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# Serotyping of *Salmonella Enterica* Isolated from Broiler Chicks in an Outbreak in Sudan

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*Study design:* A total of 52 random samples, out of 2000 i nfected broiler chicks, were cultured and identified.

The study was undertaken in the laboratories of the Veterinary Research Institute, Ministry of animal resources and fisheries, Khartoum as well as Department of Botany, Faculty of Science, University of Khartoum, Sudan during March 2014- December 2014.

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*Strictly as per the compliance and regulations of :*

# Serotyping of *Salmonella Enterica* Isolated from Broiler Chicks in an Outbreak in Sudan

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*Abstract-* *Aims:* This study investigates the first emergence of *Salmonella* outbreaks among 8.000 broiler chicks, of the 'Ross' breed, in Sudan. Mortality was observed in 25% of the total chicks; therefore, samples were taken for culturing, identification and characterization of the causative agent.

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*Methodology:* A total of 52 isolates were recovered from different organs of each chick. These isolates were characterized as *Salmonella enterica* (*S.enterica*) subspecies *enterica* using both conventional and automated methods. Vitek 2 Compact system was used as a fully automated method. Ten isolates were selected, as representative isolates, and were furthely identified using specific primers targeting each of *inv*A and *hil*A genes. The serotypes and phagetypes of each of the ten isolates were detected the examined number is very low.

*Results:* All of the 52 isolates have shown colony characteristic typical to *Salmonella* spp. Result of the Vitek2 compact system showed that isolate was typical *Salmonella enterica*. The primer pairs targeting *inv*A and *hil*A genes successfully amplified the extracted DNA giving the specific amplicons. Out of the ten representative *Salmonella enterica* isolates, nine showed the antigenic reaction of *S*. Enteritidis serovar and phagetype 3a while the tenth one was found identical to *S*. Typhimurium and phagetype 2.

*Conclusion:* The most pathogenic S*almonella* serovars, *S.* Enteritidis and *S.*Typhimurium were reported in the Sudan causing a serious economic risk in poultry farms.

*Keywords: salmonella, serovars, salmonellosis, vitek PCR, sudan.*

## I. Introduction

Avian salmonellosis represents a group of acute and a chronic diseases caused by one or more members of genus *Salmonella* [1]. *S. enterica* is one of the most important public health pathogens [2] and may be acquired by the consumption of poultry products [3] and causes a number of significant diseases in poultry and humans. *S. enteric* is recently classified into more than 2500 serovars [4]. Most poultry infected by *Salmonella* spp become carriers; infection may also be fatal, depending on the particular serovar and the age of the bird at the time of infection [5].

Salmonellosis outbreak remains as a serious economic problem in countries where control measures are not efficient or in those areas where climatic conditions, favor the environmental spread of the microbes. The economic losses are chiefly due to morbidity, mortality, reduced growth rate, reduced feed conversion efficiency, drop in egg production, decrease fertility and hatchability.

In Sudan isolation of *Salmonella* was reported by different investigators. For example, Mamoun *et al*. [6] isolated 21 *Salmonella* strains from several poultry farms in three different states, all were found to be *S. enteritidis*. The isolation of *S. enterica sub enterica* serotype San-Diego from three goats (3.84%) at Omdurman Central Abattoir was reported [7]. Yagoub *et al.* [8] isolated *Salmonella* sp. from 1.43% of raw milk samples collected from different locations. Yagoub *et al*. [9] isolated *Salmonella Paratyphi A (S. Paratyphi* A) and *Salmonella Paratyphi B* (*S*. Paratyphi B) from 6% of the white cheese samples collected from retailer shops and restaurants in Khartoum and Omdurman. Yagoub [10] reported the isolation of *Salmonella* spp from 6.2% of the fish samples (gills, intestine, skin and muscles) collected from fish markets in Khartoum State. Forty-five *Salmonella* isolates (not serotyped) were isolated from carcasses, liver, spleen, intestinal contents of chicks from a poultry farm in El Obeid (unpublished data). Recently *Salmonella* Umbadah plus other 19 new serovars were reported from different sources at Khartoum [11; 12].

The Vitek system was originated in the 1970 as an automated system for identification and antimicrobial sensitivity test (AST) which automatically performs all the steps required for identification and AST of bacteria after primary inoculums have been prepared and standardized. This system allows kinetic analysis by reading each test every 15 minutes. The optical system combines multichannel fluorimeter and photometer reading to record fluorescence, turbidity and colorimetric signals [13].

Then, the aim of this study was to isolate and characterize the *Salmonella* spp. causing broiler chicks outbreaks.

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## II. Material and Methods

Eight thousand (8,000) broiler chicks, of the 'Ross' breed, were bought for commercial benefits in March 2014. Due to mortality that was started at the first day, postmortem was done to investigate the gross lesions and taking samples from all organs. Samples were sent to Veterinary Research Institute, Ministry of animal resources and fisheries, Khartoum, Sudan for culturing, detection and identification of the causative agent.

*a) Isolation and characterization of Salmonella*

Salmonellae were isolated and identified according to the techniques recommended by the International Organization for Standardization described by Molla *et al*. [14]. Samples of broiler chicks including liver ($n$=10), intestine ($n$=10), heart ($n$=10), kidney ($n$=10), spleen ($n$=10), trachea and brain (one sample each) were each inoculated in nine ml of sterilized buffered peptone water. The inoculated buffered peptone water was incubated at 37°C for 24 hours and the resulting suspensions were cultured on selenite broth medium and then purified on nutrient, MacConkey and XLD media (Hi media, India). Cellular, colony morphology and biochemical characteristics of each isolate were tested [15].

*b) Vitek 2 Compact Automated System*

Ten representative isolates, selected from each of the examined organs, were furtherly characterized using full automated system Vitek 2 compact (bioMerieux) to confirm the species *S.enterica*. The Gram Negative card that used in Vitek2 compact was based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities and resistance [16; 17; 18]. The GN card used contained a total of 47 wells representing 47 different biochemical tests and one negative control well. Identification was done according to the manufacturer's guidelines.

*c) Molecular identification of Salmonella isolates*

PCR primers, targeting each of *inv*A (F: 5´ GTG AAA TTA TCG CCA CGT TCG GGC AA3´ and R: 5´ TCA TCG CAC CGT CAA AGG AAC C 3´ [19] and *hil*A (F: 5´-CTG CCG CAG TGT TAA GGA TA-3´ and R: 5´-CTG TCG CCT TAA TCG CAT GT-3´ [20] *Salmonella* specific genes, were evaluated to detect the isolated *Salmonella*. DNA from each isolate ($n$=52) was extracted according to the boiling – centrifugation method [21]. A single colony of a pure nutrient agar culture was grown overnight at 37°C in 1.0 ml Luria–Bertani broth. Bacterial cells were precipitated by centrifugation at 13,000 rpm for 5 min. in a micro-centrifuge (MSE, MSBo1o.cx2.5, Sanyo, UK). The supernatant was discarded and the pellet was re-suspended in 500µl deionized distilled water. The suspension was boiled for 10 min. in a water bath then immediately cooled on ice. Extracted DNA was then stored refrigerated at 4°C until used as a template for PCR amplification.

The extracted DNA was amplified by an established PCR technique [22]. PCR amplification reactions were carried out in 25 µl total volume of PCR mixture containing 5 µl of template DNA, 12.5µl of the PCR master mix (Promega) (50 unit/ml *Taq* DNA polymerase in an appropriate reaction buffer {pH 8.5}, 400 µM each dNTPs and 3mM $MgCl_2$) and 0.1 µM of each of primer pair. DNA was amplified according to reaction conditions published for each primer pair in a thermal cycler (Techne/ Flexigene - biotech). The cycling conditions were as follow: an initial incubation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 60 seconds, annealing at 64°C (*inv*A) or 62°C (*hil*A) for 60 seconds, extension at 72°C for 60 seconds, and final extension period for 7 minutes at 72°C.

Appearance of the target band specified for each primer set on the 1.2% agarose gel under specified gel electrophoresis conditions is considered as a positive amplification product.

*d) Salmonella Serotyping and Phage typing*

Ten presumptive *Salmonella* isolates (selected based on their biochemical reactions and molecular identification) were shipped to the Public Health Agency, Office International des´ Epizooties (OI´E) Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada for serotyping and phagetyping. The antigenic formulae of Popoff and Le Minor [23] were used to name the serovars. Phagetyping was performed using the standard phagetyping technique described by Anderson and Williams [24].

## III. Results

*a) Conventional biochemical tests identification*

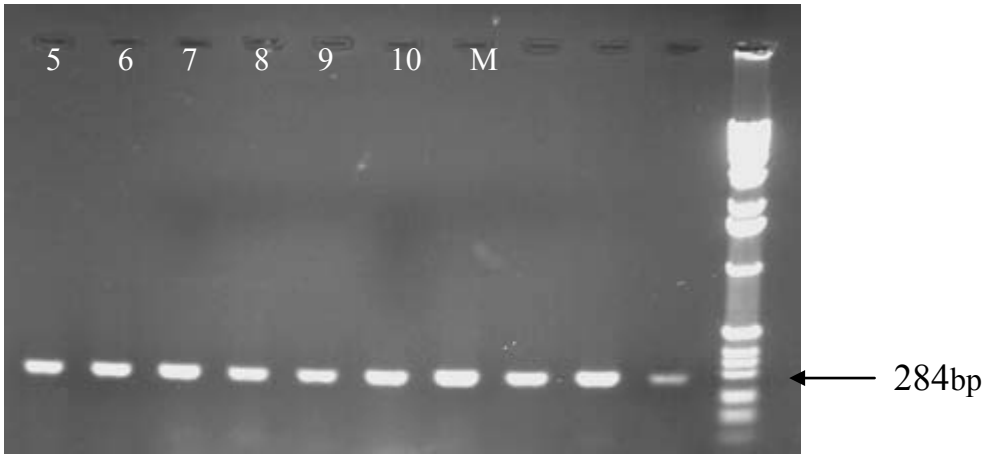
The average cumulative mortality percentage in the 8,000 broiler chicks, during the period from 1 to 8 days of age, was 25%. A total of 52 bacterial isolates, cultured from different internal organs, were recovered on selenite broth, Nutrient, MacConkey and XLD media. All of the isolates were Gram negative and have shown colony characteristic typical to *Salmonella* spp. The isolates were positive for citrate and methyl red tests and they were negative for indole, Voges-Proskauer and urease tests.

*b) Vitek 2 Compact Automted System*

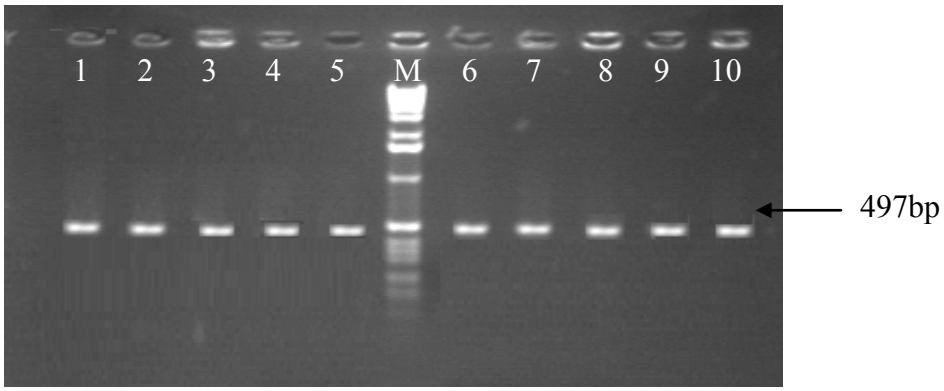
Result of the Vitek 2 compact system showed that the isolates were typical *Salmonella enterica*.

*c) Molecular identification*

DNA extracted from the isolates were used to evaluate the specificity of two primer sets to detect *Salmonella* sp. The primer pairs targeting *inv*A and *hil*A genes successfully amplified the extracted DNA giving the specific amplicons for each primer (Fig. 1).




*inv*A gene




*hil*A gene

*Figure 1 :* PCR amplification products detected for the two primer sets. Lanes 1 - 10 represent *Salmonella* isolates, M = 100bp DNA ladder

*d) Serotyping and Phagetyping*

Serotyping test showed that all of the tested isolates (*n* =10) were members of *S. enterica* subspecies enterica. Results in Table 1 show that nine of the ten isolates reported here belonged to serovar Enteritidis (9,12:g,m:-) and one isolates was serotyped as *S.* Typhimurium (4,5:i:1,2). All of the nine Enteritidis isolates were phagetype 3a while the Typhimurium isolate was phagetype 2.

*Table 1 :* *Salmonella* serotyping and phagetyping results

| *Salmonella* isolate No. | Antigenic formula | Serovar | Phagetype |
|---|---|---|---|
| 1 | 9,12:g,m:- | Enteritidis | 3a |
| 2 | 9,12:g,m: | Enteritidis | 3a |
| 3 | 4,5:i:1,2 | Typhimurium | 2 |
| 4 | 9,12:g,m: | Enteritidis | 3a |
| 5 | 9,12:g,m: | Enteritidis | 3a |
| 6 | 9,12:g,m: | Enteritidis | 3a |
| 7 | 9,12:g,m: | Enteritidis | 3a |
| 8 | 9,12:g,m: | Enteritidis | 3a |
| 9 | 9,12:g,m: | Enteritidis | 3a |
| 10 | 9,12:g,m: | Enteritidis | 3a |

The mortality rate of 8.000 chicks was 25% (2000). The other chicks which were 75 % (6000) survived under treatment using Gentadox (Avico) that contain 200mg of gentamyicin sulphate and 125 mg of doxycycline hydrochloride.

## IV. Discussion

Salmonellosis is a common bacterial infection in human. Illness caused by different *Salmonella* spp. can range from a mild to severe gastroenteritis and in some people, invasive disease, which can be fatal. Long term consequences such as reactive arthritis can also result from *Salmonella* infections [25]. Symptoms of *Salmonella* outbreak was observed on 25% of the commercial broiler chicks. It is likely due to the fact that chicks are not fully immune competent when they are below 2 weeks of age because of a lower percentage of CD4+CD8- in the thymus; CD4-CD8+ and CD4+CD8+ in the spleen [26]. Many researchers have reported that salmonellas is outbreaks in human were associated with poultry meat or eggs [27; 28]. Vertical transmission of infections from breeding hens to progeny has been an important aspect of the epidemiology of *Salmonella* species infections within the poultry industry [29; 30].

In this study *Salmonella* were identified in 52 intact samples, selected from 2000 symptomatic chicks, ten of them were serotyped as S. Enteritidis and *S.* Typhimurium are by far the two dominating serotypes isolated from poultry and poultry products [31; 32] and these two serotypes are also the most frequently isolated serotypes in humans [33; 34]. A number of *Salmonella* outbreaks reported in the world are a result of injudicious introduction of infected birds [35-38]. According to FAO/WHO reports [39], most foodborne *S.* Enteritidis infection is associated with the consumption of raw eggs and foods containing raw eggs. In the European Union-wide baseline study of *Salmonella* in commercial broiler flock, 2005-2006, the observed prevalence of positive flocks was 23.7 % while the member-state specific rates varied from 0.0 to 68.2 %. A total of 11.0% of the broiler flocks were estimated to be positive for *S.* Enteritidis and/or *S.* Typhimurium. The Member State-specific observed flock prevalence of *S.* Enteritidis and/or *S.* Typhimurium varied from 0% to 39.3% [40]. In an experimental infection done by Akhtar, *et al.* [41] showed the pathogenisity of *S.* Enteretidius phagetype 3a in a newly hatched white leghorn chicks.

## V. Conclusion

The most pathogenic species of *Salmonella, S.* Enteritidis and *S.* Typhimurium were reported in the Sudan causing a serious economic risk in poultry farms.

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