

Motile *Salmonella* serotypes causing high mortality in poultry farms in three South-Western States of Nigeria

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ABSTRACT

This study was carried out to identify the *Salmonella* serotypes causing high mortality in chickens in Lagos, Ogun and Oyo states, Nigeria. Chickens presented for postmortem examination during disease outbreaks that were characterised by high mortality (40 per cent to 80 per cent) in poultry farms in the study area were examined from January to December, 2013. Samples of the lungs, heart, liver, spleen, kidneys, proventriculus, intestine and caecum were collected from suspected cases of salmonellosis, for bacterial culture and identification. *Salmonella* isolates were confirmed using PCR and serotyped using the Kauffman-White scheme. Twenty-six day-old pullets were raised to two weeks and inoculated orally with 0.2 mL of 1×10^8 colony forming units of *Salmonella* Zega identified in the present study to determine their pathogenicity, while another 26 served as control. The *Salmonella* serotypes were *S* Zega (n=13; 35.14 per cent), *Salmonella* Kentucky (n=9; 24.32 per cent), *Salmonella* Herston (n=6; 16.22 per cent), *Salmonella* Nima (n=4; 10.81 per cent), *Salmonella* Teitelkeber (n=3; 8.11 per cent), *Salmonella* Colindale (n=1; 2.70 per cent) and *Salmonella* Tshiongwé (n=1; 2.70 per cent). Clinical signs in both natural and experimental infections were acute (70 per cent) and chronic (30 per cent), and included weakness, anorexia, yellowish diarrhoea, pasted vents, somnolence and mortality, while gross lesions showed marked pulmonary congestion and oedema, necrotic foci in the myocardium; the liver, spleen and kidneys were markedly enlarged and had subcapsular multifocal necrosis. There were catarrhal proventriculitis and enteritis, and haemorrhagic typhilitis. While most of the serotypes identified in the present study have been isolated from poultry sources from commercial farms in Nigeria, to the best of the authors' knowledge, they have not been previously reported to cause high mortality in chickens in the study area.

INTRODUCTION

Poultry farming has contributed to the economy of many countries and accounts for 25 per cent of local meat production in Nigeria.^{1,2} However, productivity of poultry is faced with various disease challenges.^{3,4} Among the poultry diseases, salmonellosis caused by

Salmonella enterica is one of the most important bacterial diseases posing serious challenge to poultry production worldwide.³⁻⁵

Salmonella species are Gram-negative, aerobic or facultative anaerobic, motile by means of peritrichous flagella or non-motile and non-spore forming rods.^{6,7} They are divided into serotypes based on the lipopolysaccharide (O), flagellar (H) and sometimes capsular (vi) antigens.⁸ There are more than 2500 known serotypes.^{6,7} The serotypes that are host-specific to poultry are non-motile which are *Salmonella* Gallinarum and *Salmonella* Pullorum.⁹⁻¹¹ While *Salmonella* Typhi is a host-restricted *Salmonella* serotype which causes enteric fever exclusively in human beings, *Salmonella* Enteritidis and *Salmonella* Typhimurium are commonly reported to cause disease in both poultry and human beings, and therefore, are of public health importance. However, there are many other motile *Salmonella* serotypes that can infect poultry and the distribution of these serotypes vary according to geographical location.^{9,10,12-14} Poultry is the main reservoir for this classic zoonotic foodborne pathogen and this organism can survive for a long time in the environment including poultry houses.¹⁵

Although a tentative diagnosis of fowl typhoid can be carried out by considering flock history, a clinical sign, mortality and pathological lesions, a definitive diagnosis by isolation and identification of the organism is required for most cases.⁹

Since vaccination is still the best preventive measure, characterising the different *Salmonella* serotypes in the study area will help in developing an effective vaccine and provide a better control programme. Therefore, there is the need to isolate and characterise the different *Salmonella* field strains to their serotypes.

In the present study, there were reports of outbreaks of diseases of unconfirmed aetiology that were characterised by high mortality in commercial poultry farms in the study area. Postmortem examinations on the carcasses suggested avian salmonellosis. The *Salmonella* serotypes that were associated with the high mortality in commercial and backyard poultry farms in Lagos, Ogun and Oyo states, within south-western Nigeria, were identified, which were not known to cause mortality in chickens in the study area. The disease was then reproduced in an experimental trial, following Koch's postulate, thereby confirming their pathogenicity.

MATERIALS AND METHODS

Study area

The study was conducted in Lagos, Ogun and Oyo states within south-western Nigeria, which lies between longitude 30°E and 7°E and latitude 4°N and 9°N in the west of the lower Nigeria and south of the Niger Trough.¹⁶

Postmortem examination

Chickens that died during outbreaks of avian diseases in commercial and backyard poultry farms in the study area were submitted for postmortem examination in the Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. The flock sizes of the farms ranged from 5000 to 160,000 birds. The study was carried out over a period of one year (January to December, 2013) during which 8 to 13 birds from each disease outbreak were examined. The total number of outbreaks, chickens, their ages, breed and type of chickens were recorded according to the type of farm, month of examination and the State from which they were submitted. In each suspected case of salmonellosis outbreak investigated, samples of the lungs, heart, liver, spleen, kidneys, proventriculus, small intestine, caecum and bile were collected from one bird. Two hundred and seventy samples were collected from 30 different suspected cases of salmonellosis in commercial poultry farms, and 54 from six cases were collected from backyard farms, making a total of 324 samples from 36 (out of 55) salmonellosis outbreaks. The samples were aseptically collected in separate sterile sample bottles, for bacterial culture and identification. In 80 per cent of the cases, the process of bacteriology were started on the same day postmortem samples were collected, while in 20 per cent, the samples were refrigerated at 4°C for 24 hours.

Bacterial culture, isolation and Identification

Bacterial culture and identification were carried out in the Department of Veterinary Microbiology and Parasitology of the Federal University of Agriculture, Abeokuta. Swabs from aseptically sectioned tissue samples of the organs and bile collected at postmortem examination were taken and separately applied into nutrient broth and buffered peptone water for pre-enrichment, and incubated at 37°C for 24 hours. Two mL of the

samples from the pre-enrichment media were inoculated into 50 mL of Rappaport-Vassiliades broth (Oxoid Basingstoke, UK) and onto Tetrathionate glucose broth (Oxoid, Basingstoke, UK) following the pre-enrichment for selective enrichment and incubated at 37°C for 24 hours. Using a sterile wire loop, a loopful of each incubated broth culture was inoculated onto xylose lysine deoxycholate (XLD) agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. The plates were examined for typical colonies of *Salmonella*. The colonies from these plates were further subcultured on XLD agar and incubated at 37°C for 24 hours. The plates from the subculture were also observed for typical colonies of *Salmonella* as described by Doughlas and others.¹⁷ Suspected *Salmonella* colonies were inoculated onto MacConkey agar for purification.

The prevalence of salmonellosis was calculated as percentage of isolation of *Salmonella* serotype from each organ.

PCR procedure

Forty-eight representative samples of the suspected *Salmonella* isolates from the 36 outbreaks were put on nutrient slant, freeze-dried and sent to the National Veterinary Research Institute, Vom, Plateau State for confirmation using conventional PCR as follows:

Oligonucleotide primers

A set of primer pair 139–141 specific for the *invA* gene from *Salmonella* species was used.¹⁸ The primer sequences were as follows: *invA*f 5-3¹GTGAAATTATCGCCACGTT CGGGCAA (sequence length of 26 bases) and *invA*r 5-3¹-TCATCGCACCGTCAAAGGAACC (sequence length of 22 bases) with amplification product (base pair) of 284. DNA extraction kit ZR Fungal/Bacterial DNA Miniprep, catalogue number D6005 was obtained commercially from Zymo Research, USA. Extraction of DNA was done according to the manufacturer's instructions.

DNA amplification

PCR amplifications were performed in a final volume of 50 µL containing 10 µL of DNA template, 25 µL of 2× PCR master mix (x10 buffer, 3 µL of 1.5 Mm MgCl₂, 3 µL each of 2.50 mM deoxynucleoside triphosphate, 0.2 µL containing 1.25 unit Taq polymerase), 2 mM forward and reverse primers and make up the volume to nuclease-free water. Amplifications were carried out in a GeneAMP PCR Applied Biosystem 9700 thermocycler (Thermacycler Applied Biosystem (AB) 9700, USA) using conditions described by Galan and others.¹⁸ Initial denaturation was at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 second, annealing at 55°C for 1 second and final extension at 72°C for 7 minutes. DNA Amplicons were sized by electrophoresis on 1:2 per cent agarose gel containing 5 µg/ml ethidium bromide with 100 bp and 50 bp ladders (GibcoBRL) as molecular weight markers as described by various workers.^{19–23}

Salmonella species serotyping

Thirty-seven *Salmonella* isolates, one from each of 35 outbreaks and 2 from 1, that were confirmed by PCR to be *Salmonella* species were inoculated into nutrient agar slope, freeze-dried and sent to the World Organization for Animal Health, Italian Reference Laboratory for *Salmonella*, Istituto Zooprofilattico Sperimentale Dellevenzie, Podava, Italy for serotyping using the Kauffman-White scheme.²⁴

Experimental study

An experimental study in line with Koch's postulate of reproducing the disease experimentally and re-isolation of putative pathogen in order to demonstrate that the serotypes isolated in the present study were the cause of the natural salmonellosis in chicken in the commercial and backyard poultry farms was carried out.

Source of birds and bacteria (*S Zega*)

A total of 52 day-old ISA brown chicks were used for the experimental study. They were purchased from a certified hatchery (Agritet, Yola, Adamawa State, Nigeria) and housed using deep litter management system, in the Poultry Pen of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU) Zaria. The poultry pen was previously cleaned, disinfected with formalin and fumigated with potassium permanganate (KMnO₄) before the arrival of the birds. The birds were infected at two weeks of age after they were examined to certify being *Salmonella*-free by taking cloacal swab samples from them before infection with *S Zega*. The cloacal swabs were cultured in the Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, ABU, Zaria which confirmed that they were *Salmonella*-free, when no growth of the organisms was observed from the culture.

The *S Zega* used in this experimental study was among other *Salmonella* serotypes isolated from the natural cases of avian salmonellosis in the present study. It was used for the experiment because it was the most predominantly isolated serotype.

Standardisation of the inoculation dose of *S Zega*

The inoculation dose (colony forming units (cfu)) was determined using Sensititre nephelometer (TREK Diagnostic Systems, UK) according to the method described by Goldman and Green.²⁵ The pure culture of *S Zega* isolated was brought from an 18-hour plate culture. Sterile wire loop was used to pick two to three colonies of the *S Zega* and emulsified in 50 mL of sterile normal saline and incubated at 37°C for 10 min to allow for bacterial growth. Two mL of the suspension containing the bacteria was put in a sterile tube and inserted into the Sensititre nephelometer after calibration, and the concentration of the bacteria was taken from the calibration. The procedure was repeated after 10 minutes each of incubation at 37°C until a concentration of 1×10⁸ was obtained.

The birds were grouped into infected (group A) and uninfected control (group B) as follows:

Group A consisting of 26 birds and each was inoculated with 0.2 ml of 1×10⁸ cfu of the *S Zega* orally and were fed commercial poultry feed and administered water (Spring Waters Nigeria Limited, Kerang Mangu local government area (LGA)) ad libitum.

Group B consisting of 26 birds was not inoculated but was fed commercial poultry feed and administered water (Spring Waters Nigeria Limited, Kerang Mangu LGA) ad libitum and was used as a control group.

All the birds in the four groups were monitored daily, from the day of treatment to day 12 post-infection (pi) to determine the incubation period and clinical signs of the disease. Two birds from each group were sacrificed on day zero before infection and continued pi at days 1 pi, 2 pi, 3 pi, 4 pi, 5 pi, 6pi, 7 pi, 8 pi, 9 pi, 10 pi, 11 pi and 12 pi and postmortem examinations were carried out on the carcasses to determine the development of pathological lesions according to days pi. Those birds that died naturally from the disease were collected and examined at postmortem as for the rest of the birds under the study. Swabs from aseptically sectioned visceral organs were taken from both infected chicks and uninfected chicks, for bacterial culture and identification to determine the day from which *S Zega* can be isolated.

Bacterial culture and identification of putative pathogen(*S Zega*)

Bacterial culture and identification of the putative pathogen (*S Zega*) was carried out using the tissue swabs collected at postmortem, according to the method described above.

RESULTS

Natural cases of avian salmonellosis in commercial poultry farms

One thousand and sixty-four birds from 104 outbreaks of diseases were submitted from commercial poultry farms while 123 from 14 outbreaks were from backyard farms, making a total of 1187 birds from 118 outbreaks that were examined. This comprised 748 birds from 72 outbreaks submitted from Ogun state; 273 from 27 outbreaks from Lagos state and 166 from 17 outbreaks from Oyo state. Out of the total number of birds examined, 547 birds from 55 outbreaks were diagnosed with salmonellosis.

Out of the 118 cases of disease outbreaks submitted for postmortem examination during the study period, 16 representing 13.56 per cent were recorded in birds within the one to three weeks age bracket. There were 28 outbreaks representing 23.73 per cent among those birds within the ages of four to eight weeks. Birds within the ages of nine weeks and above had the highest number (n=74; 62.71 per cent) of outbreaks. The ISA brown breed had the highest number of outbreaks (n=63; 53.39 per cent), followed by Nera black (n=35; 29.66 per cent), while other exotic breeds had 13 representing 11.02 per

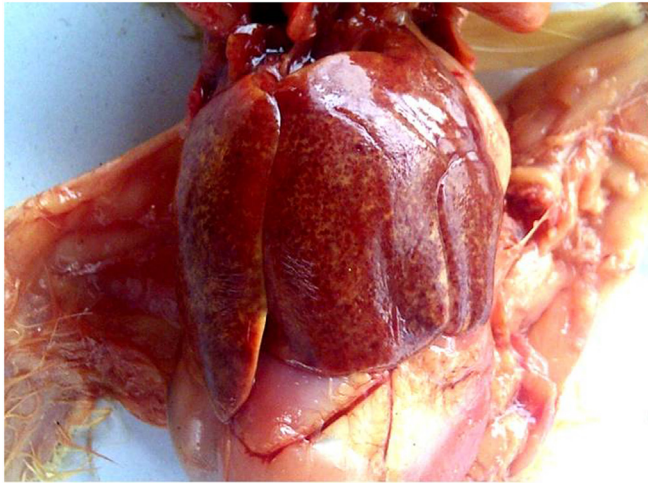


FIG 1: Liver of a two-week-old pullet with natural *Salmonella* Zega infection showing enlargement with multifocal subcapsular necrosis.

cent outbreaks. The local breed had the least number of outbreaks (n=7; 5.93). The type of bird which had the highest number of outbreaks were layers (n=66; 55.93 per cent) followed by broilers (n=27; 11.02 per cent), then pullets and cockerels, (n=13; 11.02 per cent) and (n=11; 9.32 per cent), respectively. Breeders had the least outbreak (n=1; 0.85 per cent).

The highest number of disease outbreaks in chicken in which carcasses were presented for postmortem examination were recorded in January which had 14 representing 11.86 per cent outbreaks, followed by April (n=13; 11.02 per cent), May (n=12; 10.17 per cent), June (n=12; 10.17 per cent), March (11; 9.32 per cent), July (n=10; 8.47 per cent), October (n=9; 7.63 per cent), November (n=9; 7.63 per cent), December (n=8; 6.78 per cent), February (n=7; 5.93 per cent), August (n=7; 5.93 per cent). The least number of outbreaks was recorded in November (n=6; 5.08 per cent).

Clinical signs

The disease presented with an acute form in most cases (n=39; 70 per cent) while in some cases, they showed chronic manifestation (n=16; 30 per cent). The clinical signs were in acute salmonellosis weakness, anorexia, yellowish diarrhoea, pasted vents, somnolence and mortality of 40 per cent to 80 per cent. The chronic form of the disease showed lethargy, yellowish diarrhoea with pasted vents and severe emaciation.

Postmortem findings

The disease presented with acute (n=39; 70 per cent) and chronic (n=16; 30 per cent) pathological changes in visceral organs. In the acute form of the disease, the lungs were severely congested and oedematous; there were necrotic foci on the myocardium. The livers were markedly enlarged and had multifocal subcapsular necrosis in the visceral and parietal surfaces (Fig 1). The spleen and the kidneys were severely congested and enlarged. The proventriculus contained a large amount of mucus and

TABLE 1: Percentage of isolation of *Salmonella* species from chickens submitted from Lagos, Ogun and Oyo states, Nigeria for postmortem examination in 2013

State	Number of outbreaks	Number of isolation (%)
Lagos	9	7 (77.8)
Ogun	21	20 (95.2)
Oyo	6	4 (66.7)
Total	36	31 (86.1)

the mucous membrane was hyperaemic. The intestines contained mucus admixed with diarrhoeic faeces and the mucous membranes were necrotic and hyperaemic. The mucous membranes of the caeca were necrotic and the lumen contained dark red caecal cores. While in the chronic form of the disease, most of the changes observed in the acute form have resolved, but the carcasses were emaciated with pasted vents. There were foci of necrosis in the visceral organs.

Prevalence of *Salmonella* serotypes

During the period of this study, 36 samples each of the lungs, heart, liver, spleen, kidneys, proventriculus, small intestine, caecum and bile were collected from 36 (out of the 55) suspected cases of salmonellosis, making a total of 324 samples, for bacterial isolation and identification. Out of the 36 outbreaks, 21 were from Ogun state, 9 from Lagos state and 6 from Oyo state (Table 1). Of the 324 samples of the visceral organs collected, the lungs showed the highest percentage of isolation (n=36; 86.1 per cent) followed by the kidneys (n=36; 77.8 per cent) and the bile (n=36; 77.8 per cent). The proventriculus had the lowest percentage of isolation (n=36; 55.6 per cent) (Table 2). The overall percentage of isolation was 86.1 per cent (n=324) and *Salmonella* species were isolated in all the months of examination.

TABLE 2: Organ-specific prevalence of *Salmonella* species from chicken submitted from Lagos, Ogun and Oyo states, Nigeria for postmortem examination in 2013

Organ	Number of sample	Number of isolation	Percentage
Lung	36	31	86.1
Heart	36	23	63.9
Liver	36	25	69.4
Spleen	36	24	66.7
Kidney	36	28	77.8
Proventriculus	36	20	55.6
Intestine	36	24	66.7
Caecum	36	25	69.4
Gall bladder (bile)	36	28	77.8
Total	324	228	70.4

TABLE 3: *Salmonella* serotypes isolated from chicken submitted from Lagos, Ogun and Oyo states, Nigeria for postmortem examination, in 2013

<i>Salmonella</i> serotypes	State			Total
	Lagos	Ogun	Oyo	
	N (%)			
<i>Salmonella</i> Zega	2 (40.00)	9 (31.03)	2 (66.67)	13 (35.14)
<i>Salmonella</i> Kentucky	3 (60.00)	5 (17.24)	1 (33.33)	9 (24.32)
<i>Salmonella</i> Herston	0 (0.00)	6 (20.69)	0 (0.00)	6 (16.22)
<i>Salmonella</i> Nima	0 (0.00)	4 (13.79)	0 (0.00)	4 (10.81)
<i>Salmonella</i> Teitelkebir	0 (0.00)	3 (10.34)	0 (0.00)	3 (2.70)
<i>Salmonella</i> Tshiongwe	0 (0.00)	1 (3.45)	0 (0.00)	1 (2.70)
<i>Salmonella</i> Colindale	0 (0.00)	1 (3.45)	0 (0.00)	1 (2.70)
Overall	5 (13.51)	29 (78.38)	3 (8.11)	37 (100)

N, number of serotypes

PCRs

In this study, 48 representatives of suspected *Salmonella* isolates from the 36 natural cases of avian salmonellosis were tested using PCR. Out of the 48 presumed *Salmonella* isolates, 41 were confirmed to be *Salmonella* species. The PCR result showed that *Salmonella*-positive samples and the positive control, *S* Enteritidis correspond to 284 bp on the ladder of the molecular marker, which is the base pair unique for all *Salmonella* species.

Salmonella serotypes

Thirty-seven *Salmonella* isolates were serotyped in which seven serotypes were identified. They included *S* Zega, *Salmonella* Kentucky, *Salmonella* Herston, *Salmonella* Nima, *Salmonella* Teitelkebir, *Salmonella* Colindale, and *Salmonella* Tshiongwe. The predominant serotype was *S* Zega (n=13; 35.14 per cent) followed by *S* Kentucky (n=9; 24.32 per cent), then *S* Herston (n=6; 16.22 per cent), *S* Nima (n=4; 10.81 per cent), *S* Teitelkebir (n=3; 8.11 per cent), *S* Colindale (n=1; 2.70 per cent) and *S* Tshiongwe (n=1; 2.70 per cent). Out of the 37 serotypes, 13.51 per cent were isolated from Lagos state, 78.38 per cent from Ogun state and 8.11 per cent from Oyo state. All the *S* Hertson, *S* Nima, *S* Teitelkebir as well as the *S* Tshiongwe and *S* Colindale serotypes were isolated from Ogun state (Table 3). Some of the *S* Zega and *S* Kentucky were isolated from both commercial and backyard poultry farms whereas the remaining isolates were isolated from commercial poultry farms.



FIG 2: Three-week-old chicks infected orally with *Salmonella* Zega showing somnolence and mortality, six days pi.

Experimental studies

Clinical signs

The chicks in group D (uninfected control) showed no clinical signs. The infected chicks showed signs of weakness and anorexia by day four pi while somnolence were observed from day five pi. Yellowish diarrhoea and mortality were recorded from day six pi (Fig 2).

Postmortem findings

Gross lesions were pulmonary congestion and oedema, the heart had necrotic foci on the myocardium. The liver was markedly enlarged and had multifocal necrosis in the visceral and parietal surfaces from day five pi till the end of the experiment (Fig 3). The spleen and kidneys were



FIG 3: Liver of a three-week-old chick infected orally with *Salmonella* Zega (left) showing enlargement with multifocal necrosis 10 days postinfection; and normal liver from an uninfected chick (right).

markedly congested, enlarged with subcapsular foci of necrosis. The proventriculus contained a large amount of mucus and the mucous membranes were hyperaemic. The intestines were distended with diarrhoeic faeces admixed with mucus and the mucous membranes were hyperaemic, while the caecum contained reddish caecal core and the mucous membranes were hyperaemic.

Bacterial culture and identification

S Zega was re-isolated from the infected chicks and identified as described earlier.

DISCUSSION

Motile *Salmonella* serotypes causing high mortality in commercial poultry farms in Lagos, Ogun and Oyo states, Nigeria, were identified in the present study, and the pathogenicity of *S Zega* was confirmed experimentally. The *Salmonella* serotypes reported in this study were isolated from outbreaks of salmonellosis in all age groups, breeds and types of chickens, with older birds and layers being the most affected, in both backyard and commercial poultry farms. Poppe and others²⁶ reported a high occurrence of diseases in older birds among commercial poultry farms in Canada. The reason may be attributed to the longer time the older birds have stayed on the farms, persistent contamination in poultry houses for long periods of time, consecutive generation of chickens that are being kept on the farm and laying stress in layers.^{13 26} The low percentage of diseases among young chicks may suggest that the hatcheries where day-olds were procured had strict prevention and control measures. All the seven serotypes were associated with the outbreaks in commercial poultry farms while only *S Zega* and *S Kentucky* were isolated from outbreaks in both backyard and commercial poultry farms. This may probably be due to the fact that they were the most predominant serotypes isolated in this study. *S Kentucky* has been reported to be the most predominantly isolated serotype in commercial layer farms in Nigeria.²⁷ It may also be due to the less number of samples collected from backyard farms compared with the ones from commercial poultry farms. Similarly, the isolation of *S Zega* and *S Kentucky* in all the two states may be attributed to their predominance in the study area as seen in this study; whereas the isolation of all the seven serotypes in Ogun state may be due to the larger number of samples collected from the state. *S Zega* was the most commonly isolated serotype in the present study and has been isolated from human sources in Zaire.²⁸ However, *S Kentucky* was the second most frequently isolated serotype and has been reported to infect chickens worldwide.²⁹ *S Kentucky* was also reported to be the most common serotype isolated from litters, dust, faeces, feed and water in Nigerian commercial chicken layer farms across the country.²⁷ In other separate studies, *S Herston* has been reported to be associated with diarrhoeal cases in children from the Niger Republic; and *S Nima* has caused an outbreak of salmonellosis in human beings through

contaminated chocolates, in many countries.³⁰ Whereas *S Colindale* was one of the most common *Salmonella* serotypes isolated from fresh lettuce in Burkina Faso.³¹ Barua and others³² also reported a high prevalence of motile *Salmonella* in commercial layer farms in Bangladesh. However, most of these serotypes of *Salmonella* have not been reported to cause high mortality in poultry. With the exception of *S Kentucky*, information appears to be scanty on the prevalence of these serotypes on commercial poultry farms worldwide, and reports in the literature on their pathogenicity to avian species are very rare. The result was contrary to the expectation that *S Gallinarum* and *S Pullorum* may be associated with the natural cases, since they are host-specific and highly adapted to chickens.^{33 34} While these serotypes have been reported to cause disease in human beings, the mortality most of them cause in chickens is unusual. However, motile *Salmonella* serotypes other than the ones isolated in this study have been reported by several workers to be the cause of serious mortality and pathological lesions in commercial poultry farms.^{6 12 35} Foley and others¹⁵ and Baumler and others³⁶ reported that the niche created by the eradication of *S Gallinarum* and *S Pullorum* in commercial poultry farms in the USA and Canada are likely to be filled by *S Enteritidis*, *Salmonella Heidelberg* and *S Kentucky*. Ogunleye and others (2006) reported that *Salmonella Paratyphi*, motile *Salmonella* isolated during a fulminating outbreak of salmonellosis on commercial poultry farms in Debiwise farms, Ibadan, Oyo state, Nigeria were highly pathogenic in pullets that were experimentally infected. While there is paucity of information on the pathogenicity of some of the *Salmonella* serotypes identified in the present study, the availability of draft genomes of these serotypes have earlier been reported.³⁷

The isolation of motile *Salmonella* serotypes from the outbreak of salmonellosis on commercial poultry farms is of significant public health concern.^{15 38} Motile *Salmonella* are known to infect a wide range of hosts including human beings^{15 39-42} and account for approximately 93.8 per cent of million human cases of gastroenteritis and 155,000 of deaths worldwide each year.³⁸ Contact with infected poultry and consumption of contaminated poultry products, especially undercooked meat and raw eggs are important sources of infection by this major foodborne pathogen worldwide.^{15 39-42}

The clinical signs and gross lesions observed in the experimental study were similar to the ones described in the natural cases in the present study and are consistent with those earlier reported in avian salmonellosis.^{45 9} The finding on the clinical signs and pathology of *S Zega* in experimentally infected pullets following Koch's postulate of reproducing the natural disease in experimental infection, which was fully established proves its pathogenicity, and this suggests that the other *Salmonella* serotypes identified from the natural cases in the present study may equally be highly pathogenic in chickens, since they present with similar clinical signs and lesions in the natural cases.

In conclusion, motile *Salmonella* serotypes were the cause of high mortality in commercial poultry farms. Although the serotypes identified in the present study have been previously isolated from poultry sources from commercial poultry farms in Nigeria, to the best of the authors' knowledge, this is the first report on them causing high mortality in chickens in the study area.

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Contributors Conception and design: FMM, ND-GI, SNS. Acquisition of data: FMM, AAA. Data Analysis: AKFK. Interpretation of data: FMM, ND-GI, SNS, MM. Drafting the work: FMM, PDL. Revising the work critically: ND-GI, SNS, CNK. Final approval of the version to be published: FMM. Postmortem diagnosis: FMM, ND-GI, SNS. Culture and isolation of the Salmonella: PAA, FMM, AAA. Confirmation by PCR: PDL. Participation in serotyping work: IOF. Supervision: ND-GI, SNS, CNK, MM.

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Data sharing statement The prevalence study, the pathological and immunohistochemical studies in both natural and experimental trials are not included in this article.

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