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THE ROLE OF SALMONELLA SPECIES AS A CAUSE OF OMPHALITIS IN BABY CHICKS IN ASSIUT GOVERNORATE

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ABSTRACT

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A total of 130 diseased and dead broiler chicks of 1-10 days of age obtained from different sources were examined; yolk sac samples from these chicks were cultured and the bacteria were isolated and identified on biochemical tests. Unabsorbed yolk sac was observed in 48 (36.9%) chicks associated with signs of septicemia in many cases. These unabsorbed yolk sac exhibit signs of moderate to mild inflammation indicating that the cause of death is the consequences of the infection rather than it's direct effect. The recovered 12 Salmonella isolates were: S. enteritidis 5(41.6%), S.virchow 3(25%), S.typhimurium 2(16.6%), S.heidelberg 1 (8.3%), S. kentucky 1(8.3%). Thickness of yolk sac wall and the fibroses may be the cause of un absorption of yolk, Sever changes were seen in the liver, heart, intestine and yolk sac represented by congestion, inflammatory cells infiltration.

Key words: *Salmonella, Omphalitis, Baby chicks, Assiut governorate.*

INTRODUCTION

Yolk sac infection (YSI) is a major cause of mortality in broilers during their first week of life (Bains, 1979; Coutts, 1981 and Jordan 1996). YSI occurs in all flocks resulting in decreased hatchability, increased mortality and increased cull rate due to retarded growth (Gross, 1964; Coutts, 1981; Mosqueda and Lucio, 1985). The mortality rate is about 5-10%; however the infection may cause a much higher mortality in a batch of chicks in the first week of life (Wray *et al.,* 1996).

Yolk sac infection occurs mainly due to bacterial contamination of the eggshell at the broiler breeder farm shortly after the egg is laid, while the cuticle is still moistened (Mosqueda and Lucio, 1985; Saif *et al.,* 2003). Including the contamination takes place as a consequence to certain factors lack of hygiene in the nests. Presence of eggs on the floor, incubation of dirty eggs or eggs with egg shell defects and collection of dirty and clean eggs at the same time (Coutts, 1981; Mosqueda and Lucio, 1985). Poor fertile egg storage conditions, poor egg disinfection, and humidity levels during incubation may also promote egg contamination (Banis, 1979).

Another important route of yolk sac infection is the bacterial penetration through a poorly healed navel (Coutts, 1981; Mosqueda and Lucio, 1985; Wray *et al*., 1996). Efficient absorption of yolk due to fasting was reported by Pisarsaki *et al*. (1998). The affected yolk sacs revealed signs of mild to moderate inflammation. The wall was edematous and infiltrated with a number of heterophils. The wall was also thickened due to hyperemia, accumulation of edematous fetid and infiltration with heterophils and macrophages, Asheg *et al. (2001*), saif *et al.* (2003) and suha *et al*. (2008). The pericardium, abdominal air sacs and serosal surface of intestine were mildly thickened by variable combinations of heterophils, macrophages and fibrin, in addition to presence of multifocal infiltration of inflammatory cells in hepatic tissues, Gorham *et al*. (1994), Ismail and Garo(2009).

MATERIALS and METHODS

1. BACTERIAL CULTURING MEDIA:

RVs broth (Rappaport Vassiliodis Soya broth):
SS agar (SalmonellaShigella).
Urea agar base.
Lysin Iron Agar.
Triple Sugar Iron.

2. STAINS:

Gram's stain.

3. BIOCHEMICAL REAGENTS:

Standard biochemical reagents were used for identification of the bacterial isolates as mentioned by Carter and Cole (1990) & Barrow and Felthem (1993).

METHODS:-

1. NECROPSY FINDING:
All chicks subjected to necropsy before sampling in order to record any gross lesion on their viscera with special reference to the yolk sac infections. Postmortem examination was done according to the procedure recommended for poultry by Chauhan and Roy (2007).

2. Sample Collection and Transportation: Yolk sac samples were collected aseptically using sterile plain swabs and RVs broth in a sterile test tube. The collected swab samples were labeled, packed and transported along with portable coolant (Ice Pac). The collected samples were stored in refrigeratorat + 4 °C as mentioned by Quinn *et al*. (2002).

3. BACTERIAL ISOLATION:

Following completion of the necropsy finding, all chicks were sampled with sterile cotton swab from the yolk sac dipped in sterile peptone broth and incubated for 16-18 h at 37°C after pre-enrichment, 1 ml of enriched cultures of all sample types were transferred to 9 ml of RVS and incubated at 37°C for 18-24 h. At the end of selective enrichment, the broths were plated into SS agar and incubated at 37°C for 24 h in order to isolate the suspected colonies. Primary cultures were evaluated by visual examination of the morphology of the bacterial colonies and were sub cultured. Quinn *et al*. (2002).

4. BACTERIAL IDENTIFICATION:

The identification of the isolated colonies was performed using standard bacteriological and biochemical procedures as described by Carter and Cole (1990) & Barrow and Felthem (1993).

5- Serological Identification:

Serological identification of Salmonella was carried out according to Kauffman- White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigen.

Identification of Somatic (O) antigen "Slide agglutination test":

- A dense suspension of the organism was prepared by suspending growth in 0.5 ml of saline solution.
- Using a wax pencil, 2 circles about 1 cm in diameter on a microscopic slide were marked.
- One drop of Salmonella Polyvalent "O" Antisera was put in one of the marked circles and one drop of the saline solution was put in the other circle (negative control).
- Using a clean dropper, one drop of bacterial suspension (0.05 ml) was transferred into each of the circle and mix thoroughly by gently racking for 1- 2 minutes (excessive evaporation was avoided).
- Positive reaction was adopted by rapid and complete agglutination. A delayed or partial agglutination should be considered negative.
- Salmonella group and the other somatic components of the group were also identified using by using separate "O" antisera factors.

Identification of Flagellar (H) antigen "Tube agglutination test":
Determination of Flagellar (H) antigens was carried out by using Polyvalent H antisera for both phase 1 and phase 2 in order to determine the complete antigenic formula of the isolates. A lapful of H antiserum was added to one drop of the bacterial suspension in the small agglutinating tube and mixed gently by a sterile loop. The agglutination tube was gently agitated for one minute and observed for agglutination under normal lighting conditions.

Histopathology:
Specimens from yolk sac wall, liver, heart and intestine were obtained from all chicks and kept in 10% neutral buffered formalin for atleast 24 hours. After that, these sample were exposed (according to James, 1976) for dehydration, paraffin embedding blocking, sectioning, mounting and staining with hematoxyline and Eosin for light microscopy.

RESULTS

Prevalence of unabsorbed yolk sac:

Out of the 130 dead and diseased chicks of 1-10 days of age, unabsorbed yolk sac was observed In 48(36.9%) chicks. Maximum percentage 45.8%(22\48) of unabsorbed yolk sac was observed in chicks at (5-6) day old followed by age of (3-4) day old 25% (12\48),In(7-8)day old 20.8% (10\48), and minimum percentage in (9-10) day old were 8.3% (4\48).

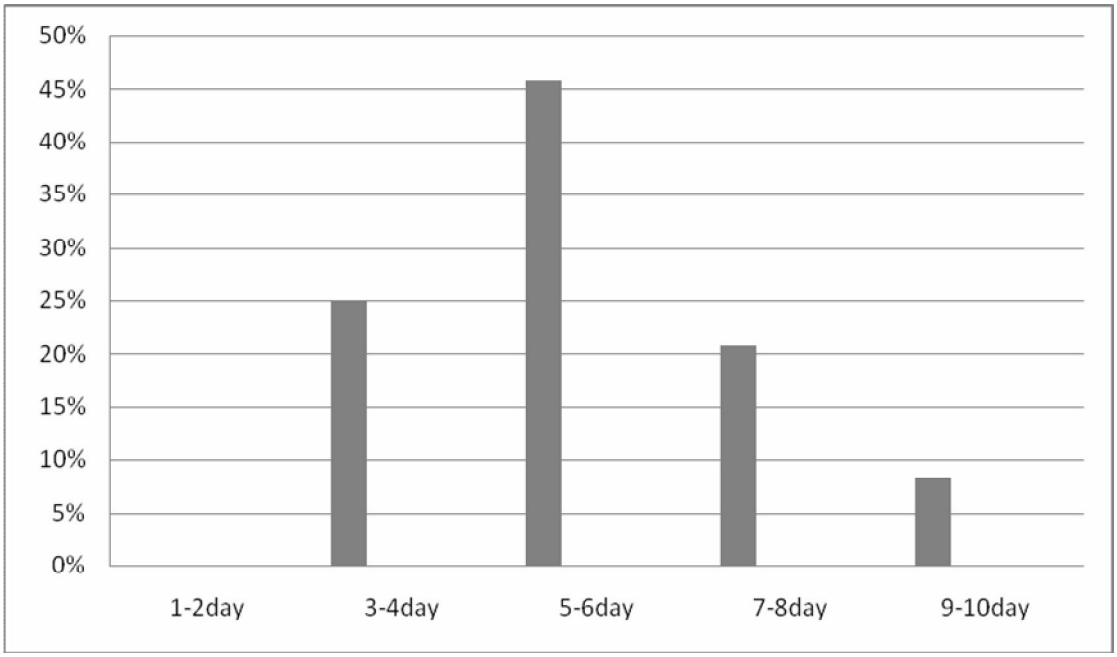


chart 1: Prevalence of YSI in correlation to age of examined chicks

although we cannot record any YSI cases at 1-2 day old of age.
Moreover, out o the 48 cases of chicks infected with yolk sac infection, Salmonella spp, were recovered from 12 cases (25%). The obtained data revealed that the recovered 12 salmonella isolates were *S.enteritidis* 5\12(41.6%), *S.typhimurium* 2\12(16.6%), *S.virchow* 3\12(25%), *S.heidelberg* 1\12(8.3%) and *S.kentucky* 1\12(8.3%).

Table 1: Serological identification of Salmonellae

NO OF Samples	Identified strains	Antigenic structure	
		O	H
3	***Salmonella Virchow***	6,7,14	r : 1,2
5	***Salmonella enteritidis***	1,9,12	g,m : 1,7
1	***Salmonella Heidelberg***	4,5,12	r : 1,2
2	***Salmonella typhimurium***	1,4,5,12	i : 1,2
1	***Salmonella Kentucky***	8,20	i : Z6

Gross lesions finding: Revealed an unabsorbed yolk sac (fig1) in 48 out of 130 chicks, (1-10 day old of age). The Putrefactive odor from the birds can be assumed as first signal for the farmers, flabbiness and distention of abdomen, moist umbilicus and change in size, consistency and appearance of yolk can be thought as indicative of yolk sac infection. The unabsorbed yolk sac was distended with yellow or yellow brown and watery, retained caseous yolk (especially in 6-7day old chicks). The affected birds also exhibit systemic manifestations such as pericarditis, peritonitis peticheal and ecchymotic hemorrhages on serosal surface of visceral organs (particularly the intestine) (in 6-9 day old chicks) and congestion and enlargement of the lungs, liver and/or kidneys were observed in 28 chicks.

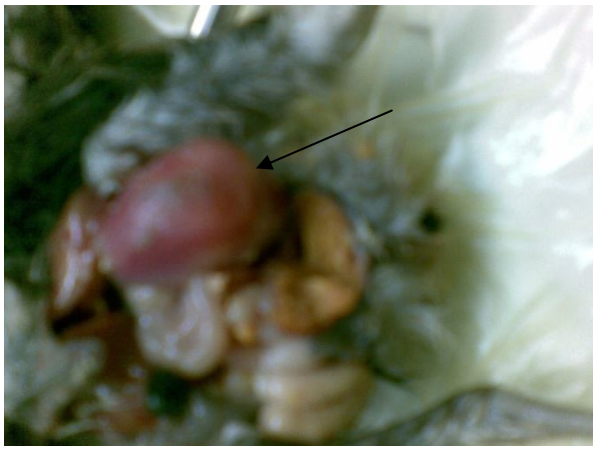
Yolk sac:
The microscopic examination of the affected yolk sacs revealed signs of mild to moderate inflammation. The wall was edematous and infiltrated with heterophils. Thickening and fibrosis were also seen in some cases (Fig5 a,b,c).

Liver:
Sever changes were seen in the liver which characterized by congestion of central vein with perivascular cellular reaction, and inflammatory cells

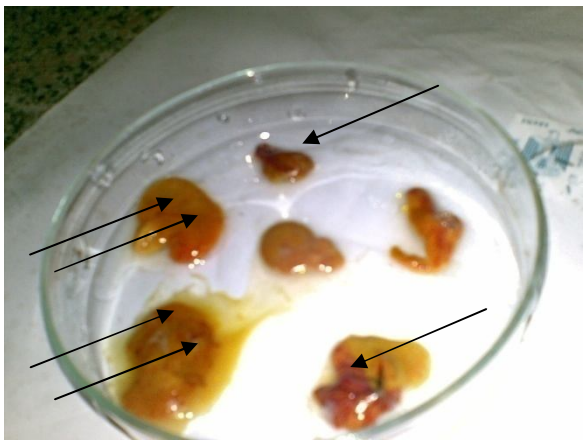
infiltration (Fig 2,a). In some cases the hepatic tissue showed salmonella granuloma within hepatic tissues (Fig 2,b). More over sub capsular hemorrhage with dilatation of bile duct were seen .Coagulative necrosis of hepatocytes was also seen in some cases.
(Fig 2,c)

Heart:
Pericardlitis with subepicardial lymphocytic cell reactions and hemorrhage were observed. Necrosis were seen in the wall of myocardium blood vessels (Fig3,a,b,c). The pepricardium and adjacent myocardium were infiltrated by numbers of heterophils (Fig 3,d).

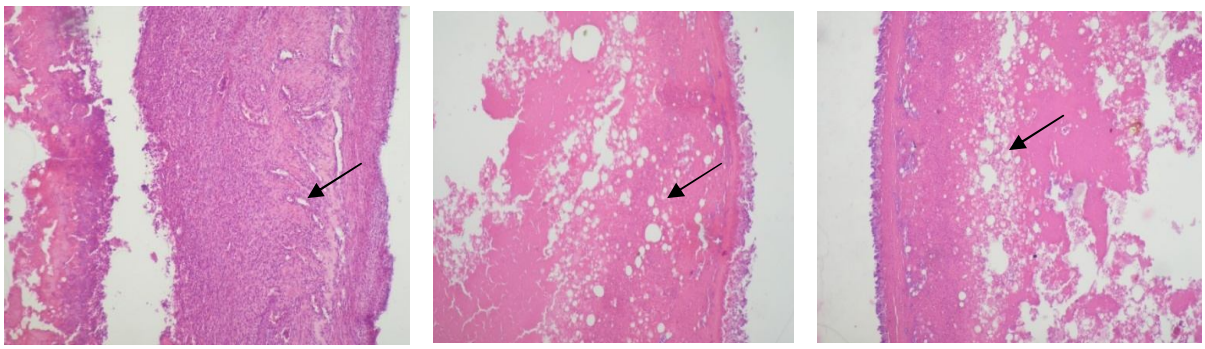
Intestine:
Intestinal serosa was mildly thickened and showing sever congestion in serosal blood vessels.The intestinal lumen filled with an aggregation of sloughed cells, necrotic debris, heterophils and macrophages (Fig 4a,b).



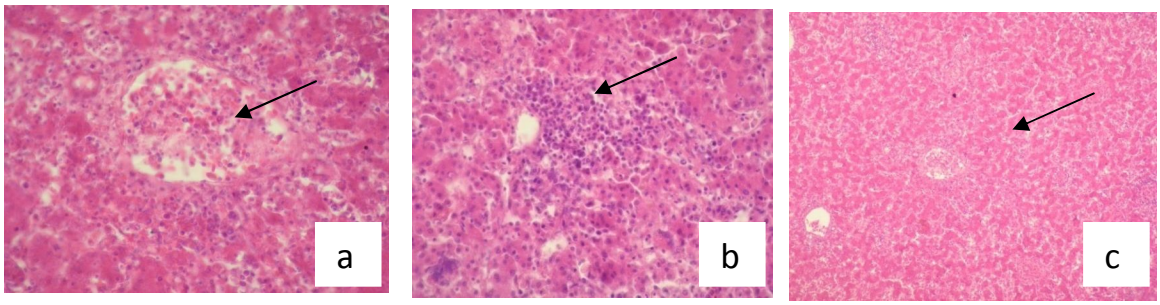
(Fig. 1): Showing unabsorbed yolk sac in 7day old broiler chick (arrow)



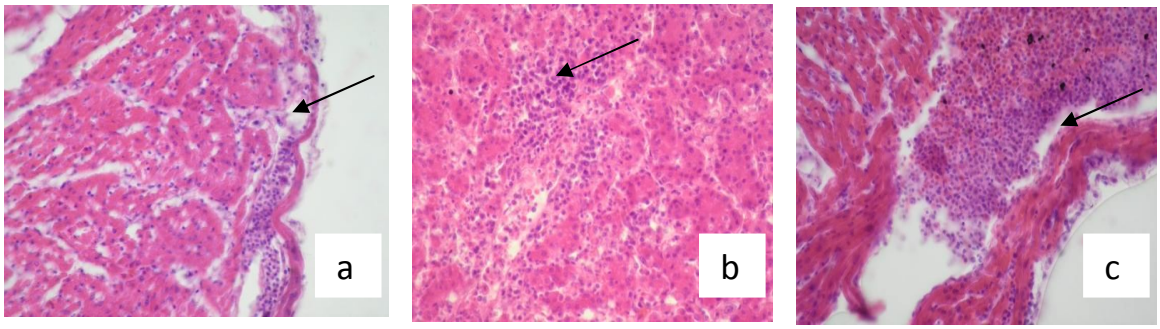
(Fig.2): Showing infected yolk sacs were larger in mass (double arrow) than uninfected (one arrow) from chicks of same age (4day old chicks)



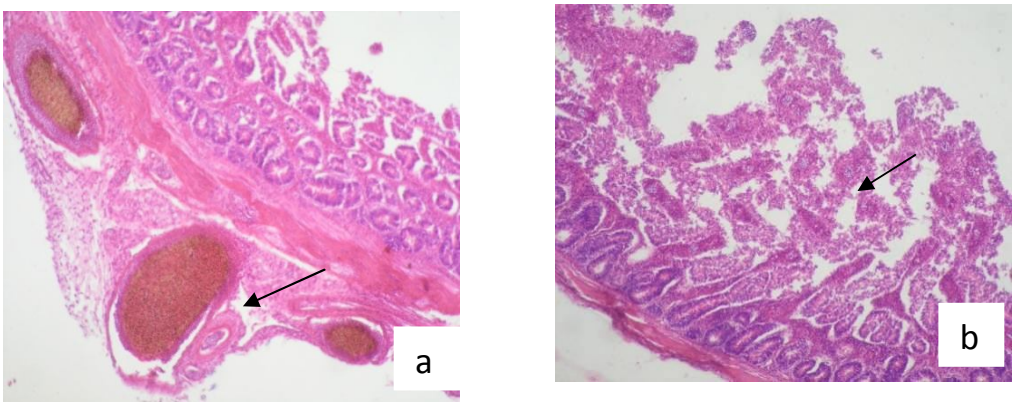
(Fig. 3 a,b,c) Yolk sac showing edematous thickened and fibrosed wall (x400)



(Fig. 4,a): Liver showing lymphocytic infilteration within hepatocyte and coagulative necrosis arrow.
(Fig. 4,b): Liver showing salmonella granuloma, arrow. X400.
(Fig. 4,c): Liver showing coagulative necrosis of hepatocytes arrow. X400.



(Fig.5,a,b) heart showing infiltration of heterophilis within pericardium and myocardium.x400
(Fig. 5c) heart showing subepicardium hemorrhage with inflammatory cells. arraw (x400).



(Fig. 6a): Intestine showing sever congestion in serosal blood vessels (x400).
(Fig. 6b): Intestine showing aggregation of sloughed cells and necrotic debris within lumen, arrow (x400).

DISCUSSION

Salmonella spp, recorded in natural cases, are the primary pathogens, the main route of infection is through unhealed navel but in some instances transmission is possible by blood stream and by contamination of yolk it is inverted into chick as reported by Khan *et al*. (2004).

Out of the 130 dead and diseased chicks, yolk sac infection was observed in 48(36.9%) dead and diseased chicks. This finding was is conformity with the reports of Rahman *et al*. (2006) that assessed 31.45% and 28.42% mortality in chicks due to yolk sac infection. Yolk sac infection mortality was highly correlated with salmonella infection, the mortality peak was observed at day 4-5(45.8 %). Similar results were reported by Abadi *et al. (*2013). Mortality rate declined thereafter until 10 days post hatch. Similar results were reported by Rosario *et al*. (2005) who indicated that the YSI mortality curve lasts 7-10 days, it peaks at 4-5 days and decreases during the following 3 to 5 days. The relatively higher first week mortality of chicks when compared to different parts of the world Ahmed *et al*. (2009). The cases of yolk retention and yolk sac infection were recorded up to 10 days of age but high rate of mortality was observed upto 3 days of age Khan *et al*. (2004).

The obtained data revealed out of the 48 cases of chicks with yolk sac infection, Salmonella spp, were recovered from 12 cases (25%), infected chicks with salmonella may die with peaks in mortality between the 4-5 day and by the 15 day with mortality rate ranging from 19 to 29%, Chen *et al*. (2002).

the obtained data revealed that the recovered 12 salmonella isolates were S.enteritidis 5\12(41.6%), S.typhimurium 2\12(16.6%), S.virchow 3\12(25%), S.heidelberg 1\12(8.3%), S.kentucky 1\12(8.3%). This results agree with those of Abd-Galil *et al. (*1994) identified Salmonella isolated from baby chicks as S.typhimurium and it to be responsible for early chicks mortality. Also Byrd *et al*. (1999) isolated a total 11 different Salmonella serotypes from baby chicks with S. Heidelberg and S.kentucky.

Postmortem examination revealed an unabsorbed yolk sac in 48 chicks. The unabsorbed yolk sac were distended and or congested with their content was yellow or yellow brown and watery, or cheesy in many cases (especially at 7 day old). The flabbiness and distention of abdomen, moist umbilicus and change in size, consistency and appearance of yolk could be thought as indicative of yolk sac infection. The affected birds also exhibit systemic manifestations such as pericarditis and peritonitis. Congestion and enlargement of the lungs, liver and/or kidneys were observed in 37 chicks. These findings are identical with those mentioned by Gorham *et al.* (1994), Bailey *et al.* (1996), wray *et al*. (1996), and Asheg *et al*. (2001).

The microscopic examination of the affected yolk sacs revealed signs of mild to moderate inflammation. The wall was edematous and infiltrated with heterophils. In some case the wall was thick and fibrosed, These microscopic lesions are identical with those mentioned by Jordan (1990), Anjum (1997) and Khan *et al.* (2004), thickness of yolk sac wall and the fibroses may be the cause of unabsorption of yolk, because this fibrosis closed the pores of the wall of yolk sac. The affection on the blood vessel wall due to Salmonella infection may be also incriminated as a cause of un absorbed yolk sac.

Sever changes were seen in the liver which characterized by congestion of central vein with perivascular lymphocytic cellular reaction. In some cases the hepatic tissue showed salmonella granulomain our opinion these lesions were attributed to the infection by salmonella which lead to unabsorbed yolk sac.

More over subcapsular hemorrhage with dilatation of bile duct were seen Coagulative necrosis of hepatocytes. These finding were also recorded by Ismail and Garo (2009).

Examination of the heart revealed Pericarplitis and subepicardial lymphocytic cell reactions and hemorrhage. Necrosis of the wall of blood vessels in myocardium, pepricardium and adjacent myocardium were infiltrated by numbers of heterophils, Similar findings were recorded by Jordan (1990), and Anjum (1997).

Intestinal serosa was mildly thickened with sever congestion in serosal blood vessels. The intestinal lumen filled with an aggregate of sloughed cells, necrotic debris, heterophils and macrophages. All these lesions were also observed by Asheg *et al*. (2001), Saif *et al*. (2003) and Suha *et al*. (2008).

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دور السالمونيلا كمسبب لالتهاب كيس المح فى كتاكيت التسمين فى محافظة أسيوط

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تم دراسة ١٣٠ كتكوت مريض ونافق عند عمر (١-١٠ أيام) تم تحديد ٤٨ كتكوت مصاب باحتباس كيس المح بنسبة ٣٦,٩% وكانت الافات التشريحية المميزة للحالات اظهر عدم امتصاص كيس المح فى كل الحالات وكانت اكياس المح كبيرة ومتورمة ومكوناتها تحتوى على مح لونة اصفر أو يميل الى اللون البنى واحيانا مائى القوام أو متجبن خاصة فى عمر ٦-٧ أيام وكانت تلك الحالات مقترنة برائحة كريهة مع أنتفاخ بطون الطيور المصابة وايضا رطوبة وعدم ألتئام السرة. تم عزل ١٢ عترة من السالمونيلا من الحالات المصابة باحتباس كيس المح (٤٨) بنسبة ٢٥% واظهرت الاختبارات السيرولوجية ان ٥ عترات بنسبة ٤١,٦% كانت S.enteritidis، ان ٣ عترات كانت S.virshow بنسبة ٢٥%، عترتان كانت S.typhimurium بنسب٦ ,١٦% عترة كانت S.heidelberg بنسبة ٨,٣%، وعترة S.kentuky بنسبة٨,٣ %. كانت اعلى نسبة أصابة بالتهاب كيس المح ٤٥,٨% فى مجموعة الطيور فى عمر ٥-٦ يوم \ وكانت نسبة حدوث المرض ٢٥% فى الطيور فى عمر ٣-٤ يوم \ثم٨ ,٢٠% فى الطيور المصابة فى عمر ٧-٨ يوم\ وأقل نسبة حدوث المرض٣ ,٨% كانت فى الكتاكيت فى عمر ٩-١٠يوم هذا ولم يتم تسجيل أى حالات فى اليومين الاول والثانى من عمر الصيصان. وأظهرت النتائج الميكروسكوبية أن هناك تغيرات شديدة فى الكبد والقلب والامعاء وكيس المح وهذة التغيرات تمثلت ف الاحتقان الشديد وارتشاح الخلايا وخاصة الخلايا الليمفاوية كما وجد تنكرز تجلطى فى خلايا الكبد وسمك فى جدار كيس المح مع تليف فى اغشيتة وتنكرز تجلطى وارتشاحات للخلايا الالتهابية وخاصة الليمفاوية وكذلك تغيرات فى الامعاء والقلب والتهاب فى غشاءالقلب وارتشاحات ليمفاوية فى عضلة القلب وتضخم وسمك وتليف فى جدار كيس المح.