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ISOLATION AND MOLECULAR IDENTIFICATION OF THE *invA* GENE OF *Salmonella* spp. IN DROMEDARY CAMELS

Ali Kadhim ALTAEE and Afaf Abdulrahman YOUSIF

Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, P.O. Box 28601 AlDawoodi, Iraq

Email: afaf.a@covm.uobaghdad.edu.iq

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ABSTRACT: This study was done to determine the percentage of *Salmonella* spp. in camels from three provinces (Karbala, Al-Najaf and AL-Muthana) in Iraq with different age and both sexes. Total of 250 fecal samples from 250 camels were collected. Diagnostic study depended upon the morphological and cultural properties of the isolates on some selective media like Xylose lysine deoxycholate (XLD) and *Salmonella Shigella* (SS) agars which were used in addition to different biochemical tests and molecular assay by PCR for detection of virulence gene invasion A (*invA*) with Phylogenetic study. The clinical signs appearing on animals infected with *Salmonella* were greenish diarrhea, loss of appetite with mild systemic reaction. Bacteriological and molecular tests revealed isolation of five *Salmonella* isolates with *invA* gene. Two of these isolates were sequenced. The results showed that the first strain *S. enterica subspecies typhimurium* (LC730846) converged with a group of global strains with one node, as it converged with the global strain that held the clade (MK017934.1 and MT460418.1). While the second local strain *S. enterica serovar enteritidis* (LC730849) appeared with a new node and it is not affiliated with any association with the world *S. enterica* strains. It is concluded that the presence of *Salmonella* spp. in camels needs monitoring in order to minimize the risks of infection exposing human beings.

Keywords: Camels, Fecal samples, *invA* gene, PCR, *Salmonella*.

INTRODUCTION

Salmonellosis is an important infectious zoonotic disease that affects the health and economic effect of industrialized and developing countries. In developing nations, salmonellosis is often a common but neglected disease (Mohammadpour et al., 2020). Members of genus *Salmonella* are ubiquitous in nature. They live in the human (Abebe et al., 2020), various animals (Afaf et al., 2010; Al Zubuidy and Yousif, 2012), animals such as goats, dogs and cows (Afaf et al., 2010; Al Zubuidy and Yousif, 2012) respectively and Pets contaminate the environment of their owners by shedding *Salmonella* intermittently in their feces (Dróżdż et al., 2021).

Salmonellosis is characterized by enterohepatic or enteric manifestations, which result in several clinical signs, that included neonatal diarrhea abortion, orchitis, pneumonia, and septicemia (Abdelwahab et al., 2019). Chronic salmonellosis in camels is characterized by diarrhea, weight loss and death within a few weeks, Humans can be infected by the consumption of contaminated foods originated from camels, infected drinking water, or close contact with infected camels (Wernery and Kaaden, 2002).

Salmonella infection of camels is reported in Sudan, UAE, Palestine, France, Somalia, North Africa, USA, Ethiopia, Egypt, and Iran (Sepehr, 2012). Sevilla-Navarro et al. (2021) reported that the prevalence of *Salmonella* in camels was 5.5% (3/54), and the only serovar isolated was *S. Frintrop* Using pulsed-field gel electrophoresis analysis which revealed low genetic diversity, and all isolates showing nearly identical pulsotype (similarity >95%), they indicate that dromedary camels seems to be a reservoir for *Salmonella* transmission especially camel riding has become as one of the main touristic attractions in different countries which increases the popularity in recent years.

The pathogenesis of salmonellosis was begins with the local colonization of bacteria, followed by bacterial spread of infection to other regions of body, which leads to different clinical signs according to the virulence of *Salmonella* strain (Robert et al., 2013). Twenty eight serotypes of *Salmonella* were isolated from camels in United Arab Emirates by Winery (1992), these serotypes were identified with the most frequent *S. saintpaul*, followed by *S. frintrop*, *S. hindmarsh* and *Salmonella typhimurium*.

Camels are an important source of *Salmonella*. It is, therefore, important to control and prevent salmonellosis in these animals and their products to decrease the transmission to a human. Therefore, this study aimed at isolating and identifies *Salmonella* species in camels with determining their virulence gene *invA* via conventional PCR assay with phylogenetic analysis.

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MATERIAL AND METHODS

Ethical committee

This study was approved by the ethical and research committee of Veterinary Medicine of College, University of Baghdad, book No. 39/D A in 7/12/ 2021.

Animals and samples

The study was performed on 250 camels at field located in three provinces in Iraq [Karbala (36), AL Najaf (191) and AL Mothana (23)]. Camels aged from one day -seven years, and were from both sexes. The study extended from January 2021 to December 2021. Data and history obtained from owner, clinical examination are recording, systemic reaction (temperature, pulse, respiratory rates), and presence or absence of diarrhea and other signs (Al-Graibawi et al., 2021). 250 fecal samples were collected from these camels and put in sterile container and kept on ice till reaching the laboratory.

Isolation and identification of *Salmonella*

Fecal samples (1 gram) were transferred in sterile container immediately to the lab in ice box. The samples were cultivated on Selenite-F Broth and MacConkey agar and incubated aerobically at 37 °C for 24- 48 hours. Microscopical examination by Gram staining was done according to Quinn et al. (2004) and the bacterial cells were examined using X100 lenses with immersion oil. After cultivating suspected colonies on Xylose lysine deoxycholate (XLD) agar and *Salmonella Shigella* (SS) agar was confirmed by Gram staining and different biochemical tests (oxidase, urease, indole tests, catalase, TSI and citrate utilization).

Antibiotic susceptibility test

Antibiotic susceptibility of isolates was determined by disc diffusion method (Bauer et al., 1966) on Muller Hinton agar. The test was done by using different antibiotic discs according to (Quinn et al. 2004, Arcan and Afaf, 2013). The antibiotic inhibition zone was estimated as mention the Clinical and Laboratory Standards Institute (CLSI, 2023).

Molecular assay: PCR and sequencing

Primers used in this study were obtained from Bioneer Company, Korea and were designed based on the sequence of the *invA* gene: Forward primer GTGAAATTATCGCCACGTTCGGGCAA and reverse primer TCATCGCACCGTCAAAGGAACC with an expected amplicones size of 280 bp (Cocolin et al., 1998).

Primer	Primer /sequence 5- to 3-		Product size (bp)
InvA	F	GTGAAATTATCGCCACGTTCGGGCAA	284
	R	TCATCGCACCGTCAAAGGAACC	

DNA extraction and PCR program

The isolation of DNA genomic from bacterial growth done according by using DNA extraction kit (Addprep bacterial genomic DNA extraction kit) from Addbio Company (Korea). The purity and concentration of the final template DNA after extraction were measured by Quantus Fluoro meter (Promega Company, Korea). Colonies suspected to be *Salmonella* by conventional phenotypic methods were placed in 500 µl of distilled water and boiled for 10 min to release the genomic DNA and subsequent detection of the *invA* gene by PCR. The strains were stored at -70°C for their future sequencing analysis. The PCR reaction cocktail was contained of 50 mM KCl, 10 Tris-HCl mM pH 9, 0.1% Triton X-100, 2 mM MgCl2, 0.01% of gelatin, 0.2 mM of each dNTP, 1 µM of *invA* primer (Bioneer, Korea), 1U of Taq DNA polymerase (Highway®) and 5 µl of DNA. The thermal profile used for the detection of the *invA* gene was detailed in (Table 1). The PCR were analyzed by electrophoresis in a 2% agarose gel in the presence of ethidium bromide. The band size was determined by comparing the products amplified with the molecular size marker DNA Ladder 100 bp (Promega®, Korea).

Table 1 - PCR thermocycler condition of *invA* gene

Thermocycling	Primer / *InvA*	Cycles
1- Initial denaturation	95°C / 5min	1
2- Denaturation	95 °C / 30 sec.	35
3- Annealing	60°C / 35 sec.	35
4- Extension	72 °C / 55 sec.	35
5- Final extension	72°C / 5 min	1
6- Hold	4°C	

Sequence analysis

All samples with positive PCR product (20 µl) were sent to (Macrogen company, Korea) for sequence analysis by using sanger macrogen analyzer for determination sequence variation among the isolates, the results was analysis by using Bioedit software version (3.1) and phylogenetic tree analysis was performed by mega 11.0. Based on the NCBI-BLAST data, Multiple Sequence Alignment Analysis of *invA* gene, phylogenetic tree, and homology sequence identity were made with the Genbank-NCBI strain/isolate (Gharban and Yousif, 2020).

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Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference–LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

RESULTS

Clinical signs

Clinical signs included diarrhea with greenish color feces (Figure 1), and with yellowish to white color (Figure 2). Other clinical signs were loss of appetite, moderate dehydration, dullness, congestion of mucous membranes and slight increase of temperature, pulse and respiration rates.

Figure 1 - Camel affected with *Salmonella* showed diarrhea with greenish color, recombanacy and fever.

Figure 2 - Camel affected with *Salmonella* showed diarrhea with yellowish to white color with dehydration.

Isolation of *Salmonella*

From 250 fecal samples collected from diarrheic and non-diarrheic camels, 5 isolates of *Salmonella* (2 %) were determined. Cultivation characteristics of *Salmonella* spp. isolates showed colorless, smooth colonies on MacConkey agar, On *Salmonella Shigella (SS)* agar the organisms were produced small sized with black pin-head, circular or round smooth, raised, colorless (Figure 3A), while the colony was pale pink with large black center on Xylose Lysine (XLD) agar (Figure 3B). The colony on smears on Gram's stain showed G-ve bacteria pink in color, the shape appeared as small rod under the microscopic. To classify the isolates of bacteria, a number of biochemical tests were conducted on *Salmonella* isolates as shown in (Table 2).

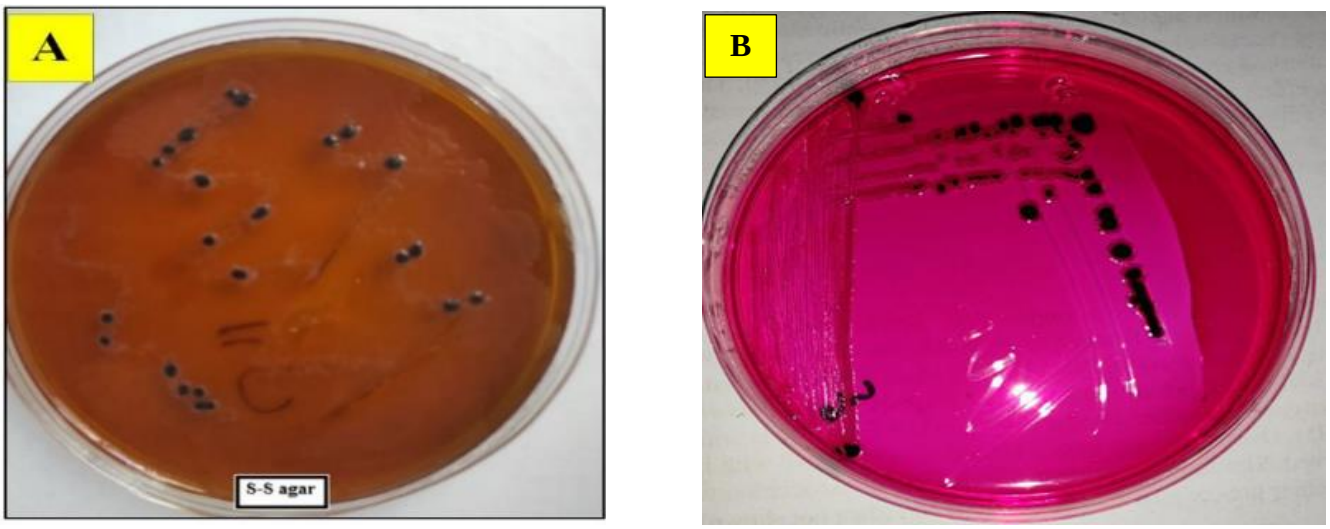

Figure 3 - Colony of Salmonella on SS agar (A), on XLD agar (B).

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Table 2 - Results of *Salmonella* isolates on biochemical tests.

Tests / Bacteria	Oxidase	Catalase	Indole	VP	Citrate	H_2S	Motility	Urease
Salmonella	-	+	-	-	+	+	+	-

Antibiotic succebitbilty of *Salmonella* spp.

The antibiotic susceptibility of *Salmonella* spp. is presented in Table 3 and Figure 4. The results revealed that the 5 (100%) of *Salmonella* spp. isolates were resistant at least 9 antibiotic; Gentamicin, Erythromycin, Tetracycline, Ciprofloxacin, Amikacin, Sulfamethoxazole, Trimethoprim, Nalidixic acid and Cefotaxime The 5 *Salmonella spp.* isolates revealed susceptibility to 4 antibiotic classes.

Table 3 - Antibiotic drugs against *salmonella spp.* isolates. S: sensitive isolates, R: resistant isolates to antibiotic

Antibiotic (concentration)	S (%)	R (%)	P-value
Ampicillin (10 µg)	12 (100%)	0 (0%)	
Amoxicillin (30 µg)	12 (100%)	0 (0%)	
Gentamicin (30 µg)	0 (0%)	12 (0%)	
Erythromycin (60 µg)	0 (0%)	12(100%)	
Tetracycline (5 µg)	0 (0%)	12(100%)	
Ciprofloxacin (10 µg)	0 (0%)	12(100%)	0.0037 **
Amikacin (10 µg)	0 (0%)	12(100%)	
Sulfamethoxazole (30 µg)	0 (0%)	12(100%)	
Trimethoprim (30 µg)	0(0%)	12 (100%)	
Chloramphenicol (30 µg)	12(100%)	0(0%)	
Nalidixic acid (30 µg)	0 (0%)	12(100%)	
Cefotaxime (25 µg)	0 (0%)	12(100%)	
P-value	0.0074 **	0.0069 **	—

**: P≤0.01.

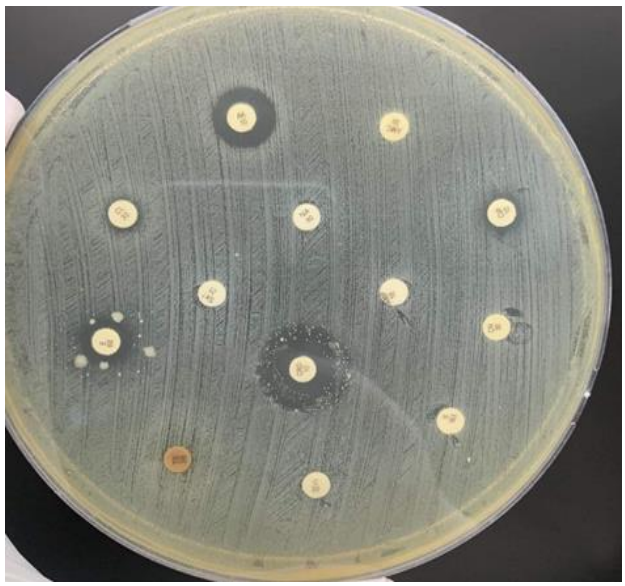

Figure 4 - The antibiotic succebitbilty of *Salmonella*

Results of molecular assay

In PCR technique, all the *Salmonella* isolates amplified a 248 bp DNA amplicones, which suggested the presence of *invA* and further confirmed that all the isolates were *Salmonella.*

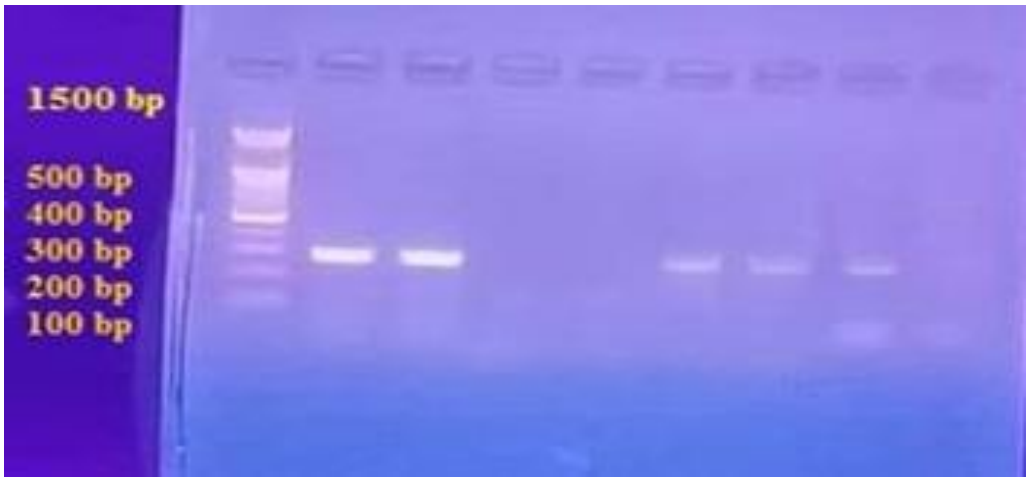

Figure 5 - Representative Agarose gel analysis of PCR assay targeting invA gene in *Salmonella* isolates. Lane M: 100 bp DNA ladder, Lane 1: Negative control, Lane 2 3, 6, 7, and 8): *InvA* gene PCR results.

Identification of clades in phylogenetic tree

Present results obtained two serotypes of *Salmonella* namely *Salmonella enteritidis* and *Salmonella typhimurium* by sequencing analysis with two accession number from gene bank websites as (LC730849, LC730846) respectively (Figure 6).

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Figure 6 - phylogenetic analysis of *Salmonella spp*. Among different isolates in different countries.

DISCUSSION

There are various studies showing the prevalence and seroprevalence of *Salmonella* spp. in camel farms, as well as the risk factors associated with the presence of the bacterium and the prevalence in slaughter house (Wernery, 1992; Bosilevac et al., 2015; Sevilla-Navarro et al. 2021).

The criteria for the cultural and biochemical identification of *Salmonella* spp. are widely described and the detection of *Salmonella* spp. by conventional culture is considered the reference method (AL-Darraji and Yousif, 2012). However, this method suffers from the disadvantage of the time required for the obtaining a result, these tests depend on the appearance of the analyzed characteristics and can be affected by variations in culture media and in the incubation conditions. Alternatively molecular methods are being used more and more; allowing a faster and simpler diagnosis (Tracogna et al., 2013)

Currently, *Salmonella* is detected by standard bacteriological, biochemical and serological techniques. These techniques are generally time-consuming, tedious and expensive as they require hundreds of antisera as well as well-trained technicians (Nora and Thong, 2010). Many researchers underlined the importance and necessity of founding a more rapid and effective detection methods as a basis of controlling the infection (Al-Zubaidy et al., 2015). Several rapid and sensitive methods have been developed for identification of Salmonella serotypes from clinical specimens (Salih and Yousif, 2018a).

All strain of Salmonella isolates were resistance to many antimicrobial agents, the findings of the current study were in accordance with Kipper et al. (2019) who found (100%) *Salmonella enterica* isolate carrying this region of resistance in the petri dish among 63 isolates. Lekagul et al., (2020) mentioned that the *Salmonella* spp. have the ability of spread the antibiotic resistance by the genes called transfer-associated, thus, so causing increase in the incidence and the severity of the disease. Also the treatment of *salmonella* in all animals was difficult due to multidrug- resistant of *Salmonella* spp.

Salmonella specific PCR with primers for *invA* is rapid, sensitive and specific for detection of *Salmonella* in many clinical samples. The detection of a 284 bp product of the *invA* gene, specific for *Salmonella* spp., was achieved by using the conventional PCR variant. This PCR technique has been shown to be reproducible, specific and sensitive for the detection of *Salmonella* spp. It is recommended that the standardization of a PCR be carried out in each laboratory. The

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qualitative comparison of the conventional PCR assay with bacteriological culture showed a greater efficiency of PCR in terms of sample processing time and the time needed for obtaining results (36 to 48 hours), compared to the culture technique (5-6 days).

The sequencing of the product obtained confirms that the PCR method detects the *invA* gene of different serovar of *Salmonella enterica*, which guarantees the specificity of the PCR assay and is also a requirement for the validation for the diagnosis of pathogens in different clinical isolates.

InvA is a gene that is shared by all *Salmonella* species; as a result, it is frequently employed as a genetic target that is selective for the detection of *Salmonella* strains (González-Escalona et al., 2012). Furthermore, it has been reported that *invA* has mutation rates comparable to housekeeping gene indicating that it is a potential candidate pertaining to tests for detection using PCR (Boyd et al., 1997). invA sequences that can be obtained from the NCBI (23 global unique sequences, with 2 additional local *S. enterica subsp. enterica typhimurium* and *S. enterica subsp. Enterica enteritidis* that were sequenced throughout the course of this research, due to the fact that sequences for these serovar were not readily accessible to the public. A phylogenetic tree was generated using the maximum likelihood method with the core genome sequences of 23 reference strains; nucleotides clustering, sequence type, serotype, country, and source are indicated at phylogenetic tree, *Salmonella* spp. isolates belonged to the following species and serovar and accession number: *S. enterica serovar Enteritidis* (LC730849) and *S. enterica subspecies typhimurium* (LC730846).

CONCLUSION

In conclusion, the PCR technique is a basic tool for the detection of *Salmonella* enterica, and its virulence factors, such as the *invA* gene, provides information ranging from the identity of a given bacterium to its virulence potential, and Sequencing of *invA* gene of *Salmonella* showed identity 99%-100% with the world level. Also, it is suggested that the presence of *Salmonella* spp. in camel needs monitoring in order to minimize the risks of infection exposed the human beings.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dr. Afaf Abdulrahman Yousif; Email: afaf.a@covm.uobaghdad.edu.iq

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

All authors contributed equally in all details of this paper.

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Consent to publish

All authors agree to publish the article "Isolation and molecular identification of *invA* gene of *Salmonella* spp. in dromedary camels" in an online journal of animal and feed research.

Competing interests

The authors declare no competing interest.

REFERENCES

Abdelwahab GE, Tigani-Asil E, Yusof MF, Abdullah ZS, Rifat JF, Al Hosani MA, et al. (2019). Salmonella enterica and Theileria co-infection in dromedary camels (*Camelus dromedarius*) in UAE. Open Veterinary Journal, 9(3): 263-268. https://doi.org/10.4314/ovj.v9i3.12 PMid: 31998621

Abebe E, Gugsa G, & Ahmed M. (2020). Review on major food-borne zoonotic bacterial pathogens. Journal of Tropical Medicine, Volume 2020. https://doi.org/10.1155/2020/4674235; PMid: 32684938

Addis Z, Kebede N, Sisay Z, Alemayehu H, Wubetie A. and Kassa T. (2011). Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. BMC Infectious Diseases, 11:222. https://doi.org/10.1186/1471-2334-11-222

AL-Darraji AJZ and Yousif AA (2012). Epidemiological study of goats infected with diarrhea caused by some bacteria of Enterobacteriaceae. Iraqi journal of Veterinary Medicine, 36(0A): 70-77. https://doi.org/10.30539/iraqijvm.v36i0A.357

Al-Graibawi MA, Yousif AA, Gharban HA and Zinsstag J (2021). First serodetection and molecular phylogenetic documentation of *Coxiella burnetii* isolates from female camels in Wasit governorate, Iraq. Iraqi Journal of Veterinary Sciences, 35: 47-52. https://doi.org/10.33899/ijvs.2021.130888.1890

Al-Zubaid AAN, Yousif AA (2013). Prevalence and antimicrobial susceptibility of *salmonella* species isolate from slaughtered cows in Iraq. The Iraqi Journal of Veterinary Medicine, 37(1): 96-101. https://doi.org/10.30539/iraqijvm.v37i1.339

Al-Zubaidy AAN, Yousif AA, Al-Graibawi MAA, Darkhan J (2015). Detection of invasion gene *invA* in *Salmonella* spp. isolated from slaughtered cattle by PCR method. The Iraqi Journal of Veterinary Medicine, 39(1): 128-133. https://doi.org/10.30539/iraqijvm.v39i1.210

Atnafie B, Paulos D, Abera M, Tefera G, Hailu D, and Kasayeand S, Amenu K (2017). Occurrence of Escherichia coli O157: H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. BMC Microbiology, 17: 24. https://doi.org/10.1186/s12866-017-0938-1; PMid: 28122502

Bosilevac JM, Gassem MA, Al Sheddy IA, Almaiman SA, Al-Mohizea IS, Alowaimer A et al. (2015). Prevalence of *Escherichia coli* O157: H7 and *Salmonella* in camels, cattle, goats, and sheep harvested for meat in Riyadh. Journal of Food Protection, 78(1): 89-96. https://doi.org/10.4315/0362-028X.JFP-14-176; PMid: 25581182

Boyd EF, Li J, Ochman H, and Selander RK (1997). Comparative genetics of the inv-spa invasion gene complex of *Salmonella enterica*. Journal of Bacteriology, 179(6): 1985-1991. https://doi.org/10.1128/jb.179.6.1985-1991.1997 PMid: 9068645

Castanheira S, López-Escarpa D, Pucciarelli MG, Cestero JJ, Baquero F, and García-del Portillo, F (2020). An alternative penicillin-binding protein involved in *Salmonella* relapses following ceftriaxone therapy. EBioMedicine, 55: 102771. https://doi.or-g/-10.1016/j.ebiom.2020.102771; PMid: 32344200

CLSI (Clinical laboratory Standard Institute) (2023). Performance Standards for Antimicrobial Susceptibility Testing, 33th edition, CLSI Supplement M100., clinical laboratory standard institute. ISBN Number: 978-1-68440-170-3. https://clsi.org/media/tc4b1paf/m10033_samplepages-1.pdf

Dróżdż M, Małaszczuk M, Paluch E, and Pawlak A (2021). Zoonotic potential and prevalence of *Salmonella* serovar isolated from pets. Infection Ecology & Epidemiology, 11(1): 1975530. https://doi.org/10.1080/20008686.2021.1975530 PMid: 34531964

Fukushima K, Yanagisawa N, Sekiya N, and Izumiya H (2020). Bacteremia caused by *Salmonella* Poona in a healthy adult in Tokyo, Japan. Internal Medicine, 59(2): 289-292. https://doi.org/10.2169/internalmedicine.3161-19 PMid: 31534082

Gharban HA, and Yousif AA (2020). Serological and molecular phylogenetic detection of Coxiella burnetii in lactating cows, Iraq. The Iraqi Journal of Veterinary Medicine, 44(E0):42-50. https://doi.org/10.30539/ijvm.v44i(E0).1020

González-Escalona N, Brown EW, and Zhang G (2012). Development and evaluation of a multiplex real-time PCR (par) assay targeting ttrRSBCA locus and *invA* gene for accurate detection of *Salmonella* spp. in fresh produce and eggs. Food Research International, 48(1): 202-208. https://doi.org/10.1016/j.foodres.2012.03.009

Kipper D, Hellfeldt RM, De Carli S, Lehmann FK, Fonseca AS, Ikuta N, et al. (2019). *Salmonella* serotype assignment by sequencing analysis of intergenic regions of ribosomal RNA operons. Poultry Science, 98(11): 5989-5998. https://doi.org/10.3382/ps/pez285 PMid: 31134273

Lekagul A, Tangcharoensathien V, Mills A, Rushton J, and Yeung S (2020). How antibiotics are used in pig farming: a mixed-methods study of pig farmers, feed mills and veterinarians in Thailand. BMJ global health, 5(2):e001918. https://doi.org/10.1136/bmjgh-2019-001918 PMid: 32180998

Marchant P, Hidalgo-Hermoso E, Espinoza K, and Retamal P (2016). Prevalence of *Salmonella* enterica and Shiga toxin-producing *Escherichia coli* in zoo animals from Chile. Journal of Veterinary Science, 17(4): 583-586. https://doi.org/10.4142/jvs.2016.17.4.583; PMid: 27030195

Mohammadpour R, Champour M, Tuteja F, and Mostafavi E (2020). Zoonotic implications of camel diseases in Iran. Veterinary Medicine and Science, 6(3): 359-381. https://doi.org/10.1002/vms3.239; PMid: 32160657

Mohammadi G, and Najafi S (2017). Salmonellosis outbreak in a herd of camel. Applied Animal *Science Research Journal*, 6(23): 7-10. https://doi.org/10.22092/aasrj.2017.115778

Nori MEE and Thong KL (2010). Differentiation of *Salmonella enterica* based on PCR detection of selected somatic and flagellar antigen. African Journal of Microbiology Research. 4(22):2451-2456. https://academicjournals.org/journal/AJMR/article-full-text-pdf/BFE312E15694

Sterzenbach T, Crawford RW, Winter CSE, Baumler AJ, Barrow PA, and Methner U (2013). *Salmonella* virulence mechanisms and their genetic basis. *Salmonella in domestic animals.* 2nd. https://doi.org/10.1079/9781845939021.0080

Sepehr S (2012). Prevalence of *Salmonella enterica* Contamination of camel milk in Iran. Research Journal of Biological Sciences, 7(4):195–199. https://doi.org/10.3923/rjbsci.2012.195.199

Sevilla-Navarro S, Cerdà-Cuéllar M, Ayats T, Jordá J, Marin C, and Vega S (2021). Characterization of *Salmonella Frintrop* isolated from dromedary camels (Camelus dromedarius). Transboundary and Emerging Diseases, 68 (2): 742-746. https://doi.org/10.1111/tbed.13737

Tracogna MF, Lösch LS, Alonso J M, and Merino LA (2013). Detection and characterization of *Salmonella* spp. in recreational aquatic environments in the Northeast of Argentina. Revista Ambiente & Água, 8: 18-26. https://doi.org/10.4136/ambi-agua.114

Wernery U (1992). The prevalence of *Salmonella* infections in camels (Camelus dromedarius) in the United Arab Emirates. British Veterinary Journal, 148(5): 445-450. https://doi.org/10.1016/0007-1935(92)90031-U

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Wernery U, and Kaaden OR (2002). Infectious diseases in camelids. The second edition, Blackwell Science Wissenschaft-Verlag, Berlin. Vienna. ISBN 3-8263-3304-7. https://doi.org/10.1002/9780470699058

Citation: Altaee AK and Yousif AA (2023). Isolation and molecular identification of the *invA* gene of *Salmonella* spp. in dromedary camels. *Online J. Anim. Feed Res.*, 13(6): 443-450. DOI: https://dx.doi.org/10.51227/ojafr.2023.61