table II).

The organism was recovered from the liver, spleen, heart and heart blood, and bone marrow of the infected birds. No clinical sign or gross lesion or pathogen was observed from the negative control.

**DISCUSSION**

It was not possible to monitor the body weight of the experimental birds beyond the initial data because of the fulminating disease produced by *Salmonella Paratyphi* A. This was unlike the 1.8–12.6% reduction in weight recorded over a period of 28 days p.i. by Dhillon et al. (8). In an earlier study these authors recorded 30.7, 7.6 and 15% mortality for *Salmonella Pullorum*, *Salmonella enteritidis* PT5A and *Salmonella enteritidis* PT4 (chicken CA), respectively, at a dose of 1.0 x 10⁶ CFU/ml in specific pathogen-free chickens. This is lower than the 95% recorded in the present investigation. These findings confirm the earlier report that both morbidity and mortality in salmonellosis are highly variable in chickens and are influenced by age, strain susceptibility, nutrition, flock management, and characteristics of exposure (22). *Salmonella* species are known to vary in virulence. For instance *Salmonella enteritidis* PT4 is reported to produce higher mortalities than other *Salmonella enteritidis* phage types (3, 10). The higher mortality found in the current investigation compared to the values recorded elsewhere (8) suggests that the *Salmonella Paratyphi* A may be more virulent than *Salmonella Pullorum* and *Salmonella enteritidis* PT5A. Hence, the organism could be more devastating as experienced in the flock from which it was recovered during the outbreak.

Enteric fever in humans is caused by *Salmonella Typhi* and occasionally by *Salmonella Paratyphi* A, *Salmonella Schottmuelleri* and *Salmonella* Hirschfeldii. The typhoid fever is caused by *Salmonella Typhi*, while the paratyphoid fever is caused by *Salmonella Paratyphi* A, *Salmonella Schottmuelleri* and *Salmonella* Hirschfeldii (7, 15, 20, 23). The clinical features of the paratyphoid fever and typhoid fever are similar but the former is a milder disease (11). In Nigeria, salmonellosis due to *Salmonella Gallinarum*, *Salmonella Pullorum* and *Salmonella Typhimurium* have been reported in poultry (2, 18, 19). However, *Salmonella Paratyphi* A has not been previously reported. It produced a fulminating disease in infected pullets, as found in this study, similar to the clinical observations made in infected birds at Debiwise Poultry Farm in Nigeria. Similarly,
a large outbreak of *Salmonella enterica* serotype Paratyphi B infection caused by goats’ milk cheese was reported in France (7).

From a consumer’s viewpoint, efforts should be made to reduce the spread of this organism in poultry and poultry products in Nigeria in light of the findings of this investigation. The paratyphoid is a zoonotic disease; food products from infected birds pose a risk to human health, and thus hinder public acceptance of poultry products in general, as was the case in the United States of America, Prince Edward Island and Canada (1, 12).

The involvement of *Salmonella* Paratyphi A in poultry disease outbreaks is of public health concern against the backdrop of food safety since this organism has been incriminated in human typhoid (1, 7, 15, 23). This is because *Salmonella* Paratyphi A affects humans; hence infected birds can spread the disease to exposed human beings. *Salmonella* Paratyphi A has been incriminated in human typhoid fever in Nigeria (Akingbola, pers. commun.). It is therefore possible that this organism might have been introduced to the poultry farm from infected farm attendants or contaminated feed and/or water. The fish pond located on the farm may have contributed to the outbreak because a recent report linked aquacultural practices to reservoirs of *Salmonella enterica* Paratyphi B with resultant gastroenteritis in humans (14). Hence it is suggested that livestock farm workers be screened for both typhoid and paratyphoid infections regularly to reduce the incidence of typhoid fever in humans and livestock.

**Acknowledgments**

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**REFERENCES**


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**Table II**

Histopathological findings in dead pullets infected (orally) with 0.5 ml of 1.3 × 10⁸ CFU of *Salmonella Paratyphi A* in Pullets

<table>
<thead>
<tr>
<th>Organ</th>
<th>Lesions</th>
<th>Severity (num. of pullets affected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Congestion of the sinosoids / central veins</td>
<td>++ (7)</td>
</tr>
<tr>
<td></td>
<td>Hepatocyte necrosis</td>
<td>+++ (10)</td>
</tr>
<tr>
<td></td>
<td>Perivascular / parenchymal mononuclear cellular infiltration</td>
<td>+++ (10)</td>
</tr>
<tr>
<td>Intestines</td>
<td>Villous stunting</td>
<td>+++ (6)</td>
</tr>
<tr>
<td></td>
<td>Increased leucocytic infiltration of lamina propriae</td>
<td>+++ (6)</td>
</tr>
<tr>
<td></td>
<td>Focal area of mucosa hemorrhages</td>
<td>+ (3)</td>
</tr>
<tr>
<td>Spleen</td>
<td>Lymphoid hypoplasia around splenic arterioles</td>
<td>++ (12)</td>
</tr>
<tr>
<td></td>
<td>Lymphoid hypoplasia throughout the parenchyma</td>
<td>++ (2)</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>Glandular necrosis / mononuclear cell infiltration</td>
<td>+ (9)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Congestion of interstitial vessels</td>
<td>+++ (4)</td>
</tr>
<tr>
<td></td>
<td>Tubular epithelial necrosis</td>
<td>++ (2)</td>
</tr>
<tr>
<td>Heart</td>
<td>Congestion of coronary vessels</td>
<td>– (18)</td>
</tr>
</tbody>
</table>

– Absent  
+ Weak  
++ Moderate  
+++ Marked  
* Lesion observed in the last bird (euthanized) that survived until day 21 postinoculation
La pathogénicité de Salmonella Paratyphi A, isolée dans l’élevage de volailles de Debiwise lors d’un foyer aigu, a été testée. Vingt poules semaines âgées ont été inoculées oralement avec 1,3 x 10^8 UFC/volaille de Salmonella Paratyphi A, et vingt autres du même âge ont servi de témoins non infectés. Quatre jours après l’inoculation, les animaux infectés ont présenté de l’apathie, des plumes ébouriffées ou entremêlées, de la somnolence, des diarrhées vert jaunâtre, une baisse de la prise d’eau et d’aliments ; la mortalité a été de 95 p. 100, seize jours après l’inoculation. Des lésions importantes se sont développées dans les poules infectées. À l’examen histopatologique, le proventricule a présenté de la nécrose glandulaire focale, infiltrations de cellules mononucléaires et périvasculaires. Une hémarragie de la muqueuse a été observée, ainsi qu’un ternissement des villosités intestinales avec réduction de leur taille, et la présence de débris épithéliaux dans la lumière avec augmentation de l’infiltration leucocytaire dans la lamina propria. Dans le foie, une congestion modérée et diffuse de la sinusoides et de la veine centrale était présent, ainsi que de nombreux foyers de nécrose hépatocytaires, des infiltrations mononucléaires, et cellulaires et pérovascuaires. Il y avait une dépression lymphoïde diffuse dans le nodule et autour des artérioles liliales, ainsi que sur tout le parenchyme. Les reins étaient congestionnés avec des zones de nécrose épithéliale tubulaire caractérisées par une caryorrhexis des noyaux. Salmonella a été trouvée dans le foie, la rate, le cœur, le sang du cœur et la moelle osseuse des oiseaux infectés. Ni signe clinique, ni lésion macroscopique, ni agent pathogène n’ont été observés chez les témoins.


Pathogénicité de Salmonella Paratyphi A chez des poulettes

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La pathogénicité de Salmonella Paratyphi A, isolée dans le élevage de volailles de Debiwise lors d’un foyer aigu, a été testée. Vingt poules semaines âgées ont été inoculées oralement avec 1,3 x 10^8 UFC/volaille de Salmonella Paratyphi A, et vingt autres du même âge ont servi de témoins non infectés. Quatre jours après l’inoculation, les animaux infectés ont présenté de l’apathie, des plumes ébouriffées ou entremêlées, de la somnolence, des diarrhées vert jaunâtre, une baisse de la prise d’eau et d’aliments ; la mortalité a été de 95 p. 100, seize jours après l’inoculation. Des lésions importantes se sont développées dans les poules infectées. À l’examen histopatologique, le proventricule a présenté de la nécrose glandulaire focale, infiltrations de cellules mononucléaires et une infiltration leucocytaire périvasculaire modérée. Une hémorragie des muqueuses a été observée, ainsi qu’un ternissement des villosités intestinales avec réduction de leur taille, et la présence de débris épithéliaux dans la lumière avec augmentation de l’infiltration leucocytaire dans la lamina propria. Dans le foie, une congestion modérée et diffuse de la sinusoides et de la veine centrale était présent, ainsi que de nombreux foyers de nécrose hépatocytaires, des infiltrations mononucléaires, et cellulaires et pérovascuaires. Il y avait une dépression lymphoïde diffuse dans le nodule et autour des artérioles liliales, ainsi que sur tout le parenchyme. Les reins étaient congestionnés avec des zones de nécrose épithéliale tubulaire caractérisées par une caryorrhexis des noyaux. Salmonella a été trouvée dans le foie, la rate, le cœur, le sang du cœur et la moelle osseuse des oiseaux infectés. Ni signe clinique, ni lésion macroscopique, ni agent pathogène n’ont été observés chez les témoins.


Resumen

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Se examinó la patogenicidad de Salmonella Paratyphi A, aislada de la Cría de Debiwise durante un brote fulminante. Veinte pollas de 16 semanas fueron inoculadas oralmente con 1,3 x 10^8 UFC/ave de Salmonella Paratyphi A, mientras que otras 20 pollas de la misma edad sirvieron como controles no infectados. Al día cuatro post inoculación (p.i.) se observaron mareos, plumas erizadas y mal cuidadas, somnolencia, diarrea verde-amarrilla, disminución en el consumo de agua y alimento en las aves infectadas; la mortalidad fue de 95% al día 16 p.i. Importantes lesiones patológicas se registraron entre los días 7 y 14 p.i. El hígado estaba moderadamente aumentado, con focos necróticos múltiples y coloración bronce a bronce oscuro. Los riñones estaban hinchados, con zonas necróticas focales dispersas, mientras que el bazo estaba ligeramente aumentado. Histopatológicamente, el proventriculo mostró necrosis glandular focal, infiltraciones de células mononucleares e infiltraciones leucocitarias perivasculares moderadas. Se observó una hemorragia en las mucosas, aplastamiento de las vellosidades intestinales con reducción de la altura de las vellosidades y presencia de restos epiteliales en el lumen con infiltración leucocitaria aumentada en la lámina propia. El hígado mostró congestión difusa moderada de las venas centrales y sinusoidais, así como múltiples focos de necrosis de hepatocitos, infiltración mononuclear e infiltración celular perivascular. Se observó una reducción linfoidé difusa en el nódulo y alrededor de las arterias del bazo, así como en el parénquima. Los riñones estaban congestionados con necrosis epitelial tubular, caracterizada por cisternes del núcleo. El organismo se recuperó en el hígado, bazo, corazón, sangre del corazón y médula ósea de las aves infectadas. No se observaron signos clínicos o lesiones macroscópicas o patológicas en las aves control negativas.

Palabras clave: Pollo – Salmonella Paratyphi A – Patogenicidad – Nigeria.