Egyptian Journal of Veterinary Sciences

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Diagnostic Study for Salmonella Infection In Broiler Farms In Kirkuk Province

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The aim of the study is diagnosis Salmonella infection in broiler chickens in Kirkuk province. For this purpose, 120 samples were collected from broiler chicken organs, which included form the cecal tonsils, liver, spleen, and gallbladder. ELISA, PCR, Culture methods, whole blood agglutination tests, and slide agglutination tests were all used for Salmonella infection diagnosis. The study recorded the rate of Salmonella isolated from the cecal tonsils ,liver ,gallbladder and spleen 39 (61.9%) ,28(44.4%),16(25.4%) and 13 (20.6%) respectively, while the results showed that 52.5% of the broiler chickens had Salmonella bacteria isolated from them, as it was found that the cecal tonsils are the best place to isolate that bacteria, and the results also showed that *Salmonella enteritidis* is the most prevalent species. The ELISA test resulted 60.3%, whereas other widely used tests produced a result of 100%. The test of ELISA , the test of slide agglutination and the test of whole blood agglutination for *Salmonella enteritidis*, with the test of slide agglutination results for *Salmonella typhimurium* recorded 68.4%,71.9%, 62.3%,73.9% ,83.8% and 71.4%, respectively. The study concludes that the best place to isolate the bacteria is the cecal tonsils. In addition, the most common type of Salmonella that was diagnosed in the samples studied was the *Salmonella enteritidis*.

Key words: *Salmonella* infection, broiler farms, Kirkuk Province.

Introduction

Salmonellosis is one of the enterobacterial pathogens , an important source for morbidity and mortality in the world [1]. It is gram-negative bacteria, it is one of the problems that can be transmitted through food, and it is considered the most dangerous among these problems, as it can infect several hosts such as humans, animals, birds, and insects [2]. Salmonella infection in broiler can be caused dehydration, gasping, drooping wings, depression, sleepiness, weakness, and decreased appetite, among other clinical symptoms Lameness, swelling in the joints, and blindness are occasionally possible [3]. Salmonella has a numerous of virulence factors dispersed throughout its chromosomes, known as salmonella pathogenicity islands (SPIs). They are successively located at centisomes 63, 31, 82, 92, and 25 [4]. The largest gene in spI-1 is Salmonella enterotoxin gene (Stn) which will binding with host-microbe interactions and responsible for bacterial invasion of endothelial cells. The plasmid-encoded fimbriae (pef) known as the pef operon will facilitated *S. enteriditis* adherence with the small intestine [5]. The SipB is linked to the entry into non-phagocytic cells and the eradication of macrophages. SpiA is connected to macrophage survival, whereas OrgA is connected to host recognition/invasion [6].

Material and Methods

The study was carried out in the Kirkuk province (Dibis, Taza, Daquq, Laylan, Hawija and Tuz Khurmatu regions) between September and December of 2022. used 120 samples from 35000 birds in different flocks From each samples taken the liver, spleen, gallbladder, cecal tonsils, and blood for bacterial isolation and serology test.

Culture methods

Peptone water (HIMEDIA-INDIA) was used to cultivate each sample as a liquid selective enrichment medium before being sub-cultured in Selenite F broths for 24 hours at 43°C. then sub-culturing for 24 hours at 37°C on specific marketed medium (MacConkey, Brilliant Green, and Xylose

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(Received 11/12/2023, accepted 08/01/2024)
DOI: 10.21608/EJVS.2024.254791.1720

Lysine Deoxycholate Agar) [7]. The biochemical tests were carried out. It is feasible to determine the serotype of a Salmonella isolate using Salmonella antisera (Diagnostic Pasteur, Paris, France), which are divided into 2 groups: Salmonella flagellar polyvalent H Polyvalent and Salmonella Antisera Somatic O Antisera. These testing validated the Salmonella species [8,9].

Antibiotic sensitivity test

Antibiotic sensitivity test work according to [10].

Create the DNA blueprint

In order to create the DNA blueprint for the PCR test, bacteria are cultured in the brain-heart infusion broth to reactivate them. The DNA is then recovered using the boiling lysis procedure in accordance with [11].

Serology tests

ELISA: for detection antibodies level against invasive strains of *Salmonella enteritidis, Salmonella typhimurium, Salmonella pullorum,* and *Salmonella gallinarum* in chicken and turkeys, an ELISA test was carried out using a kit (Gulid Hay Ltd., UK). used 0.2 ml from the blood with 0.2 ml from the antigen of salmonella (Nobillis, Intervet, Holland).

Whole blood agglutination test

In the whole blood agglutination test, when agglutination appears within two minutes, the test is deemed successful. This test is used for detection *S. pullorum* and *S. gallinarum.* The somatic and flagellar antigens for *S. enteriditis* were prepared and used in the current study's slide agglutination test (for *S. enteriditis* identification). Somatic antigen is made by heating to 100 °C and in depending in [12]. Used the formalin for prepared the flagellar antigen (BDH, England) accordance with [13]. *S. enteritidis* was detected by using a slide agglutination test in this study, for prepare the entire bacteria antigen for *Salmonella enteritidis* were sonicated for fifty minutes at time at rate of forty MHZ per second in a water-cooled sonicator according to [14].

Statistical analysis

All statistical calculation were carried out with the according to [15, 16].

Result and Discussion

Salmonella was isolated from broilers at a rate of 52.5% (63:120) depending on PCR technique, biochemical test, and colony morphology. Table 1 and figure 1. (Salmonella isolated from the liver, spleen, gallbladder and cecal tonsils is considered a positive case for infection).

TABLE 1. Primer as for detection of *Salmonella . enteritidis* [17].

Primer		Sequences	Out product size
S. enteritidis	*R*	*CAG GGC ATT TGC TGA TTC TTC*	568
	R	*TCATCGCACCGTCAAAGGAACC*	

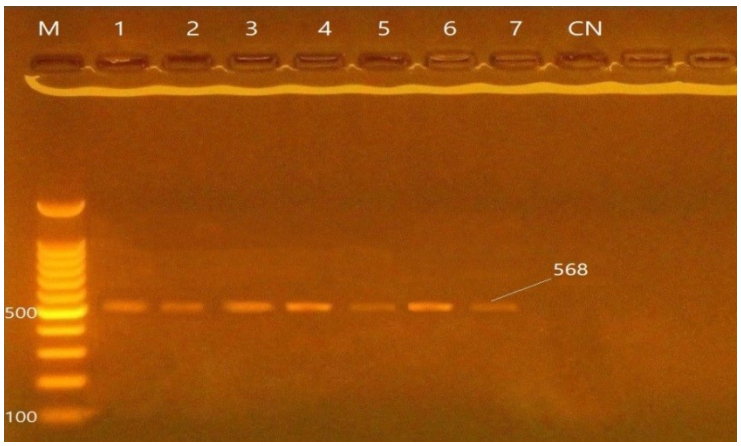

Fig. 1. Shown the results of agarose gel electrophoresis for PCR products vallue at M: 100 bp DNA ladder, whereas the positive result for *Salmonella enteritidis* in lines (1-7) at 568bp.

TABLE 2.Primers group used for detection of *S. enteritidis* virulence factors 17 [18].

Gene	Sequences		Out product size
sipB	F	GGACGCCGCCCGGGAAAAACTCTC	734
	R	TTTTTGGCAATGCATCAGGGA	
spiA	F	GCGTAACAAAGAACCCGTTTGCGTGAGATG	500
	R	CCAGGGGTCGTTAGTGTACAGTGATGGATT	
orgA	F	ACACTCCCGTCGCCGCCTTCACAA	268
	R	GGACGCCGCCCGGGAAAAACTCTC	

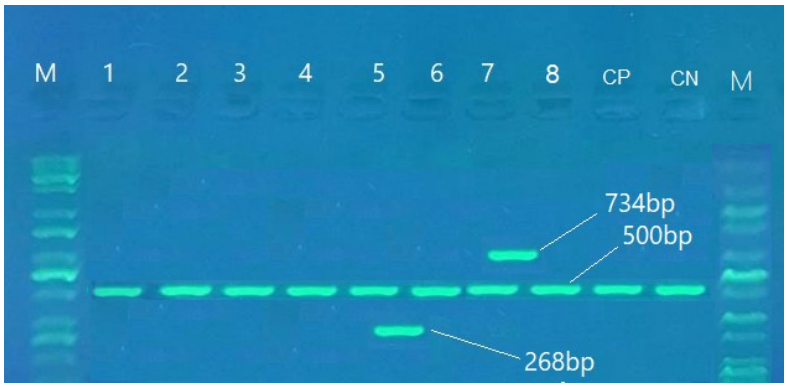

Figure 2: Shown the 2% agarose gel electrophoresis with ethidium bromide staining the PCR results. SipB: 734 bp, spiA: 550 bp, and orgA: 268 bp are the *Salmonella enteriditis* virulence factors that were isolated from broiler. M :DNA marker, CP control positive, CN for control negative, and lines (1–8) for positive results.

Compared to other studies, the rate of Salmonella isolation in this study was high. While in other studies [19, 20], they isolated Salmonella at a rate of 34.7% and 41.2%, respectively. That might be because our study used the samples from clinically sick broilers, whereas other studies used survey samples. In this study, the rates of Salmonella isolation from the cecal tonsils, liver, gallbladder and spleen were (39) 61.9%, (28) 44.4%, (16) 25.4%, and (13) 20.6% respectively (Table 3).

TABLE 3. The Salmonella isolation depending on the organ

Organs	Number of Salmonella isolated	Rate of Salmonella isolated
Cecal tonsils	39/ 63	61.9%
Liver	28 / 63	44.4%
Gallbladder	16/ 63	25.4%
Spleen	13 / 63	20.6%

The tonsils in the cecum and liver had a high isolation rate in this result concurs with [21]. The reason for this case may be due Salmonella invasted the mucous membranes and Lenning-Bayer patches during the early stages of the sickness and then be transported by macrophages to the vital organs, particularly the liver [22]. Four species of *Salmonella, Salmonella enteritidis, Salmonella typhimurium, Salmonella Pullorum* and *Salmonella gallinarum*, were identified in this study by biochemical tests and antisera with agglutination [23]. Xylose, Arabinose, Trehalose, and Maltose, were all fermented by *Salmonella enteritidis* also generated gases. whereas Inosito was not, and it agglutinated with anti-flagellar (g, m) and anti-somatic antibody 1,9,12. [24]. *Salmonella typhimurium* fermented the Trehalose, Arabinose and Xylose with produced the gases and acid , additionally, it agglutinates with anti-flagellar antibodies 1,2. and antisomatic antibody 1,4,5,12 [25]. *Salmonella gallinarum* is non-motile and did not produce any gases while fermenting Xylose, Arabinose, Trehalose, and maltose. It also agglutinated with an anti-somatic antibody. *Salmonella pullorum* had a immobile appearance and fermented Trehalose, Arabinose and Xylose with produce the gases and acid in addition to agglutinating with an anti-somatic antibody [26]. Table 4 describe the percentage of *Salmonella spp*. which isolated from samples of this study.

TABLE 4. Explain the isolated rate for Salmonella species

Salmonella species	Number of isolated	The rate of isolation
Salmonella enteritidis	23/ 120	19.2%
Salmonella typhimurium	16/ 120	13.3%
Salmonella Pullorum	14/ 120	11.7%
Salmonella gallinarum	10/ 120	8.3 %
Total	63/ 120	- 52.5%

High occurrence of *Salmonella enteritidis* and *Salmonella typhimurium* in this research compared with another type of Salmonella. This result concur with the result of [27]. The ratio for *S. pullorum* compared with *S. gallinarum* agreement with other results studies [27]. Salmonella resistant to antibiotics appear different ratio depending on salmonella serotype and the type of antibiotic, as in table 5.

TABLE 5. Describe resistance the Salmonella isolates to antibiotic types with Percentage.

Types of Antibiotic	Types of Salmonella isolation			
	Salmonella enteritidis no. of isolate 23/ 63	***Salmonella typhimurium*** no. of isolate 16/ 63	***Salmonella pullorum*** no. of isolate 14 / 63	***Salmonella gallinarum*** no. of isolate 10/ 63
Ampicillin	8 / 23 (34.8%)	9 / 16 (56.3%)	3/ 14 (21.4%)	4/ 10 (40%)
Ciprofloxacin	6 / 23 (26.1%)	5/ 16 (31.2%)	0 (0%)	0 (0%)
Streptomycin	7 / 23 (30.4%)	5 / 16 (31.2%)	4/ 14 (28.6%)	3 /10 (30%)
Nalidixic acid	8 / 23 (34.8%)	7/ 16 (43.8%)	8/ 14 (57.1%)	6 /10 (60%)
Tetracycline	10 / 23 (43.5%)	6 / 16 (37.5%)	4/ 14 (28.6%)	5 / 10 (50 %)
Cefotaxime	5 / 23 (21.7%)	3 / 16 (18.8%)	0 (0%)	0 (0%)
Chloramphenicol	6 / 23 (26.1%)	4 / 16 (25%)	3 / 14 (21.4%)	2 / 10 (20%)
Trimethoprim	9 / 23 (39.1%)	7 / 16 (43.8%)	4 / 14 (28.6%)	4 / 10 (40%)

The high rate of antibiotic resistance in this study may be caused by widespread use of certain antibiotic types and the transfer of resistant genes amongst bacteria this concur [28]. The sensitivity specificity positive and negative predictive values of the ELISA test were, respectively, 60.3 %, 68.4%, 67.8%, and 39.1% when compared to the results of the culture test, according to table 6.

TABLE 6. Shown the bacterial culture with ELISA test results for Salmonella species isolated

The culture test's result		The ELISA test's result			
		Positive		Negative	
The results of culture	The samples number	The number	Percentage (%)	The number	Percentage (%)
The positive results for culture	63	38	60.3%	25	39.7%
The negative results for culture	57	18	31.6%	39	68.4%
Total results	120	56	46.7%	64	53.3%

Positive isolation case in this study produced negative results in ELISA test. It is caused by early infection by weak immune system [29]. Moreover, the ELISA kit which used for identify IgG, which presented 10 days post infection [30]. The sensitivity, specificity, positive and the whole blood agglutination test's negative predictive values were 100%,71.9%, 62.5%, and 39.6%, respectively, when compared to bacterial culture (for detection of *Salmonella. Pullorum* and *Salmonella gallinarum*). As describe in Table 7.

TABLE 7. Compares the results of a bacterial culture with a whole blood agglutination test.

The culture test's outcome		The Whole blood agglutination tests result			
The results of culture	No.	Positive		Negative	
		No.	%	No.	%
The negative culture for the species of Salmonella.	57	16	28.1%	41	71.9%
The positive culture for *Salmonella Pullorum* with *Salmonella gallinarum*	25	25	100%	0	0%
The positive culture for *Salmonella enteritidis* with *Salmonella typhimurium*	38	27	71.1%	11	28.9%
Total positive culture for Salmonella spp	63	40	63.5%	23	36.5%

Slide agglutination test's sensitivity ,positive and negative predictive value were all 100%, 62.3%, 73.9%, and 100%, respectively, when compared to bacterial culture for detection *S. enteritidis.* According to Table 8.

TABLE 8. Compares results of the slide agglutination test with bacterial cultures that are positive for *Salmonella enteritidis*.

The culture tests result	No.	Slide agglutination tests result			
		Positive		Negative	
		No.	%	No.	%
The negative culture for Salmonella species.	57	24	42.1%	33	57.9%
The positive culture for *Salmonella enteritidis*	28	28	100%	0	0%
The positive culture for *Salmonella gallinarum*, *Salmonella Pullorum, Salmonella typhimurium*	35	15	42.9%	20	57.1%
Total positive culture to *Salmonella spp*	63	10	15.9%	53	84.1%

Slide agglutination test's sensitivity, specificity, positive predictive values, and negative predictive values were all 100%, 83.8%, 71.4%, and 100%, respectively, when compared to bacterial culture's when detection *S. typhimurium*. As shown in Table 9.

TABLE 9. Compares the test of slide agglutination with a bacterial culture that positive for *Salmonella typhimurium.*

The culture tests result		The test of slide agglutination results			
The results of culture	No.	Positive		Negative	
		No.	%	No	%
The negative results of culture for Salmonella species.	57	21	36.8%	36	63.2%
The positive results of culture for *Salmonella typhimurium*	18	18	100%	0	0%
The positive results of culture for *Salmonella gallinarum*, *Salmonella pullorum, Salmonella enteritidis*	45	13	28.9%	32	71.1%
Total positive culture for *Salmonella species*	63	18	41.9%	61	58.1%

The presence of a positive result in a serology test after a negative result in a culture is caused by intermittent bacterial shedding, a low bacterial population, or antibiotic treatment [31]. Many factors, including immunization, carrier birds, endemic areas, and cross-reaction with other similar bacteria, might result in false positive results in serology tests (poor specificity) [31]. The sensitivity and specificity of serological testing varied in the current investigation. It could occur as a result of the antigens employed in the test of serology (bacteria cell whole or parts). Due to the cross reactivity between salmonella and other bacteria, particularly Enterobacteriaceae, slide agglutination assays have a low specificity [32].

Conclusions

We conclude from this study that the cecal tonsils are the best place to isolate these bacteria, and that infection with *Salmonella enteritidis* is the most common type of salmonella. The percentage of the ELISA test was 60.3%, compared to 100% for the other tests used. The ELISA test , slide agglutination test and the test of the test whole blood agglutination for *Salmonella enteritidis*, and the test of slide agglutination for *Salmonella typhimurium.* 68.4%, 71.9%, 62.3% and 71.4%,respectively.

Acknowledgments

The authors thanks to vet. med. college, Tikrit ,University. The authors are very grateful to Dr. Qusai Saleh un limited support throughout the experiment.

Conflict of interest

There are no conflicts of interest to be declared.

Funding statement

The article was not financially supported.

Author contributions

Conceptualization, study design, sample

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دراسة تشخيصية لعدوى السالمونيلا في مزارع الدجاج اللاحم في محافظة كركوك

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خلاصة

الهدف من الدراسة هو تشخيص الإصابة بالسالمونيلا في الدجاج اللاحم في محافظة كركوك. ولهذا الغرض تم جمع 120 عينة من أعضاء الدجاج اللاحم والتي شملت شكل اللوزتين الأعور والكبد والطحال والمرارة. تم استخدامELISA ، PCR، طرق الثقافة، اختبارات تراص الدم الكامل، واختبارات تراص الشرائح لتشخيص الإصابة بالسالمونيلا. وسجلت الدراسة نسبة السالمونيلا المعزولة من اللوزتين الأعور والكبد والمرارة والطحال 39 (61.9%)، 28 (44.4%)، 16 (25.4%) و13 (20.6%) على التوالي، بينما أظهرت النتائج أن 52.5% من دجاج التسمين تم عزل بكتيريا السالمونيلا منها، حيث تبين أن اللوزتين الأعورتين هي أفضل مكان لعزل تلك البكتيريا، كما أظهرت النتائج أن السالمونيلا المعوية هي أكثر الأنواع انتشارا. وقد نتج عن اختبار ELISA نسبة 60.3%، في حين أعطت الاختبارات الأخرى المستخدمة على نطاق واسع نتيجة 100%. اختبار الاليزا واختبار تراص الشرائح واختبار تراص الدم الكامل لـ*Salmonella enteritidis* ، بينما سجلت نتائج اختبار تراص الشرائح لـ*Salmonella typhimurium* 68.4% ، 71.9%، 62.3%، 73.9%، 83.8%، 71.4%، على التوالى. وخلصت الدراسة إلى أن أفضل مكان لعزل البكتيريا هو اللوزتين الأعوريتين. بالإضافة إلى ذلك، فإن النوع الأكثر شيوعًا من السالمونيلا الذي تم تشخيصه في العينات التي تمت دراستها هو *Salmonella. Enteritidis*

الكلمات الدالة: عدوى السالمونيلا، مزارع الدجاج اللاحم، محافظة كركوك.