Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

Molecular Detection of *Salmonella enterica serovars Typhimurium* in Diarrheic Calves and Sheep

Ahmed Madhloom Jari[1], Hameedah Hamzah Ajeel[2]*, Hamed Abbas Hassan Aljabory[1]

[1]Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq; [2]Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq.

Abstract | This study employs a range of methods, including culturing, the VITEK 2 system, and PCR fingerprinting, to confirm Salmonella infections in sheep and calves. The study included 200 animals (100 sheep and 100 calves) with diarrhea, and a total of 200 rectal swab samples were colleved from individual animals. Samples were cultured on various differentiation and selective Salmonella media, such as Salmonella-Shigella agar (SS agar) and Xylose Lysine Deoxycholate (XLD) agar. After culturing of the 200 rectal samples, the present study's findings showed that 170 (85%) of the samples had growth. In contrast, 30 (15%) of the samples showed no growth. Out of 100 sheep rectal samples, 55(55%) showed colonies of *Salmonella spp.*, while 15 (15%), 5 (5%), and 15 (15%) showed *E. coli*, *Staphylococcus*, and *Shigella*, respectively, with 10 (10%) of the samples showing no bacterial growth. The calf's rectal samples also revealed that 45 (45%) out of 100 samples had colonies of *Salmonella spp.*, while 20 (20%), 5 (5%), and 10 (10%) had colonies of *E. coli, Staphylococcus,* and *Shigella*, respectively. Twenty (20%) of the samples showed no bacterial development. The percentage of diarrheic sheep and calves is as follows: 100 (50%), 35 (17.5%), 10 (5%), and 25 (12.5%) for *Salmonella*, *E. coli*, *Staphylococcus*, and *Shigella*, respectively. The diagnosis was confirmed using PCR and then using the 16S rRNA gene to confirm bacterial isolates. In this study, we utilized the adhesion genes of *S. typhimurium* (TolC and SipC genes), which were designed using primers (IDT, Canada). The results showed that SipC genes produced nine positive isolates (two from calves and seven from sheep), whereas TolC genes produced 19 positive isolates (seven from calves and 12 from sheep). The study provides valuable information on the isolation, diagnoses, and molecular detection of Salmonella species in animals with diarrhea, indicating the bacterium is critical organisms in diarrheic animal and opens up potentila treatment options

Keywords | *Salmonella typhimurium*, Diarrhea, PCR, *SipC* gene, *TolC* gene

Received | July 15, 2025; **Accepted** | August 28, 2025; **Published** | September 03, 2025
***Correspondence** | Hameedah Hamzah Ajeel, Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq; **Email:** hameedahhamza@vet.uoqasim.edu.iq
Citation | Jari AM, Ajeel HH, Aljabory HAH (2025). Molecular detection of *Salmonella enterica serovars Typhimurium* in diarrheic calves and sheep. J. Anim. Health Prod. 13(s1): 259-267.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.259.267
ISSN (Online) | 2308-2801

INTRODUCTION

Salmonellosis is concerned as one of the most prevalent diseases in small ruminants (Ferreras *et al.*, 2007; Farouk *et al.*, 2021). *Salmonella* spp. can affect cattle at any age, but calves between 4 and 28 days are particularly at risk (Holschbach and Peek, 2018). These bacteria are major zoonotic agents responsible for causing infections and clinical cases of salmonellosis (Ehuwa *et al.*, 2021). While *Salmonella* spp. can occasionally cause asymptomatic infections, clinical salmonellosis results from the pathogenicity of these organisms mediated through their virulence genes (Casaux *et al.*, 2023). Calves affected with salmonellosis exhibit high body temperature, weakness,

anorexia, and Bloody or mucoid diarrhoea (Shehta *et al*., 2022; Nikkhah *et al*., 2023). Salmonellosis in calves is a global health issue with significant economic implications, primarily due to high mortality rates, poor growth, and the potential for the infection to spread to humans (Holschbach *et al*., 2018). Salmonellosis consequences The consequences of salmonellosis to animals and humans and its implications have on food safety, public health, and economy which include both prevention and treatment expenses; production losses or even deaths that are caused to animals and humans (Ali *et al*., 2021; He *et al*., 2023; Sanni *et al*., 2023).

Several factors contribute to the dissemination of *S. typhimurium* in sheep, including husbandry conditions, environmental circumstances, and the presence of other pathogen species (Tanner and Kingsley, 2018). Young animals, particularly lambs, are often exposed to a higher risk of infection and greater disease risk compared to older ones (Napoleoni *et al*., 2021; Mosa *et al*., 2025). The novel BssS/BssR two-component system (TCS) functions as a novel biofilm-promoting factor in *S. typhimurium*. for *S. typhimurium* to form biofilms, it must sense and respond to cues within and outside the host via the BssS/BssR TCS. These pathways are widespread in bacteria and are essential modes of communication that enable the bacteria to sense and respond to their environment. Usually, they are composed of a sensor histidine kinase, in this case, BssR, as implied by its name, and a response regulator, BssS, which is also inferable from its name (Lv *et al*., 2022). SipC contributes significantly to allowing bacteria to enter the host cells and is a key player in the initiation of infections (Giovagnoni *et al*., 2020). TolC functions as the outer membrane factor of several resistance-nodulation-division (RND) efflux systems (Smith and Blair, 2014). The tolC gene is also of importance, as it can provide information on the multidrug resistance of *Salmonella typhimurium*, since it codes for a protein that is an essential component of the efflux pumps. These pumps function to remove antibiotics from the cytoplasm or the inner leaflet of the inner membrane, thereby reducing their intracellular concentration and diminishing their effect (Honeycutt *et al*., 2020). This study aimed to Examining the frequency of *Salmonella* spp. in sheep and cattle with diarrhea from various ages and seasons with identifying *Salmonella* spp. by using VITEK 2 system and determining the resistance of isolates to a variety of antibiotics as well as identify a few virulence genes by using PCR technique.

MATERIALS AND METHODS

Samples collection

Two hundred faecal sample swabs (100 from calves and 100 from sheep) were collected over a period from September 2024 to March 2025. These were swabs from individuals of either sex (both male and female) at any age, particularly young ones with suspected diarrhea. Swabs were taken from all sheep and calves with diarrhea, clinically diagnosed by veterinarians.

Salmonella isolation and identification

The conventional bacteriological methods were used to isolate Salmonella from the samples. The samples were inoculated into the nutrient broth and incubated at 37°C for 24 hours. Fecal samples were subjected to initial nutrient pre-enrichment and then incubated. Subsequently, 0.5 mL was transferred to 10 mL of Tetrathionate Broth (Merck) and incubated at 37°C for 24 hours. Pre-incubated Salmonella-Shigella agar and Xylose Lysine Deoxycholate (XLD) agar were streaked with a loop of each enrichment broth and incubated. The plates were then analyzed for the existence of Salmonella colonies. Suspected colonies were inoculated into TSI (Triple Sugar Iron agar), peptone water, Simmon's Citrate, Urea medium, and MR-VP (Khan *et al*., 2022; Al-Roomi and Ajeel, 2024).

Extraction of bacterial DNA

DNA was extracted from the samples, and the concentration levels ranged from 50 to 150 ng/µL. The process for obtaining DNA from bacteria utilized an AMB DNA purification kit, following the manufacturer's instructions (Geneaid, Taiwan). Store the DNA at -20°C for future use. The absorbance ratio at 260 nm to 280 nm (A260/A280) is often used to evaluate the purity of DNA and RNA samples (Wilfinger *et al*., 1997). This method, which measures absorbance, offers a rapid and straightforward way to assess potential protein contamination in nucleic acid samples. Generally, an A260/280 ratio near 1.8 is seen as a sign of relatively pure DNA, whereas a ratio of approximately 2.0 is accepted as pure RNA (Syafiradewi *et al*., 2024).

Application of PCR for detection of *Salmonella*

Polymerase chain reaction, commonly referred to as PCR, is a widely used technique for identifying Salmonella due to its exceptional sensitivity and specificity (Deb *et al*., 2024). The PCR analysis was performed to detect the Salmonella invasion genes (SipC and TolC) according to the manufacturer's instructions (IDT/Canada). A series of temperature changes, known as thermal cycling, is crucial in the PCR process to enhance the amplification of target DNA. The initial step, known as denaturation, involves heating the DNA to a high temperature (typically between 94°C and 95°C) to separate the double-stranded DNA into its single strands. The subsequent step, referred to as annealing, cools the temperature (around 50-65°C) to enable primers to attach to the single-stranded DNA. The

final step, called extension, raises the temperature (typically to 72˚C) to allow the DNA polymerase to create new DNA strands that are complementary to the target sequence. This cycle of three steps is repeated several times (typically 25-40 cycles) to exponentially amplify the amount of target DNA, making it possible to detect even minute amounts of Salmonella DNA. The PCR analysis was performed to detect the Salmonella invasion gene. Universal primers targeting the *16S rRNA* gene are commonly used to amplify variable regions for the detection of *Salmonella*, providing a broad-range approach for identifying diverse *Salmonella* species (Maynard *et al*., 2005) Table 1.

Statistical analysis

When utilizing the computer program (SPSS), the ANOVA analysis determines the statistical significance of the data, with a p-value of <0.05 (Al-Rawi and Khalaf Allah, 2000).

RESULTS

Direct examination of collected samples

The identification of bacterial isolates relied on various diagnostic methods, including diverse culture media, such as *Salmonella-Shigella* Agar and XLD Agar, as well as biochemical and analytical profile index tests. In this study, 100 rectal samples were collected from sheep and 100 rectal samples from calves suffering from diarrhea, of both sexes (males and females), at any age in both species (sheep and calves) in Babylon Province, Iraq.

The results of the present study, illustrated in Figure 1, show that out of 200 rectal samples, 170 (85%) exhibited growth, while 30 (15%) showed no growth (Table 2). Out of 100 rectal samples of sheep, 55(55%) exhibited colonies of *Salmonella* spp. while 15(15%), 5(5%), 15(15%) revealed *E. coli*, *Staphylococcus*, and *Shigella,* respectively. Ten samples showed no bacterial growth. Also, the rectal samples of calf showed that out of 100 sample 45 (45 %) appeared colonies of S. spp. while 20(20%), 5(5%), 10(10%) exhibited colonies of (*E. coli, Staphylococcus, and Shigella* respectively while 20(20%) did not show any bacterial growth. In total, the highly percent of diarrheic sheep and calves in the present study as follow 100(50%), 35(17.5%), 10 (5%), 25(12.5%) for *E. coli*, *Staphylococcus, Shigella* respectively, while 30(15%) don't showed any bacterial growth. The statistical analysis showed that *Salmonella* spp. is significantly increased (P<0.01) in number as compared with other isolated bacteria in both sheep and calves, as shown in Table 2.

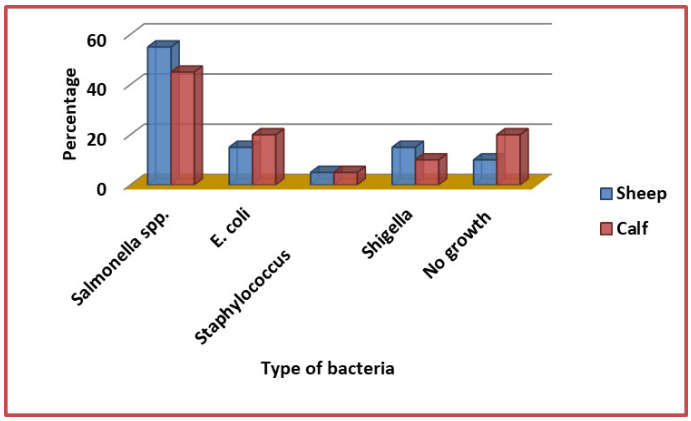

Figure 1: The percentage of growth in the culture sample.

A study on dairy cattle farms in northern Italy discovered an alarming frequency of *Salmonella* in calf carcasses and organs, with 61.67% of the samples tested positive. The most often discovered serotypes in these dairies were S. Dublin (38. 33%), *S. typhimurium* (23. 33%), and the monophasic variant of *S. typhimurium* (14. 17%), indicating the wide variety of *Salmonell*a serotypes affecting calf health in this area (Parolini *et al*., 2024). In Hamedan, Iran, it was discovered that 18. Thirty-three percent of stool samples collected from industrial farms tested positive for Salmonella Typhimurium (Asl *et al*., 2022). In the Babylon region,

Table 1: Specifications of the used primers.

Gene		Sequence	Fragment length	Ref
16SrRNA	F	AGAGTTTGATCCTGGCTCAG	1500 bp	(Karunakaran *et al*., 2010)
	R	CTACGGCTACCTTGTTACGA		
BssS gene	F	GTCATTCAGACCCATCCGCT	118 bp	(Esmat *et al*., 2018)
	R	CTTCAGTCCCTTCCGGTTCC		
BssR gene	F	TGCGAAAAGCGAAGGGGTAT	111 bp	(Lories *et al*., 2020)
	R	GATCCAGTCCCTGCCGTAAA		
Tolc gene	F	GCAGACGCTGATCCTCAATAC	623 bp	(Honeycutt *et al*., 2020)
	R	TTGCGCCGACGAAGTTATAC		
SipC gene	F	GTCAGTGACCTGGGGTTGAG	425bp	(Cheng, 2017)
	R	AGTGATCCCCAACTGAAGCG		

Table 2: The percentage of *Salmonella* spp isolates and other bacteria from sheep and calf.

Type of bacteria	Sheep (100 samples)	Calf (100 samples)	Total isolated No.	X2	P value
Salmonella spp.	55(55%)	45(45%)	100(50%)	2	0.157(NS)
E. coli	15(15%)	20(20%)	35(17.5%)	0.866	0.352(NS)
Staphylococcus	5(5%)	5(5%)	10(5%)	0	1(NS)
Shigella	15(15%)	10(10%)	25(12.5%)	1.14	0.285(NS)
No growth	10(10%)	20(20%)	30(15%)	3.92	0.048(S)
X^2	100	42.54	151.56		
P value	<0.0001(HS)	<0.0001(HS)	<0.0001(HS)		

NS: No significant difference at P<0.05, S: Significant difference at P<0.05, HS: Highly considerable difference at P<0.01.

it was discovered that 50 (71. 4%) of the 75 rectal swab samples taken from diarrheic calves were identified as Escherichia coli. In comparison, 20 (28.5%) revealed Gram-positive bacteria, including 8 (32%) isolates of *Staphylococcus* spp. (Abdulzahra and Ali, 2025). One investigation in El-Behira province, Egypt, discovered a 8% detection rate of *E. coli* in diarrheic calves (Shaaban *et al*., 2018). Another study found that *Staphylococcus aureus* had a prevalence of 1.7% in calf feces in Latvia (Terentjeva *et al*., 2019). A study in northern Italy highlighted that Salmonella was most commonly identified in calf carcasses and organs, with a prevalence of 61.67%. The serotypes most frequently detected in dairies were *S. dublin* (38.33%), *S. typhimurium* (23.33%), and *S. typhimurium* monophasic variant (14.17%) (Parolini *et al*., 2024). In Hamedan, Iran, a study identified *Salmonella typhimurium* in 18% of samples. 33% of stool samples collected from industrial farms, indicating a relatively high prevalence rate of *S. typhimurium* infection as the only detected serotype in the region (Asl *et al*., 2022). In El-Behira province, Egypt, a study found a 10% detection rate of Salmonella in diarrheic calves. Serological identification revealed the presence of *S. enteritidis, S. typhimurium, S. meleagridis, S. anatum,* and *S. lagos*. The study also confirmed the significant role of diarrheic calves as sources of human infection with diarrheagenic agents, necessitating the need for establishing a plan to control infectious diarrhea in calves (Shaaban *et al*., 2018). In one study, the average percentage of both fecal and wool samples that tested positive for *E. coli* O157:H7 was found to be 9% and 18%, respectively (Edrington *et al*., 2009). Sub-clinically, the prevalence of Shiga toxin-producing *E. coli* (STEC) infection in sheep can be high, with one study finding that 66. A percentage of feces samples from sheep were reported to be positive (Ekiri *et al*., 2014; Oporto *et al*., 2019; Abed *et al*., 2024). We suspected that *Staphylococcus* may be involved in the etiology of calf diarrhea; however, its role might be less critical than that of other infectious agents, such as *E. coli* and *Salmonella*. Additional investigations are required to determine the distribution and significance of *Staphylococcus* species within the rectal microbial community of sheep (Nedbalcov *et al*., 2015; Tola, 2018; Gelalcha *et al*., 2023). Studies generally focused on the exploration of enteric pathogen content in livestock do not typically show the presence of Shigella in rectal samples (Harrington *et al*., 2015; Sabah *et al*., 2021).

Molecular characterization of *S. typhimurium* isolates

Detection of infection using 16S gene primers set

The PCR-based analysis showed that the DNA amplified for the *16S rRNA* gene was 1,500 bp, as illustrated in Figure 2. All samples tested positive for the *16S rRNA* gene.

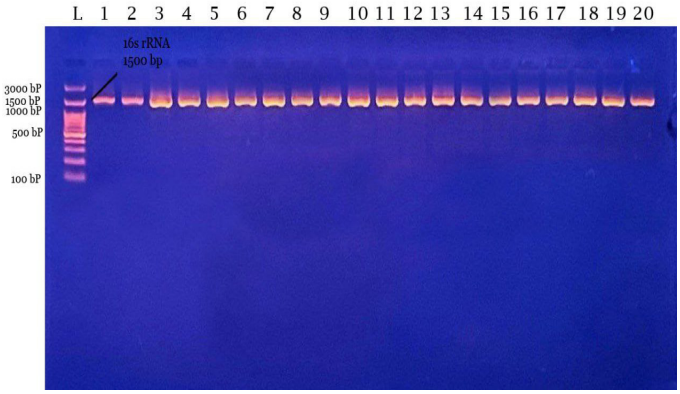

Figure 2: DNA amplification for a bp of partial sequences in local *Salmonella* spp. Detecting *16Sr RNA* gene using PCR. Lanes 1-20 positive results, Lane L: 3000 bp marker (ladder). Lanes 1, 2, 3, 4, 8, 9 and 11 indicate positive isolates from calf whereas lanes 5, 6, 7, 10, 12, 13, 14, 15, 16, 17, 18, 19 and 20 indicate positive isolates from sheep.

These primers are designed to target unique DNA sequences within the *16S rRNA* gene or other genomic regions that are specific to *Salmonella enterica* subsp. enterica or *Typhimurium*. The use of particular primers minimizes the amplification of non-target DNA, reducing the risk of false-positive results. The increased specificity and Sensitivity of specific primers make them ideal for detecting Salmonella in complex samples, such as food matrices or clinical specimens. Multiplex PCR assays often include 16S rRNA primers as an internal amplification control, ensuring the reliability and accuracy of the PCR reaction (Sandrasaigaran *et al*., 2023). The PCR technique

was an accurate method for identifying *S. typhimurium* bacteria through the detection of the *16S rRNA* gene. The results of the *16S rRNA* gene in *Salmonella* isolated from sheep and calves showed that all samples harbored the current gene. These findings are in agreement with a study that utilizes diverse sample types, including ileum mucosa, feces, and blood, and can be employed for *16S rRNA* sequencing to detect *Salmonella typhimurium* (Argüello *et al*., 2018; Rutanga *et al*., 2020). The selection of appropriate sample types depends on the specific objectives of the study and the nature of the infection being investigated. Feces can provide a non-invasive method for following Salmonella shedding. The efficiency of DNA extraction is crucial for obtaining an accurate representation of the microbial community in the sample (Oakley *et al*., 2014). The primers are designed to anneal to regions of conserved sequence on either side of the divergent areas of the *16S rRNA* gene, allowing for the amplification of DNA that contains sufficient information to differentiate the species of Salmonella in the sample. This amplified DNA can then be sequenced or subjected to DGGE. Applying universal primers also makes it a low-cost and broad-spectrum method for Salmonella detection, which is valuable when a specific serovar is unidentified; for example, specific primers can be designed from *Salmonella enterica* subsp. Enterica, serovars such as *Typhimurium* can further enhance the specificity and sensitivity of Salmonella detection (Sandrasaigaran *et al*., 2023).

Detection of BssS and BssR genes of *Salmonella typhimurium*

Figure 3 show 20 positive detection of *BssS* gene where lanes 1, 2, 3, 4, 8, 9, and 11 indicate to rectal samples swab collected from calf whereas lanes 5, 6, 7, 10, 12, 13, 14, 15, 16, 17, 18, 19 and 20 indicate rectal samples swab collected from sheep. After amplification of the gene by PCR technique, visibility was achieved with electrophoresis using UV ray (Figure 4). The result showed the 20 positives for *BssR* gene (1, 2, 3, 4, 8, 9, 11 are from calf whereas lanes 5, 6,7, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20 are from sheep). Samples were visualized through electrophoresis using UV ray after amplification by PCR. The regulatory control of bssR and bssS, in conjunction with other players that influence film development in Salmonella Typhimurium, is highly complex, involving a complex crosstalk of signal and response (Lories *et al*., 2020). These genes are regulated by a multitude of environmental stimuli, including nutrient status, temperature, and the presence of various signaling molecules. Additionally, the bssR and bssS mRNAs may be modulated by other regulators, resulting in a hierarchical regulatory network that dynamically adjusts biofilm formation in response to environmental changes.

The *bssR* and *bssS* genes play a pivotal role in regulating the production of the extracellular matrix, a hallmark of (Lories *et al*., 2020). The *bssS* gene, in particular, is known for its role in biofilm formation by Enterobacteriaceae species, highlighting the conserved nature of biofilm-related determinants and their crucial role in bacterial survival and adaptation. The ability to detect the bssS gene can provide insights into the capacity of *S. typhimurium* isolates to form strong biofilms, thereby offering a broader understanding of their persistence and pathogenic potential (Esmat *et al*., 2018).

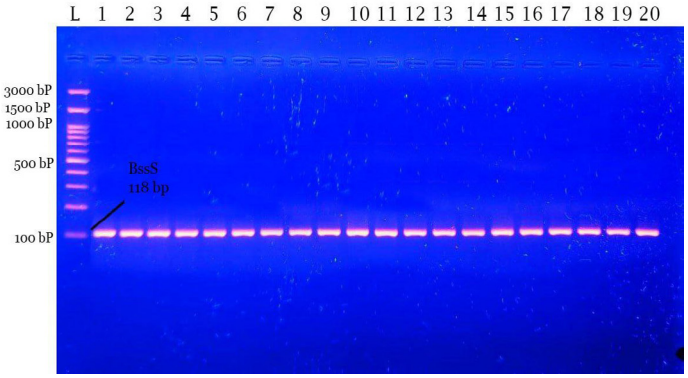

Figure 3: DNA amplification for the bp of *S. typhimurium* to detect *BssS* gene. Lanes 1-20 are showing positive samples where lanes 1, 2, 3, 4, 8, 9 and 11 indicate isolates from calf whereas lanes 5, 6, 7. 10, 12, 13, 14, 15, 16, 17, 18, 19 and 20 show the samples collected from sheep.

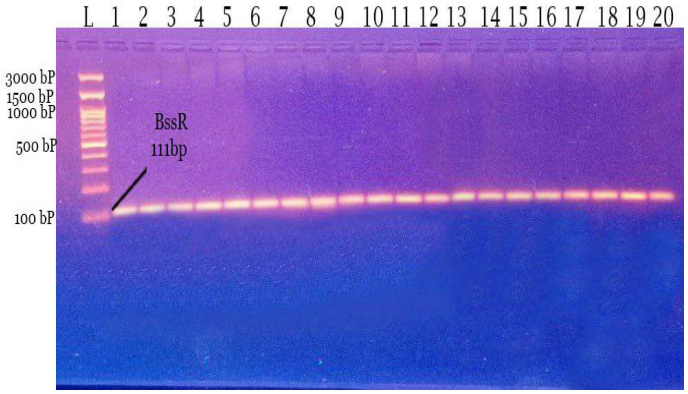

Figure 4: DNA amplification for a bp of *S. typhimuruim* to detecte *BssR* gene. Lanes 1 to 20 are positive samples where lane L indicate 3000bp ladder (marker). Lanes 1, 2, 3, 4, 8, 9 and 11 indicate positive isolates from calf whereas lanes 5, 6, 7, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20 indicate positive isolates from sheep.

Detection of *SipC* and *TolC* genes of *Salmonella typhimurium*

In Figure (5) showen DNA amplification for a bp of S. typhimurium to detect the SipC gene(425bp). Lanes 1 to 20 are positive samples where lane L indicate 3000bp ladder (marker). Lanes 1, 2, 3, 4, 8, 9 and 11 indicate positive isolates from calf whereas lanes 5, 6, 7, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20 indicate positive isolates from sheep.

On the other hand, (Figure 6) showed the tolcgene (623bp)Lane (1: 19) positive results, except for Lane 6, which corresponds to the 3000bp adder (marker). Lanes 1, 2, 3, 4, 8, 9 and 11 indicates positive isolates from calves and lanes 5, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 indicates positive isolates from sheep.

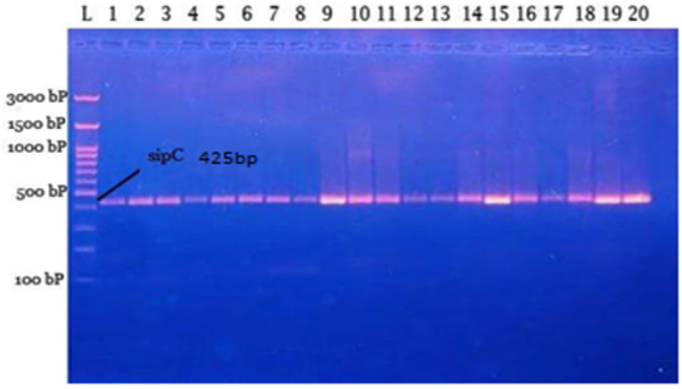

Figure 5: DNA amplification for a bp of *S. typhimurium* to detect the *SipC* gene. Lanes 9, 10, 11, 14, 15, 16, 18, 19, 20 indicate positive results. Lane L is a 3000bp ladder (marker). Lanes 9 and 11 indicate positive isolates from calves and lane 10, 14, 15, 16, 18, 19, and 20 suggests positive isolates from sheep.

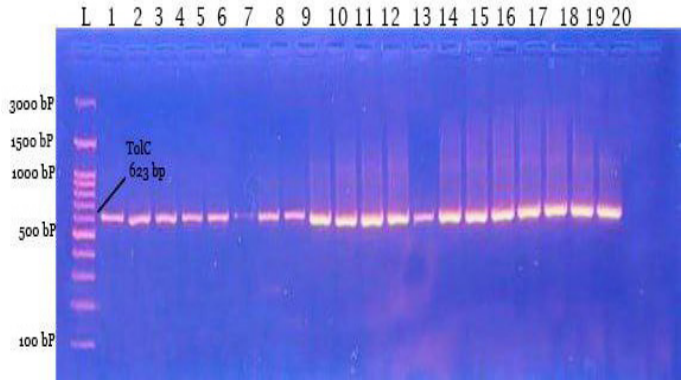

Figure 6: DNA amplification for a bp of *S. typhimurium* to detect the TolC gene. Lanes 1 to 20 indicate positive results. Lane L is 3000bp ladder (marker). Lanes 1, 2, 3, 4, 8, 9 and 11 indicates positive isolates from calves. Lanes 5, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 indicates positive isolates from sheep.

Studies will elucidate the role of TolC in *Salmonella's* ability to cause disease and survive (Buckley *et al.*, 2006). *S. typhimurium* secretes T3SS1 as one of its key effector proteins, SipC. The T3SS1 is a dedicated machinery for the injection of effector proteins into the host cells, perturbing cellular responses to allow invasion and survival. This system, encoded on Salmonella Pathogenicity Island 1 (SPI-1) and working in concert with other T3SS1 effectors, such as SipB, enhances Salmonella's entry into non-phagocytic cell types. In particular, SipB and SipC act as translocons that form a pore in the host cell membrane, through which other effector proteins are translocated (Kim *et al.*, 2018). An OM channel protein, TolC, is a common partner of various MDR efflux systems in *S. typhimurium* (Horiyama *et al.*, 2010). By pumping an array of molecules, including antibiotics, out of the bacterial cell, these efflux pumps confer resistance to several drugs. The *tolC* gene is required for the function of numerous efflux systems, including AcrAB-TolC, AcrDTolC, and AcrEF-TolC. PCR tests can be designed to specifically amplify the *tolC* gene, which means that the presence of this gene in bacterial isolates can be detected. Such assays may also be used to search for mutations or alterations in the *tolC* gene that affect its function. Efflux pump tests, which are indirect estimates of a bacterium's ability to efflux particular substrates, provide a test of TolC function. A simple method to establish the contribution of TolC to environmental survival, antibiotic resistance, and other phenotypes is to knock out the *tolC* gene and analyze the resulting phenotype. These screening assays often include fluorescent dyes or different chemical compounds that are actively transported out of the cell by efflux pumps. Such studies may elucidate the precise contributions of TolC to the virulence and colonization of *Salmonella* (Buckley *et al.*, 2006).

CONCLUSION

Salmonella enterica serovar Typhimurium remains a significant pathogen in sheep and calves, causing substantial morbidity, mortality, and economic loss in the animal industry. Infected animals, especially young lambs and calves may exhibit symptoms such as diarrhea, fever, in appetence, and dehydration, which rapidly progress to septicemia and potential death without prompt treatment. The emergence of antibiotic-resistant strains of *S. typhimurium* further complicates the issue, and antibiotic and integrated management strategies should be applied judiciously. Effective control is a combination of biosecurity measures, regular surveillance, cleanliness, and, where appropriate, vaccination. Addressing these issues requires the application of a One Health approach, recognizing the interconnections among animal health, human health, and environmental health, to minimize the impact of Salmonella infections in animals and the broader community.

ACKNOWLEDGMENT

Authors want to express sincere gratitude to the college for its excellent academic environment, facilities, and the wisdom imparted by professors. Thank you for your help along the way, whether it was through your encouragement, assistance, or simply believing in us.

NOVELTY STATEMENT

This study presents that assessing the prevalence of Salmonella species in sheep and cattle suffering from diarrhea at different ages, identifying the species using the VITEK 2 system, figuring out the isolates' resistance to different antibiotics, and identifying a few virulence genes using the PCR technique.

AUTHOR'S CONTRIBUTION

Ahmed Madhloom Jari: Collected samples, performed laboratory examinations, interpreted results, and completed all research requirements. Hameedah Hamzah Ajeel: Designed the study, supervised all stages of the research, and reviewed the final version of the manuscript. Hamed Abbas Hassan Aljabory: supervised the research, and reviewed the final version of the manuscript.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdulzahra AN, Ali RA (2025). Isolation and identification of *Escherichia coli* that cause diarrhea in neonatal calves. SAR J. Pathol. Microbiol., 6I02: 67-69. https://doi.org/10.36346/sarjpm.2025.v06i02.003

Abed DA, Hamzah KJ, Abdul-Kareem S, Mehrzad J (2024). Bacteriological and molecular study of some urinary tract infections bacteria in human and cows in Babylon province. Pak. Vet. J., 44(4).

Ali RA, Khudhur HR, Hasan AA (2021). Microbiology and histological study of gallbladder among acute and chronic cholecystitis in Babylon City, Iraq. Rev. Res. Med. Microbiol., 32(2): 95-101. https://doi.org/10.1097/MRM.0000000000000233

Al-Rawi, K. M., Khalaf Allah, A. M. (2000). Design and Analysis of Agricultural Experiments. University of Mosul. Ministry of Higher Education and Scientific Food Safety and Food Handling Practices. Foods (Basel, Switzerland), 10(5), 907. Doi/10.3390/food1005

Al-Roomi NM, Ajeel HH (2024). Antibacterial effect of green synthesis of selenium nanoparticles against *Salmonella typhi* in small buffalo. Uttar Pradesh J. Zool. 45(5): 80-90. https://doi.org/10.56557/upjoz/2024/v45i53933

Argüello H, Estellé J, Zaldívar-López S, Jiménez-Marín Á, Carvajal A, López-Bascón MA, Crispie F, O'Sullivan O, Cotter PD, Priego-Capote F, Morera L, Garrido JJ (2018). Early *Salmonella typhimurium* infection in pigs disrupts Microbiome composition and functionality principally at the ileum mucosa. *Sci. Rep.*, 8(1): 7788. https://doi.org/10.1038/s41598-018-26083-3

Asl MN, Mahmoodi P, Bahari A, Goudarztalejerdi A (2022). Isolation, molecular identification, and antibiotic resistance profile of *Salmonella typhimurium* isolated from calves fecal samples of dairy farms in hamedan. J. Med. Microbiol. Infect. Dis., 10: 42-47. https://doi.org/10.52547/JoMMID.10.1.42

Buckley AM, Webber MA, Cooles S, Randall LP, La Ragione RM, Woodward MJ, Piddock LJ (2006). The AcrAB-TolC efflux system of *Salmonella enterica* serovar *Typhimurium plays* a role in pathogenesis. Cell. Microbiol., 8(5): 847–856. https://doi.org/10.1111/j.1462-5822.2005.00671.x

Casaux ML, Neto WS, Schild CO, Costa RA, Macías-Rioseco M, Caffarena RD, Silveira CS, Aráoz V, Díaz BD, Giannitti F, Fraga M (2023). Epidemiological and clinicopathological findings in 15 fatal outbreaks of salmonellosis in dairy calves and virulence genes in the causative *Salmonella enterica Typhimurium* and Dublin strains. Braz. J. Microbiol., 54(1): 475-490. https://doi.org/10.1007/s42770-022-00898-9

Cheng S, Wang L, Liu Q, Qi L, Yu K, Wang Z, Wu M, Liu Y, Fu J, Hu M, Li M, Zhou D, Liu X (2017). Identification of a novel *Salmonella* type III effector by quantitative secretome profiling. Mol. Cell. Proteome., 16(12): 2219–2228. https://doi.org/10.1074/mcp.RA117.000230

Deb J, Gupta S, Debnath S (2024). A small review on polymerase chain reaction for the detection of *Salmonella* species. J. Appl. Pharma. Sci., 4(8): 41-51. https://doi.org/10.7324/JAPS.2024.177634

Edrington TS, Long M, Ross TT, Thomas JD, Callaway TR, Anderson RC, Craddock F, Salisbury MW, Nisbet DJ (2009). Prevalence and antimicrobial resistance profiles of *Escherichia coil* O157:H7 and *Salmonella* isolated from feedlot lambs. J. Food Prot., 72(8): 1713–1717. https://doi.org/10.4315/0362-028X-72.8.1713

Ehuwa, O., Jaiswal, A. K., Food Safety and Food Handling Practices. Foods (Basel, Doi/10.3390/food1005

Ekiri AB, Landblom D, Doetkott D, Olet S, Shelver WL, Khaitsa ML (2014). Isolation and characterization of shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O113, O121, O145, and O157 shed from range and feedlot cattle from postweaning to slaughter. J. Food Prot., 77(7): 1052–1061. https://doi.org/10.4315/0362-028X.JFP-13-373

Esmat M, Galal A, Hassan H, Mohamed N (2018). Biofilm producing bacteria in cases of urinary tract infection at Sohag University Hospital. Egypt. J. Med. Microbiol., 27(4): 87-98. https://doi.org/10.21608/ejmm.2018.285756

Gelalcha BD, Gelgie AE, Kerro Dego O (2023). Prevalence and antimicrobial resistance profiles of extended-spectrum beta-lactamase-producing *Escherichia coli* in East Tennessee dairy farms. Front. Vet. Sci., 10: 1260433. https://doi.org/10.3389/fvets.2023.1260433

Giovagnoni G, Rossi B, Tugnoli B, Ghiselli F, Bonetti A, Piva A, Grilli E (2020). Thymol and carvacrol downregulate the expression of *Salmonella typhimurium* virulence genes during an *in vitro* infection on Caco-2 Cells. Microorganisms, 8(6): 862. https://doi.org/10.3390/microorganisms8060862

Harrington SM, Buchan BW, Doern C, Fader R, Ferraro MJ, Pillai DR, Rychert J, Doyle L, Lainesse A, Karchmer T, Mortensen JE (2015). Multicenter evaluation of the BD max enteric bacterial panel PCR assay for rapid detection of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. (*C. jejuni* and *C. coli*), and Shiga toxin 1 and 2 genes. J. Clin. Microbiol., 53(5): 1639–1647. https://doi.org/10.1128/JCM.03480-14

He Y, Wang J, Zhang R, Chen L, Zhang H, Qi X, Chen J (2023).

Epidemiology of foodborne diseases caused by Salmonella in Zhejiang Province, China, between 2010 and 2021. Front. Publ. Health, 11: 1127925. https://doi.org/10.3389/fpubh.2023.1127925

Holschbach, C. L., Peek, S. F. (2018). Salmonella in Dairy Cattle. The Veterinary clinics of North America. Food animalpractice, 34(1): 133–154. doi.org/0.1016/j.cvfa.2017.10.005

Honeycutt JD, Wenner N, Li Y, Brewer SM, Massis LM, Brubaker SW, Chairatana P, Owen SV, Canals R, Hinton JCD, Monack DM (2020). Genetic variation in the MacAB-TolC efflux pump influences pathogenesis of invasive Salmonella isolates from Africa. PLoS Pathog., 16(8): e1008763. https://doi.org/10.1371/journal.ppat.1008763

Horiyama T, Yamaguchi A, Nishino K (2010). TolC dependency of multidrug efflux systems in *Salmonella enterica* serovar *Typhimurium*. J. Antimicrob. Chemother., 65(7): 1372–1376. https://doi.org/10.1093/jac/dkq160.

Juan GD, Dina M, Luca G, Beniamino C-GSS, Filipe S, Cristina S, Juan A (2024). *Salmonella* spp. in domestic ruminants, evaluation of antimicrobial resistance based on the one health approach. A systematic review and meta-analysis. Vet. Sci., 11(7): 315. https://doi.org/10.3390/vetsci11070315

Karunakaran R (2010). Molecular evolution of cell division proteins FtsA, FtsL, and FtsZ in bacteria: A phylogenetic analysis. Malaysian Soc. Microbiol., 6: 94-98.

Khan NU, Usman T, Sarwar MS, Ali H, Gohar A, Asif M, Rabbani F, Khan RU, Sultana N, Khan NA, Mobashar M, Shah AA, Wanapat M (2022). The prevalence, risk factors analysis and evaluation of two diagnostic techniques for the detection of Cryptosporidium infection in diarrheic sheep from Pakistan. PLoS One, 17(7): e0269859. https://doi.org/10.1371/journal.pone.0269859

Kim SI, Kim S, Kim E, Hwang SY, Yoon H (2018). Secretion of salmonella pathogenicity island 1-encoded type III secretion system effectors by outer membrane vesicles in *Salmonella enterica* serovar Typhimurium. Front. Microbiol., 9: 2810. https://doi.org/10.3389/fmicb.2018.02810

Lories B, Roberfroid S, Dieltjens L, De Coster D, Foster KR, Steenackers HP (2020). Biofilm bacteria use stress responses to detect and respond to competitors. Curr. Biol., 30(7): 1231–1244.e4. https://doi.org/10.1016/j.cub.2020.01.065

Lv M, Ye S, Hu M, Yang X, Liang Z, Zhou X, Zhang L, Zhou J (2022). Two-component system arcba modulates cell motility and biofilm formation in dickeyaoryzae. Front. Med., 13: 2022. https://doi.org/10.3389/fpls.2022.1033192

Mac Faddin JF (2000). Biochemical tests for identification of medical bacteria. 3rd ed. Baltimore (Md.): Williams and Wilkins. SN -683053183

Maynard C, Berthiaume F, Lemarchand K, Harel J, Payment P, Bayardelle P, Masson L, Brousseau R (2005). Waterborne pathogen detection by use of oligonucleotide-based microarrays. Appl. Environ. Microbiol., 71(12): 8548–8557. https://doi.org/10.1128/AEM.71.12.8548-8557.2005

Mosa AH, Aljabory HAH, Rabeea AHMH, Waheed LS (2025). Molecular identification of Helicobacter pylori in local goats from selected regions in central Iraq. J. Anim. Health Prod., 13(1): 65-70. https://doi.org/10.17582/journal.jahp/2025/13.1.65.70

Mosa AH, Hamzah KJ, Aljabory HA (2022). First study on the molecular prevalence of caprine arthritis encephalitis virus in goats in Babylon, Iraq. Vet. World, 15(4): 1129. https://doi.org/10.14202/vetworld.2022.1129-1133

Napoleoni, M., Villa, L., Barco, L., Busani, L., Cibin, V., Lucarelli, C., Enter-Net and Enter-Vet Peripheral Laboratories Referents for Marche Region. (2021). A strong evidence outbreak of Salmonella Enteritidis in central Italy linked to the consumption of contaminated raw sheep milk cheese. Microorganisms, 9(12), 2464.

Nedbalcov K, Nechvtalov K, Kuerov Z (2015). Determination of the minimum inhibitory concentration (MIC) and mutant prevention concentration (MPC) of selected antimicrobials in bovine and swine *Pasteurella multocida, Escherichia coli*, and *Staphylococcus aureus* isolates. J. Univ. Vet. Sci. Brno, Czech Republ., 84(2). https://doi.org/10.2754/avb201584020083

Nikkhah A, Alimirzaei M, Kazemi H (2023). Salmonellosis in young calves: A perplexing problem beyond diarrhea. J. Vet. Physiol. Pathol., 2(2): 5-8. https://doi.org/10.58803/jvpp.v2i2.22

Oakley BB, Buhr RJ, Ritz CW, Kiepper BH, Berrang ME, Seal BS, Cox NA (2014). Successional changes in the chicken cecal microbiome during 42 days of growth are independent of organic acid feed additives. BMC Vet. Res., 10: 282. https://doi.org/10.1186/s12917-014-0282-8

Oporto B, Ocejo M, Alkorta M, Marimón JM, Montes M, Hurtado A (2019). Zoonotic approach to Shiga toxin-producing *Escherichia coli*: Integrated analysis of virulence and antimicrobial resistance in ruminants and humans. Epidemiol. Infect., 147: e164. https://doi.org/10.1017/S0950268819000566

Parolini F, Ventura G, Rosignoli C, Rota Nodari S, D'incau M, Marocchi L, Santucci G, Boldini M, Gradassi M (2024). Detection and phenotypic antimicrobial susceptibility of *Salmonella enterica* serotypes in dairy cattle farms in the Po Valley, Northern Italy. Animals, 14(14): 2043. https://doi.org/10.3390/ani14142043

Pincus T, Miles C, Froud R, Underwood M, Carnes D, Taylor SJ (2011). Methodological criteria for the assessment of moderators in systematic reviews of randomised controlled trials: A consensus study. BMC Med. Res. Methodol., 11: 14. https://doi.org/10.1186/1471-2288-11-14

Rutanga JP, Block TD, Vandelannoote K, Puyvelde S, Cafmeyer J, Hardy L, Muvunyi C, Jacobs J, Deborggraeve S (2020). A 16S ribosomal RNA TaqMan real-time reverse transcription PCR for specific detection of Salmonella in blood. Reaserch gate. https://doi.org/10.21203/rs.2.22017/v1

Sabah AY, Hamzah AH, Bakir Al-Hilli Z (2021). Effects of supplementation of brassica juncea seed extract in drinking water on intestinal histomorphometry, bacteriology, and serum biochemistry parameters of broiler chicken. Arch. Razi Inst., 76(4): 925-934.

Sandrasaigaran P, Kuan CH, Radu S, Gobal D, Abidin, UFUZ, Rukayadi Y, Hasan H (2023). Multiplex touchdown polymerase chain reaction for rapid detection of *Salmonella enterica* subsp. enterica serovars enteritidis and typhimurium in food. Rynnye Lyan Resources. Food Res., 7(2): https://doi.org/10.26656/fr.2017.7(2).829

Sanni AO, Onyango J, Rota AF, Mikecz O, Usman A, PicaCiamarra U, Fasina FO (2023). Underestimated economic and social burdens of non-*Typhoidal salmonella* infections: The one health perspective from Nigeria. One health (Amsterdam, Netherlands), 16: 100546. https://doi.org/10.1016/j.onehlt.2023.100546

Shaaban SI, Ayoub MA, Ghorbal SH, Nossair MA (2018). Calves as a reservoir of some diarrheagenic agents for human contacts in El-Behira province. Alex. J. Vet. Sci., 56(2): 48-

53. https://doi.org/10.5455/ajvs.285867
Shehta A, El-Zahar H, Mansour A, Mustafa, B., Shety, T. (2022). Clinical, Hematological and some biochemical alterations during diarrhea in friesian calves naturally infected with *E. coli* and *Salmonella*. Beni-Suef Univ. J. Basic Appl. Sci., 11(1): 128. https://doi.org/10.1186/s43088-022-00309-w
Smith HE, Blair JM (2014). Redundancy in the periplasmic adaptor proteins AcrA and AcrE provides resilience and an ability to export substrates of multidrug efflux. J. Antimicrobe. Chemother., 69(4): 982–987. https://doi.org/10.1093/jac/dkt481
Syafiradewi Y, Romi ZI, Mansyur, Estria FP (2024). Analisis konsentrasi dan kemurnian Deoxyribo Nucleic Acid (DNA) dari rumput pakan ternak. Agrivet: Jurnal Ilmu-Ilmu Pertanian Dan Peternakan (J. Agric. Sci. Vet.), 12(2): 167–179. https://doi.org/10.31949/agrivet.v12i2.10618
Tanner, J. R., Kingsley, R. A. (2018). Evolution of Salmonella within hosts. Trends in microbiology, 26(12), 986-998.
Terentjeva M, Streikiša M, Avsejenko J, Trofimova J, Kovaļenko K, Elferts D, Bērziņš A (2019). Prevalence and antimicrobial resistance of *Escherichia coli, Enterococcus* spp. and the major foodborne pathogens in calves in latvia. Foodb. Pathogens Dis., 16(1): 35–41. https://doi.org/10.1089/fpd.2018.2523
Tola EH (2018). Isolation and Identification of *Salmonella* from apparently healthy pet dogs in Holeta Town, Western Shoa, Ethiopia. Current Trends in Biomedical Engineering and Biosciences.
Tsega KT, Maina KJ, Tesema NB (2023). Characterization of potential probiotics *Lactobacillus* species isolated from the gastrointestinal tract of Rhode Island Red (RIR) chicken in Ethiopia. Heliyon, 9(7): e17453. https://doi.org/10.1016/j.heliyon.2023.e17453
Wilfinger WW, Mackey K, Chomczynski P (1997). Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques, 22(3): 474–481. https://doi.org/10.2144/97223st01