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Virulence Range and New Pathological Pictures of *Salmonella enteridits* and *Salmonella typhimurium* Isolated from Ducklings in Experimental Infected Chicks

Hend K. Sorour*, Heba Badr, May Fathy Abdelaty, Heba Roshdy, Ahmed Abd El-Halem Mohammed and Mona Aly Abdelhalim AbdelRahman

Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute (AHRI, Agricultural Research Center (ARC), Dokki P.O. Box 246, Giza 12618, Egypt

***Corresponding Author:** Hend K. Sorour, **E-Mail:** drhendsorour@gmail.com

ABSTRACT

Salmonellosis is a major global pathogen in the poultry industry and is a significant public health concern. Ducks are known to be carriers of *Salmonella*. Therefore, monitoring salmonellosis is the most important strategy for preventing the disease. An experimental design was planned to study the pathogenicity of two *Salmonella* strains. One hundred and fifty chicks were divided into three groups; group one was inoculated with the Salmonella enteritidis strain, group two was inoculated with the Salmonella typhimurium strain, and group three was UN inoculated. Symptoms, postmortem lesions and mortality rate were recorded. The chick growth performance parameters were also determined. Using ANOVA for statistical analysis, there was a significant difference in body weight, body gain, feed consumption, and feed conversion ratio between the two infected groups and the blank group (uninoculated group). In this study, the prevalence of Salmonella enteritidis was (1.73%) and Salmonella typhimurium (0.43%) in imported ducklings in Egypt. Both *Salmonella* strains were subjected to an antimicrobial sensitivity test. It showed that Salmonella enteritidis had a 60% antimicrobial resistance profile and Salmonella typhimurium had a 20% antimicrobial resistance profile. Furthermore, genotypic characterization was performed and the seven virulence genes(stn, avrA, sopB, ompF, invA, Mgtc, Ssaq) were found. New pathological lesions of *Salmonella* infection were discovered, such as skull hemorrhage at 3 days and 6 days of age, and a liver similar to a button shape in necropsied infected chicks with Salmonella typhimurium at 21 days of age. Furthermore, hemorrhagic spots were observed on the duodenum. In the presence of *Salmonella*, Clostridium perferingens was discovered in a bacteriological investigation of duodenal lesions samples from infected chicks. At 30 days of age, administration of acetic acid (1%) as an alternative tool for controlling *Salmonella*. In conclusion, salmonellosis is a risk factor for necrotic enteritis, and using acetic acid to eliminate salmonella infection is insufficient.

Keywords: Imported ducks, Multidrug resistance, Salmonella enteritidis, Salmonella typhimurium, Pathogenicity, Virulence genes.

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INTRODUCTION

Salmonella is a major foodborne pathogen, causing 715,000 deaths related to diarrhea each year, with foodborne diseases accounting for one-third of these cases, according to the World Health Organization **(Besser, 2018).** *Salmonella* species are spread from poultry to humans, most often through the consumption of contaminated food or water, poultry and poultry products, or through direct contact with infected people or animals **(Knodler and Elfenbein, 2019).**

Salmonellosis in ducks causes significant economic loss as well as a public health concern because infected duck flocks serve as a reservoir for *Salmonella* that can be transmitted to humans **(Kim *et al.*, 2021).** The main strategies for preventing salmonellosis outbreaks are to monitor *Salmonella* in poultry and to practice good hygiene **(Heba *et al.*, 2021).** *Salmonella* virulence attributed to the invasion,

survival, and extraintestinal spread genes are found on *Salmonella* pathogenicity islands (SPIs), which are thought to have been acquired via horizontal gene transmission.

17 SPIs have been identified; the type III secretion system (T3SS) encoded by SPI-1 is considered the most important virulence factor for *Salmonella* because it delivers effector proteins required for enteritis invasion and exhibiting. SPI1 and SPI2 are involved in virulence, and a better understanding of the SPI regulatory network can lead to drug discovery and infection prevention **(Sarika and Navneet, 2021).** However, in 1995, European Union countries banned antibiotic growth promoters in the food animal industry due to increased concern about the spread of antimicrobial resistance genes. Many other countries adopted a strategy, creating an emergency demand for alternative treatment keys **(Mashael *et al.*, 2020a).**

Organic acids have demonstrated significant antimicrobial activity against a wide range of intestinal pathogens **(Huyghebaert *et al.*, 2011).** The early age of infected chicks with Salmonella typhimurium may be essential to change the susceptibility to necrotic enteritis **(Shivaramaiah *et al.*, 2011). Gulbeena *et al.*, (2016**) studied acetic acid (1%) supplementation as an aid in decreasing S. Pullorum Count in the cecum of infected birds and the severity of these gross lesions. However, 1.5% acetic acid supplementation also showed adverse effects (diarrhea and poor growth performance in birds challenged with S. Pullorum).

The goal of this research was to investigate the virulence ranges of Salmonella typhimurium and Salmonella enteritidis isolated from ducklings, as well as to assess the pathway of two serovars in experimentally infected one-day-old chicks and the role of acetic acid as an alternative tool for the control of salmonellosis.

MATERIALS AND METHODS

1. Ethics approval

In this study, birds were handled, infected, and treated according to the regulations for the care and husbandry of experimental animals, which were approved by the Animal Care Committee of the Animal Health Research Institute (AHRI) Dokki, Giza, Egypt.

2. Sample collection

From January to December 2019, we collected 231 paper linings from imported ducklings, which contained 30 chicks each., *Salmonella* was isolated and identified using **(ISO 6579-1:2020)**. The samples were enriched with ISO buffer peptone water (Oxoid, UK) and incubated aerobically at 37 ° C for 16-18 hours.

They then added 0.1 ml and 1 ml of incubated buffer to Modified Semisolid Rappaport Vassiliadis medium (MSRV, ʿLabM, UKʾ) and MKTTn broth (LabM, UK), respectively, and incubated at 41.5 ° C for 24 hours and 37 ° C for 24 hours. After culture of both tubes in XLD agar medium (LabM, UK) and S.S. agar medium (Oxoid, UK), which was then incubated at 37 ° C for 24 hours, a selected colony was collected for further identification (urea agar, triple sugar iron, Lysin medium broth) (Oxoid, UK).

3. Serotyping identification of Salmonella enteridits and Salmonella typhimurium

We used the slide agglutination test with *Salmonella* antiserum (Sifin Co., Japan) to serotype isolated *Salmonella* spp. **(ISO 6579-3: 2014).**

4. Antimicrobial susceptibility test

The disc diffusion test was performed on all isolated Salmonella enteritidis and Salmonella typhimurium on Mueller Hinton agar (Oxoid, UK) against 13 antibiotic discs; Ampicillin (10 µg), Apramycin (15 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Colistin sulfate (10 µg), Levofloxacin (5 µg), Lincomycin (10 µg), Nalidixic acid (30 µg), Norfloxacin (10 µg), Streptomycin (10 µg), Tetracycline (10 µg) and Trimethoprim-sulfamethoxazole (1.25/23.75 µg). Furthermore, inhibition zones were interpreted following the Clinical and Laboratory Standards Institute guidelines **(CLSI, 2021).**

5. Molecular detection of *Salmonella* Pathogenicity Island genes (SPI)

A QIAamp DNA Mini Kit was used to extract DNA from culture broth (Qiagen, Germany, GmbH Catalogue no.51304). The extracted DNA was used in subsequent polymerase chain reaction (PCR) assays for species confirmation and gene detection using Emerald Amp MAX PCR Master Mix (Emerald, Amp G.T. (2 premixes), Japan) and specific oligonucleotide primers supplied by Metabion (Germany), which are listed in Table 1. The ProFlex thermal cycler-PCR machine was used to amplify the specific gene (Applied Biosystems Thermo Scientific, USA). Agarose gel electrophoresis was used to detect gene-specific PCR amplicons.

Table 1: Primers sequences, target genes, amplicons sizes, and cycling conditions:

Target genes	Primers Sequences	Amplified segment (bp)	Annealing	References
stn	TTGTGTCGCTATCACTGGCAA CC ATTCGTAACCCGCTCTCG TCC	617	59˚C 40 sec.	Murugkar *et al.*, 2003
avrA	CCT GTA TTG TTG AGC GTC TGG AGA AGA GCT TCG TTG AAT GTC C	422	58˚C 40 sec.	Huehn *et al.* 2010
sopB	TCAGAAGRCGTCTAACCACTC TACCGTCCTCATGCACACTC	517	58˚C 40 sec.	Thung et al., 2018
ompF	CCTGGCAGCGGTGATCC TGGTGTAACCTACGCCATC	50	519	
invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	55	284	Salem et al., (2017)
Mgtc	TGACTATCAATGCTCCAGTGAAT ATTTACTGGCCGCTATGCTGTTG	58	655	Huehn et al.,(2010)
Ssaq	GAATAGCGAATGAAGAGCGTCC CATCGTGTTATCCTCTGTCAGC	58	677	

6. Experimental design

The following protocol was approved by (ARC-IACUC) committee, Animal Health Research Institute and IACUC protocol Number was ARC-AH-22-24.

6.1. Chicks Husbandry

The challenge involved 100-sixty commercial Cobb broiler chicks that were one day old. We randomly selected ten chicks and used PCR techniques to test for the absence of bacterial pathogens such as *Salmonella*, M.G., MS, and E. coli infection **(Wafaa *et al.*, 2012).** Furthermore, 150 chicks were introduced to the challenge. The challenged model was housed in batteries in a biosafety level-2 experimental room at AHRI. Chicks were raised in temperature-controlled chambers. The chickens were fed commercially balanced diets, free of pathogens or medicinal additives. A random sample of the diet was examined for *Salmonella* before introducing two types of feed to the chicks: starting feed (1-15j) and finishing feed (15-35j). The birds received water and food without *Salmonella* ad libitum.

6.2. Management

Work has been carried out in three groups to investigate the virulence and pathogenicity of Salmonella typhimurium and Salmonella enteritidis strains in chicks. A total of 150 chicks were divided into three groups, each group containing 50 chicks, the first group inoculated with Salmonella enteritidis strain, a second group inoculated with *Salmonella typhimurium*, and the third group was unchallenged (negative control). The first and second groups were questioned orally with a single dose of approximately 0.5 ml of 1 x 10^9 CFU/ml of Salmonella enteritidis and Salmonella typhimurium at one day old age, respectively **(Okamoto *et al.*, 2007),** and this trial was continued until 35 days old. After 30 days after inoculation (P.I.), we divided the remaining chickens into seven groups to introduce 1% acetic acid **(Gulbeena *et al.*, 2016)** and sulfa drug (applied according to the disc diffusion test).

Later, at 30 days (P.I.), seven groups were identified: group 1 was the Salmonella enteritidis group; group 2 was the Salmonella typhimurium group; group 3 was *Salmonella enteritidis* treated with Acetic Acid; group 4 was Salmonella typhimurium treated with Acetic Acid, group 5 was Salmonella enteritidis treated with the sulfa drug; group 6 was Salmonella typhimurium treated with the sulfa drug; and finally, group 7 was an uninoculated group, which is a negative control.

6.3. Inoculum Preparation Challenge

Salmonella typhimurium and *Salmonella enteritidis* isolates were stored at -50°C in 13% glycerol stock in the RLQP inventory. First, culture the stored *Salmonella*, then separate a colony to prepare the inoculation by suspending it in sterile normal saline and adjusting the solution to the appropriate concentration **(Mashael *et al.*, 2020b).**

6.4. Salmonella typhimurium and Salmonella typhimurium capability for infectiousness induction

Transient tests were conducted for selected Salmonella enteritidis and Salmonella typhimurium and were carried out in six 1day-old chicks (three chicks for each strain) by inoculation with

approximately 1 x 10^5 CFU/ml. Additionally, selected Salmonella typhimurium and Salmonella enteritidis reisolated from the internal viscera were grouped by(**Francesca *et al.*, 2014)** and **(ISO 6579-1 2020).**

6.5. Preparation of the agent used

Route of administration of acetic acid in drinking water (1%) At 30 days of P.I., we introduced the sulfa drug at 0.5 ml/ Kg orally, which was added daily for five days.

6.6. Growth performance

Weighing all birds at variable intervals and feed intake to determine feed consumption, body weight gain, and feed conversion ratio were calculated according to **(Pratima *et al.*,2020).**

6.7. Virulence study

Clinical signs and mortality were observed and recorded throughout the experiment **(Sanak *et al.*, 2019).** The postmortem lesion (PM)was performed on three necropsied chicks twice weekly (at 3 days,6 days,9 days,12 days,15 days,18 days,21 days,24 days,27 days and 30 days).

Internal organs (liver, cecum, heart, spleen, lung, and gall bladder) were collected from euthanized chicks during PM, along with litter samples (once/week) for Salmonella shedding analysis **(Wafaa *et al.*, 2012 and ISO 6579-1: 2020).**

Take a sample from the duodenum with hemorrhagic spots at 21 days of age for Clostridium perfringens testing. Cooked meat medium (Becton Dickinson and Company, Sparks, Maryland, USA), 10% sheep blood agar with neomycin (40 g / mL), and tryptose-sulfite-cycloserine agar plates (TSC) were used for isolation. They were incubated in an anaerobic jar containing GasPak TM (Oxoid Limited, Thermo Fisher Scientific Inc., U.K.). Egg yolk agar (Oxoid Limited, Thermo Fisher Scientific Inc., U.K.) to detect lecithinase activity and litmus milk medium (Oxoid Limited, Thermo Fisher Scientific Inc., U.K.) to detect the stormy fermentation reaction to characterize the Clostridium periferingens **(Tessari *et al.*, 2014).**

Furthermore, the enumeration of *Salmonella* strains from liver and cecum samples was performed at 33 and 35 days to determine the efficacy of acetic acid as a tool to control infection.

7. Statistical analyses

Data were analyzed using the ANOVA test.

RESULTS

Salmonellosis was 9% (21/231) in imported duckling flocks in Egypt in 2019, *Salmonella enteritidis* and *Salmonella typhimurium* were, accounting for 1.73 % (4/21) and 0.43 % (1/21) of the total. The phenotypic antimicrobial susceptibility test was applied to Salmonella enteritidis and Salmonella typhimurium strains against thirteen commercial antibiotic discs. Salmonella enteritidis is resistant to lincomycin, clindamycin, ampicillin, nalidixic acid, streptomycin, tetracycline, cefotaxime, and ciprofloxacin and intermediate resistance to norfloxacin and levofloxacin, but sensitive to Colistin, trimethoprim-sulfamethoxazole, and apramycin. Salmonella typhimurium was resistant to lincomycin, clindamycin, and nalidixic acid and was intermediate resistant to streptomycin and ciprofloxacin but sensitive to ampicillin, norfloxacin, Colistin, levofloxacin, trimethoprim-sulfamethoxazole, apramycin, tetracycline, and Cefotaxime. 100% resistance to lincomycin, ampicillin, clindamycin and nalidixic acid was reported. Salmonella enteritidis had 61% resistance to multidrug resistance, while Salmonella typhimurium had 20% resistance**.** Variable SPI genes (stn, avrA, sopB, ompF, mgtc, Ssaq, and invA) were found in *Salmonella* strains (Table 2).

Table 2: Antimicrobial resistance profile and virulence genes of Salmonella Enteridits and Salmonella Typhimurium

Antimicrobial drugs	**Zone of inhibition interpretation**			**Antibiotic Resistance profile**	**Detection of Virulence Genes by PCR**
	Resistant	Intermediate -resistant	Sensitive		
Salmonella enteridits	8/13 (61%)	2/13 (15%)	3/13 (20%)	Ampicillin,Cefotaxime, Ciprofloxacin,Clindamycin, Lincomycin, Nalidixic acid, Streptomycin, and Tetracycline.	Stn, avrA, sopB, ompF, invA, Mgtc and Ssaq were detected.
Salmonella typhimurium	3/13 (20%)	2/13 (15%)	8/13 (61%)	Clindamycin, Nalidixic acid Lincomycin	Stn, avrA, sopB, ompF, invA, Mgtc, and Ssaq were detected.

The symptoms of the chicks in an experiment are described. The infected groups showed dullness, depression, diarrhea, pasty vent, and gasping until the end of the experiment, while the control group showed no signs. The mortality rate in the Salmonella typhimurium group was 4% (2/50) on the 18th day, with no evidence of outbreaks in the other two groups. Postmortem lesions of the infected groups (Table 3) showed unabsorbed yolk atrophied and liver necrosis, congestion, lung congestion, and hemorrhage. On 3days of P.I., the Salmonella typhimurium group had skull bleeding**,** ascites, internal visceral bleeding, and clotted blood in the abdomen. The unabsorbed yolk sac, skull bleeding, and yellowish-coated abdominal material were also observed in the Salmonella enteritidis group at 6 days P.I. Pericarditis and petechial bleeding, pneumonia, air sacculitis, splenomegaly, ballooning of the intestine, clear fluid in the intestine, undigested food in the intestine, and an enlarged cecum were among the other reported lesions. Breast muscle bleeding was demonstrated, and the bronze liver was in the Salmonella enteritidis group. Furthermore, from 21day to 30 days of P.I., there were obvious hemorrhagic spots on the duodenum and catarrhal enteritis in infected groups. Liver congestion was observed at 6 days P.I., liver necrosis at 9 days P.I., liver haemorrhage at 18 days P.I., and spleen and lung congestion and haemorrhage were recorded at 3 days P.I., and the intestinal lesion was discovered at 3 days P.I. At 21 days P.I., a new button shape was seen in the liver of infected groups**.** At 15 days of P.I., the small intestine revealed mild catarrhal enteritis, congestion, and severe hemorrhages throughout the intestinal tract (Fig.1).

Table 3: Postmortem lesions scoring in experimental groups

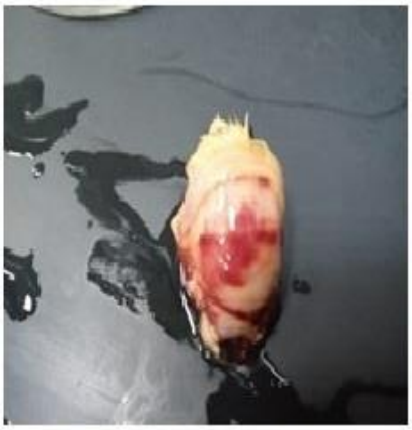
Postmortem Lesions / selected Days	**Inoculated groups**																					
	ST	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**	**ST**	**SE**
Days	**0**	**0**	**3**	**3**	**6**	**6**	**9**	**9**	**12**	**12**	**15**	**15**	**18**	**18**	**21**	**21**	**24**	**24**	**27**	**27**	**30**	**30**
Atrophied yolk			**3/3**	**3/3**																		
Unabsorbed yolk						**1/3**																
Lung congestion			**3/3**							**2/3**												
Lung H.				**3/3**																		
Pneumonia							**3/3**		**3/3**		**1/3**		**3/3**									
Liver necrosis			**3/3**	**3/3**			**3/3**	**1/3**	**1/3**		**1/3**		**2/3**						**2/3**		**2/3**	
Liver H.													**1/3**									
Liver congestion					**2/3**	**3/3**		**2/3**	**1/3**		**1/3**			**2/3**			**3/3**		**1/3**		**1/3**	
Bronze liver												**2/3**		**1/3**		**3/3**	**3/3**			**3/3**		**3/3**
Per hepatitis										**2/3**												
Skull hemorrhage			**3/3**		**3/3**																	
Skull congestion						**3/3**				**2/3**												
Enteritis					**3/3**		**3/3**	**3/3**														
Froth cecal content			**3/3**																			
Clear fluid ceal content			**3/3**				**3/3**						**3/3**		**3/3**		**3/3**		**3/3**		**3/3**	
Enlarged cecum								**1/3**						**3/3**		**3/3**		**3/3**		**3/3**		**3/3**
Undigested food								**1/3**														
Ballooning intestinal tract							**3/3**						**3/3**		**3/3**		**3/3**		**3/3**		**3/3**	
Ascitis			**3/3**																			
Pericarditis						**1/3**	**3/3**		**3/3**		**3/3**		**3/3**		**3/3**							
Pericardial hemorrhage							**3/3**															
Internal hemorrhage			**3/3**																			
Yellowish chessy material						**1/3**																
Airsaculitis							**3/3**		**3/3**		**3/3**		**3/3**		**3/3**		**3/3**		**3/3**		**3/3**	
Splenomegaly							**2/3**															
Breast muscle H.										**2/3**	**3/3**		**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**
h.patches on duodenum															**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**
Button shape in liver															**2/3**							

ST= Salmonella typhimurium SE= Salmonella enteridits

Three chicks from each group were exposed for postmortem examination through-out the experiment in group at (3 day,6 day,9 day,12 day,15 day,18 day,21 day,24 day,27 day and 30 day).

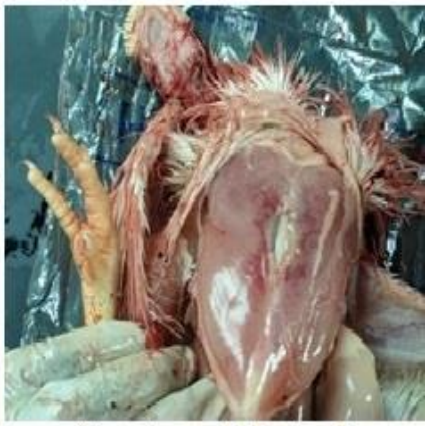
Number from 1 to 3 indicate number of necropsied chicks showed lesion

Figure 1: salmonellosis gross lesions

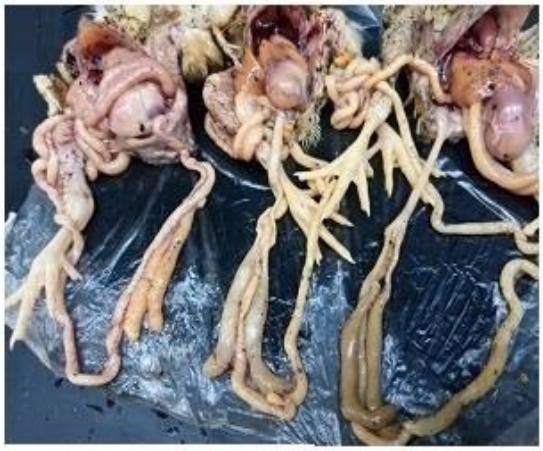


a: Skull hemorrhage

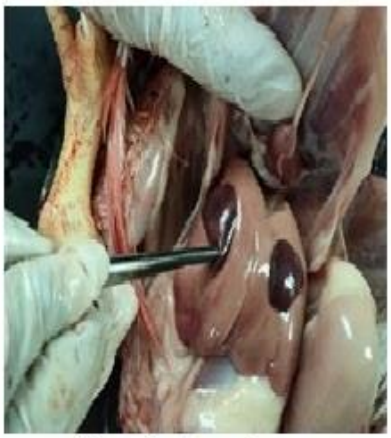
b: Clotted blood



c: Breast muscle hemorrhage

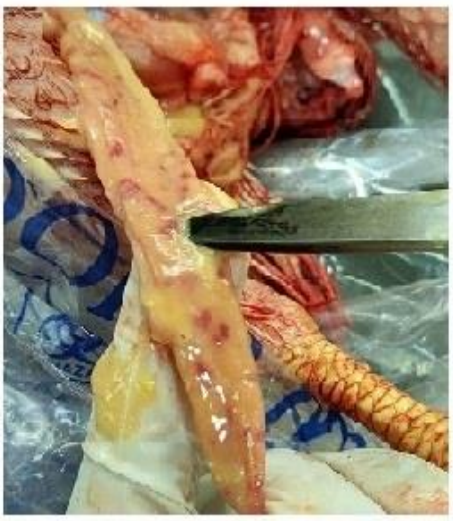


d: Intestinal lesions



e: Liver button shape

f:Hemorrhagic spots on duodenum



g: Catarrhal enteritis and hemorrhagic spots on duodenum

Clostridium perfringens from the duodenum was phenotypically characterized in infected chicks, accompanied by salmonella infection (Table 4).

Table 4: Intercurrent of *Salmonella* and *Clostridium perfringens*:

Days of experiment	Duodenal lesions		Liver lesion * Liver button shape		Clostridium perfringens *isolation* from the duodenum**			Salmonella isolation (Cecum and litter)		
21 days of age										
	S.E. group	S.T. group	S.E. group	S.T. group	S.E. group	S.T. group	Control group	S.E. group	S.T. group	Control group
Number of examined necropsied chicks	3	3	No lesion	2	Detected	Detected	Not detected	Detected	Detected	Not detected
24 days of age										
Number of examined necropsied chicks	3	3	No lesion	No lesion	Detected	Detected	Not detected	Detected	Detected	Not detected
27 days of age										
Number of examined necropsied chicks	3	3	No lesion	No lesion	Detected	Detected	Not detected	Detected	Detected	Not detected
30 days of age										
Number of examined necropsied chicks	3	3	No lesion	No lesion	Detected	Detected	Not detected	Detected	Detected	Not detected

Salmonella strains were isolated in varying percentages from organs of infected groups and recovered from the litter weekly but not from the negative control group. The growth performance of the chicks is determined weekly using body weight, weight gain, feed consumption, and feed conversion ratio characteristics until they are 30 days old. Infected birds showed decreased body weight, weight gain, increased feed intake, and less efficient conversion rates (Fig.2).

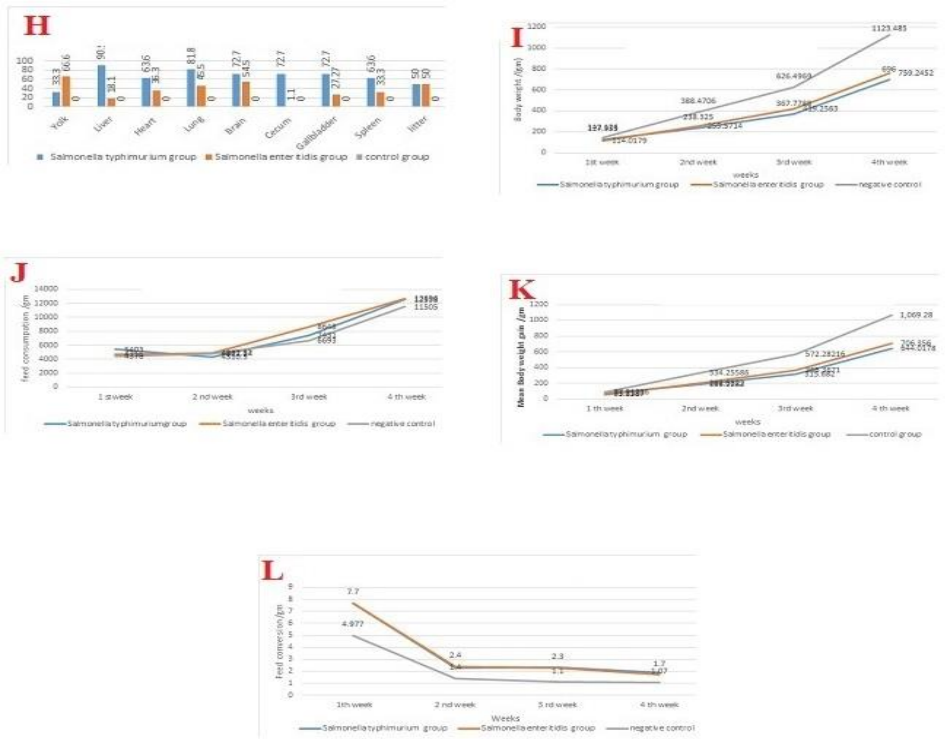


Fig.2: Growth parameters

Finally, viable *Salmonella* counts from the liver and cecum were used to determine the antibacterial activity of 1% acetic acid administration. After 5 days of P.I., the *Salmonella* cell count was higher in the acetic acid-treated groups than in the PI-treated groups after 3 days of P.I. This explains why acetic acid has bacteriostatic activity against Salmonella, while the Sulfa drug-treated group outperformed the treatment group (Table 5).

Table 5: Mean of most probable number (MPN) of salmonella count after sulfa and acetic acid treatment.

The organ used for salmonella count	Grouping of chickens for treatment						
	S.E. group	S.T. group	S.E. with the acetic acid group	*S.T. with Acetic acid group	S.E. with sulfa group	S.T. with sulfa group	Blank group
**liver $_a$ After 3 ays	150x10^1 CFU/gm	300x10^1 CFU/gm	1x10^1 CFU/gm	*1x10^1* CFU/gm	Less than x10^1CFU/g	Lessthanx10^1 CFU/gm	Zero
**cecum $_a$ After 3 ays	450 x10^1 CFU/gm	750 x10^1 CFU/gm	4.3x10^1 CFU/gm	*34x10^1* CFU/gm	Less thanx10^1 CFU/gm	Less than x10^1CFU/gm	Zero
***Liver $_b$ After 5 day	66.6x10^1 CFU/gm	133.33x 10^1 CFU/gm	100x10^1 CFU/gm	*166.6x10^1* CFU/gm	Less thanx10^1 CFU/gm	Less thanx10^1 CFU/gm	Zero
***Cecum After 5 days	100x10^1 CFU/gm	150.6x1 0^1 CFU/gm	250x10^1 CFU/gm	*148x10^1* CFU/gm	Less thanx10^1 CFU/gm	Less thanx10^1 CFU/gm	Zero

DISCUSSION

Salmonellosis is a foodborne disease that can be fatal to humans and animals. It also contains virulence genes on *Salmonella* pathogenicity islands (SPI), responsible for *Salmonella* invasion and pathogenesis in host cells. Studying and detecting genes encoded on Salmonella pathogenicity islands is an important procedure for understanding disease pathogenicity mechanisms and planning salmonellosis control and prevention in poultry.

Salmonella infection in imported poultry can serve as a source of bacterial evolution. The horizontal transmission of virulence genes results in new pathogenic strains that spread to new locations and hosts. Salmonella enteritidis and Salmonella typhimurium were isolated from imported ducklings, similar to what we discovered **(kamelia *et aL.*, 2014; Mohamed *et al.*, 2017).**

Furthermore, this result differs from that of **El-gaos *et al.*, (2020)** who revealed that Salmonella typhimurium had the highest percentage. In this work, Salmonella enteritidis was found to be completely resistant to lincomycin, clindamycin, ampicillin, nalidixic acid, streptomycin, tetracycline, cefotaxime, and ciprofloxacin. At the same time, *Salmonella typhimurium* was completely resistant to lincomycin, clindamycin, and nalidixic acid**.** However, multidrug-resistant Salmonella strains have been established and spread globally due to widespread antibiotic use, posing a serious threat to global public health (**Gong *et al.*, 2013).**

Engy *et al.*, (2021) reported that Salmonella typhimurium (93.3%) and Salmonella infantis (6.7%) isolates were 100% resistant to cephradine and amoxicillin but resistant to colistin sulfate (80%), streptomycin (60%), chloramphenicol (33.3%), ampicillin, and neomycin (26.7% of each) discovered that Salmonella isolated from poultry were more resistant to penicillin than ampicillin.

Our results reported that different virulence genes (stn, avrA, sopB, ompF, invA, Mgtc, Ssaq) encoded variable SPI genes (SPI1, SPI2, SPI3,and SPI5) detected by PCR strains of Salmonella typhimurium and Salmonella enteritidis. **Siddiky *et al.*, (2021)** discovered virulence genes in *Salmonella typhimurium* and *Salmonella enteritidis* strains and reported that isolates carried virulence genes invA.

The Salmonella pathogenicity island 2 contains the type III secretion system, which plays an important role in the pathogenesis of Salmonella infections. SPI genes encode many functional proteins involved in cell

invasion and host-bacterial cell interaction **(Bhowmick *et al.*,2019)**.

The invasion gene (InvA) is responsible for stimulating Salmonella invasion in epithelial cells **(El sharkawy *et al.*, 2017)**. It is the only exhibit found in Salmonella and is used for Salmonella genotyping **(Fekry *et al.*, 2018).** *Salmonella* species contain invA and inv*a*E/A, *ssa*Q, *mgt*C, *spid*R and *sop*B genes **(Nabila and Dina, 2017).**

Furthermore, **Saleh, (2019)** reported that numerous virulence markers and determinants, including flagella, capsule, plasmids, adhesion systems, and type 3 secretion systems encoded on the Salmonella pathogenicity island (SPI)-1 and SPI-2 and other SPIs, have been demonstrated to play pivotal roles in its pathogenesis.

Ernes *et al.*, (2019) found that the 27 Salmonella enteritidis strains tested positive for virulence genes such as invA, ssaQ, and mgtC in Salmonella. **Asmaa *et al.*, (2016)** discovered the presence of InvA, the ssaQ gene, and mgtC. The InvA, OmpA, and Stn Genes genes play important roles in the pathogenicity of salmonellosis in poultry **(Fekry *et al.*, 2018).**

Diarrhea was evident in the Salmonella typhimurium and Salmonella enteritidis groups, as reported by **Mashooq *et al.*, (2019).** On the 18th day, the mortality rate in groups challenged with Salmonella typhimurium was 4%, but no other deaths were recorded in groups inoculated with Salmonella enteritidis, in contrast to **Muna *et al.*, (2016),** which recorded 25% mortality in both groups *(Salmonella enteritidis* and *Salmonella typhimurium*). Liver lesions were observed as liver congestion at 6-day-PI, liver necrosis at 9-day P.I., liver hemorrhage at 18-day P.I., and lesions of the spleen and lung (congestion and hemorrhage) were found at 3-day-PI. The intestinal lesion was found at 3 days P.I., similar to PM lesions mentioned by **Shah *et al.*, (2013)** as lung and spleen congestion and hemorrhage.

Furthermore, in Salmonella-infected chicks, **Sharkawy *et al.*, (2017)** observed pasty diarrhea and decreased body weight**. Sherrill**, **(2019)** stated that salmonellosis lesions in poultry were found to be friable and stained with bile, with or without necrotic foci in the liver, swollen spleen and kidneys, anemia, and enteritis**. Mashoomq *et al.*, (2019)** found severe liver congestion, hemorrhages, and hepatomegaly up to 15-PI, with liver necrosis occurring at 9-day-PI. Congestion and haemorrhages in the spleen and lung tissues can occur up to 15 days P.I. New pathological images were recorded. At 3-days of P.I., the first signs of skull haemorrhage were observed in both the *Salmonella enteritidis* and *Salmonella typhimurium* infected groups.

At 21 days of P.I., the shape of the liver button in the Salmonella typhimurium group. Salmonella has the ability to induce necrotic enteritis, as demonstrated by **Shivaramaiah *et al.*, (2011).** Salmonella infection triggers Clostridium perfringens proliferation in experimental chicks, so necrotic enteritis is believed to be a sequela of salmonellosis.

Salmonella infection is caused by a pathogen with a complex tolerance action in the presence of organic acids like acetic acid **(Raquel *et al.*, 2014).** The failure of 1% acetic acid to eliminate the infection in this study, Salmonella Typhimurium can produce organic acid tolerance, raises concerns about food safety because this serotype is one of the most commonly recorded deaths and cases of food poisoning (**Hendriksen *et al.*, 2011). Gulbeena *et al.*, (2016)** reported that 1% acetic acid supplementation demonstrated partial protection by showing better growth performance, lower feed conversion ratios, and lower gross and histopathological change rates.

In this study, the infected chicks showed a decrease in body weight, a gain in body weight, and increased feed consumption, and the feed conversion ratio is less valuable in comparison with uninoculated chicks, which is consistent with **Sanak *et al.*, (2019).**

CONCLUSION

Monitoring salmonella species in imported and native birds are the main routine work and control strategy for disease prevention; furthermore, decontamination of Salmonella-positive flocks is the policy in Egypt to protect the poultry industry. *Salmonella enteritidis* and *Salmonella typhimurium* infection play a role in the horizontal transmission of virulence genes (SPI) and the generation of new pathogenic bacteria that will be adapted to be located in new areas and new hosts. Salmonellosis causes a new pathological lesion that significantly increases broiler susceptibility to necrotic enteritis, implying that controlling salmonellosis in broilers is economically important. Due to a complex tolerance mechanism, use caution when using acetic acid to control infection.

Conflict of interest

The authors declare that they are not involved in any potential conflicts of interest.

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