Pathology of broiler chicks naturally infected with *Salmonella enteritidis* (*S. enteritidis*) &*Salmonella typhimurium* (*S. typhimurium*) during an outbreak in Sudan.

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Abstract:

Aims of the study: To isolate and identify bacteria causing the disease, Characterize the bacterial isolates uses the automated machine vitek 2 compact, serotyping and Phage typing of bacterial isolates and study the histopathological finding due to the causative agents.

Place of Study: The samples collected from poultry farm included liver, intestine, kidney, spleen, heart, trachea and brain. Then transported immediately on ice to the Veterinary Research Institute, Soba for isolation, identification and characterization of bacteria.

Study design: A total of 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 liver, intestine, kidney, spleen, heart, trachea and brain.

Methodology: 52 samples from that organs were Isolated and identified according to bacteriological standard methods.

Automated system Vitek 2 compact was used to confirm and characterize the isolates.

Serotyping and phagetyping of isolates were done as further characterization.

Gross and histopathological lesions on different tissues were studied. All the histopathological pictures were found similar to those done by the previous researchers.

1. Introduction:

Among the food-borne pathogens the genus *Salmonella* is one of the most common causes of foodborne infections worldwide [1, 2]. More than 2,500 different serovars of *Salmonella enterica* had been identified and most of them had been described as the cause of human infections, but only a limited number of serovars are of public health importance. Most reports have mentioned *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis as the most common causes of human salmonellosis world wide [3, 4]. *S. enteritidis* was the most prevalent serovar isolated from patients and food preparations in a survey conducted in southern Brazil from 1999 to 2008 [5]. It was estimated that approximately 75% of human Salmonella infection cases were due to contaminated food products derived from beef, pork, poultry and eggs [6] Poultry often become infected through the consumption of contaminated feed, cross-contamination in breeding houses, or during slaughter and processing [7]. An infection with Salmonella usually starts by ingestion, followed by colonization in the intestine. After colonization, Salmonella is able to penetrate the mucosal epithelium which results in a systemic infection, with colonization of the spleen and liver [8]. With increasing regulatory pressure placed on poultry and livestock processors to reduce pathogen contamination in processed meats, more emphasis is likely to be focused on reducing pathogen contamination on farms [9]. Therefore, development of a

rapid and sensitive method to Salmonella spp and their Serovars is desirable. Several techniques for improving the detection of Salmonella Serovars in fecal material such as the use of a selective culture medium and enzyme-linked immunosorbent assay have been developed [10,11]. However, problems remain with sensitivity and specificity that have limited routine use of these procedures. In general, these methods are laborious and time-consuming, in contrast with molecular methods that reduce the time of diagnostic with the same efficiency [12, 13].

1.1 The objectives:

The objectives of the study was to isolate and identify bacteria causing the disease, Characterization of the bacterial isolates uses the automated machine vitek 2 compact, serotyping and Phage typing of bacterial isolates and study the histopathological finding due to the causative agents.

2. Material and methods:

A total of eight thousand (8,000) broiler chicks, of the 'Ross' breed, were bought for commercial benefits in March 2014. Mortality was observed in 2,000 (25%) of the total chicks; therefore, 52 samples were taken from different organs for culturing, identification and characterization of causative agents. Due to mortality that was started at the first day, postmortem was done to investigate the gross lesions and taking samples from liver, intestine, kidney, spleen, heart, trachea and brain. All tissue samples were collected and handled aseptically to prevent cross contamination using sterile sampling materials.

2.1 Bacterial isolation and identification:

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 selenite broth medium and incubated at 37°C for 18-24 hours then purified on nutrient agar, macconkey agar and xylose-lysine-desoxycholate agar

(XLD). Cellular, colony morphology and biochemical characteristics of each isolate were tested.

Conventional identification was done according to [14].

2.2 Characterization of bacteria using Vitek 2 Compact:

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; 1 8]. 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.

2.3 Salmonella Serotyping and Phage typing:

Ten presumptive *Salmonella* isolates (selected based on their biochemical reactions and vitek 2 compact results) were shipped to the Public Health Agency, Office International des´ Epizooties (OI´E) Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada of serotyping and phagetyping. The antigenic formulae Of Popoff and Le Minor [16] were used to name the serovars. Phagetyping was performed using the standard phagetyping technique described by Anderson and Williams [1].

2.4 Histopathological method:

Tissue specimen collected for histopathological examination were fixed in 10%formalin solution, processed by standard paraffin embedding technique; microtetomy of the embedded tissue to 5-6 micron thick sections was carried out.

The sections were placed onto glass slides, dried and stained with hematoxylin and eosin (H&E) .

3. Results:

3.1 Conventional biochemical tests identification:

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, H_2S and methyl red tests and they were negative for indole, Voges-Proskauer and urease tests. The identity of suspected black colonies from XLD and pale colonies from macConkey agar were biochemically confirmed.

3.2 Vitek 2 Compact Automated System:

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

3.3 Serotyping and Phagetyping:

Serotyping test showed that all of the tested isolates (*n* =10) were members of *S. enteric* 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 *S. enteritidis* isolates were phagetype 3a while the *S. 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.

3.4 pathological finding:

3.4.1 Grossly:

The freshly dead birds showed discoloration and enlargement of liver, spleenomegaly, inflammation and thickening of intestinal mucosae. Necrotic foci on the surface of the spleen and liver, other changes included mild grayish nodular areas on the heart.

3.4.2 Histopathologically:

Liver:

Liver showed congestion, haemorrhage, focal degeneration and necrosis, inflammatory cells infiltration locally at perivascular areas and thrombi in central vein. hepatocytes with hydropic vaculation. Complete necrosis in some areas where debris replaced hepatocytes. Dilatation of sinusoids (Fig 1). Also thickening of liver capsule in some section and loss of liver cord appearance (Fig 2).

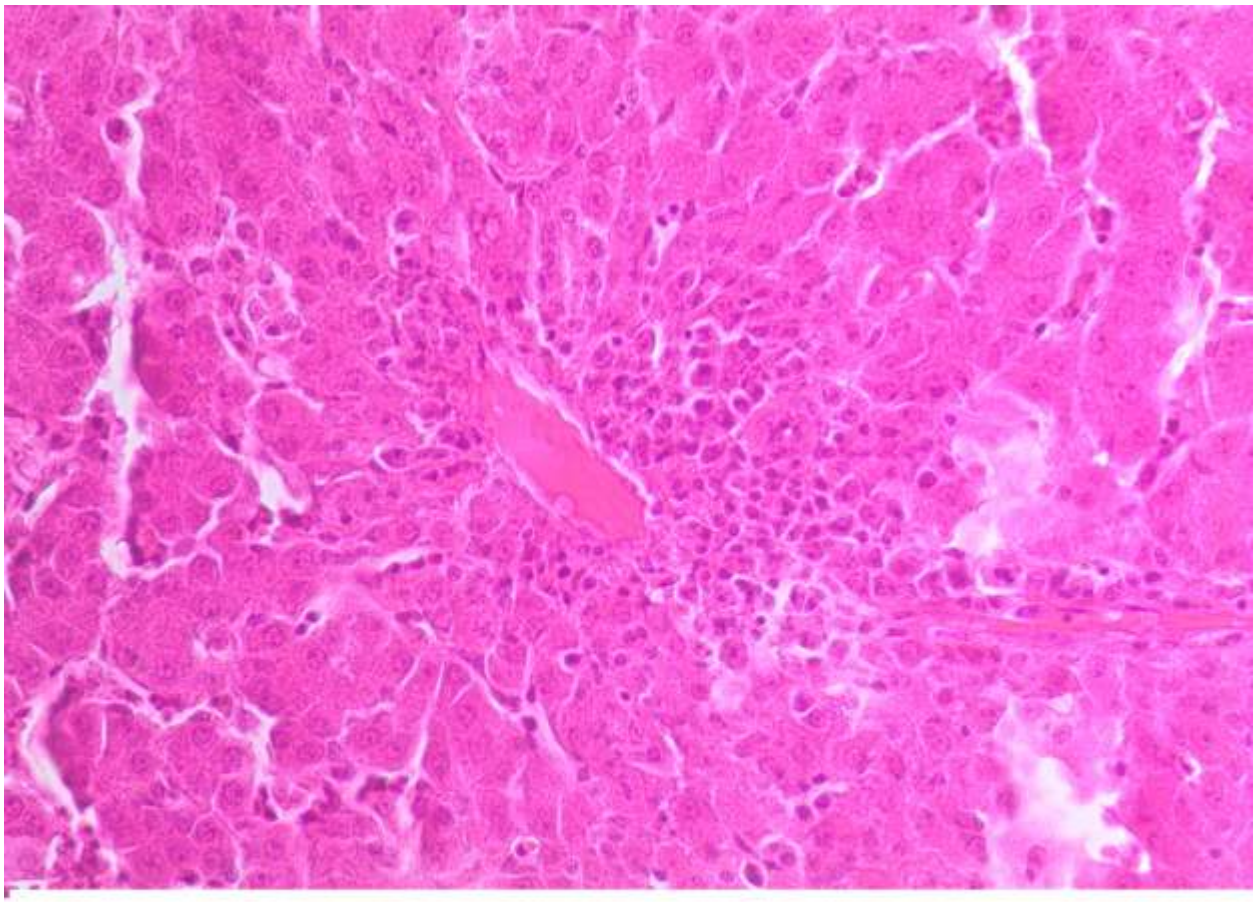

Fig 1:

liver section shows thrombi in central vein, necrosis, prevascular cuffing, dilitation of , sinusoid, vaculation, haemorrhge and infilltration of inflamatory cells.

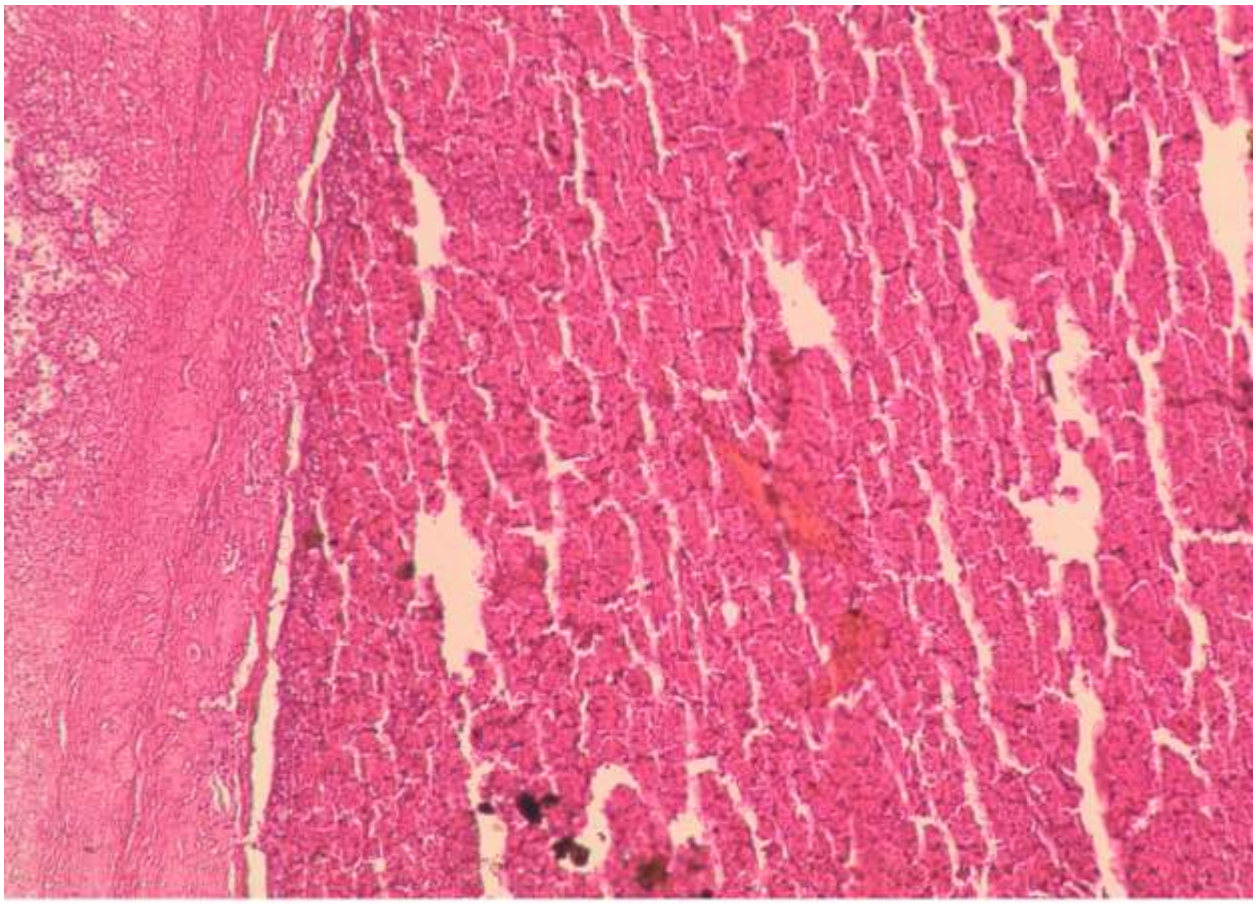

Fig 2:
liver section shows thickining of capsule, vaculations and loss of liver cord appearance.

Intestine:

In the intestine there was desquamation of mucosal epithelium resulting in denaturated villi where the lumen filled with necrotic masses (Fig 3). Severe Infiltration of inflammatory cells and atrophied of intestine glands (Fig4).

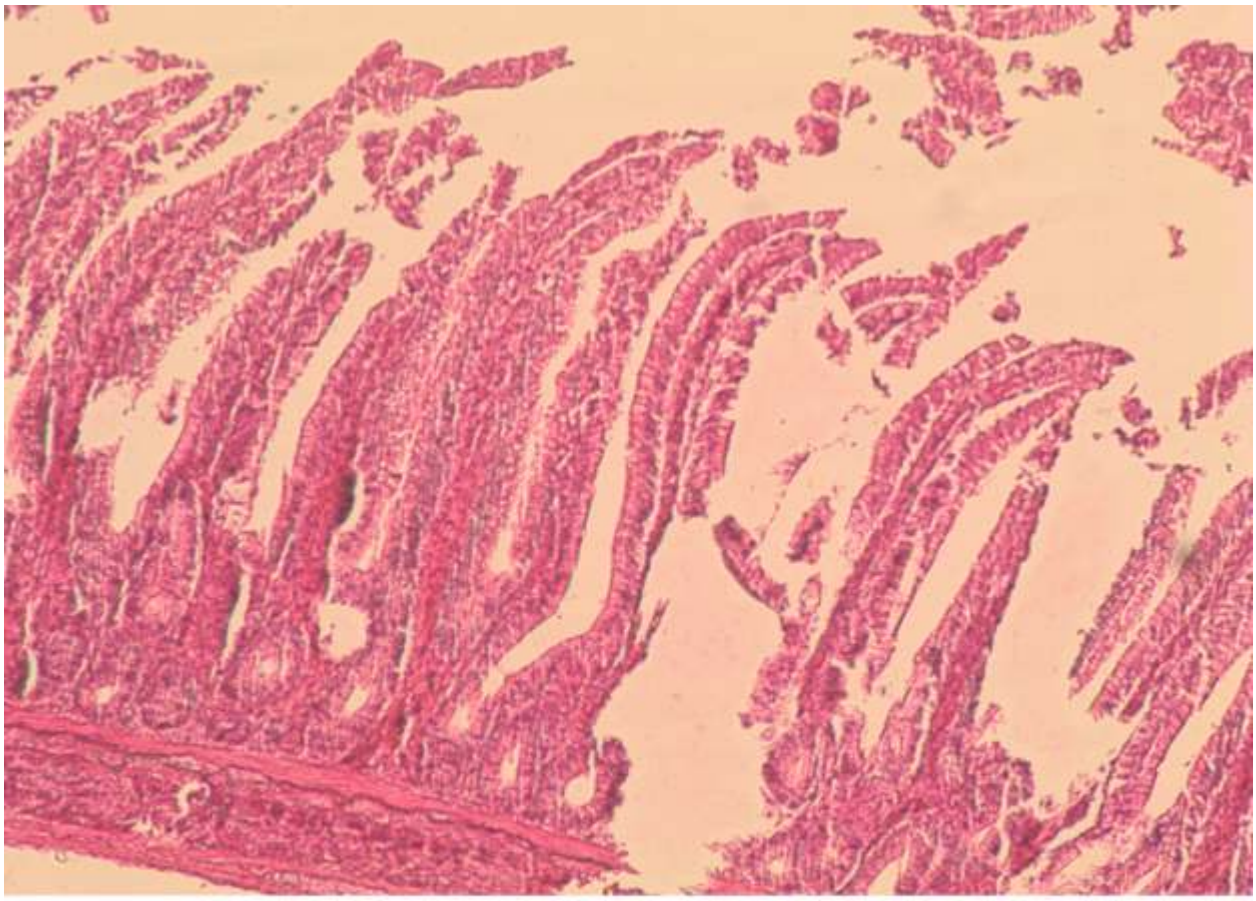

Fig 3:

inestine section shows sloughing of villi, necrrotic masses of intestinal lumen and infilltration of inflamatory cells.

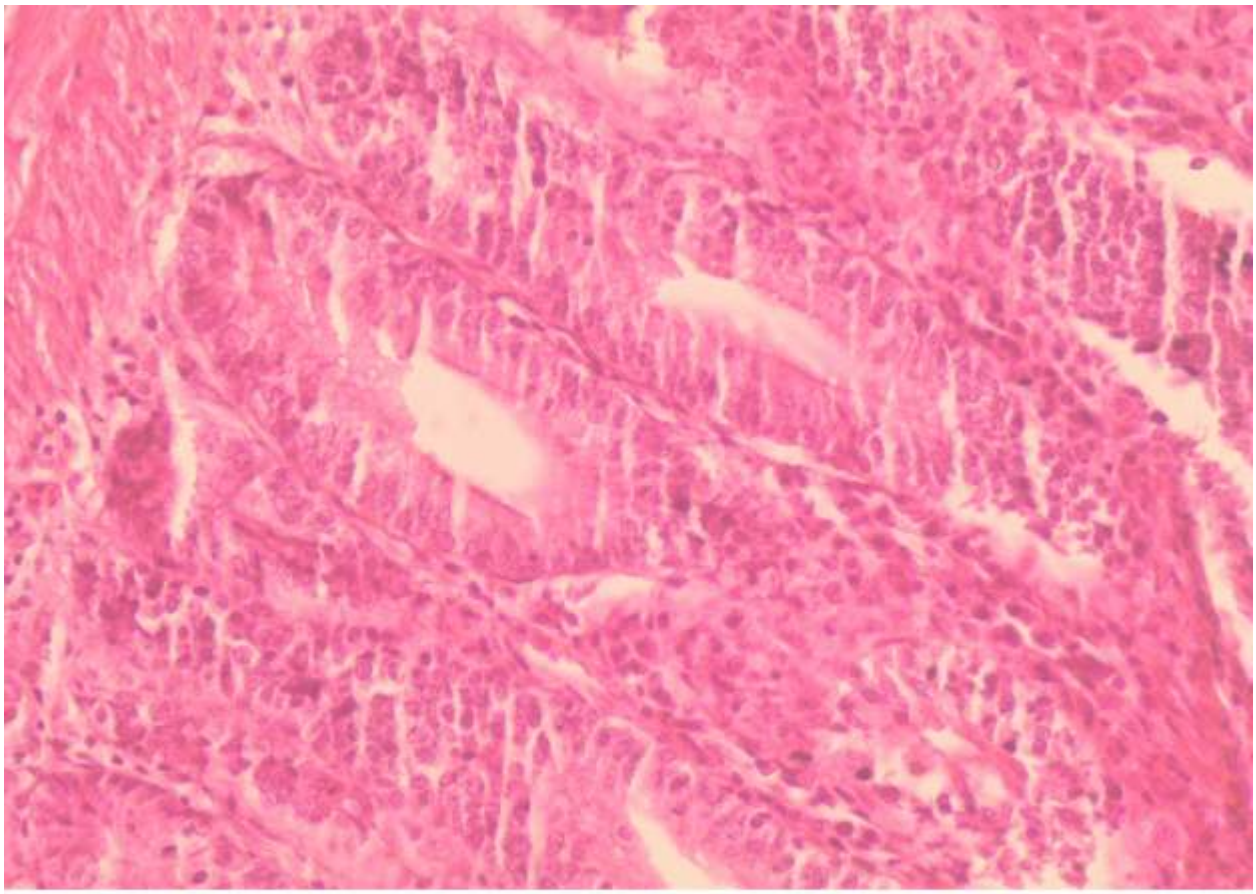

Fig 4:
intestine section shows severe infilltration of inflamatory cells and atrophied of intestinal glands.

Proventricular:

There is sloughing of epithelial layers and necrosis (Fig 5).

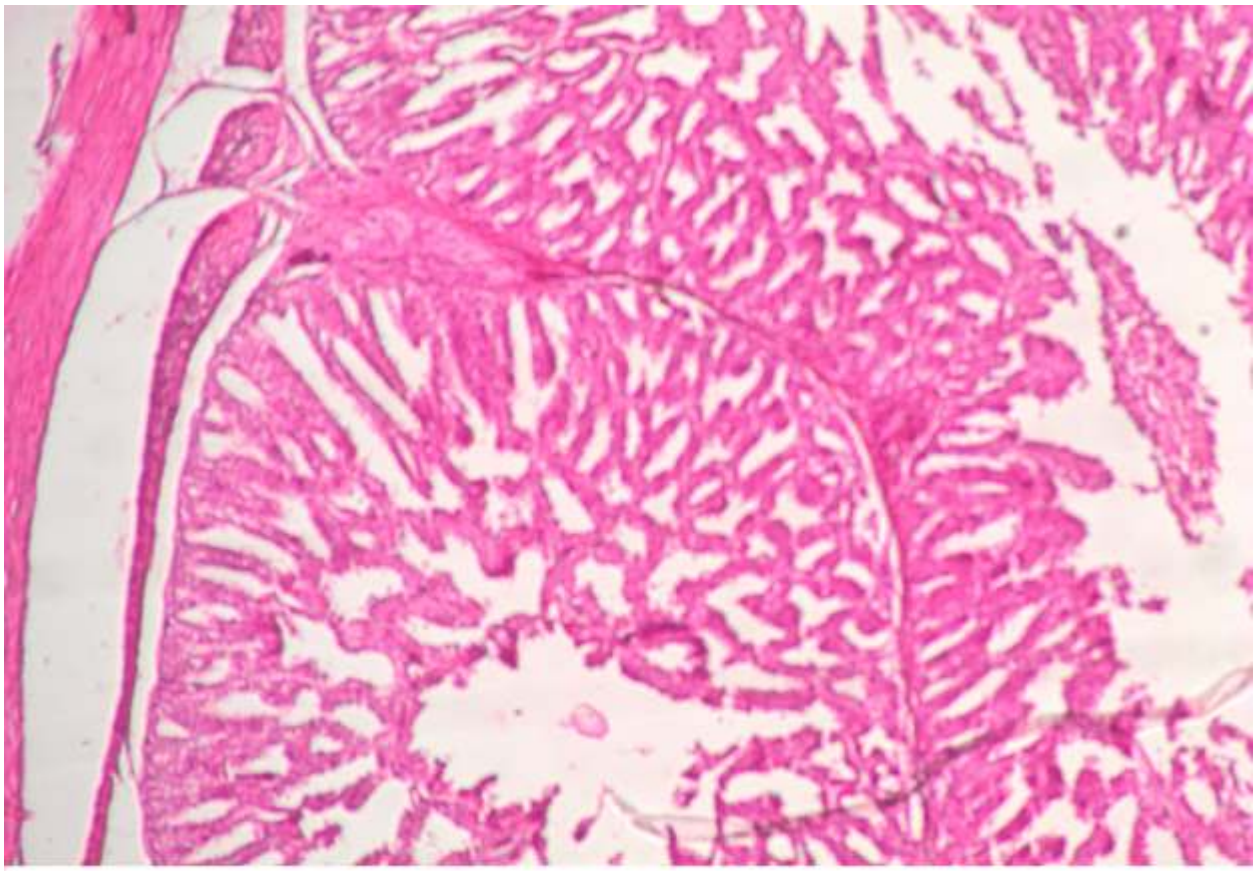

Fig 5:
proventriculus section shows sloughing of epithelial layers and necrosis.

Brain:

The brain showed vaculation, necrosis, haemorrhage, congestion of blood vessels and infiltration of inflammatory cells (Fig 6, 7).

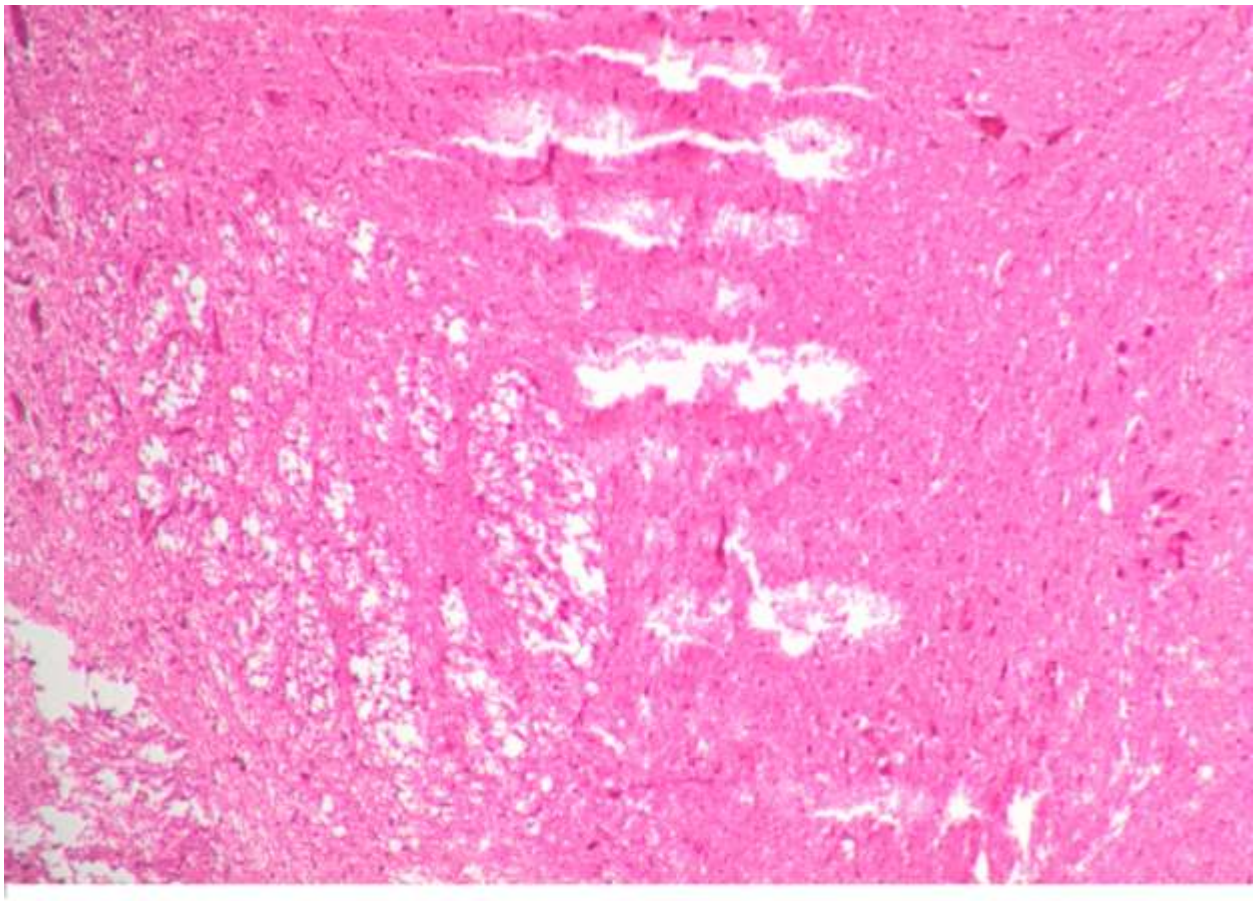

Fig 6:
brain section shows vaculation and necrosis.

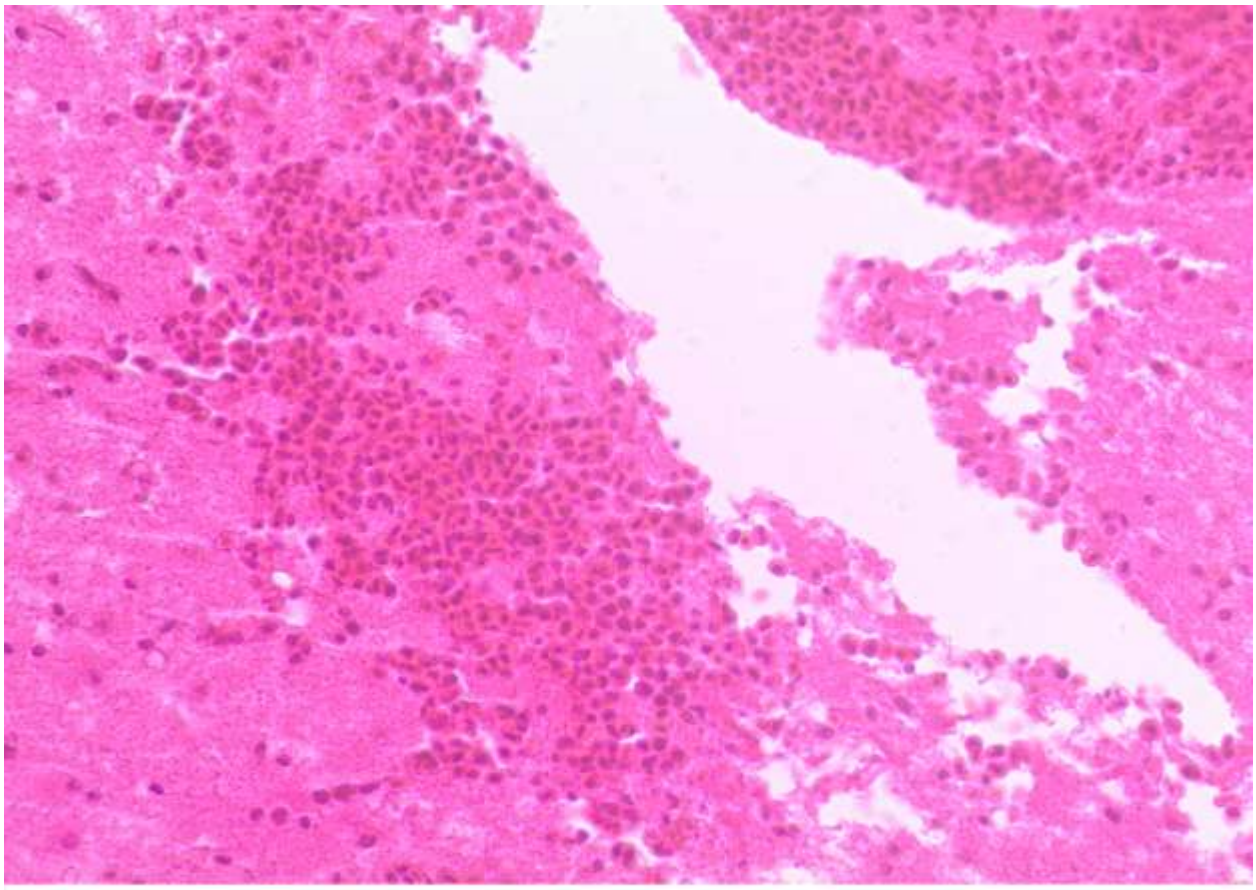

Fig 7:
brain section shows severe haemorrhge and necrosis.

Spleen:

The spleen showed haemorrhage, congestion, depletion of lymphocytes and round vaculation scatter along the spleen section (may be fatty changes) (Fig 8).

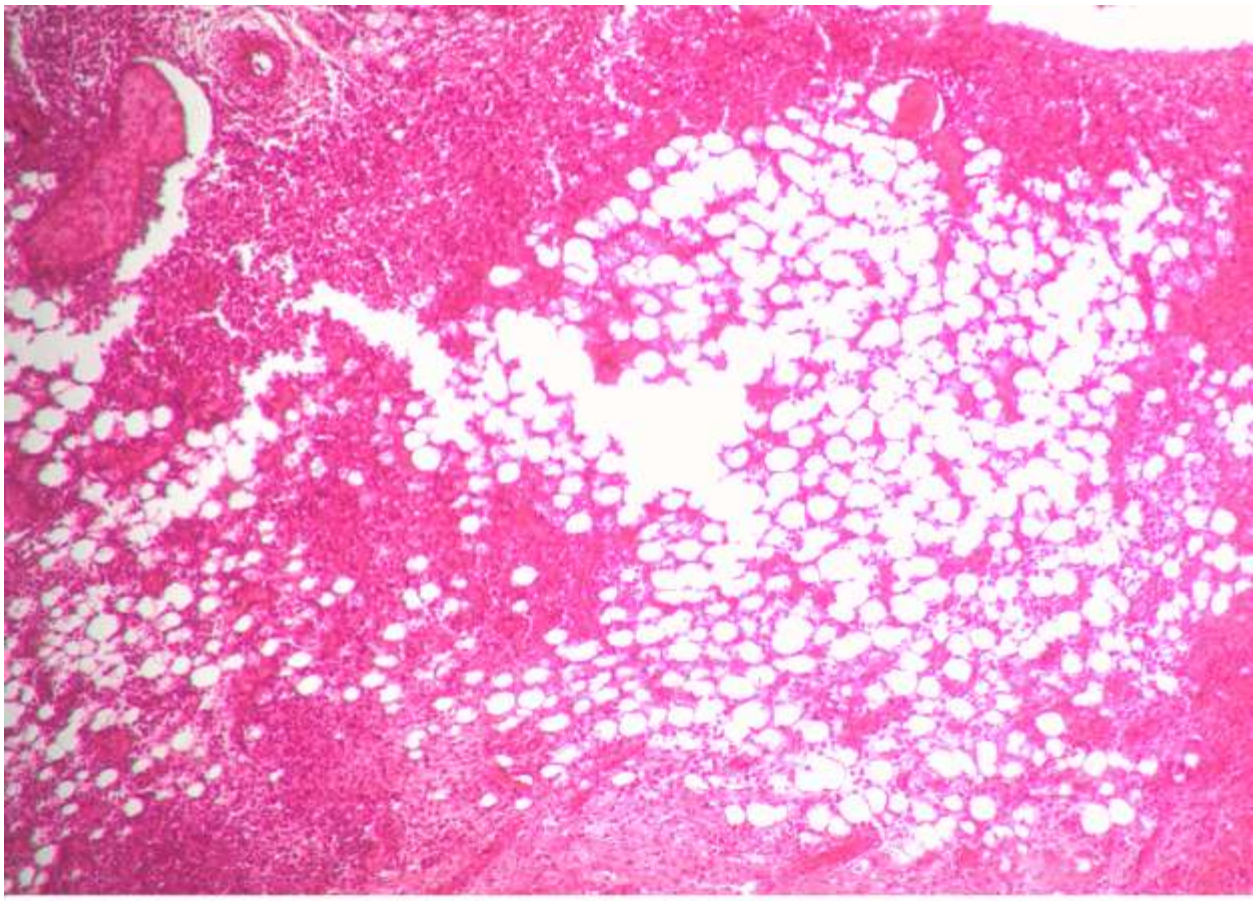

Fig 8:
spleen section shows haemorrage, congestion, depletion of lymphocytes and vacules.

Heart:

The heart section showed muscle congestion, fragmentation of myocardial muscle and fiber leucocytic infiltration (Fig 9)

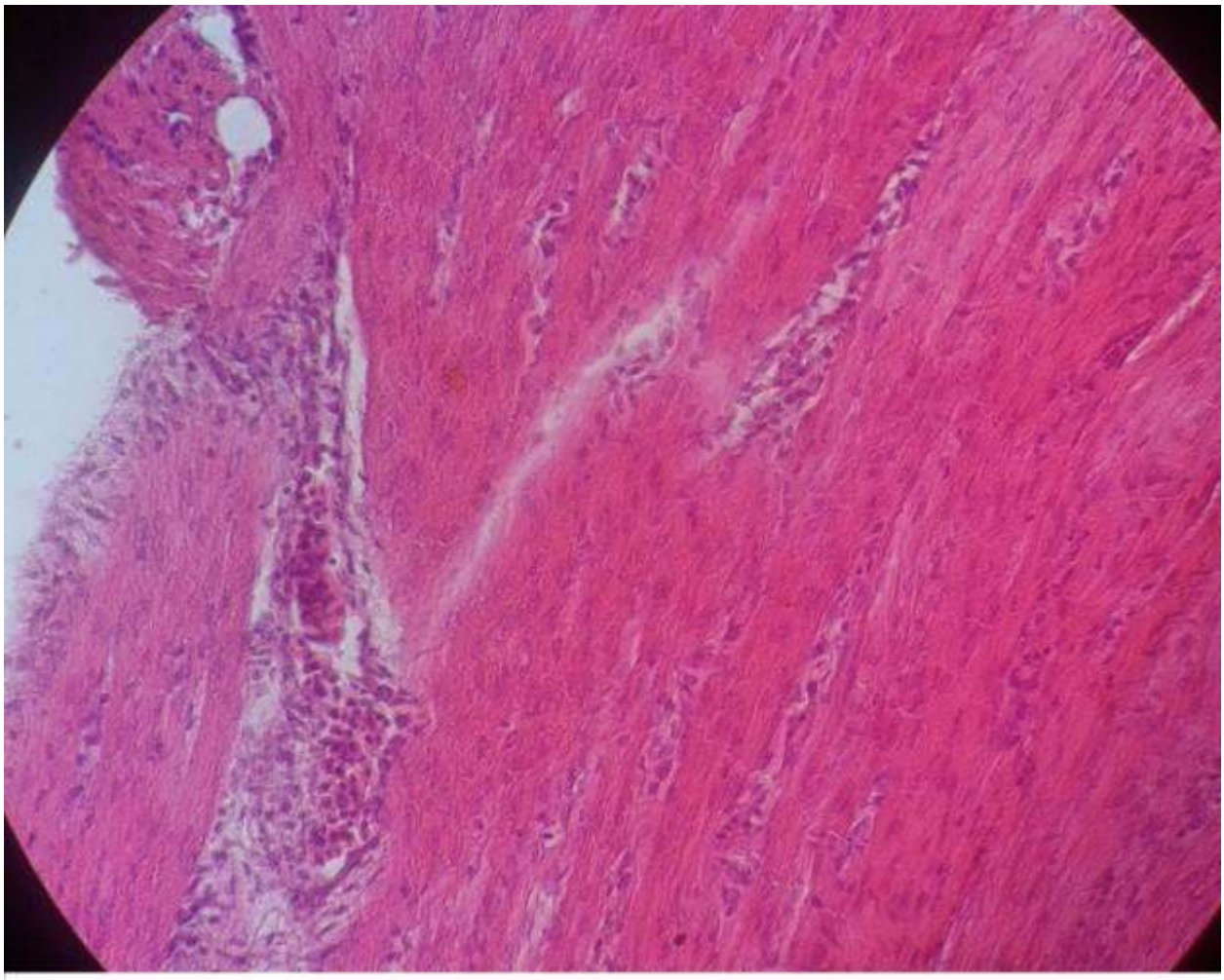

Fig 9:
heart section shows severe heamorrage, degeneration of myocadial muscle fiberand infilltration of inflamatory cells.

4. Discussion:

In this study *Salmonella* spp were identified in 52 samples out of 2000 chicks, ten of them were serotyped as *S. enteritidis* [n=9] and *S. typhimurium* [n=1] . These two serotypes are the most frequently isolates in poultry and poultry products and humans [18, 19, 20, and 21]. using of Vitek 2 Compact was significant as it was a full automating system that contain 64 biochemical tests to which is quite enough

to confirm the organism. In this study mortality started in day 1, this may due to vertical transmission of infections which has been an important aspect of the epidemiology of *Salmonella* species within the poultry industry [22; 23].

In 1980's *S. enteritidis* outbreaks dramatically increased globally and the pathogen emerged as serious threat for poultry industry and public health [24,15]. Since then the infections continued increasing over time, worldwide [26,27,28] and still continues to rise even though the overall incidence of *Salmonella* in general has decreased [29,28].There are various phage types of SE [30].The prevalent and dominant status of different phage types varies in different countries and may change in a country over time[31,32,33].. There is variation in the virulence among the various phage types and even within the various isolates of the same phage type [34, 35].The variation in virulence has also been reported among the same phage types being isolated from different locations [36]. *S. enteritidis* infection in adult chickens produces few clinical signs [37], but in young broiler chickens it may cause increased mortality and the culling of large numbers of chickens[38]. [39] studied The pathogenicity of *S. enteritidis* in Malaysia after experimental infection in newly hatched chicks it was determined on the basis of clinical signs of disease, mortality rate, body weight gain, bacterial isolation and, observations of gross and histopathological changes. he reported that the infection with SE PT3A and PT 35 caused 10% and 5 mortality, respectively during first week of age only, this is less than The mortality rate in this study which was 25% and near to [40] who reported 21%.

The gross lesions and histopathological findings observed in this study were consistent with previous studies in chickens [41]. The gross lesions of hepatomegaly, spleenomegaly and congested liver observed in this study were also similar to those in chickens reported by previous researchers [41, 42]. The gross lesions are highly indicative of septicaemic infection. The histopathogical

findings (cellular infiltration of the liver and heart, congested liver) in this study were also similar to previous works in chickens [43, 44].

5. Conclusions:

-Good hygiene must be applied in the hatcheries to avoid vertical transmission of salmonellosis.

-using automated system in identification of *Salmonella* spp is very important to get reliable and accurate results.

- Serotyping and phagetyping must be done to confirm the Serovar that causes the disease.

- High mortality can be observed specially in young broiler chickens when infected with pathogenic species like *S. enteritidis* and *S. typhimurium.*

- The isolation of organism from the liver, spleen, heart and intestine implying a septicemic condition.

- The histopathogical findings are similar in most species.

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