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CLINICAL AND DIAGNOSTIC STUDY OF BOVINE DIARRHEA CAUSED BY *SALMONELLA ENTERICA* SEROVAR *TYPHIMURIUM* IN BASRAH, IRAQ

ALI B. ALDEEWAN [1]; ALYAA S. JASIM [1]; KAMAL M. ALSAAD [2] AND NAWRES N.JABER [1]

[1] Department of Microbiology and Parasitology, University of Basrah, College Of Veterinary Medicine, Iraq

[2] Department of Internal and Preventive Medicine, University of Basrah, College of Veterinary Medicine, Iraq

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ABSTRACT

Bovine salmonellosis is an important contagious disease that is usually endemic to a particular region. the present study aimed to study the clinical cases, diagnostic tools and the specific gene responsible for the pathogenesis in Basrah, Iraq. The study was conducted on 2-4 years-old (88) native cow breeds from November 2022 to July 2023. Nearly, 38.63% of cows were positive for *Salmonella* by clinical signs, culture and confirmed using polymerase chain reaction (PCR). Diseased cows suffered from diarrhea, dehydration and pyrexia. Hematological analysis indicated leukocytosis, neutrophilia and significantly higher erythrocyte sedimentation rate and clotting factor indices. Results also indicated hypoglycemia, decreased total protein, and increased blood urea nitrogen. Fecal samples (1gm) were taken directly from the rectum of all suspected animals and transmitted immediately to the diagnostic lab in a cooling box. The PCR amplification and sequencing results of the 16S rRNA gene from *Salmonella* isolates revealed that 10 out of 19 isolates exhibited distinct bands of approximately 1500 bp corresponding to the *Salmonella enterica* serovar *typhimurium.* The results of *cdtb* and *pltB* genes amplification exhibited clear bands of approximately 819 bp and 518 bp, respectively. According to the phylogenetic analysis, our local isolates represented a close relationship to the other published strains in the NCBI database. In conclusion, bovine salmonellosis is considered a serious disease due to its negative effects on the infected animals of all ages. Therefore, early elimination and control of this disease could be the right way to protect farm animals.

Keywords*:* *Salmonella typhimurium,* Cows, PCR, Basrah, Iraq

INTRODUCTION

Salmonellosis refers to an infection of the digestive canals caused by the species *Salmonella enterica*, which has more than two thousand strains. Moreover, cows were always infected with some of those species (Adem and Bushra, 2016; Constable *et al*., 2017). The most important *Salmonella* species infecting cattle were classified as B, C, D, and E groups, including *Salmonella typhimurium, Salmonella dublin, Salmonella. montevideo, and Salmonella anatum,* respectively (Craig and James, 2006).

Corresponding author: Kamal M. Alsaad
E-mail address: kamalsad58@yahoo.com
Present address: Department of Internal and Preventive Medicine, University of Basrah, College Of Veterinary Medicine, Iraq

It was documented that *Salmonella enterica* of the family *Enterobacteriaceae* is a non-sporulating aerobic or facultatively anaerobic rod-shaped organism, gram-negative and motile. Furthermore, six important subspecies were recognized, including *S. enterica: S. enteric enterica (I), S. enterica salamae (II), S. enterica arizonae (IIIa), S. enterica diarizonae (IIIb), S. enterica houtenae (IV),* and *S. enterica indica (VI).* On the other hand, there are different serovars have been registered for *S. enterica* species in both healthy and sick animals (Jones *et al*., 2007; Carey *et al*., 2023).

It was shown that *Salmonella* is a widespread organism that exists anywhere in dirty farms and different animal species such as mammals, birds, insects, reptiles, and even humans. Moreover, *Salmonella* is known to be an opportunistic organism, that could infect and multiply when the immune system is depressed, especially when the bacteria of the digestive system are absent or in the case of young animals. However, it can also infect healthy animals upon exposure to high doses of infected organisms (Davison, 2005; Danielle, 2006).

It has been thought that *S. enterica* can infect different animals and even birds, and in general, salmonellosis could cause high mortalities in different species, including domestic and laboratory animals and even humans (Craig and James, 2006; Jones *et al.*, 2007; Adem and Bushra, 2016).

Salmonella infection is either in a carrier state of animals (apparently healthy) or an acute state of infection. However, a series of clinical manifestations ranging from apparently subclinical to the more obvious clinical salmonellosis (Costa *et al*., 2012; Kemal, 2014).

In calves, salmonellosis mostly infects those deprived or deficient from colostrum. Hereby, diseased animals showed pyrexia, fluid mixed with mucous or blood diarrhea, moderate to severe dehydration, and finally death, which could occur within 24-72 h (Mohler *et al*., 2009; Adem and Bushra, 2016). On the other hand, in adult cows, salmonellosis was manifested with acute intestinal problems preceded mostly by stress factors. The diseased animal shows signs of fever, loss of appetite, varying degrees of dehydration, and bad-smell diarrhea mixed mostly with mucus and/or blood. Moreover, decreased milk production in lactating cows, and abortion during different stages of pregnancy could also be indicated. However, death, which is the final result could be indicated according to the severity of infection and the pathogenicity of *Salmonella* serotype (McEvoy *et al*., 2003).

Bovine diarrhea caused by *Salmonella enterica* serovar *typhimurium* infection has always been suspected clinically in Basrah, Iraq. Therefore, clinical and diagnostic study, as well as detection of the specific gene responsible for the pathogenesis were the main aims of the present study.

MATERIALS AND METHODS

Samples collection:

The study was conducted on 2-4 years-old (88) local native cow breeds at Basrah (Alqurna, Basrah city center, Hartha, AlZubair, Aboalkhaseeb, Modaina and Aldeer districts). Iraq suspected cows exhibited different clinical signs such as diarrhea, weakness, and loss of appetite. Clinical examinations have been applied for both unhealthy suspected and control groups (Constable *et al*., 2017). Fecal samples (1gm) were taken directly from the rectum of all suspected animals, placed immediately in a sterile tube containing buffered peptone water, and transmitted immediately to the diagnostic lab. The study started from November 2022 to July 2023. Twenty-five clinically healthy local cow breeds were served as the control group.

Blood samples, hematological and biochemical analysis:-

Ten ml of the blood was collected from the jugular vein of each animal. Three (3) milliliters mixed with Ethylenediamine-tetraacetic acid (EDTA), and three (3)

milliliters mixed with Trisodium citrate (using plasma), for CBC estimation using a hematological analyzer (USA) and evaluation of clotting factor indices. Moreover, Giemsa-stained blood smears was performed for the assessment of the absolute differential leukocyte count according to Harvey (2012). Furthermore, the erythrocyte sedimentation rate (ESR) was estimated via the Wintrobe tube method (Weiss and Wardrop, 2011). Serum samples were collected and used for biochemical analysis, including estimation of glucose, blood urea nitrogen (BUN), and total protein, through the spectrophotometric method using kits from Biolabo, France (CLp. Regulation *EC) No. 1272).

Bacterial Isolation and Diagnosis

Pre-enrichment and enrichment: -

The buffered peptone water that contained the fecal sample was incubated at 37ºC for 24 hours and then cultured in tetrathionate broth and incubated at 42ºC for 24 hours. (Flowers *et al*., 1992)

Samples culture: -

A loopful of incubated tetrathionate broth was inoculated on Xylose Lysine Deoxycholate (XLD) and brilliant green agar (BGA) agar plates and incubated overnight (18-24 h) at 37 °C. The suspected *Salmonella* colonies were subjected for morphological and biochemical confirmation of *Salmonella*. (WHO, 2010)

Identification of *Salmonella* isolates and Biotyping

The bacterial isolates were identified as *Salmonella* species using the following methods: Catalase test, Oxidase test, Lactose fermentation, Urease test, Indole test, Api-20Esystem (Analytical profile index for *Enterobacteriaceae* test) (Macfaddin, 2000).

DNA extraction

DNA was extracted from bacterial cultures using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega from USA) and the manufacturer's instructions were followed and the extracted DNA was eluted and stored at -20 °C until the time of use.

DNA sequencing of 16S rRNA gene

Macrogen Company (South Korea) has sequenced the PCR results produced using universal primers (F: 5′-AGAGTTTGATCCTGGCTCAG-3′, R: 5′-CTACGGCTACCTTGTTACGA-3′) as listed in Table 1, (Hou *et al*., 2018). The Snapgene commercial application was utilized to evaluate the DNA sequences (SnapGene® software from Dotmatics; available at snapgene.com). NCBI BLAST was used to verify the results and detect any DNA modifications in the gene's sequence.

Genotypic detection of virulence genes:

Primers:

The used primers in the present study (Macrogen, Korea), were prepared according to the manufacturer's instructions by adding deionized distilled water (ddH_2O) to the lyophilized primers, depending on the volume fixed to the tube containing the primers. They were then thoroughly mixed using a vortex mixer to create stock solutions with a concentration of 100X (Pico moles per microliter), which were then stored at -20°C. Ten microliters of the stock solution were put into the RNase-DNase-free tube. Next, 90 microliters of dd H2O were added, and a vortex mixer was used to mix them. This made a 10x concentration of primer, which was then kept at -20°C. Primers used to detect the 16S rRNA, *cdtB*, and *pltB* genes are listed in Table 1.

Reaction mixture of PCR

For all PCR reactions, the mixture contained 25µl GoTaq® G2 Colorless PCR Master Mix (Promega, USA), 5µl of the extracted DNA, 1,5 µl of each primer, and nuclease-free water that was added to a final volume of 50µl. The cycling conditions for PCR of this study are listed in the Table (2)

Table 1: Primers used in this study

Gene	Primer Sequence	Size bp	Reference
16S	F: 5′-AGAGTTTGATCCTGGCTCAG-3′ R: 5′-CTACGGCTACCTTGTTACGA-3′	1500	(Hou *et al.*, 2018).
cdtB	F:5'-GTTTCCAGACAAAGAGCGGATAATG-3' R: 5'- GGCGAGATGCGACAGTTG-3'	819	den Bakker *et al*., 2011
pltB	F: 5'-ATAAAAACGGTTAAAGCTAACGGT-3' R: 5'-AATAGTACGCTCTGGCTATAGC-3'	518	den Bakker *et al*., 2011 Designed based on CP085809.1

Table 2: PCR program

Monoplex genes and product size (bp)	Temperature (°C)/ Time Cycling condition					Cycle number
	Initial denaturation	Denaturation	Annealing	Extension	Final extension	
16S rRNA 1500	95ºC 5 min	95ºC 30 sec	49 ºC 1min	72ºC 1.5 min	72ºC 10 min	35
Cdtb 819	95ºC 5 min	95ºC 30 sec	58ºC 30 sec	72ºC 1 min	72ºC 5 min	35
Pltb 518	95ºC 5 min	95ºC 30 sec	58ºC 30 sec	72ºC 1 min	72ºC 5 min	35

Statistical analysis:

Student *t*-test representing the SPSS program was used in the current study, according to Leech *et al.* (2013) for the estimation and calculation of the statistical difference comparing between diseased and control groups. The data was presented as the mean ± standard error. The differences were considered significant when $P<0.05$.

RESULTS

Out of 88 suspected local cow breeds, 34 animals (38.63%) were found positive for *Salmonella* based on the clinical signs. Out of 34 positive *Salmonella* samples, 19 were diagnosed as *Salmonella* based on morphological and biochemical tests. Ten isolates were diagnosed as *Salmonella enterica* serovar *typhimurium* based on the 16S rRNA sequencing results. Seven sequences were selected from seven different districts for the NCBI deposition and registration.

Diseased cows showed acute different clinical manifestations, including stringy diarrhea mixed with mucous and/or blood with signs of acute enteritis, including abdominal pain, grinding of teeth, and arching back, which was seen in (100%). Moreover, moderate to severe dehydration manifested by sunken eyes as well as rough and dry skin (97.05%), loss of appetite (94.11%), a dirty perineal region as well as emaciation and weakness were seen in (88.23%) of diseased cows. Milk production declined in (38.23%) of lactating diseased cows and (29%) were suffering from tenesmus (Table 3).

Hematological analysis of diseased cattle infected with *Salmonella enterica serovar typhimurium* indicates leukocytosis due to a significant ($P<0.05$) increase in the absolute count of neutrophils, compared to the control healthy cows. On the other hand, the erythrocyte sedimentation rate (ESR) values were significantly ($P<0.05$) high. Furthermore, a significant difference has been encountered in clotting factor indices and low values of total platelets count were detected in diseased cows. On the contrary, the distribution width of platelet and its mean volume, prothrombin time, activated partial thromboplastin time, and fibrinogen time rose significantly in diseased cows, compared to the healthy animals (Table 5).

Table 3: Clinical manifestations of (34) cows infected with *Salmonella.*

Clinical manifestations	No. of affected cows	%
Stringy diarrhea with signs of acute enteritis	34	100
Dehydration of different degrees (moderate to severe)	33	97.05
Loss of appetite	32	94.11
Dirty perineal region	30	88.23
Emaciation and weakness	30	88.23
Decrease milk production in lactating cows	13	38.23
Tenesmus	10	29.41

Concerning the vital clinical signs of infected cows, Results indicated a significant rise (P<0.05) in body temperature, respiratory as well as heart rate in the diseased cows group than in controls (Table 4).

Table 4: Clinical parameters of diseased cows with *Salmonella* and control group

Clinical parameters	Control healthy cows n=25	Diseased cow group n=34	P value
Body temperature /	38.4± 2.11	40.2± 3.35*	P<0.05
Heart rate /mint	76.45± 3.68	112.43± 6.57*	P<0.05
Respiratory rate /mint	28.41± 4.11	82.56± 10.34*	P<0.05

Mean ± standard error of the mean. The differences were considered significant at P<0.05.

Table 5: Blood analysis and clotting factor indices difference of diseased cows with *Salmonella* and control group

Parameters	**Control healthy cows n=25**	**Diseased cow group n=34**	**P value**
Hematological parameters analysis			
Total leukocytes count $\times 10^3$	11.19± 1.33	15.72±2.12*	P<0.05
Neutrophils/absolute	4981 ± 163.21	9871 ± 205.11*	P<0.05
Lymphocytes/absolute	4942 ± 116.78	4781.145 ± 0.81	P<0.05
Monocytes/absolute	597 ± 161	582 ± 220	P<0.05
Eosinophils/absolute	492 ± 22	491 ± 25	P<0.05
Basophils/absolute	91±51	92±61	P<0.05
ESR mm/24hr	3.11±1.34	18.43± 5.67*	P<0.05
Clotting factor indices analysis			
Total platelets count $x10^3$ g/ L	434.34 ± 65	301.11 ± 88*	P<0.05
The platelet distribution width %	12.112 ± .261	24.165 ± 6.83*	
The mean platelet volume/fL	13.141 ± 2.58	18.252 ± 5.71 *	P<0.05
Prothrombin time /sec	14.16 ± 2.51	34.41 ± 8.11 *	P<0.05
The activated partial thromboplastin time/sec.	45.31±4.21	71.63± 12.53 *	P<0.05
Fibrinogen time /sec	14.33± 3.73	21.11± 7.49*	P<0.05

Mean ± standard error of the mean. The differences were considered significant at P<0.05.

In the current study, the infection with *Salmonella enterica serovar typhimurium* in cows affected significantly (P<0.05) some biochemical parameters. For example, hypoglycemia, decreased total protein values, and increased BUN in diseased cows, compared with the healthy control group (Table 6).

Table 6: Biochemical changes of diseased cows with *Salmonella* and control group

Biochemical parameters	Control healthy cows n=25	Diseased cow group n=34	P value
Glucose mg/dl	88±5.56	42± 8.93*	P<0.05
Total protein g/dl	6.11±1.76	4.82± 1.78*	P<0.05
BUN mg/dl	27.34± 4.33	52.45±6.67*	P value

Detection of 16S rRNA Gene:

Salmonella species isolated from animal fecal materials (19) were subjected to PCR assay to amplify the 16S rRNA gene. The results of PCR amplification and sequencing showed that 10 out of 19 isolates were *Salmonella enterica* serovar *typhimurium*. Figure 1 shows PCR bands for ten isolates for the 16S rRNA gene (1500bp).

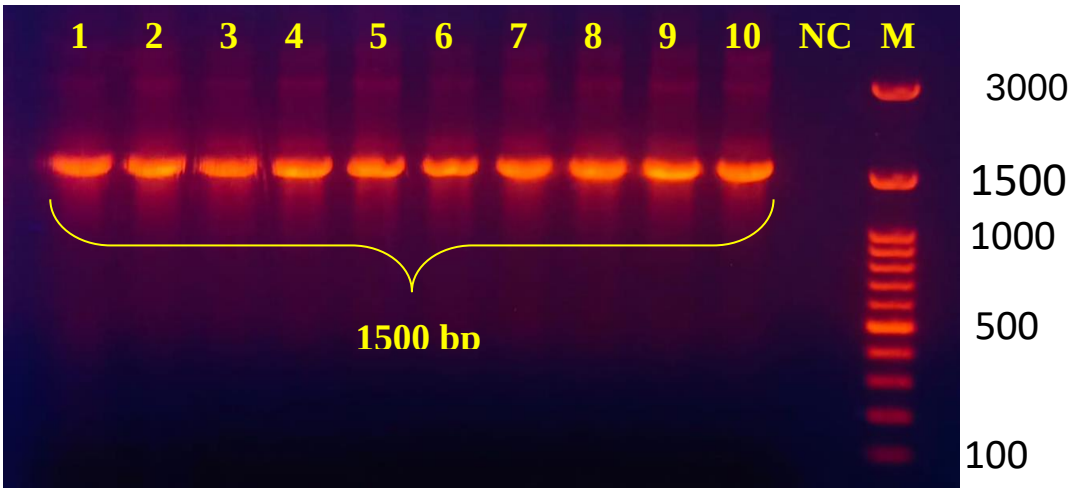

Figure 1: Agarose gel electrophoresis of PCR-amplified 16S rRNA gene of *Salmonella enterica* serovar *typhimurium* isolates. Lanes 1- 10: 16S rRNA gen1500 bp, Lane M: DNA marker and Lane NC: Negative control.

Molecular Detection of *Cdtb* Gene in *Salmonella* Species

Results of the *cdtb* gene of *Salmonella* isolates in animals showed that 10 out of 10 (100%) of samples showed clear bands of approximately 819 bp (Figure 2)

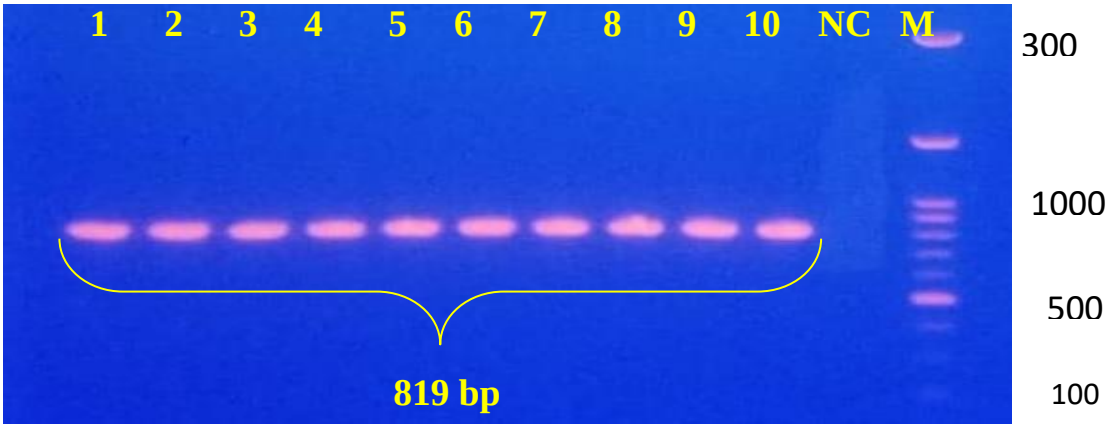

Figure 2: Agarose gel electrophoresis of PCR-amplified *cdtB* gene of *Salmonella enterica* isolates. Lanes (1-10) *cdtB* gene 819bp, Lane M: DNA marker. Lane NC: Negative control.

Molecular Detection of *pltB* Gene in *Salmonella* Species

The *pltB* PCR amplification was successful and revealed clear bands at 518 base pairs (Figure. 3) where 10 out of 10 (100%) samples were positive.

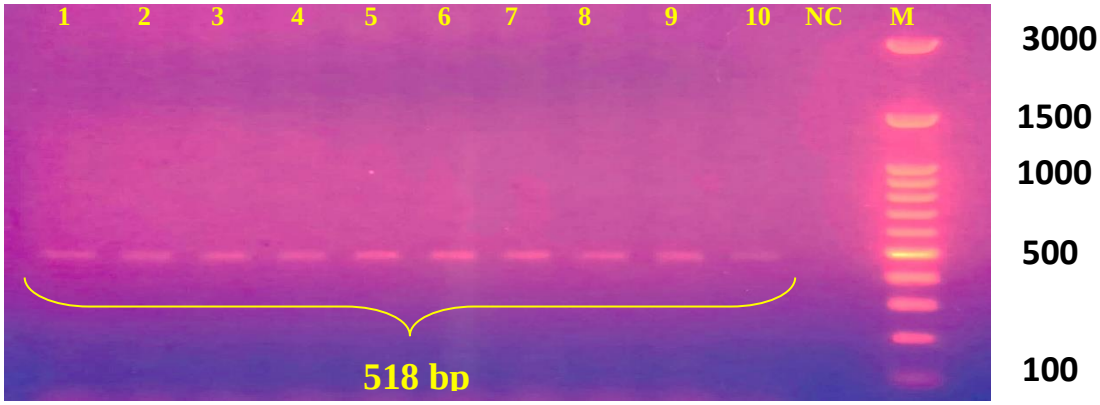

Figure 3: Agarose gel electrophoresis of PCR-amplified for *pltB* gene of *Salmonella enterica* serovar *typhimurium* isolates**.** Lanes 1- 10: ***pltB* gene** 518 bp, Lane M: DNA marker and Lane NC: Negative control.

Phylogenetic analysis from *Salmonella* Species

The evolutionary history was deduced using the Neighbor-Joining method. We selected 7 isolates, one from each district (however, there are three of the isolates that are the same) for sequencing based on the geographical distribution of the isolates (Alqurna, Basrah city center, Hartha, AlZubair, Aboalkhaseeb, Modaina, and Aldeer districts). The sequences were deposited in the NCBI database as follow; (PP856650, PP856651, PP856652, PP856653, PP856654, PP856655, PP856656). The phylogenic tree includes a total of 17 nucleotide sequences (7 from our local *Salmonella* isolates and the rest are closely related isolates from the NCBI database). The Neighbor-Joining method was used to determine evolutionary history, with the most optimal tree shown.

Figure 4: Phylogenetic tree analysis for the local *Salmonella* isolates and other closely related isolates from the NCBI database. The Neighbor-Joining method was used to determine evolutionary history, with the most optimal tree shown. The bootstrap test (with 1000 repetitions) shows the proportion of duplicate trees where the related taxa are grouped. This information is shown below the branches (located above the branches). Red squares indicate the diagnosed and NCBI-registered isolates used in this study.

DISCUSSION

It has been documented that bovine salmonellosis is an important contagious disease that affects all domestic animals and even humans and is usually endemic to a particular farm. Furthermore, salmonellosis of large animals could vary clinically in accordance with the management and housing system. The epidemiologic criteria played major roles for different *Salmonella* species (Constable *et al*., 2017). The disease can always affect all body tissues, particularly the digestive system, resulting in acute and/or sub-acute enteritis; however, carrier states were also prominent (Santos *et al*., 2002b; Santos *et al*., 2002d; Craig and James, 2006; Adem and Bushra, 2016).

Workers (Santos *et al*., 2002b,c; Jones *et al.*, 2007; Adem and Bushra, 2016) proved that acute enteritis caused by *Salmonella enterica* serovar *typhimurium*, which is confirmed in the current study can present in all animal species of different ages, causing high pyrexia with severe enteritis manifested by a putrid smell of diarrhea mostly mixed with mucus and dysentery. However, in terminal stages, the diseased animals could feel painful tenesmus with rarely followed by rectal prolapse. On the other hand, researchers described cows suffering from *Salmonella enterica* serovar *typhimurium* with complete anorexia, increasing thirst due mostly to moderate to severe dehydration. Furthermore, affected animals show signs of shallow rapid respiration with tachycardia and strong heartbeats, as well as congested mucus membrane, especially of the eyes, the pregnant animal might abort as well (Craig and James, 2006). Constable *et al*., (2017) added that severely affected animals show severe dehydration with sunken eyes, dry, rough skin, dirty perineal region, recumbency, and finally die. Notwithstanding, animals surviving from the acute stages might squall with polyarthritis or pneumonia. All mentioned clinical manifestations were also indicated in the current study, which was also indicated by (Santos *et al*., 2002b, c; Smith, 2002; Costa *et al*., 2012; Constable *et al.*, 2017).

In the present work, *Salmonella enterica* serovar *typhimurium* isolates were confirmed by PCR. However, this test was applied to detect the existence of the genes usually related to the virulence of *Salmonella typhimurium*, where results indicated that *cdtB* gene was observed in (100%) of *Salmonella typhimurium* isolates length of (819 bp) as reported by AL-Oqaili, (2019), who assured that *ctdB* gene was present in 100% of *Salmonella typhimurium* isolates. At the same time, Thakur *et al.* (2019) in their scientific work also documented and indicated that the *ctdB* gene was found in *Salmonella typhimurium* in all isolates. The 100% positivity of our PCR-tested samples agreed with (Taddele *et al*., 2011; Yang *et al.*, 2016; El-Sebay *et al*., 2017)

It was shown that the liberation of the typhoid toxin, which is encoded on *SPI11*, is unique to *Salmonella typhimurium*, which is only expressed when *S. Typhi* entered the intracellular macrophages and localized inside the *Salmonella*-containing vacuole (SCV) (Jones *et al*., 2007). However, the cytolethal distending toxin (*cdtB*) gene encodes toxins, that cause apoptosis of infected cells (Holschbach and Peek, 2018; Ben Hassena *et al.*, 2021). It was proved that typhoid toxin is unusual compared to other *CDTs*, in that *S. Typhi* lacks the *CdtA* and *CdtC* subunits. Rather, typhoid toxin is a complex composed of one *CdtB* molecule, one *PltA* molecule, and multiple *PltB* molecules (Jones *et al*., 2007).

The hematological analysis of the present study indicated leukocytosis and neutrophilia which were also indicated by (Wray, 1980). It was documented that neutrophilic leukocytosis, which occurred as a result of bacterial infection, was a defense mechanism against bacterial infection and played a good role in the small inflammatory processes, moreover, it can release proteins such as defensin and lysozyme, which have

antibacterial activity (Santos *et al*., 2002a). On the other hand, neutrophils are considered the first responders to microbial infection, as their activity and death in large numbers reflect the formation of pus as they become more active in the phagocytosis mechanism for engulfing the foreign bacteria. In addition, although neutrophils are not able to renew their lysosomes used in digesting microbes, they die after having phagocytosed a few pathogens (Costa *et al*., 2012; Marjory *et al*., 2022). On the contrary, Santos *et al*. (2002c) observed a decrease in leukocyte count (leucopenia) of the transitory type, which is characterized by decreased lymphocyte count (lymphopenia) as well as decreased neutrophil count (neutropenia).

Results also indicate a significant increase in ESR in diseased cows compared to the control group. It was shown that the rate of erythrocyte precipitation becomes faster in diseased animals with anemia or tissue damage (intestinal damage) compared to normal, healthy animals. Hereby, the significant rise in values of ESR indicated in the current study of infected cows might reflect the obvious inflammatory reaction induced by enteritis. The reported high fibrinogen levels in the blood (hyperfibrinogenemia) in this study played an important role in erythrocytes clumping and increasing sedimentation rate rapidly (Weiss and Wardrop, 2011; Harvey, 2012).

Indices of clotting factor were also different induced by *Salmonella typhi* infection in diseased cows, which could reflect the occurrence of a disseminated intravascular coagulation syndrome and a bleeding tendency of infected animals, which could be indicated in tissues of dead carcasses whereby, although, reports concerning acute salmonellosis in cattle does not refer to severe bleeding tendency in diseased cows. However, the severity of this coagulation mechanism depends on the intensity and duration as well as the ischemic and inflammatory problems induced by the disease (Bick, 2003). It was believed that the depression of thrombocyte count and activities with decreased clotting time could reflect the appearance of hemorrhagic patches indicated on different body tissues, especially the intestinal tissue, which may be explained by the release of a special moderator such as platelet-activating factor reflected by the inflammatory disturbances (Rebar *et al*., 2005).

The cell death CDT-dependent could occur because of a response of apoptotic mechanism either in fibroblastic, epithelial, or lymphoblastic cells (Pons *et al*., 2019). Further, Jones *et al*. (2007) and Ben Hassena *et al. (*2021), added that the toxin can play a major role in the development of chronic *S. typhi* infection. Those researchers confirmed that typhoid toxin is responsible for the development of clinical manifestations as well as the shift from acute to chronic typhoid fever and could be a powerful target for confirming those signs.

It was shown that because of the special nature of the infections caused by *Salmonella,* there was always a demand to promote a new, fast, as well as reliable test for fast detection of these infections to initialize fit control measures. One of the limitations of phenotypic methods for bacterial identification is the inability to identify the bacterium on a species level in some cases (Santos *et al*., 2002d). Most biochemical profiles didn't lead to accurate bacterial identification in most cases; reproducibility of the result is not guaranteed and depends mainly on the metabolic fingerprint of the isolates, which in turn varies based on the physiological status of the isolate at the time of carrying out the assay (Zhang *et al*., 2011).

In this study, all isolates contained the typical 16S rRNA of a molecular weight of 1500 bp, similar to previous results (Taddele *et al*., 2011; Yang *et al.*, 2016; El-Sebay *et al*., 2017), where all *Salmonella* isolates species were positive for the 16S rRNA gene. It was shown that the objective target genes for the identification of the *Salmonella*

species were a convenient factor for the fast diagnosis of these microorganisms.

Concerning the sequencing in this study, the local *salmonella* isolates had variable similarity to isolates from other geographical areas around the globe. This highlights the ability of *Salmonella* to spread and integrate in wide geographical areas.

Results of some biochemical changes of diseased cows with *Salmonella enterica* serovar *typhimurium* infection showed significant hypoglycemia, and hypoproteinemia, as well as increased values of BUN compared with the controls.

Hypoglycemia could occur when the consumption of food is decreased by the diseased animal and becomes lower than normal, that insufficient for the energy needs. Thereby, there will be inadequate ruminal production of digestive acids such as propionic acid, which is considered the main source of glucose in cows reflecting hypoglycemia (Santos *et al*., 2002c) and anorexia. Consequently, dehydration arises due to the loss of body fluids and electrolytes (Smith, 2002).
Hypoproteinemia detected in the infected cows might be a result of intestinal loss of protein related to deep necrotizing enteritis, in which a large amount of protein effusion occurs in the intestinal lumen (Wray, 1980). The decreased protein levels during *S. typhimurium* infection are attributed to digestive disorders, protein destruction during pyrexia, as well as less production from the liver (Santos *et al*., 2002c; Constable *et al*. 2017). On the other hand, dehydration in the current study was assisted in the renal ischemia with insufficient perfusion and consequently reflected on the increased values of blood urea nitrogen (BUN) in the diseased cows, which could explain the indirect damage to renal tissue (Smith, 2002; Holschbach and Peek 2018).

CONCLUSIONS

It is known that salmonellosis is a serious infectious disease that causes high rates of infection and sometimes deaths of infected animals. Therefore, correct and efficient diagnostic methods to limit the spread of the disease among animals could be an important way to control it.

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Ethical approval

The Animal Ethics Committee of the College of Veterinary Medicine, University of Basrah, Iraq, allowed and permitted this research to be conducted.

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دراسة سريرية وتشخيصية للاسهال البقري المتسبب عن جراثيم Salmonella enterica serovar typhimurium في البصرة، العراق

علي بلبول الديوان[١]، علياء سبتي جاسم[١]، كمال الدين مهلهل السعد [٢]، نورس نوري جبا ر
[١]فرع الاحياء المجهرية والطفيليات ، كلية الطب البيطري، جامعة البصرة ، العراق
[٢] فرع الطب الباطني والوقائي ، كلية الطب البيطري ، جامعة البصرة ، العراق

Email: kamalsad58@yahoo.com Assiut University web-site: www.aun.edu.eg

في هذه الدراسة تم تشخيص مرض السالمونيلا في أربع وثلاثين بقرة (٣٨,٦٣٪) من خلال العلامات السريرية والزرع البكتيري وتم تأكيدها باستخدام تفاعل البلمرة المتسلسل (PCR) حيث أجريت الدراسة على ثمان وثمانون بقرة من سلالات الأبقار المحلية بعمر ٢-٤ سنوات في البصرة، العراق. أذ تم أخذ عينات براز (١ جرام) مباشرة من مستقيم جميع الحيوانات المشتبه في اصابتها ونقلها فوراً إلى مختبر التشخيص. بدأت الدراسة في الفترة من نوفمبر ٢٠٢٢ إلى يوليو ٢٠٢٣. وأظهرت الأبقار المريضة علامات سريرية مختلفة أهمها الإسهال والجفاف والحمى. واشار تحليل الدم إلى زيادة عدد كريات الدم البيضاء بالإضافة إلى ذلك، كان معدل ترسيب كريات الدم الحمراء مرتفعا بشكل ملحوظ. وتمت ملاحظة اختلاف كبير في مؤشرات عوامل التخثر. كما أشارت النتائج إلى نقص السكر في الدم، وانخفاض البروتين الكلي، وزيادة اليوريا نيتروجين في الدم في الأبقار المريضة. كشفت نتائج فحص البلمرة المتسلسل لجين16S r RNAمن عزلات السالمونيلا أن ١٠ من أصل ١٩ عزلة ايجابية في النطاق الصحيح للتفاعل عند ١٥٠٠ قاعدة زوجية لتتوافق مع نوع السالمونيلا المعوية serovar typhimurium. ومن ناحية أخرى، أظهرت نتائج تضخيم الجينات *cdtb* و*pltB* حزم واضحة عند ٨١٩ و٥١٨ قاعدة زوجية على التوالي. كما، أظهر التحليل الوراثي أن العزلات المحلية لها علاقة وثيقة مع السلالات الأخرى المنشورة في قاعدة بيانات NCBI.واستنتج من هذه الدراسة إلى أن داء السالمونيلا البقري يعد مرضا خطيرا لما له من آثار سلبية على الحيوانات المصابة في جميع الأعمار. ولذلك، فإن القضاء على هذا المرض والسيطرة عليه في وقت مبكر يمكن أن يكون الطريقة الصحيحة لحماية حيوانات المزرعة

الكلمات المفتاحية: جراثيم السالمونيلا، الابقار، تفاعل البلمرة المتسلسل، البصرة، العراق