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The effect of salmonella pullorum on broiler chicken in Iraq

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Abstract : Salmonella pullorum is classified under serogroup D, with Salmonella gallinarum and other organisms like Salmone enteritidis,S. typhi and S. dublin. PD is a major threat to poultry production in developing countries. IL-6 plays a key role in both the body's innate defenses and the maturation of the adaptive immune system. The mortality rate for PD in chicks can reach 100%, peaking between the ages of two and three weeks. Serological testing may be utilized to determine whether a flock is infected. Adaptive and innate immune mechanisms play important roles in the body's ability to defend against various pathogens. Clinical signs could include decreased feed intake, huddling close to heat sources, and diarrhea. Good hygiene, biosecurity, serological testing, and slaughter regulations are all necessary to eliminate Salmonella. result after 10 days after exposure to an infection, the mortality is highest in the third and fourth groups of chicks. Anorexia, diarrhea, dehydration, depression, huddling, ruffled feathers, weakness, decreased feed consumption, decreased feeding and pasting of the vent feathers are some of the clinical signs that were noted throughout the experimental period. Young birds are especially susceptible to S. pullorum colonization, which can result in either a severe clinical illness or a disease-free persistence of infection. Both the kidney and liver were impacted by S. pullorum in 2-day-old chickens. the liver primary lymphoid tissues where bacteria located and grow showed the most severe histopathological abnormalities. Microscopically the lesions in the liver, kidney, were less severe in (G1 and G4) compared to (G2and G3).

Keyword: salmonella pullorum ,liver, kidney

Introduction

Salmonella pullorum primary targets is poultry, where it causes a debilitating illness called pullorum disease (PD, white diarrhea).(Guo et al., 2019). PD is a major threat to poultry production in developing countries. IL-6 plays a key role in both the body's innate defenses and the maturation of the adaptive immune system.(Kaiser and Staheli, 2008 and Al-Kaissy, G. A. 2006). Enterobacteriaceae family consists of numerous gram-negative aerobic or facultatively anaerobic rods. Yersinia, Salmonella, Escherichia coli, Shigella, and Citrobacter are some of the genera that belong to this category. Many clinical problems, such as typhoidal and non-typhoidal infections, have been linked to these bacteria. (Ryan and Ray, 2004 and Jaffer, M. R. 2013) .Salmonella pullorum is classified under serogroup D, along with Salmonella gallinarum and other organisms like Salmone enteritidis, S. typhi, and S. dublin. Small, round, glistening, smooth, translucent, and slightly elevated colonies are formed after 24 to 48 hours of incubation on nutritional or blood agar.(CFSPH, 2009) The mortality rate for PD in chicks can reach 100%, peaking between the ages of two and three weeks. Pullorum disease resistance is higher in leghorn chickens and other smaller breeds than in larger breeds. Some birds are able to make a full recovery from their injuries. (CFSPH, 2009) Salmonella's LPS can stimulate the production of pro-inflammatory cytokines ,(Cui, 2013). which can lead to inflammation and death. LPS interactions with the host immune system are critical for pathogenesis and pathogenicity. Salmonella enter the small intestine via the proventriculus , which has an acidic environment when it is consumed by humans.(Marcus et al., 2000) Salmonella can be transmitted vertically, by an egg-associated (trans-ovarian)

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transmission to offspring, and horizontally to other hosts, including humans, as well as from a variety of sources, such as parent birds, feed, rodents, wild birds, and other vehicles (Cui, 2013).
Adaptive and innate immune mechanisms play important roles in the body's ability to defend against various pathogens.(Beal et al., 2004b). Young chickens' caecum and spleen responses to S. enteritis infection have been shown to vary depending on breed genetics.(Cheeseman et al., 2007). Salmonella-resistant chicken lines have macrophages with higher levels of the pro-inflammatory cytokines IL-18 and IFN- compared to susceptible birds.(Wigley et al., 2006),. Many gut reactions are induced when newly hatched chickens are infected with S.pullorm (Withanage et al., 2004). The immune clearance of S. Typhimurium infection from the gut may also be dependent on cellular responses, particularly Th1.(Withanage et al., 2005b). When infected with the highly invasive S. enteritidis serotype, the chicken caeca began to express higher levels of immune mediators.(Desmidt et al., 1998; Beal et al., 2006).Cellular immune mechanisms appear to be affected by chemical bursectomy, which decreases the ability of chickens to clear the intestinal infection, but systemic clearance was not affected. S. enteritidis and S. Typhimurium infections in chickens with bursts provide additional evidence for the importance of cellular immunity in Salmonella infection.(Beal et al., 2006) Pullorum illness typically affects infants and young chikensunder the age of three and infrequently affects adults. Clinical signs could include decreased feed intake, huddling close to heat sources, and diarrhea with urate staining of the vent. Serological testing may be utilized to determine whether a flock is infected.(Rahman et al., 2016). Unabsorbed yolk sacs, typical gray nodules in the lungs, heart, and gizzard, cheesy material in the ceca, swollen and congested spleens, and large and enlarged kidneys are common in young birds. Ovarian follicles may be atrophic and regress in adult carriers, and ovaries may be reduced or deformed.(Shivaprasad, 2000)Good hygiene, biosecurity, serological testing, and slaughter regulations are all necessary to eliminate Salmonella (Andino and Hanning, 2015 and Al-Khatib, G. M. 2005).

Methodology

Experimental design

The study conducted to evaluate vaccinal immunity against NDV and AIV with Pullorum infection, Using two hundred and fifty of broiler chick (Ross 308). The broiler chickens were divised into five groups (50 chicks per group).Each group was given the following care:
First group: infected with *S. Pullorum* and vaccinated against (NDV, AIV). Second group: infected with *S. Pullorum* and treated by (ciprofloxacin 10% 1ml / liter or 20 % 0,5 L for 7day and colistin 200million IU/0.5L for 7 days) also vaccinated against (NDV, AIV). Third group: infected with *S. Pullorum* and treated by (ciprofloxacin 10% 1ml / liter or 20 % 0,5 L for 7day and colistin 200million IU/0.5L for 7 days) only. Fourth group: infected with *S.Pullorumm* only as positive control. Fith group: uninfected and unvaccinated as negative control.

Histopathological examination

Internal organ specimens measuring (1x1x1) cm were collected at 42 days, including (liver, kidney). After removal, the tissues were immediately fixed in a 10% buffer formaldehyde solution. The specimens were cleaned with tap water after 72 h of fixation standard procedure by soaking in a series of progressively stronger alcoholic concentrations (from 70% to absolute 100%) for 2 hours at each concentration to extract the water from the tissues. The samples were cleared with xylol, then infiltrated twice with semi-liquid paraffin wax at 58 °C. The samples were embedded in paraffin wax blocks, and subsequent sections of all tissues were cut to a thickness of 5µm using a rotary microtome. Hematoxylin and Eosin stain (H and E) was used to color all tissues, and a light microscope was used to observe the histopathological changes (Luna, 1968).

Result

Clinical signs and mortality

Table (1-1) displays the clinical signs that were noted throughout the experimentalperiod Respiratory signs include anorexia, diarrhea, dehydration, depression, huddling, ruffled feathers, weakness, decreased feed consumption, and huddling near heat sources with pasting of the vent feathers (Figure 1). After 10 days following the infection, the mortality is highest. A dead chick may be found lying on his abdomen, postmortem

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examination dead chicks show swollen and congested spleen and enlarged kidney, while the liver was friable, congested, echomatic hemorrhage also severe congestion of thymus (Figure 2).

In contrast to the first groups' mild clinical signs and low mortality, the fourth group had high rates of morbidity and mortality, while the third and second groups was recorded low morbidity and no mortality but the fifth group were not recorded any morbidity and no mortality because not exposed to any infection.

Table 1-1 Development of clinical signs and mortalities during 20 days post infection with local *Salmonella Pullorum* at one days old.

Index / Groups	Morbidity %	Mortality %
G1	80% (40) **a**	14% (7) **b**
G2	50% (25) **c**	8% (4) **c**
G3	40% (20) e	6% (3) **c**
G4	90% (45) **a**	18% (9) **a**
G5	0% (0) **d**	0% (0) **d**

The small letters appear on the percent of morbidity and mortality of the same column refer to significant differences at ($P < 0.05$) among groups.

Figure 1: A and B shows the clinical signs in chicks at 7-day-old reveled diarrhea with urate staining of the vent, pasting of the vent feathers.

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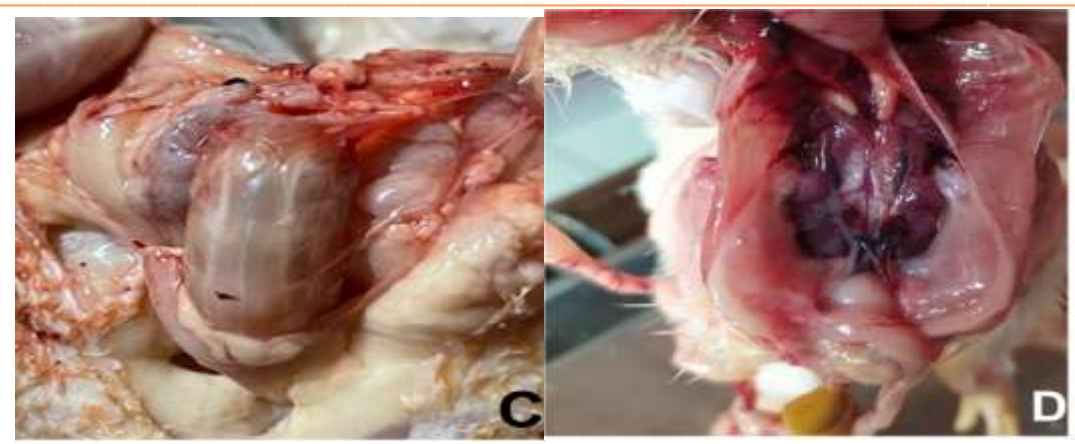

Figure 2: A, B, shows friable and echyomatic hemorrhage in liver. C shows severe congestion of thymus. D show congestion and enlargement kidney in chicks at 14-day-old.

Histopathological examination

Liver:

The microscopically findings of G1 characterized by venous dilation with eosinophilic edematous substance associated with necrotic finding of adjacent periportal hepatocyte and focal mononuclear cells infiltration portal region (Figure 3). In G2 group, there was focal aggregation of lymphocyte with heterophil accompanied with mild suggestion of adjacent hepatic sinusoidal and vessels (Figure 4). G3 group, showed main hepatic lesion showed perivascular mononuclear cell infiltration with degeneration finding of adjacent hepatocyte (Figure 5). G4 group, showed aggregation of mononuclear cell mainly around central vein (Figure 6). G5 group the liver sections showed normal structural.

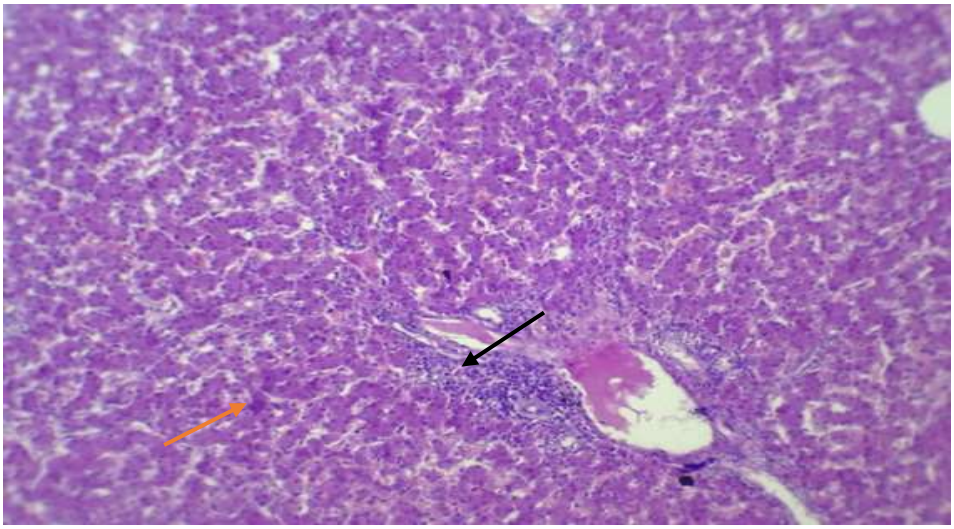

Figure 3: Histopathological section in the liver of G1 group at 14-day-old chicks shows venous dilation with eosinophilic edematous substance associated with necrotic finding of adjacent periportal hepatocyte and focal mononuclear cells infiltration portal region (H&E stain 10X)

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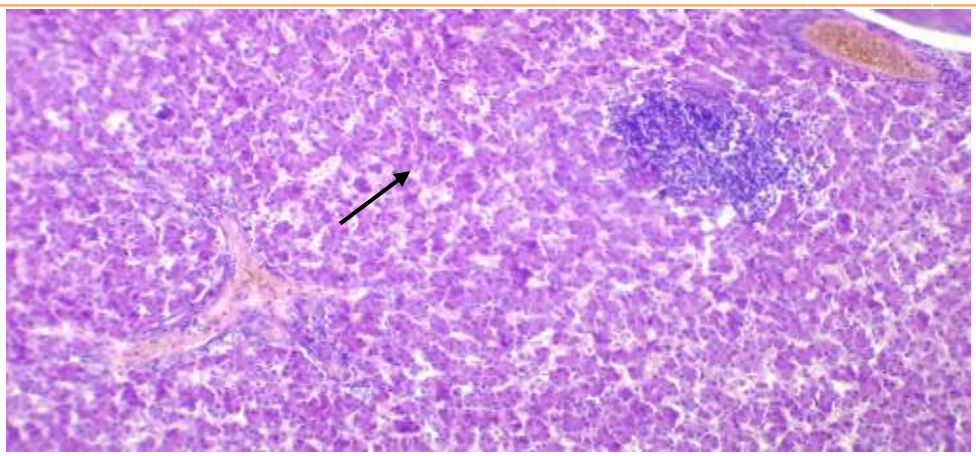

Figure 4: Histopathological section in the liver of G2 group at 14-day-old chicks shows focal aggregation of lymphocyte with heterophil accompanied with mild suggestion of adjacent hepatic sinusoidal and vessels (H&E stain 10X).

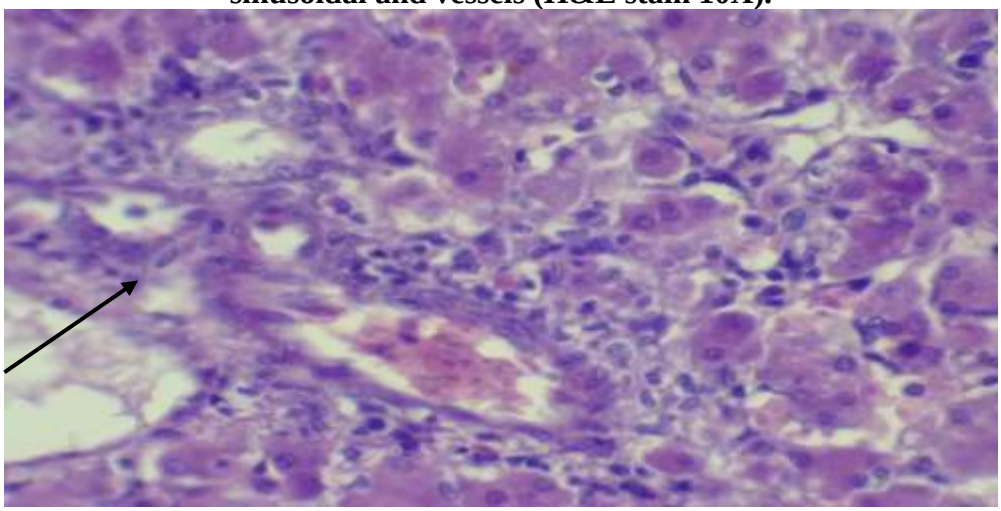

Figure 5: Histopathological section in the liver of G3 group at 14-day-old chicks shows main hepatic lesion showed perivascular → mononuclear cell infiltration with degeneration finding of adjacent hepatocyte (H&E stain 40X).

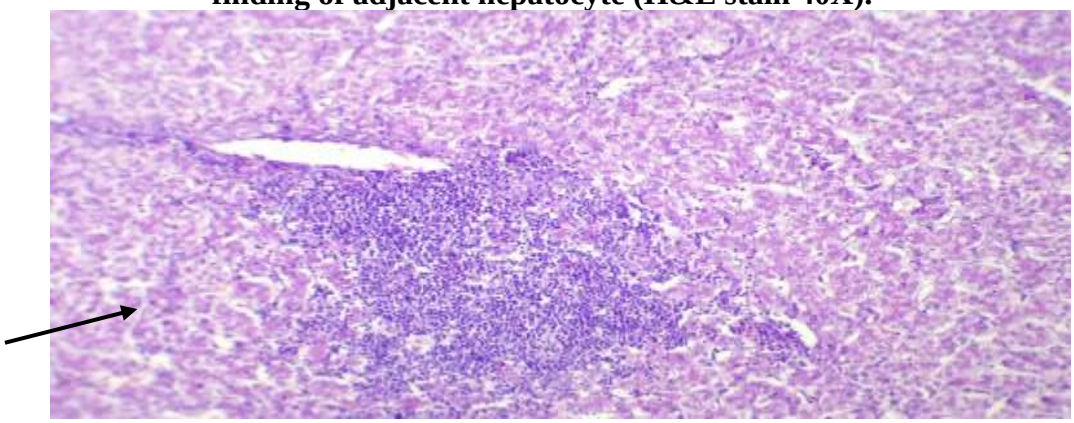

Figure 6: Histopathological section in the liver of G4 group at 14-day-old chicks shows aggregation of mononuclear cell mainly around central vein (H&E stain 10X).

Kidney

The histopathological findings of kidney in G1 group were mild interstitial hemorrhagic with few mononuclear cell infiltration between degenerated tubules (Figure 7). G2 group showed mild interstitial hemorrhage with infiltration of leukocyte with prominence basophilic tubules (Figure 8). G3 group showed marked cellular swelling of renal tubules with hyaline cast appearance in some dilated tubule and prominence of basophilic degenerated tubule (Figure 9). G4 group showed marked cellular swelling of renal tubules with hyaline cast appearance in some dilated tubule and prominence of basophilic degenerated

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tubule (Figure 10). G5 the kidney sections showed edematous substance in Bowman space of some glomeruli.

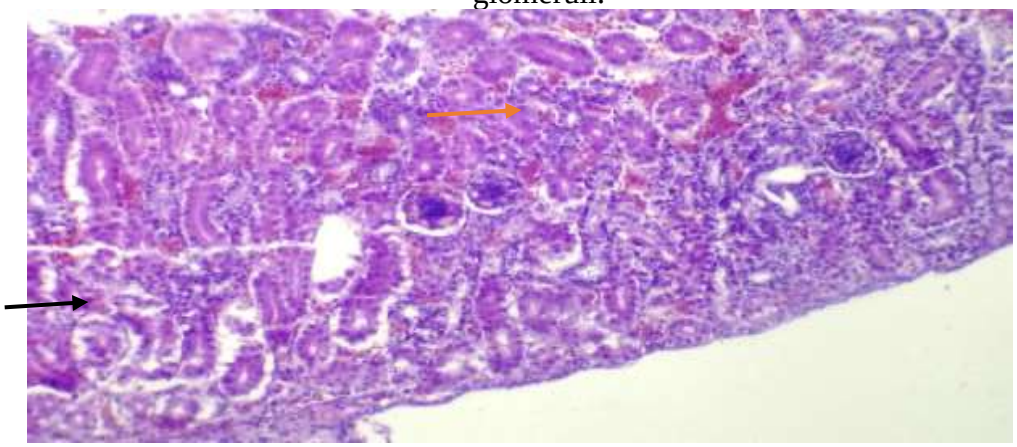

Figure 7: Histopathological section in the kidney of G1 group at 14-day-old chicks shows mild interstitial hemorrhagic with few mononuclear cell infiltration between degenerated tubules (H&E stain 40X).

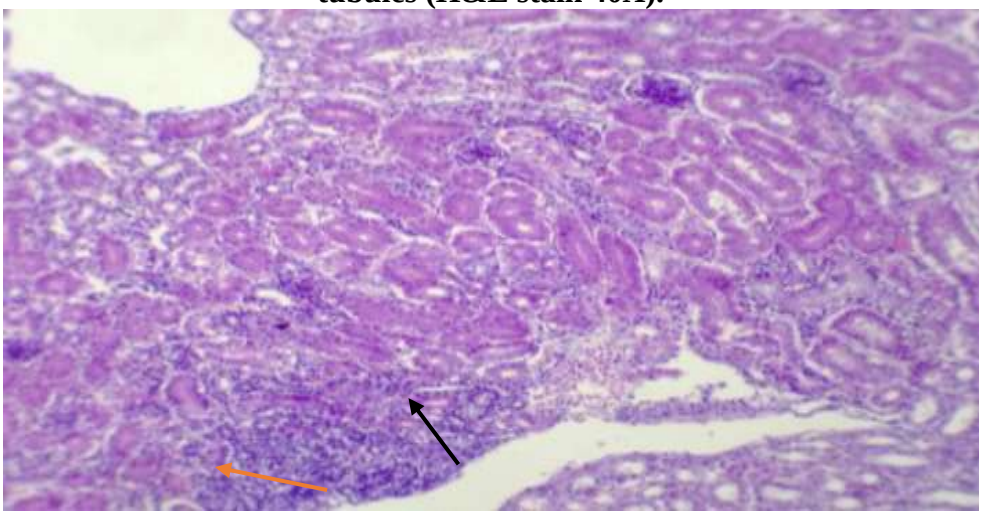

Figure 8: Histopathological section in the kidney of G2 group at 14-day-old chicks shows mild interstitial hemorrhage with infiltration of leukocyte with prominence basophilic tubules (H&E stain 10X).

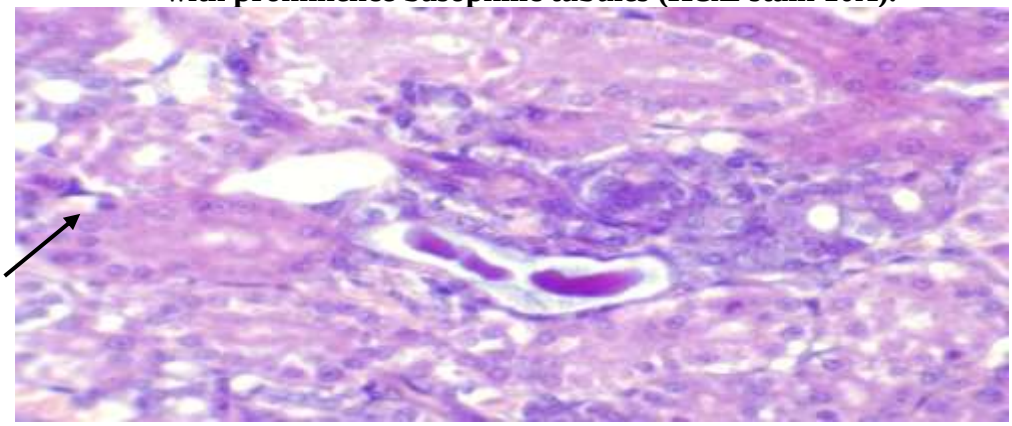

Figure 9: Histopathological section in the kidney of G3 group at 14-day-old chicks shows marked cellular swelling of renal tubules with hyaline cast appearance in some dilated tubule and prominence of basophilic degenerated tubule (H&E stain 10X).

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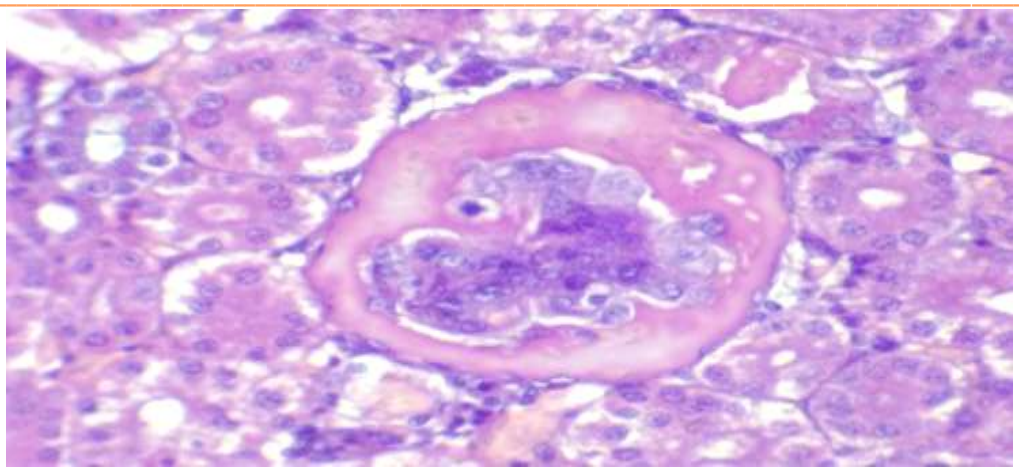

Figure 10: Histopathological section in the kidney of G4 group at 14-day-old chicks shows edematous substance in Bowman space of some glomeruli (H&E stain 40X).

Discussion

In the current investigation, mortality was not observed in the first week (0–7 days of age) or before the S. Pullorum challenge, but it was seen on 3 consecutive days in week 2 after oral challenge. Maximum mortality from S. pullorum was found in G4, then G1, and then G2. Young birds are especially susceptible to S. Pullorum colonization, which can result in either a severe clinical illness or a disease-free persistence of infection., like white diarrhea and a high mortality rate (Kogut and Arsenault, 2017). The gross lesions gradually disappear three weeks after infection (Shivaprasad, 2000). Although this disease is not pathognomic, pullorum-infected birds' liver and spleen lesions may resemble "white spots," which are uncommon with gallinarum (OIE, 2010). These findings are consistent with other studies (Kumar*etal.*,2010), which suggests that young chicks lack full immunocompetence due to a decreased proportions of CD4+CD8- and CD4-CD8+ cells in the spleen and thymus, respectively. (Erf*etal.*,1998). Additionally, at 1-2 weeks of age, due to the lack of maternal antibodies that provide protection (Lee *et al.*, 2001). S. Pullorum induced bacteremia and colonized to varying degrees in the liver, lungs, heart, kidney, gut, spleen, and ceca of chicks, according to the findings of the oral route of inoculation (Wigley, *et al.*, 2005). Young birds are particularly vulnerable to S. pullorum infection, and because of their immature immune systems, they have trouble resisting bacterial invasion. We investigated the S. pullorum organ dissemination pattern in 2-day-old chickens and discovered that the liver and kidney were primarily affected. According to (Barrow, 2011), the liver primary lymphoid tissues where bacteria located and grow and showed the most severe histopathological abnormalities. Our observations support this theory (Lee *et al.*, 2005). Both the kidney and the liver were impacted; in fact, both organs displayed the highest levels of infection in this investigation, which is similar with the findings of a previous study (Nazir *et al.*, 2014).

Microscopically, the lesions in the liver, kidney, were less severe in (G1 and G4) at 14 day than in (G2 and G3). The leucocytic infiltration at the perivascular areas of the liver was accompanied by hydropic vacuolation in the hepatocytes, and there were numerous necrotic foci along with Kupffer cell hyperplasia. Hepatocyte necrosis was only seen in a few spots, with focal macrophage, lymphocyte, and heterophil aggregation. Previous reports of similar degenerative, necrotic, and infiltrative lesions (Shivprasad, 2000; Sujatha *et al.*, 2003; Chauan and Roy, 2007; Ahmed *et al.*, 2008; Holt *et al.*, 2010; Saha *et al.*, 2012). The kidney's microscopic section is characterized by minor interstitial hemorrhage, few mononuclear cells between deteriorated tubules, leukocyte infiltration, prominence of basophilic degenerated tubules, and hyaline cast appearance in some dilated tubules. Similar degenerative and infiltrative changes have been described in the kidneys of birds affected by S. Pullorum (Shivprasad, 2000. Desmukh *et al.*, 2007), which highlight abnormalities in the kidneys of affected birds' renal tubular epithelium.

Conclusion

Salmonella Pullorum is a common component of the field infections. The isolate was fixed and introduced into the NCBI after being isolated from young chicks.When compared to groups that had received treatment

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with ciprofloxacin and colistin, which resulted in more immunopathological changes and an immunosuppression, S. Pullorum-infected groups showed higher immunopathological changes in the histological sections of the liver, kidney.

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