

Original research paper

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

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 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 phagotyping 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:

30 Among the food-borne pathogens the genus *Salmonella* is one of the most
31 common causes of foodborne infections worldwide [1, 2]. More than 2,500
32 different serovars of *Salmonella enterica* had been identified and most of them had
33 been described as the cause of human infections, but only a limited number of
34 serovars are of public health importance. Most reports have mentioned *Salmonella*
35 *enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis as the
36 most common causes of human salmonellosis world wide [3, 4]. *S. enteritidis* was
37 the most prevalent serovar isolated from patients and food preparations in a survey
38 conducted in southern Brazil from 1999 to 2008 [5]. It was estimated that
39 approximately 75% of human *Salmonella* infection cases were due to contaminated
40 food products derived from beef, pork, poultry and eggs [6] Poultry often become
41 infected through the consumption of contaminated feed, cross-contamination in
42 breeding houses, or during slaughter and processing [7]. An infection with
43 *Salmonella* usually starts by ingestion, followed by colonization in the intestine.
44 After colonization, *Salmonella* is able to penetrate the mucosal epithelium which
45 results in a systemic infection, with colonization of the spleen and liver [8]. With
46 increasing regulatory pressure placed on poultry and livestock processors to reduce
47 pathogen contamination in processed meats, more emphasis is likely to be focused
48 on reducing pathogen contamination on farms [9]. Therefore, development of a
49 rapid and sensitive method to *Salmonella* spp and their Serovars is desirable.
50 Several techniques for improving the detection of *Salmonella* Serovars in fecal
51 material such as the use of a selective culture medium and enzyme-linked
52 immunosorbent assay have been developed [10,11]. However, problems remain
53 with sensitivity and specificity that have limited routine use of these procedures. In
54 general, these methods are laborious and time-consuming, in contrast with
55 molecular methods that reduce the time of diagnostic with the same efficiency [12,
56 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:

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; 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.

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 phagotyping. The antigenic formulae Of Popoff and Le Minor [16] were used to name the serovars. Phagotyping was performed using the standard phagotyping 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; microtomy 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, H₂S 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 Phagotyping:

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 Phagotyping Results

<i>Salmonella</i> 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 gentamycin 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, splenomegaly, 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).

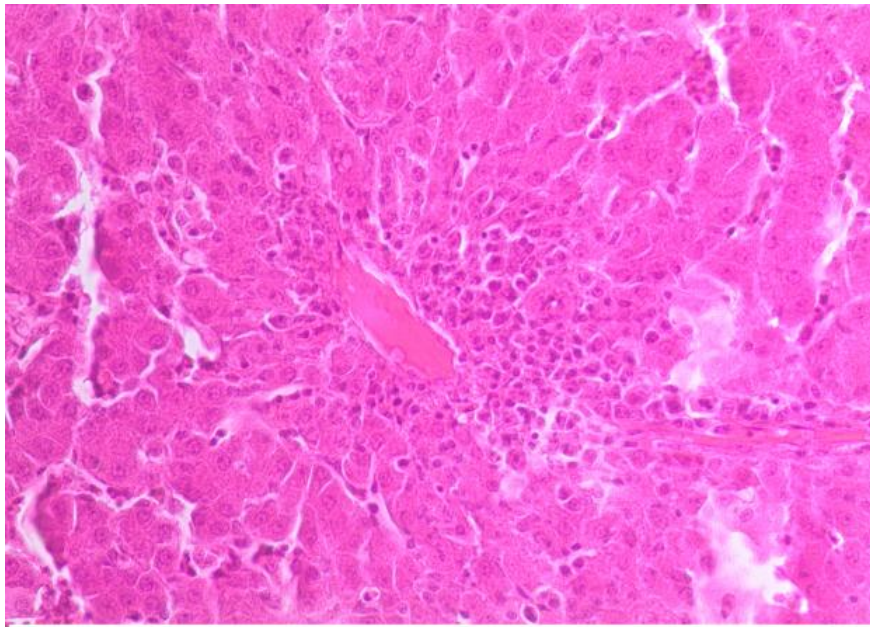


Fig 1: liver section shows thrombi in central vein, necrosis, prevascular cuffing, dilatation of sinusoid, vaculation, haemorrhage and infiltration of inflammatory cells.

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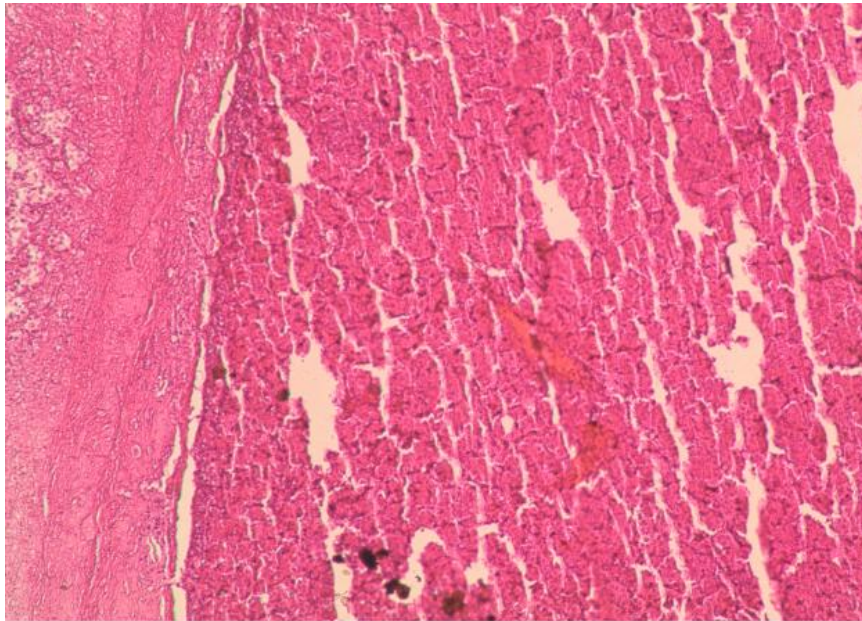


Fig 2:
liver section shows thickening of capsule, vacuations and loss of liver cord appearance.

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142 **Intestine:**

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

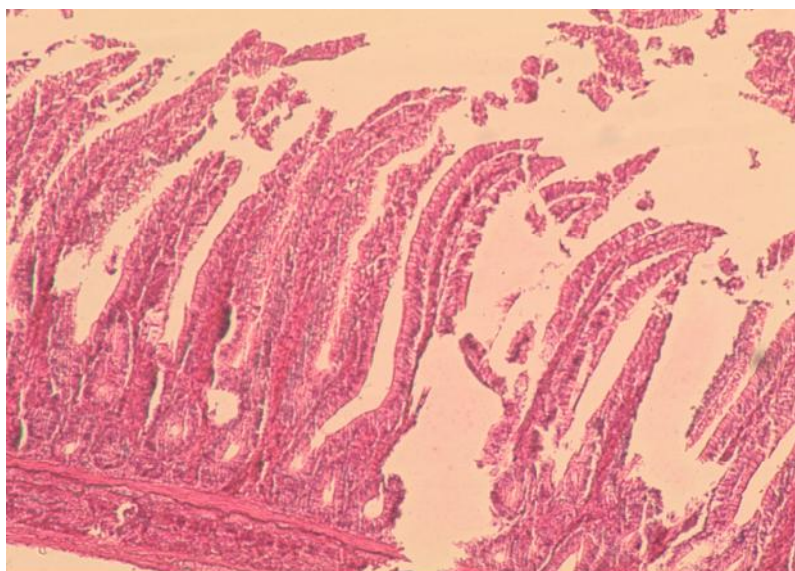


Fig 3:
intestine section shows sloughing of villi, necrotic masses of intestinal lumen and infiltration of inflammatory cells.

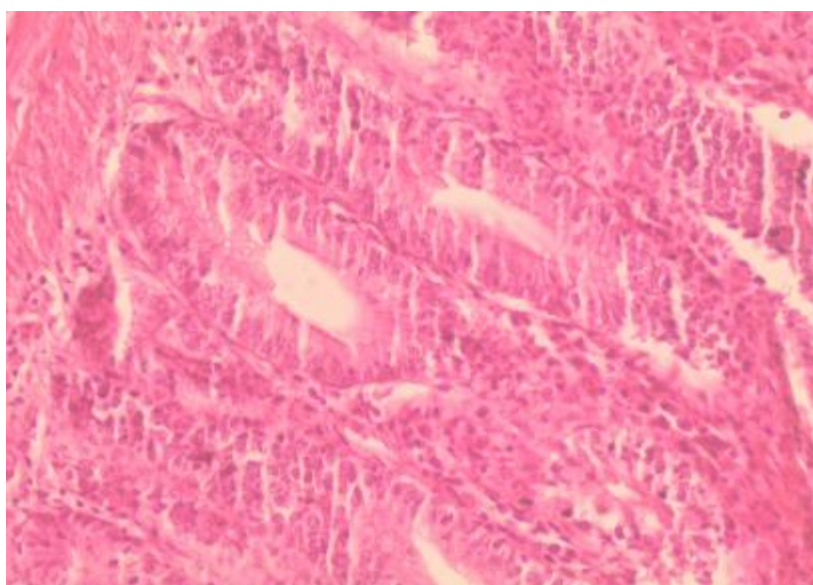


Fig 4:
intestine section shows severe infiltration of inflammatory cells and atrophied of intestinal glands.

Proventricular:

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

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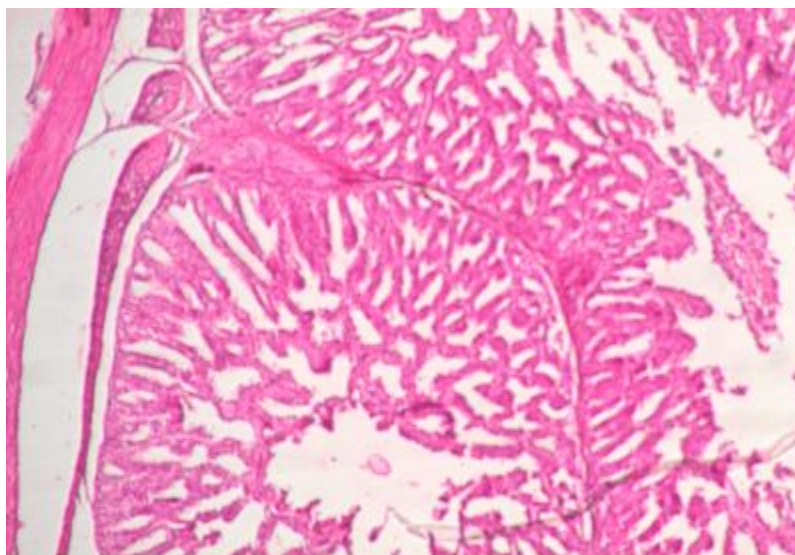


Fig 5:
proventriculus section shows sloughing of epithelial layers and necrosis.

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Brain:

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

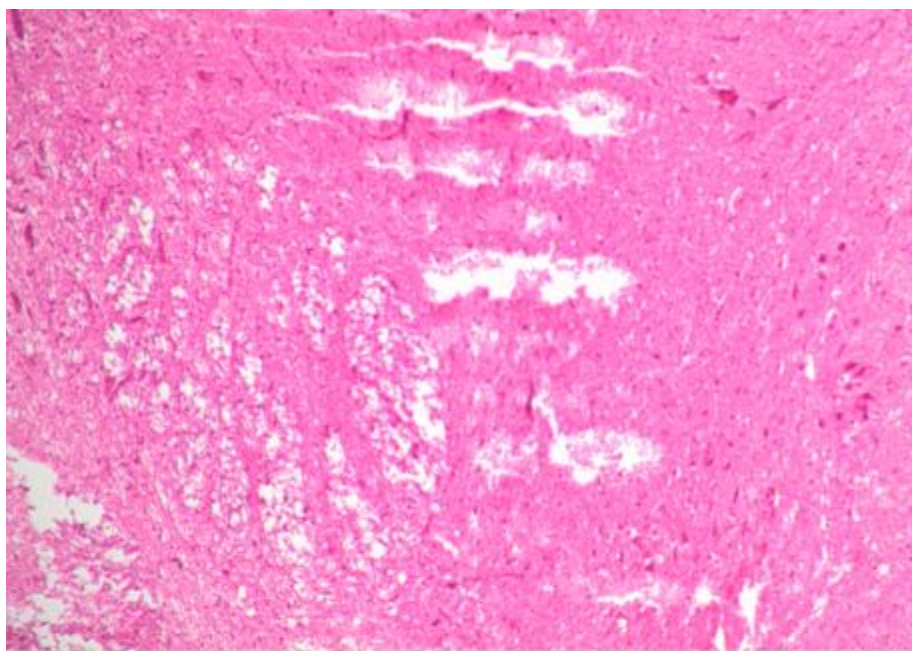


Fig 6:
brain section shows vacuolation and necrosis.

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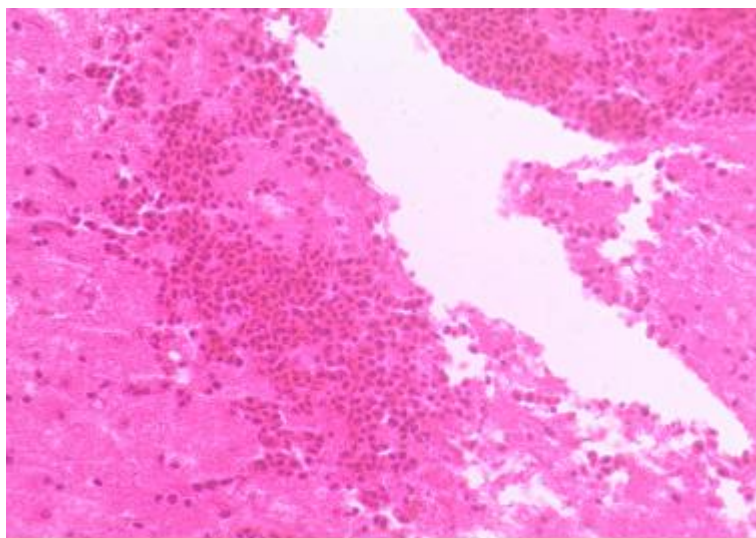


Fig 7:
brain section shows severe haemorrhage and necrosis.

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Spleen:

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

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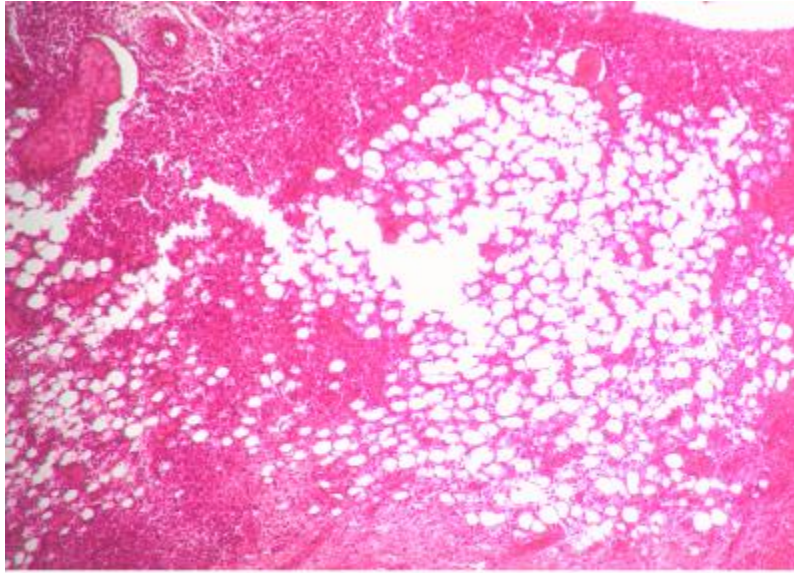


Fig 8:
spleen section shows haemorrhage, congestion, depletion of lymphocytes and vacuoles.

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Heart:

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

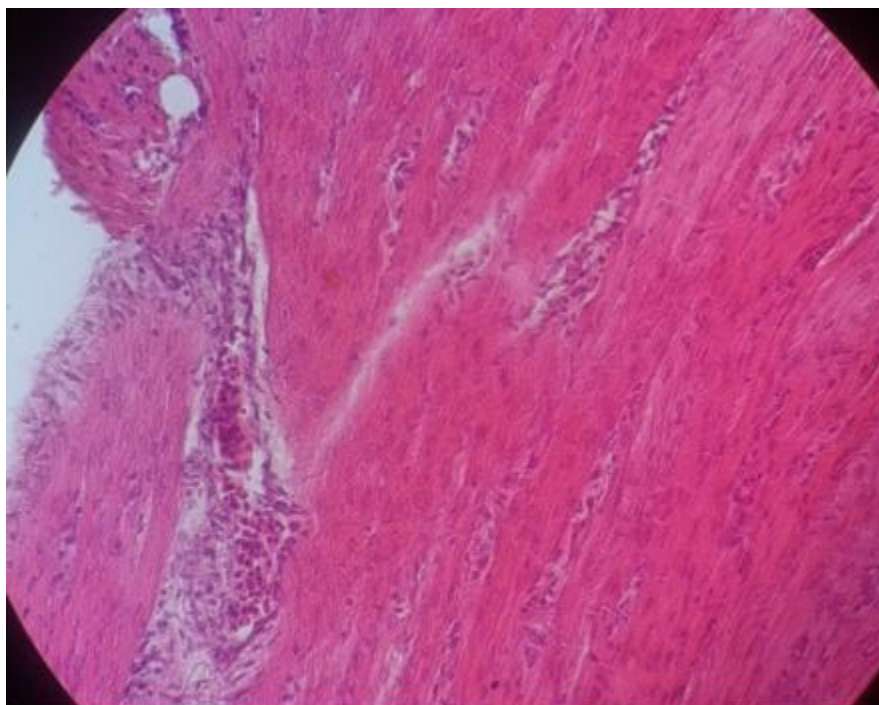


Fig 9:
heart section shows severe heamorrhage, degeneration of myocadial muscle fiberand infilltration of inflammatory cells.

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171 **4. Discussion:**

172 In this study *Salmonella* spp were identified in 52 samples out of 2000 chicks, ten
173 of them were serotyped as *S. enteritidis* [n=9] and *S. typhimurium* [n=1] . These
174 two serotypes are the most frequently isolates in poultry and poultry products and
175 humans [18, 19, 20, and 21]. In this study mortality started in day 1, this may due
176 to vertical transmission of infections which has been an important aspect of the
177 epidemiology of *Salmonella* species within the poultry industry [22; 23].

178 In 1980's *S. enteritidis* outbreaks dramatically increased globally and the
179 pathogen emerged as serious threat for poultry industry and public health [24,15].
180 Since then the infections continued increasing over time, worldwide [26,27,28] and
181 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, splenomegaly 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 histopathological 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:

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

207 - Serotyping and phagotyping must be done to confirm the Seroovar that causes
208 the disease.

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

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

213 - The histopathological findings are similar in most species.

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