Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024
Journal Homepage: https://djvs.uodiyala.edu.iq
https://doi.org/10.71375/djvs.2024.02406

A Comparative Analysis of the Conventional Versus Molecular Detection of Salmonella *spp.* Infection, with Particular Emphasis on the Histopathologic Changes Observed in Layer Hens

Sameer Shawket Hameed[1] , Khalid Ibrahim Abd Alkhazraji[2] , Ghassan H. Jameel[3]

[1,3] Department of Veterinary Microbiology, College of Veterinary Medicine, University of Diyala

[2] Department of Anatomy, College of Medicine, University of Diyala

[*]**Corresponding Author: E.mail:** Khalid.i@uodiyala.edu.iq

Abstract

This research was carried out to investigate the presence of Salmonella SPP in layer poultry farms through various microbiological tests . A total of 585 samples (from 150 suspected birds) included (liver (150), ceca (50), heart (48), spleen (37), oviduct (150), ovary (150) were obtained from flocks in Diyala. Determination of *salmonella spp* was carried out via PCR technique, biochemical tests, serotyping by O antigen and H antigen, API 20 and Viteck. Liver samples were examined microscopically to detect any changes. The results revealed that the highest number of Salmonella spp. isolates was seen in liver (34) (25 Salmonella ohio, 4 *Salmonella Kentucky* and 5 *Salmonella enteritidis)*. and the rate of infection reach to (22.66%) followed by ovary (15) (10%)(13 *Salmonella ohio and 2 Salmonella enteritidis)*, oviduct (6) (4%)(5 Salmonella ohio and 1 Salmonella enteritidis). The histological examination of the liver showed necrosis of hepatocytes, hemorrhage, aggregation of inflammatory cells.

In conclusion, *S. ohio*, *S. enteritidis*, and *S.Kentucky* were the main causative agent for Salmonellosis in laying hens at Diyala governorate .

Keywords: Salmonella, histological, chicken, PCR, liver, API 20, Vitek

Introduction

Among many diseases in poultry such as influenza, Gumboro, Marek, Newcastle, E- Coli, the Salmonella also consider as main cause of mortality and egg reduction in layers which lead to economic losses (1). Salmonella according to (2), Salmon and Smith were the first to isolate Salmonella

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

from pigs in 1885. A significant genus within the Eenterobacteriaceae family is Salmonella. The genus's Salmonella members live in both humans and animals' digestive tracts and are facultative anaerobes that are Gram-negative (3&4).They can be recovered from a variety of hosts, including humans, pigs, poultry, and food sources. Salmonella genus members can be harmful to wild or Humans with domesticated animals(5). Food-borne outbreaks have been linked to this disease, which is significant to the food sector. Enteric fever, gastroenteritis, and septicemia are among the human pathogenic conditions caused by Salmonella (6).

Salmonellosis in poultry results in significant financial loss because of mortality and low manufacturing (7). Salmonellosis has grown to be a major issue around the world as poultry farming has expanded (8). The fact that the disease's causative agents are passed vertically from parent to child makes it especially important. The natural hosts of S. pullorum and S. gallinarum are chickens (9). Pullorum illness often strikes between two and three weeks of age and can occasionally affect adults (10). It's common knowledge that fowl typhoid affects adult birds, but reports of significant mortality rates in young chicks also exist . Farms with laying hens of varying ages, housing arrangements, and flock size are risk factors linked to salmonellosis infection in laying hens (11).

It is typically advised to use conventional bacteriological techniques to identify Salmonella spp. from various references (12). Salmonella spp. surveillance has been made possible by the development of molecular tools like PCR (13). The internal and external poisoning of eggs by Salmonella spp. during laying hen rearing is a serious problem. Therefore, it is exceedingly challenging to implement appropriate salmonellosis management methods (14). Egg contamination can happen through either a vertical or horizontal path. Horizontal transmission results from exterior egg shell contamination and may enter when the egg shell cracks, while vertical transmission is caused by bacterial agent colonization in the female reproductive system and oviduct prior to egg creation of shells (15)

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

. Salmonellosis in multilayer chicks as well as hens need to be assessed in order to carry out efficient disease control strategies (16).

Materials and Methods

Geographical distribution of the study regions:

The experiment included the following areas of Diyala Governorate: Al-Hawish, Al-Jadida, Al-Khalis, Al-Muqdadiya, Al-Sindiya, Baqubah, Baladruz, Kan Bani Saad, Kanaan, and Mandali.

Collections of samples

Five hundred and eighty-five samples were collected from laying chicken in Diyala Governorate based on clinical signs, including samples of various organs (liver, oviduct, ovary, ceca, spleen, heart) in order to identify *Salmonella spp*, for the period from October 2023 to April 2024

Bacterial isolation and culturing

- **Pre-enrichment and enrichment**

All specimens were processed in a sterile manner. One gram of each specimen was placed onto (9 ml) non-selective broth (nutrient broth) then underwent for one day/ at degree of 37 to incubation. Following this, one ml of enriched sample was transferred into nine ml of selective media (F Selenite). They were then subjected to the same incubation time and temperature as before used (17).

- **Culturing**

Salmonella-shigella agar (SS agar), agar of xylose lysine deoxycholate (XLD), and agar of Mac-Conkey had streaked with a loop-full of enriching broth (with selenite F soup). The media left for 24-hour for incubation at 37°C, then examined to detected the growth of salmonella as colonies

Traditional biochemical examinations

Applying Urase test, Oxidase test, Catalase test, Triple sugar iron test (TSI), Simmons citrate test (citrate utilization), Indole test, Ornithine-decarboxylase test, Carbohydrates fermentation test.

API 20E System.

The system of this test composed of twenty-five strips, and each one of them was contained twenty microtubules that formed of (lower tube and upper cupule). Every microtubule was acting as biochemical test and contained substrate of dehydrated material.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

The API 20E test was done based on the manufacturer's directions (BioMerieux, France).

Vitek2 System

The Vitek2 system is a fully automatic system, with its identification card was used to recognize bacterial colonies suspected of being Salmonella. Sample was planted on XLD agar, then incubating for one day at 37 C. In later day, and by using of sterile inoculate loop, a clear colony was picked up and mixed into (a tube approved by the company) with normal saline. The Vitek-2 (DensiChek) spectrophotometer was used to measuring the density of the mixture in the tube (the standard turbidity of device is 0.5 McFarland).

Molecular detection

After incubating the colonies for 24 hours, the DNA of bacteria was extracted. The needed primers were prepared depending on instruction of manufacturing. Preparing all requirements of PCR as reaction mixture and amplification conditions. The invA gene was investigated and searched for in all positive isolates of Salmonella bacteria.

Conventional PCR

The conventional (uniplex) PCR process include the following; Reaction mixture (A tube of PCR was a prepared to fill with 20µl of reaction mixture), Amplification condition (The condition of PCR is includes the temperature, time and cycle for each step from denaturation to extension), and the electrophoresis and preparation of gel agarose**.**

Serological tests (Serotyping)

All samples which appeared positive result for salmonella spp. by previous techniques as (biochemical, APi 20 , PCR), were transmitted on TSI media to the laboratories of the central public health/ Ministry of health, Baghdad, Iraq (done Slide agglutination test and serotyping diagnostic technique was made using the Anti-Salmonella test reagents according manufactures of (Sifin diagnostic gmbh, Germany).

Histological examination

The histological procedures were performed according to steps followed by (18).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

Specimens preparation : The chicken in this experiment were sacrificed by dislocating of cervical vertebrae. The abdomen of each bird was opened anatomically (19), and the liver was examined in situ. Then the liver removed from the abdomen for preparation of its specimens to histological examination through applying tissue processing routinely. The Hematoxylin and Eosin stain was used to staining the sections of tissues of the liver and examined under light microscope (Olympus) and pictures were taking via a digital camera (20).

Results

As shown in Table (1), which displays the samples and the number of suspected birds from layer chicken in Diyala province decenter. After being grown on chromogenic agar specifically designed for Salmonella, the colonies showed variations in size, rounded shape, and violet color (Figure 1).

The morphological features of the Bacteria:

Isolates were viewed as gram-negative, tiny rod-shaped things that were grouped in lone or coupending on the clinical signs and necropsy findings.

***Salmonella* spp. isolation and characterization**

Morphology of the Colonies:

Different Salmonella species produced different qualities in different media. The broths used for enrichment and pre-enrichment initially had cloudy or opaque. Salmonella colonies on solid medium had a black core and appeared small, flat, globular, and clear on SS agar due to the production of hydrogen sulphide. All of Salmonella's colonies on MacConkey agar plates appear translucent and colorless because the bacteria are unable to digest lactose. The colonies on XLD agar appeared smooth, with a black

pleds under a compound light microscope Figure (2).

Biochemical and serological results

Every isolate was catalase positive and oxidase negative. Further, its positive for Simmon's citrate, lysine, and ornithine decarboxylase, but negative for urease and indole Figure (3).

Api 20E results:

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

This examination revealed the occurrence of a color change in the test strips, which is represented here by the numerical appearance (6120010), which indicates that the isolate is Salmonella spp (Figure 4).

Molecular identification (PCR) result

The target band of 284 bp was formed in the current study's invasion gene screening by PCR using primer (invA). The PCR result showed a distinct band on all positive samples, indicating the band a coupled with this salmonella gene. Figure (6) Salmonella could be found in the liver, oviduct, and ovary in each of the tissues that were analyzed.

Vitek system result

The findings of Vitek system show positive result for salmonella spp. (figure 5). the Identification of gave positive results that showed Salmonella partially resistant to Gentamycin, Ciprofloxacin and Levofloxacin, and sensitive to Amikacins and Imipenem for antibiotics used in Vitek.

Serotyping results

The results of serotyping tests that conducted at the Ministry of Health/Central Public Health Laboratory showed that the diagnosed bacteria were *Salmonella ohio*, *Salmonella kentucky*, and *Salmonella enteritidis*.

Histological Results

Histological findings of the liver of chicken

A. Control chicken

The histological examination of the liver showed that its parenchyma consists of hepatocytes that arranged in cords or chain radiated from the central vein and form liver lobules (Fig. 7). Numerous sinusoids found in between the hepatic cords. Each liver cells were taken polyhedral shape.

B. Salmonella infected chicken

The microscopic observation of liver revealed necrosis of hepatocytes that formed spaces in liver parenchyma associated with presence of hemorrhage in affected area (Fig.8). It was detected aggregation of inflammatory cells in blood vessel lumen. However, some of these inflammatory cells were penetrated the blood vessel wall toward adjusted hepatocytes. The Infiltration of inflammatory cells (mostly mononuclear cells) found near central vein as well as aggre-

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

gated as cluster to fill the space of necrotic hepatocyte (Fig.9)

Table (1): The regions and samples of suspected layer affected with salmonella spp.

Regions	No. of samples	Suspected birds
Al Huwesh	50	21
Al Jududa	50	24
Al khalis	50	15
Al mugadia	50	10
Al sindia	50	19
Baqubah	50	15
Buldroz	50	13
Kan beni sad	50	14
Kanaan	50	11
Mindlei	50	8
Total	500	150

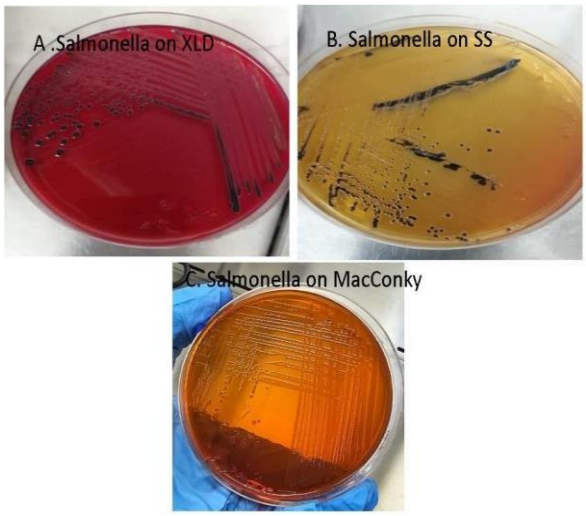

Figure (1) A: Salmonella on XLD agar; B: Salmonella on SS agar; C: Salmonella on MacConkey agar

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

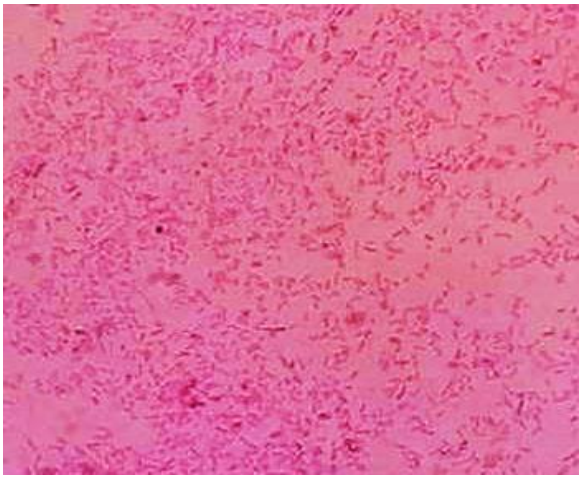

Figure (2): rod shaped Salmonella which appear in pink color.

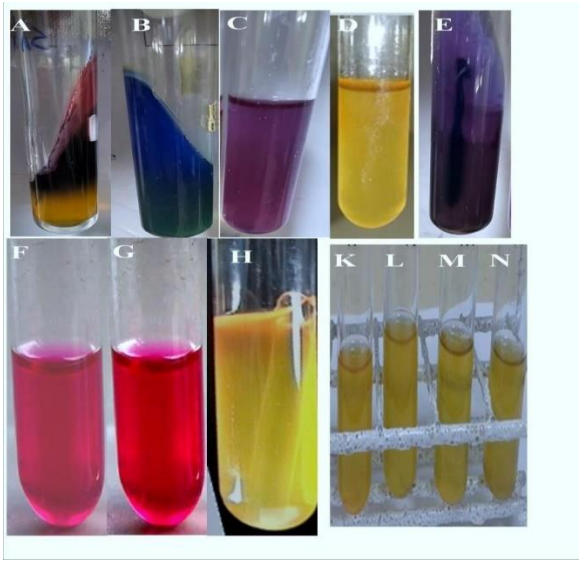

Figure (3) A: TSI ; B: Simmons citrate test ; C:ornithine decarboxylation ; D: indole test ; E: lysine decarboxylation ; F: lactose fermentation; G: sucrose fermentation; H: rhamnose fermentation; K: glucose fermentation; L: maltose fermentation M: mannitol fermentation ; N: dulcitol fermentation

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

Figure (4): A &B show the Api 20 E Test positive for Salmonella

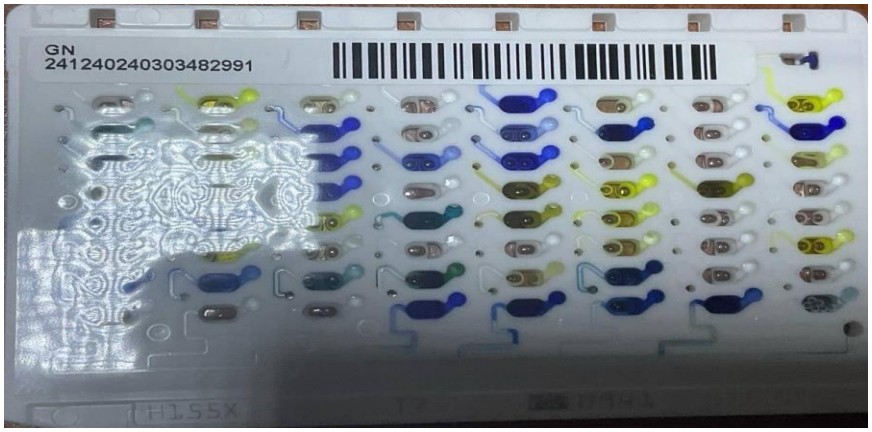

Figure (5): Vitek Test positive for Salmonella

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

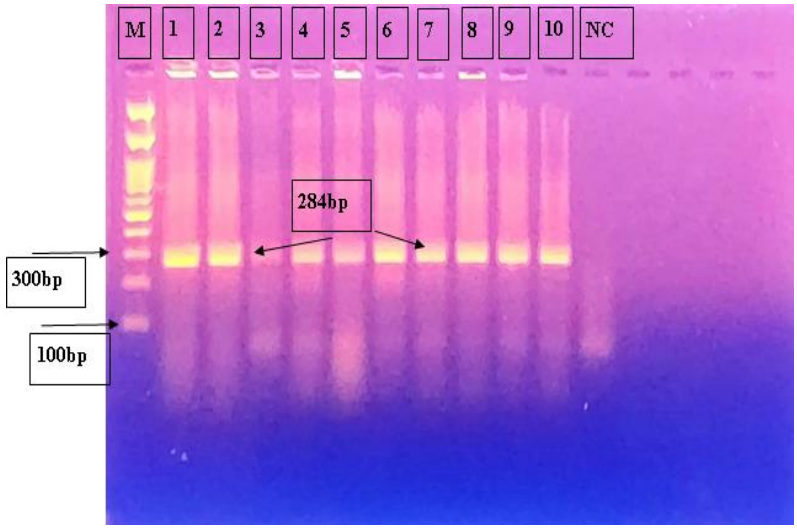

Figure (6): The isolated genomic DNA samples were subjected to gel-electrophoresis using 1% agarose and 3vol/cm in TBA buffer. By using the primer (invA) for PCR screening of the invasion gene, a 284 bp target band was obtained. Samples in Lanes 1 through 11 had positive results (Salmonella spp.). PCR markers (1-3 oviducts), (4-6 ovaries), and (7-10 liver) Lane were seen in.

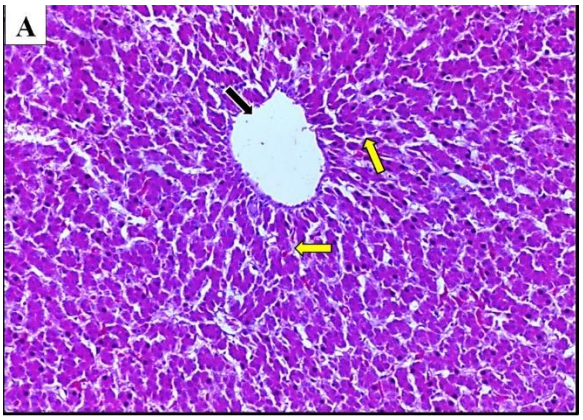

Figure (7) A : Liver photomicrograph in control group chicken showed:Normal hepatic histological architectures. Note central vein (black arrow) and hepatocyte chains (yellow arrow) that surrounded the central vein. (100x) stain H&E.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

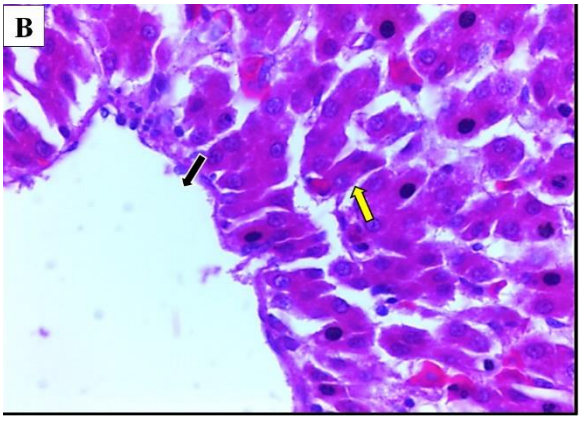

Figure (7) B : Liver photomicrograph in control group chicken showed: Normal hepatic histological architectures. Note central vein (black arrow) and hepatocyte chains (yellow arrow) that surrounded the central vein. (400x) stain H&E.

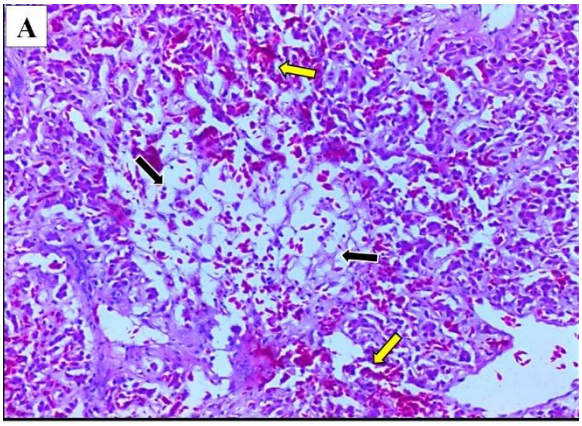

Figure 8: Liver photomicrograph in Salmonella infected chicken showed: Necrosis of hepatocytes that formed spaces (black arrow) in liver parenchyma was observed with presence of hemorrhage in affected area (yellow arrow). (100x), stain H&E.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

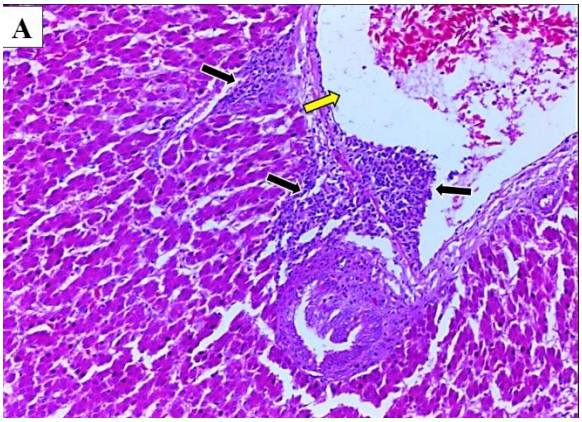

Figure 9: Liver photomicrograph in Salmonella infected chicken showed: Aggregation of inflammatory cells (black arrow) in blood vessel lumen (yellow arrow), however some of these inflammatory cells were penetrated the blood vessel wall toward adjusted hepatocytes (black arrow). H&E. A: 100x

Discussion

At the present study, All of Salmonella's colonies on MacConkey agar plates appear translucent and colorless because the bacteria are unable to digest lactose. The colonies on XLD agar appeared smooth, with a black center. After being grown on chromogenic agar specifically designed for Salmonella, the colonies showed variations in size, rounded shape, and violet color. These findings corresponding to description of (21 & 22). The result of Biochemical and serological tests that including catalase, oxidase, Simmon's citrate, lysine, and ornithine decarboxylase positive, but negative for urease and indole in current experiment were comparable to that data detected by (23) for salmonella characterization. The positive result of Api 20E test for isolates in the current experiment was support that suggestion of (24) who stated highest value result may associated with using of APi 20E . Further, the findings The findings of Vitek system show positive result for salmonella spp. in present study which also showed the Salmonella partially resistant to Gentamycin, Ciprofloxacin and Levofloxacin, and sensitive to Amikacins and Imipenem for antibiotics were similar to finding of (25) through using of Vitek

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

2 system. The PCR result showed a distinct band on all positive samples, indicating the band a coupled with this salmonella gene. Similar finding by (26) was detected in chicken samples when examined by PCR technique which proven to presence of salmonella bacteria. In addition, (27) reported that poultry products as meat showed positive reaction for gene invA which refer to presence of salmonella when used of the technique of PCR. As well as, (28) reported in Saudi Arabia, that invA gene which amplifies 284 bp fragments was a target to identify all the examined Salmonella serovars, while negative results were given in all non-Salmonella serovars. These results were in agreement with the results of this study. At previous study on chicken carcasses by (29) which detected 40 Salmonella isolates, then all these strains were subjected to invA gene and clear the product of 284 bp DNA fragment, and these results were in same to that observed in present study

By using of analysis of PCR, (30) recorded at 270 sample of pork about 37 Salmonella isolates which were displayed positive reaction to gene invA gene. These findings were corresponding to these detected in this study. The results of present research were in disagreement with the results that reported by (31) who show 5 isolate out of 8 isolate of salmonella were contain the gene of invA in captive wildlife. Salmonella's usual characteristics, which were in line with findings from previous study (32). The conventional approach of detecting *Salmonella gallinarum* was found to have rapid and definite fidelity and discriminatory capacity, as demonstrated by the molecular identification of the isolates using a serotype-specific PCR assay (33).The results of serotyping tests that conducted at the ministry of health/central public health laboratory showed that the diagnosed bacteria were *Salmonella ohio*,

Salmonella kentucky, and *Salmonella enteritidis*.This test confirmed that the bacterial isolate was *Salmonella* genus by causing clear agglutination when adding polyvalent antisera H and O separately. These finding were confirmed what that (34) said about the outer surface of bacteria contain O and H antigen. Depending on the Kauffmann-White scheme, H and O antigens serve as the basis

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

for immunological identification and classification methods for Salmonella (35 & 36). In the present examination, the liver in control chicken showed the normal architectures and arrangement of hepatocytes, cells shape, and the liver lobules. These finding is corresponding with that reported of other authors (37 & 38). The histological lesions that observed in the liver of chickens infected with salmonella were corresponded with the that mentioned by (39, 40 & 41) in chickens.

In conclusion, *S. ohio*, *S. enteritidis*, and *S.Kentucky* were the main causative agent for Salmonellosis in laying hens at Diyala governorate.

References

Dar MA, Ahmad SM, Bhat RA, Ahmed R, Ganai NA. Salmonella typhimurium in poultry: a review. World's Poultry Sci. J. , 2017;73(2): 345-354.

1. Ryan G, Roof S, Post L, Wiedmann M. Evaluation of rapid molecular detection assays for Salmonella in challenging food matrices at low inoculation levels and using difficult-to-detect strains. Journal of food protection. 2015 September; 78(9): 1632-1641.
2. Holt PS, Buhr RJ, Cunningham DL, Porter RE. Effect of two different molting procedures on a Salmonella enteritidis infection. Poultry Science. 1994 August; 73(8): 1267- 1275.
3. Gast RK, Dittoe DK, Ricke SC. Salmonella in eggs and egg-laying chickens: Pathways to effective control. Critical Reviews in Microbiology. 2024; 50(1): 39-63
4. Stathas L, Aspridou Z, Koutsoumanis K. Quantitative microbial risk assessment of Salmonella in fresh chicken patties. Food Research International. 2024; 178: 113960.
5. Higuera-Ciapara I, Benitez-Vindiola M, Figueroa-Yanez LJ, Martinez-Benavidez E. Polyphenols and CRISPR as Quorum Quenching Agents in Antibiotic-Resistant Foodborne Human Pathogens (Salmonella Typhimurium, Campylobacter jejuni and Escherichia coli 0157: H7). Foods. 2024; 13(4): 584.
6. Aragaw K, Terefe L, Abera M. Prevalence of Salmonella infection in intensive poultry farms in Hawassa and isolation of Salmonella species from sick and dead chickens. Ethiopian Veterinary Journal, 2010; 14(2): 115-124.
7. Hossain J, Attia Y, Ballah FM, Islam S, Sobur A, Islam A, Ievy S, Rahman A, Nishiyama A, Islam S, Hassan J, Rahman T. HOSSAIN, M. J. et al. Zoonotic significance and antimicrobial resistance in Salmonella in poul-

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

try in Bangladesh for the period of 2011–2021. Zoonotic Disease, 2021, 1(1): 3-24.

8. Xiong Z, Wang S, Huang Y, Gao Y, Shen H, Chen Z, Bai J, Zhan Z, Wen J, Liao M, Zhang J. XIONG, Z. et al. Ciprofloxacin-resistant Salmonella enterica serovar Kentucky ST198 in broiler chicken supply chain and patients, China, 2010–2016. Microorganisms. 2020; 8(1): 140.
9. Penha Filho RAC, Ferreira JC, Kanashiro AMI, de Costa Darini AL, Junior AB. Antimicrobial susceptibility of Salmonella Gallinarum and Salmonella Pullorum isolated from ill poultry in Brazil. Ciência Rural. 2016; 46(3): 513-518
10. Sasaki Y, Ikeda A, Ishikawa K, Murakami M, Kusukawa M, Asai T, Yamada Y. Prevalence and antimicrobial susceptibility of Salmonella in Japanese broiler flocks. Epidemiology & Infection. 2012; 140(11): 2074-2081
11. Humphrey TJ. Public-health aspects of Salmonella infections. In: Wary C, Wary A, editors. Salmonella in domestic animals. Wallingford, United Kingdm: CABI publishing , CAB international. 2000 : 245-263.
12. Bell RL, Jarvis KG, Ottesen AR, McFarland MA, Brown EW. Recent and emerging innovations in Salmonella detection: a food and environmental perspective. Microbial biotechnology, 2016 May; 9(3): 279-292
13. Mane S, Khaire P. Role of Beneficial Microbes in Sewage Sludge Management. In: Sustainable Management and Utilization of Sewage Sludge. Springer International, 2022; 183-203.
14. Johnson R, Mylona E, Frankel G. Typhoidal Salmonella: Distinctive virulence factors and pathogenesis. Cellular microbiology, 2018; 20(9): 12939
15. Gast RK, Porter Jr RE. Salmonella infections. In: Diseases of poultry. Wiley , 2019 November; 675-736.
16. Osman KM, Marouf SH, Zolnikove TR, Alatfeehy N. Isolation and characterization of Salmonella enterica in day-old ducklings in Egypt. Pathogens and global health. 2014 Jan; 108(1): 37-48.
17. Rashidi A, Alizadeh A, Sharifi SD, Najafi A. The histopathological changes of liver and testis of Japanese quail chicks fed different levels of dietary L-valine. Veterinay Animal Science, 2023 Jul: 21: 100302.
18. Farhan NA, Hussein, AA. Morphometric comparative study of the pancreas between the owl bird and the moorhen bird. Diyala Journal for Veterinary Sciences. 2024; 2(3): 75-83.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

19. Mahmood MA. Histopathological effect of pecan oil (carya illinoinensis) against ibuprofen induced kidney injury in local male rabbits. Diyala Journal for Veterinary Sciences. 2024; 2(3): 57-66.
20. Ramya P, Madhavarao T, Rao LV. Study on the incidence of Salmonella enteritidis in poultry and meat samples by cultural and PCR methods. Vet World, 2012; 5(9), 541-545.
21. Islam MJ, Mahbub-E-Elahi ATM, Ahmed T, Hasan MK.. Isolation and identification of Salmonella spp. from broiler and their antibiogram study in Sylhet, Bangladesh. J Appl Biol Biotechnol, 2016; 4, 046-051.
22. Sedeik ME, El-shall NA, Awad AM, Elfeky SM, Abd El-Hack ME, Hussein EOS, Alowaimer AN, Swelum AA. Isolation, conventional and molecular characterization of salmonella spp. from newly hatched broiler chicks. AMB Express, 2019 Aug; 9: 136.
23. Yang X, Wu Q, Zhang J, Dong X, Liu S. Biochemical and serological characterization salmonella from retail meats in Guangdong Province. Wei Sheng Yan Jiu. 2013 July; 42(4): 615-618.
24. Livermore D M, Struelens M, Amorim J, Baquero F, Bille J, Canton R, Henning S, Gatermann S, Marchese A, Mittermayer H, Nonhoff C, Oakton KJ, Praplan F, Ramos H, Schito GC, Van Eldere J, Verhaegen J, Verhoef J, Visser MR. Multicentre evaluation of the VITEK 2 advanced expert system for interpretive reading of antimicrobial resistance tests, Journal of Antimicrobial Chemotherapy. 2002; 49(2): 289–300
25. Shanmugasamy M, Velayutham T, Rajeswar J. Inv A gene specific PCR for detection of Salmonella from broilers. Veterinary World, 2011; 4(12), 562.
26. Karmi M. Detection of virulence gene (invA) in Salmonella isolated from meat and poultry products. Int. J. Genet, 2013; 3(2), 7-12.
27. Moussa IM, Aleslamboly YS, Al-Arfaj AA, Hessain AM, Gouda AS, Kamal RM. Molecular characterization of Salmonella virulence genes isolated from different sources relevant to human health. J. Food Agric. Environ, 2013; 11(2), 197-201.
28. Sharma I, Das K. Detection of invA gene in isolated Salmonella from marketed poultry meat by PCR assay. Journal Food Process Technology. 2016; 7(3): 564.
29. Chaudhary JH, Nayak JB, Brahmbhatt MN, Makwana PP. Virulence genes detection of Salmonella serovars isolated from pork and slaughterhouse

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

environment in Ahmedabad, Gujarat. Veterinary world, 2015; 8(1), 121.

30. Oladapo OD, Onifade AK, Bayode MT. Direct detection of iro B, stn and hil A virulence genes in Salmonella enterica serovar typhimurium from non-ripened cheese. Bulletin of the National Research Centre, 2022; 46(1), 175.
31. Echioda-Ogbole M, Godwin E. Occurrence and Antimicrobial Susceptibility Profiles of Salmonella species among Chickens of some Commercial Poultry Farms in Benue State, Nigeria. UMYU Journal of Microbiology Research, 2019 ; 4(1), 39-44.
32. Adamu A, Reyer F, Lawal N, Hassan AJ, Imam MU, Bello MB, Kraiczy P. Aetiologies of bacterial tick-borne febrile illnesses in humans in Africa: Diagnostic limitations and the need for improvement. Frontiers in Medicine, 2024; 11:1419575.
33. Liu B, Knirel YA, Feng L, Perepelov, AV, Senchenkova SN, Reeves PR, Wang L. Structural diversity in Salmonella O antigens and its genetic basis. FEMS microbiology reviews. 2014; 38(1), 56-89.
34. Fitzgerald AC, Edrington TS, Looper ML, Callaway TR, Genovese KJ, Bischoff KM, Nisbet DJ. Antimicrobial susceptibility and factors affecting the shedding of

35. E. coli O157: H7 and Salmonella in dairy cattle. Letters in Applied Microbiology, 2003; 37(5), 392-398.
36. Iankov ID, Petrov DP, Mladenov IV, Haralambieva IH, Ivanova R, Velev V. R, Mitov IG. Production and characterization of monoclonal immunoglobulin A antibodies directed against Salmonella H: g, m flagellar antigen. FEMS Immunology & Medical Microbiology, 2002; 33(2), 107-113.
37. Al-Khazraji KIA. Morpho-histological study of the liver in whit-eared bulbul (pycnonotus leucotis), mallard duck (Anas platyrhynchos), and gull (Larus canus). Iraqi J. Vet. Med. 2017; 41(2): 94-99.
38. Tarek K, Amin B, Eddine RD, Abdelhafidh M, Hanane A. Morpho-histological comparisons of liver between the broiler chickens and wild boar in Algeria. Adv. Anim. Vet. Sci., 2019; 7(1): 24-29.
39. Prasanna K, Paliwal OP. Experimental fowl typhoid and pullorum disease in chickens, clinical and pathomorphological studies. Indian J. Vet. Path. 2003; 26: 27-29.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

40. Akhtaruzzaman M, Islam MN, Rashid SMH, Juli MSB, Parvez NH, Islam MS. Salmonellosis in layer chickens : molecular detection and histopathological features of salmonella spp. From laying hens. J. Ento. Zoo. Stud. 2020; 8(2): 169-174.
41. Hu Z, Ojima S, Zhu Z, Yu X, Sugiyama M, Haneda T, Okamura M, Ono HK, Hu DL. 2024 Salmonella pathogenicity island-14 is a critical virulence factors responsible for systemic infection in chickens caused by Salmonella gallinarum. Frontiers Veterinary Science, 2024; 11: 1401392.