



Present Situation on Incidence of Paratyphoid Infection in Kafrelsheikh Chicken Farms, Egypt



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THIS work aimed to investigate the epidemiology and methods of diagnosis and treatment of *Salmonella* paratyphoid infection in chickens during the period from March to December 2020. For this purpose, a total of 200 suspected diseased chicks 2-14 days old were collected from 10 chicken farms located in Kafrelsheikh Governorate, Egypt. These chicks were examined, and then samples were taken from the lung, liver, intestine, and gall bladder to investigate the current situation of the incidence of *Salmonella* paratyphoid. These samples were examined bacteriologically, biochemically and serologically and histopathological examination with treatment of experimentally infected chicks. The results revealed 36 isolates with an incidence of (18%). The prevalence of *Salmonella* paratyphoid in the internal organs was highest in the intestine (55.6%), followed by liver (25%), gall bladder (13.9%), and lowest in lung (5.6%). Serotyping of *Salmonella* isolates revealed that two different serotypes (*S. Kentucky* and *S. rechovot*) with incidence of 28(77.8%) and 8(22.2%) respectively. The antibiogramme of isolated salmonella strains revealed sensitivity to thiamphenicol, amoxycillin and sulphatrimithoprine, respectively, but, resistant to flomoxone, neomycin and clindamycin. Conclusion: *Salmonella* paratyphoid is a serious infectious disease to all poultry farms due to high morbidity and mortality. Prevention and control can be achieved by adopting the principles of HACCP (Hazard Analysis Critical Control Point). Hygiene and biosecurity should be part of the overall management of the farm.

Keywords: Antibiogramme, Epidemiology, *Salmonella paratyphoid*, Treatment.

Introduction

In many countries, poultry production is considered as one of the most highly developed segments of food animal production [1-4]. It is very essential not only for the national economy but also, for welfare of human beings, particularly in Egypt. Recently, this industry is intensely affected by many constraints especially; low productivity, diseases, poor husbandry and feed shortage which affect the optimal performance of this industry.

Genus *Salmonella* is one of the most common infectious agents, especially in areas with poor hygiene [5]. *Salmonella* spp. are Gram negative, rods shaped, non-sporulating, non-capsulating, aerobic and

facultative anaerobic organisms belong to Enterobacteriaceae family [6]. More than 2300 *Salmonella* serotypes have been identified but only about 10% had been isolated from poultry. Paratyphoid infection results from poultry infection by a non-host adapted motile *Salmonellae*. Generally, it is present as a subclinical infection responsible for many cases of food borne illness all over the world [7]. Chickens can be infected with many serotypes of *paratyphoid Salmonellae*, especially *S. typhimurium*, *S. enteritidis* and *S. heidelberg* which are worldwide in distribution and have high economic and public health importance [8,9]. Regarding to the impact on health in young chickens, paratyphoid causes diarrhea and dehydration with high mortality rates, meanwhile

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in adult chickens, it doesn't cause significant clinical signs or mortality [10,11]. Concerning to the public health significance, salmonellosis is one of the most important zoonotic disease [12].

Animal or human feces or urine are the main sources of *Salmonella* infection; it contaminates feed and drinking water, as well as, flies and dust [13]. *Salmonella* can be transmitted horizontally or vertically. Vertical transmission is caused by transovarian infection as a result of a systemic infection to the mother bird resulting in ovary infection and egg production in the oviduct [14]. In naturally infected chickens, *paratyphoid Salmonellae* can localize in the ovary or oviduct and contaminate the egg contents constituting insidious risks for public health [12]. Furthermore, workers, vehicles, clothes, equipment, feed, water, wild birds, insects, pets and rodents that can transmit *Salmonella* in chicken farms [15].

Therefore, identifying the nature of the infectious diseases, risk factors and conducting the patterns of diseases are very important in designing control and preventive measures. So, the present study was designed to investigate present situation on incidence of paratyphoid infection in Kafrelsheikh chicken farms.

Material and Methods

Samples collection

The present study was conducted in the March-December period of 2020. A total of 200 chicks (2-14 days old) were collected from 10 commercial layers and broiler farms in different places of Kafrelsheikh Governorate, Egypt. These chicks were examined and then samples were taken from the lung, liver, intestine and gall bladder for isolation and identification of *Salmonella paratyphoid* in the Bacteriology Laboratory, Animal Health Research Institute, Kafrelsheikh branch.

Bacterial isolation

On the surface of MacConkey and *Salmonella-Shigella* agar media, a subculture from each of the enrichment broth was made. The plates were incubated at 37 °C for 24 hr. The suspected colonies were picked up and inoculated into soft agar as stock medium and into slant agar according to the base of ISO 6579 technique [16].

Biochemical identification

Each purified isolated culture was biochemically identified according to the scheme stated by Finegold and martin [17].

Serological identification

This was carried out by the slide agglutination test according to Kauffman-White scheme [18] for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

Antibiogram

One colony of *Salmonella paratyphoid* strain was cultured on selenite F. broth and incubated at 37°C for 6 hrs. One hundred microliters of the culture were spread uniformly onto the Muller Hinton agar then antibiotic discs were spread uniformly onto culture and the plates were incubated for 8 hours at 37°C, then the inhibition zones were measured according to the reference [19].

Pathogenicity test:

One hundred 1-day old broiler chicks (Avian Elhalwany, Gamassa, 44gm free from *Salmonella*) apparently healthy were floor reared and fed on antibiotic-free ration (Rabeh starter 23% protein). These chicks were randomly divided into five groups each group contains twenty chicks as illustrated in Table 3. Chicks were kept for 14 days for observation and recording of the clinical signs, post-mortem findings and mortalities in infected groups and compared with the non-infected group. Re-isolation trials were carried out from dead, moribund and apparently healthy chicks at the end of the experiment.

Histopathological examination:

The tissue was trimmed, washed and processed in ascending grades of alcohol, cleared in chloroform, embedded in paraffin, sectioned using a microtome and stained with hematoxylin and eosin (H&E) stain as illustrated by Luna [20]. Photomicrography was taken using a photomicrographic camera.

Statistical analysis

Statistical analysis of the obtained results was carried out according to Petrie and Watson [21].

Results

The obtained results revealed that 36 samples were positive for *Salmonella paratyphoid* with a prevalence of 18%, while 164 samples with a prevalence of 82% were *Salmonella paratyphoid-free*. The intestine had the highest isolation rate (55.6%), followed by liver and gall bladder (25% and 13.9%), while the lowest isolation rate was recorded in lung (5.6%). On the other side, 44.4%, 75%, 86.1% and 94.4% of the examined intestine, liver, gall

bladder, and lung, respectively were *Salmonella paratyphoid* free.

S. paratyphoid colonies grew on S.S agar media and appeared as white colonies with black center. These colonies appeared after 48 hrs (Fig. 1). On

MacConkey agar, *Salmonella* colonies appeared as colorless translucent colonies, but sometimes, these colonies had dark centers. Smears stained with Gram's stain from the suspected colonies were Gram negative, rod-shaped and non-spore forming bacilli.

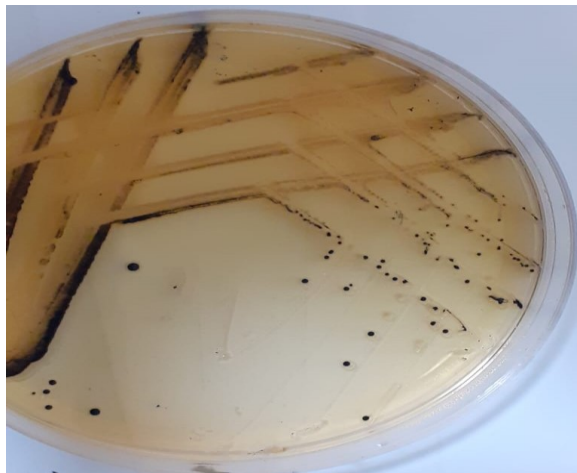


Fig. 1. *S. paratyphoid* colonies on Salmonella – Shigella agar media.

The isolated micro-organisms were catalase-positive and oxidase, indole, phenol red, sucrose, Voges –Proskauer and urease negative. While, methyl red, H₂S production, citrate utilization and glucose positive (Table 1). The isolates were subjected to serotyping using polyvalent and monovalent somatic

(O) and flagellar (H) Salmonella antisera via slide and tube agglutination test according to Kauffman Kauffman [18]. Results revealed two different serotypes *S. Kentucky* (77.8%) and *S. rechovo* (22.2%).

TABLE 1. Biochemical identification of *S. paratyphoid*.

Test	Result
Triple Sugar Iron	Yellow butt
Lysine decarboxylase	Purple butt
Hydrogen Sulphide	Blackening
Urease	No color change
Lysine decarboxylase broth	Purple color
Phenol red dulcitol broth	Yellow color with gas
Malonate broth	No color change
Indole test	Yellow surface
Poly valenat flagellar test	Agglutination
Poly valenat somatic test	Agglutination
Phenol red lactose broth	No color, no gas
Phenol red sucrose broth	No color, no gas
Voges-Proskauer	No color change
Methyl red	Diffuse red color
Simmons citrate	Growth, blue color

Antimicrobial susceptibility of the isolated *S. paratyphoid* strains was tested against 7 antibiotics using the disc diffusion method. The isolated strains were 31(86%), 25(69.4%), and 22(61.1%) sensitive to thiamphenicol, amoxicillin and sulphatrimithoprine, respectively. While, the resistant rate was 31(86.1%),

22(61.1%), and 22 (61.1%) to flomoquine, neomycin and clindamycin, respectively (As illustrated in Table 2 and (Fig. 2)). The clinicopathological picture of the experimentally infected chicks were ruffled feather, huddled together, inappetence, dropping of wing, dullness and diarrhea with pasty vent (Fig.3).

TABLE 2. Antibiotic sensitivity of *Salmonella paratyphoid* (No.=36).

Antibiotic	Disc potency	Inhibition Zone (mm)			R	I	S
		R	I	S			
Thiamphenicol (T)	20 ug	17	18-21	22	5(13.9%)	0	31(86%)
Amoxicillin (AML)	25 ug	22	23-30	31	11(30.6%)	0	25(69.4%)
Sulphatrimithoprine (SXT)	25 ug	17	18-21	22	12(33.3%)	2(5.6%)	22(61.1%)
Colstine (CL)	10 ug	8	9-10	11	18(50%)	4(11.1%)	14(38.9%)
Clindamycin (DA)	2 ug	14	15-20	21	22(61.1%)	1(2.8%)	13(36.1%)
Neomycin (N)	10 mg	12	13-16	17	22(61.1%)	4(12.5%)	10(27.8%)
Flomoquine (UB)	20 ug	13	14-17	18	31(86.1%)	0	5(13.9%)

No.: Number of isolates

R: Resistant, (I): Intermediate, (S): Sensitive

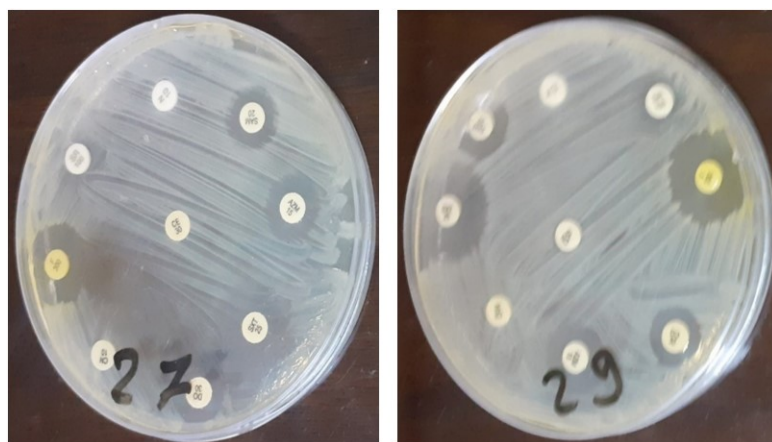


Fig. 2. Antibiotic sensitivity test *S. paratyphoid* onto the Muller Hinton agar.



Fig.3. Clinical signs of experimentally infected chicks with *S. paratyphoid* showing ruffled feather, inappetence, dropping of wing, dullness and diarrhea with pasty vent.

The post-mortem finding of the dead and sacrificed chicks was illustrated in Fig. 4. During the first day after challenges septicemia manifested by severe congestion of the liver, spleen, heart and kidney was detected. Meanwhile, the post-mortem finding of birds died two days after challenges was pericarditis, perihepatitis and ascites (Fig. 4A). After six days of challenge, the post-mortem findings of the dead birds were enlarged liver with yellow coloration, distended and enlarged gall bladder (Fig. 4B), congested lung with hemorrhagic patches, congested

heart with engorged blood vessels and yellowish white intestinal contents. After the seventh day, severe enteritis, enlarged liver with distended gall bladder, fibrinous pericarditis and perihepatitis as well as an unabsorbed yolk sac was observed post-mortem (Fig. 4C, D). While, after 8 days, severe enteritis, enlarged liver with yellowish discoloration, distended gall bladder and the two ceca were distended with yellowish-white contents, when compared with the non-infected control group which was still clinically healthy (Fig. 4C).

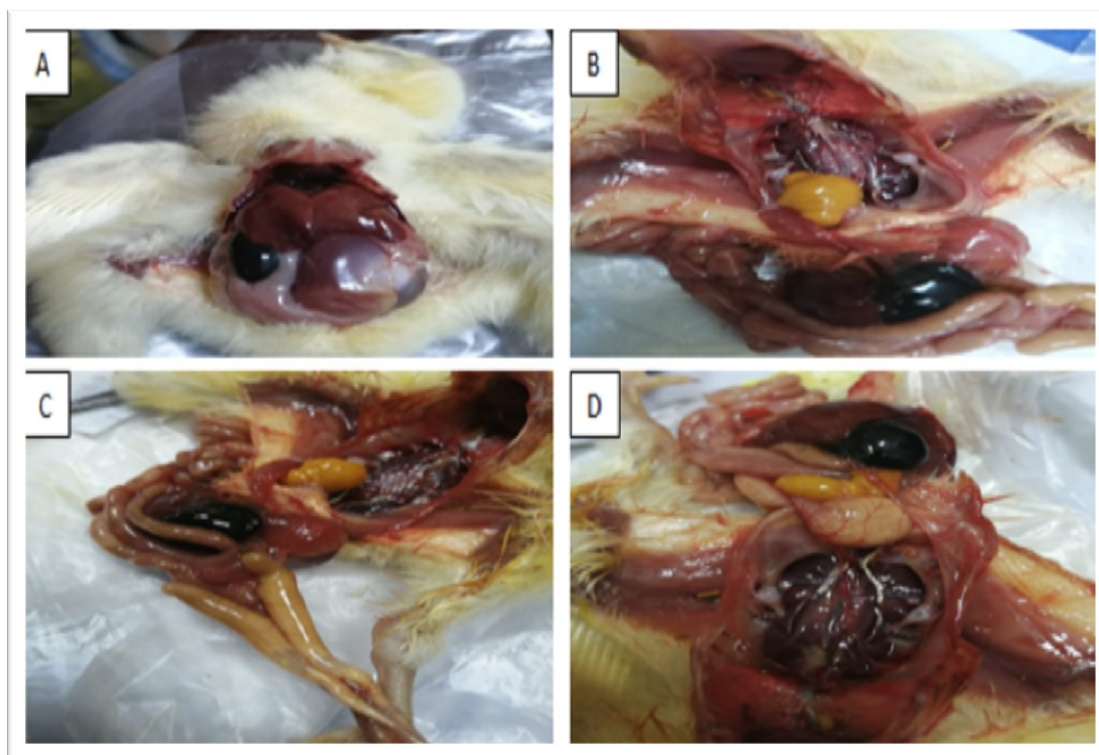


Fig. 4. Post mortem findings of experimentally infected chicks with *S. paratyphoid*; (A) shows septicemia manifested by congestion of liver, spleen, heart and kidney; (B) shows sever enteritis, enlarged liver with distended gall bladder; (C) shows distended two cecai with yellowish white contents; and (D) shows sever enteritis, congested kidney and unabsorbed yolk sac.

The experimentally infected chicks with *S. paratyphoid* were divided into five groups as illustrated in Table 3. Group one was inoculated orally with sterile saline 0.5 ml and it was clinically normal. The mortality rate in experimentally infected untreated birds was high (50% for *S. Kentucky* and 20% for *S. rechovot*) compared with the uninfected

and treated group. The treatment trial of experimentally infected chicks was in line with the result of the tested antibiogram of the isolated *S. Kentucky* and *S. rechovot*. Treatment of infected chicks by thiamphenicol 20% resulted in a low mortality rate (10%) compared with 50% and 20% in infected untreated chicks.

TABLE 3. Treatment trail in experimentally infected chicks with *S. paratyphoid*.

G.	No. of birds	Treatment	Clinical signs	Morbidity	Mortality
1	20	Ssterile saline 0.5ml orally	-	-	-
2	20	Infected with <i>S. kentucky</i> , 0.5ml (4x10 ⁸ CFU)	Ruffled feather, huddled together, respiratory manifestation and stunting growth	55%	50%
3	20	Infected with <i>S. rechovot</i> , 0.5ml (4x10 ⁸ CFU)	Whitish diarrhea, pasty vent and stunting growth	20%	20%
4	20	Infected with <i>S. kentucky</i> , 0.5ml (4x10 ⁸ CFU) and treated with thiamphenicol 20% (1.25ml/L)	Whitish diarrhea, inappentance, huddling around light	10%	10%
5	20	Infected with <i>S. rechovot</i> 0.5ml (4x10 ⁸ CFU) and treated with thiamphenicol 20% (1.25ml/L)	Diarrhea and loss of appetite	10%	10%

G: group No: Number CFU: Colony forming unit

The histopathological examination of experimentally infected chicks was illustrated in Fig. 5. Group one had normal liver, gall bladder, intestine, heart and lung tissues (Fig. 5A). Group two which was infected with *S. Kentucky*, revealed vacuolar degeneration in hepatocytes with congestion in hepatic blood vessels and some hepatocytes showing necrobiotic changes. In addition, focal congestion in pre-alveolar blood capillaries was detected in lung tissue with severe edema in cardiac muscles and myositis. Also, severe necrosis in intestinal epithelium and severe atrophy in intestinal villi was found (Fig. 5B). Group three which was infected with *S. rechovot*, revealed severe congestion in portal blood vessels and central veins, some hepatocytes showing vacuolar degeneration while some hepatocytes showing necrobiotic changes, presence of thrombus in central vein, in addition to glandular epithelium showing necrobiotic changes in gall bladder (Fig. 5C). Some alveoli had necrotic changes with fibrin exudate in its lumens with mono nuclear inflammatory cells infiltration as well as severe

cardiac edema with severe myositis and some myocytes showing necrotic changes was also found, severe atrophy of intestinal villi with severe necrosis in intestinal epithelium was seen (Fig. 5C). Group four which was infected with *S. Kentucky* and treated with thiamphenicol 20%, revealed severe vacuolar degeneration in hepatocytes with congestion in central veins as well as necrobiotic changes in the glandular epithelium of gall bladder (Fig. 5D). Severe cardiac edema with severe myositis and some myocytes showing necrotic changes and some parts of intestine showing severe hyperplasia in intestinal villi while other part showing desquamation of intestinal villi (Fig. 5D). Group five which was infected with *S. rechovot* and treated with thiamphenicol 20%, revealed congestion in portal blood vessels and hepatic sinusoids as well as multiple focal congestion in prealveolar capillaries with mononuclear inflammatory cell infiltration with mild edema between cardiac muscle bundles and some nuclei of myocytes showing pyknosis (Fig. 5E).

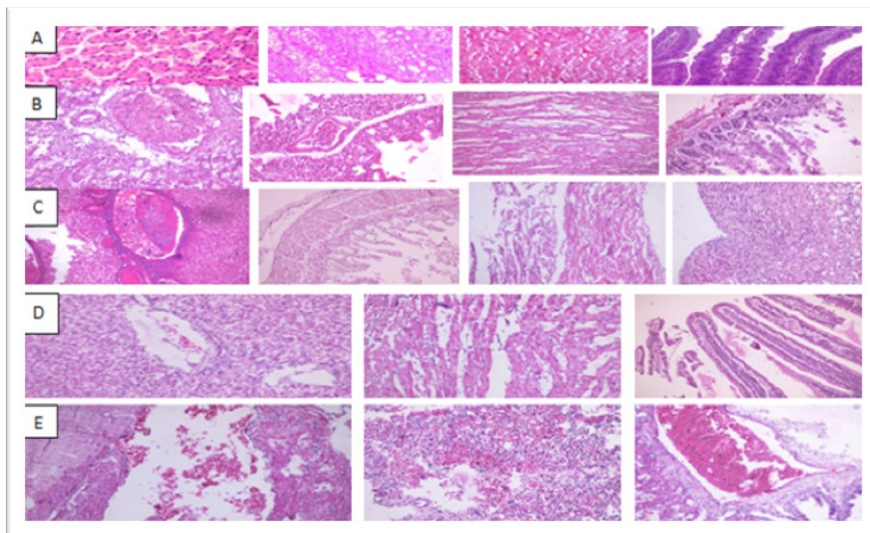


Fig. 5. Histopathological examination of experimentally infected chicks with *S. paratyphoid*. (A) Group one with normal structure of liver, lung, heart and intestine. (B) Group two infected with *S. kentucky*, with vacuolar degeneration in hepatocytes, congestion in pre alveolar capillaries, severe edema in cardiac muscles with myositis, and necrosis in intestinal epithelium with atrophy in its villi. (C) Group three infected with *S. rehovot* showing severe congestion in portal blood vessels and central veins, vacuolar degeneration, and thrombus, and necrobiotic changes in gall bladder; necrotic changes with fibrin exudate in alveoli lumens; severe cardiac edema with myositis; and atrophy of intestinal villi. (D) Group four infected with *S. kentucky* and treated with thiamphenicol 20%, revealed severe vacuolar degeneration in hepatocytes with congestion in central veins and necrobiotic changes in gall bladder; severe cardiac edema with myositis; and hyperplasia in intestinal villi. (E) Group five infected with *S. rehovot* and treated with thiamphenicol 20%, revealed multiple focal congestion in prealveolar capillaries with mononuclear cell infiltration, edema between cardiac muscles and some nuclei of myocytes showing pyknosis.

Discussion

Salmonella spp. particularly, *S. paratyphoid* is considered one of the most important pathogens for broiler and layer farms. *Salmonella paratyphoid* is implicated in many disease conditions within poultry farms, resulting in severe economic losses due to mortality and reduced production. Concerning public health, salmonellosis is a significant zoonotic disease causing many health hazards especially food poisoning cases. Therefore, identification and serotyping of *Salmonella spp.* in poultry is necessary to understand and control the associated infections.

Our results revealed that 18% of samples were positive for *Salmonella paratyphoid* and 82% were *Salmonella paratyphoid*-free. This result was in line with what has been recorded by many authors [7,22-24], while lower results (2%, 2.5%, 11.99%, 10%, 10.7%) were reported by some authors [25-30]. Higher results (30%, and 25% were recorded by some literatures [31,32]. These differences in prevalence may be related to many factors including, environment, management system, biosecurity,

antibiotics, the strain of chickens and the resistant of these chickens to *S. paratyphoid* [33].

The isolation rate was higher from the intestine than the liver followed by gall bladder, while the lowest isolation rate was recorded in the lung. These results were in agreement with some researchers [34-38].

The morphological identification of the isolated microorganism was in line with some reports [39,40] as Gram-negative small rod shape appearance and arranged in single on smears stained with Gram's stain. *S. paratyphoid* colonies grew on S.S agar media and appeared as white colonies with black centers. These colonies appeared after 48 hrs. On MacConkey agar, *Salmonella* colonies appeared as colorless translucent colonies, but sometimes, these colonies had dark centers.

Results of biochemical identification of the isolated microorganism was in accordance with many studies [24,41-43]. Our results regarding serotyping identification (*S. Kentucky*, 77.8% and *S. rehovot*, 22.2%) was in line with some authors[24,30] who

isolated *S. kentucky* from different poultry farms. However, this result differed from some studies [44,45] who reported that *S. enteritidis* and *S. typhimurium* were the most common serotypes isolated from poultry. Also, Asawy and Abd El-Latif [46] who recorded three different serovars including *S. typhimurium*, *S. derby* and *S. enteritidis* from private farms at the Dakahlia Governorate.

As a result of the widespread of multi-drug resistant *S. paratyphoid* and for an accurate and proper treatment as well as avoiding complications of the infection, antimicrobial susceptibility of the *S. paratyphoid* strains was 31(86%), 25(69.4%), and 22(61.1%) sensitive to thiamphenicol, amoxycillin and sulphatrimithoprine, respectively. While, the resistant rate was 31(86.1%), 22(61.1%), and 22 (61.1%) to flomoquine, neomycin and clindamycin, respectively. This result agreed with [47-49]. While different resistance rates were reported by many authors [24,50,51].

The pathogenicity of the isolated *S. paratyphoid* strains to one-day-old broiler chicks was illustrated. The clinico-pathological picture of the experimentally infected chicks with *S. kentucky* and *S. rehovot* were ruffled feathers, huddled together, inappetence, dropping of wing, dullness and diarrhea with pasty vent (Fig. 3). These findings were in accordance with what had been reported by some works [30, 38, 52].

Concerning the post-mortem finding of the dead and sacrificed chicks in our results came in line with what has been reported by several studies [7, 30, 38, 40].

The experimentally infected chicks with *S. paratyphoid*, group one which was inoculated orally with sterile saline 0.5 ml (it was clinically normal). The mortality rate in experimentally infected untreated birds was high (50% for *S. Kentucky* and 20% for *S. rehovot*) compared with the uninfected and treated group. These findings were in line with many investigators [10,11,23], while lower mortality rate was reported by Srinivasan *et al.* [7]. The treatment trial of experimentally infected chicks was in line with the result of the tested antibiogram of the isolated *S. Kentucky* and *S. rehovot*. Treatment of infected chicks by thiamphenicol 20% resulted in a low mortality rate (10%) compared with 50% and 20% in infected untreated chicks. This result agreed with some studies [53-55].

The histopathological examination of experimentally infected chicks agreed with some reports [56-58] who reported nearly similar results. In the gall bladder, there was a hemorrhage between the glandular epithelium with necrobiotic changes. This result was in agreement with [58]. Heart thickening of the pericardium, congestion and hemorrhage especially below the epicardium in addition to edema

in cardiac muscles with myositis were detected. Also, some researchers [58,59] reported similar result. The intestines in affected birds generally revealed congestion of mucosal vessels, marked goblet cell hyperplasia mild to moderate infiltration of heterophils and mononuclear cells in the lamina propria of the villi, in chronic cases of paratyphoid the lesions in caeca comprised of congestion and hemorrhage with degeneration and desquamation of lining epithelium and mononuclear cell infiltration in the mucosa and sub-mucosa which resulted in atrophy of intestinal glands. These findings were consistent with the findings of many studies [37,58,60]. of the study for example.

Limitations:

Lack of molecular approach using PCR.

Conclusion

Salmonella paratyphoid is a serious infectious disease to all poultry farms due to high morbidity and mortality. Prevention and control can be achieved by adopting the principles of HACCP (Hazard Analysis Critical Control Point). Hygiene and biosecurity should be part of the overall management of the farm.

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Conflict of interest

The authors declare that there is no conflict of interest.

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الوضع الحالي لحدوث عدوى الباراتفويد في مزارع الدواجن بكفر الشيخ

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تهدف الدراسة إلى معرفة الوضع الحالي لحدوث السالمونيلا باراتفويد. تم جمع 200 كتكوت من عدة مزارع تجارية للبياض والدجاج في أماكن مختلفة بمحافظة كفر الشيخ، مصر. تم فحص هذه الكتاكيت ومن ثم أخذ عينات من الرئة والكبد والأمعاء والمرارة. تم فحص العينات بكتيريًا وكيميائيًا وسيرولوجيًا، بالإضافة إلى فحص المضاد الحيوي للكائن الدقيق المعزول. أظهرت النتائج أن 36 (18%) عينة كانت موجبة للسالمونيلا باراتفويد بينما كانت 164 (82%) خالية من السالمونيلا باراتفويد. كان معدل وجود بكتيريا السالمونيلا باراتفويد من الأعضاء المختلفة أن الأمعاء سجلت أعلى معدل عزل (55.6%)، تليها الكبد والمرارة (25% و13.9%)، بينما سجلت أقل نسبة عزل في الرئة (5.6%). التتميط السيرولوجي لفصيلة السالمونيلا المعزولة كشف عن عزل نمطين مختلفين سيرولوجيًا (*S. kentucky* و *S. rehovot*) من العينات المفحوصة بنسبة انتشار 28 (77.8%) و 8 (22.2%). كانت السلالات المعزولة 86% و69.4% و61.1% حساسة للثيامفينيكول والأموكسيسيلين والسلفاتريميثوبرين على التوالي، بينما كانت المقاومة 86.1% و61.1% و61.1% للفلوموكين والنيومايسين والكلنداميسين على التوالي. لذلك، فإن ثيامفينيكول 20% دواء فعال في برامج مكافحة في المزارع المصابة بالسالمونيلا.

الكلمات الدالة: المضادات الحيوية، الوبائيات، السالمونيلا باراتفويد، العلاج.