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Determining the Virulence of Poultry-Derived *Salmonella enterica* Isolates *in vivo*

Alinur T Toleukhan[1,2], Bolat A Yespembetov[1], Zhumagul S Kirkimbayeva[2], Arman Zh Zhylkaidar[2], Bekzat B Yerzhigit[1], Azamat R Abdimukhtar[1], Nazym Syrym[1], Sabira E Alpysbayeva[1], Makhpal K Sarmykova[1], Eldos B Serikbay[1], Aktoty M Anarbekova[1], Kali K Tileukhanov[1], Meruyert M Maulenbayeva[1], Nadezhda N Zinina[1], Aslan A Kerimbayev[1], Kuandyk D Zhugunissov[1], Akbope A Abdykalyk*[1]

[1]Research Institute for Biological Safety Problems, 080409 Gvardeiskiy, Kazakhstan, [2]Faculty of Veterinary and Zoo Engineering, Department of Microbiology, Virology and Immunology, Kazakh National Agrarian Research University, 050010 Almaty, Kazakhstan

***Correspondence**:
a.abdykalyk@biosafety.kz
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ABSTRACT

Non-typhoidal *Salmonella enterica* continues to be a significant pathogen of global relevance. However, there is limited available comparative experimental data on the virulence of S. *enterica* strains that are isolated from poultry and circulating in Central Asia. This study aimed to investigate the virulence of four poultry-derived *S. enterica* isolates recovered in Kazakhstan using a standardized murine model of systemic infection. Outbred mice were inoculated intraperitoneally with graded bacterial doses ranging from 10^3 to 10^6 CFU and the pathogenicity was assessed by determining the median infectious dose (ID_{50}) and median lethal dose (LD_{50}) according to the Reed-Muench method. Systemic infection was confirmed by bacteriological re-isolation and real-time PCR. Mortality occurred primarily within 18-72 h post-inoculation and followed a clear dose-dependent pattern. Two *S. enterica* serovar Infantis strains showed relatively low LD_{50} (3.16×10^5 CFU) compared to the other isolates (1.78×10^6 CFU). This indicates relatively high virulence potential of the two *S.* Infantis strains compared to the others. Similar results were seen in the case of the ID_{50}, where the same strains showed relatively high infectivity potential compared to the others. It is noteworthy that the systemic infection was seen with doses that were not lethal to the host, indicating some dissociation between virulence and infectivity potential.

Keywords: *Salmonella enterica*, virulence assessment, mouse infection model, poultry-associated isolates

INTRODUCTION

Non-typhoidal *Salmonella enterica* remains a major bacterial cause of foodborne illness worldwide and continues to pose serious risks to both human and animal health (**1**). Poultry production is a key part of epidemiology, as contaminated meat, eggs, and farm environments can all serve as routes of transmission to humans (**1,2**). In poultry flocks, infection is often subclinical, which allows infected birds to shed the bacterium for extended periods without obvious signs of disease. This hidden persistence promotes environmental contamination and makes farm-level surveillance and control more difficult (**3**).

The pathogenic potential of *S. enterica* varies considerably among serovars and individual strains and is influenced by host susceptibility and infectious dose (**4,5**). Poultry-associated serovars such as *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Infantis are especially important because they are linked to systemic infection, persistence in host tissues, and antimicrobial resistance (**3-5**). Experimental work has shown that *Salmonella* can survive and multiply inside macrophages, this supports systemic spread and prolonged carriage (**6**). At the same time, phenotypic traits and antimicrobial resistance patterns do not consistently reflect the actual in vivo pathogenicity of field isolates (**3,4,7**).

In Kazakhstan, surveillance studies have reported the circulation of multidrug-resistant poultry-associated *S. enterica* strains, including isolates resistant to quinolones, tetracyclines and β-lactams (**7,8**). Whole-genome analyses have also shown substantial genetic diversity among circulating isolates that were linked to Eurasian lineages (**9**). In addition, environmental monitoring of industrial poultry enterprises has further indicated persistent contamination and biosecurity gaps that may facilitate long-term bacterial circulation within production systems (**10**). Despite these findings, comparative experimental data describing the in vivo virulence of poultry-derived isolates circulating in the region remain limited.

Experimental infection models provide a reproducible approach for assessing bacterial virulence under controlled conditions. Murine systemic infection models enable standardized evaluation of dose-response relationships, systemic dissemination and host mortality (**11**). Determination of median infectious dose (ID_{50}) and median lethal dose (LD_{50}), commonly estimated using the Reed-Muench method, remains a widely accepted approach for quantifying pathogenic potential in experimental bacteriology (**12**).

Therefore, the objective of the present study was to comparatively evaluate the in vivo virulence of poultry-derived *S. enterica* isolates circulating in Kazakhstan by determining their ID_{50} and LD_{50} values using a standardized murine infection model supported by bacteriological re-isolation and molecular confirmation of the systemic infection.

MATERIALS AND METHODS

Bacterial Isolates

Four *S. enterica* isolates (Kaz_6/2025, Kaz_7/2025, Kaz_8/2025 and Kaz_9/2025) were included in the study. Two isolates (Kaz_6/2025, Kaz_7/2025) were identified as serovar Infantis. These isolates were recovered in 2025 from poultry farms and associated environments in the Almaty and Zhetysu regions of Kazakhstan during routine epizootic surveillance. Standard bacteriological methods were followed for primary isolation and identification of the isolates. The phenotypic and genomic characteristics of these isolates were previously reported and are currently available in publicly accessible databases (**9**).

Preparation of Bacterial Inoculum

Each isolate was cultured on glucose fish-meat agar (FBRI SRCAMB, Obolensk, Russia) at 37°C for 24 h. Bacterial growth was harvested and suspended in sterile physiological saline. The turbidity of each suspension was adjusted to a 0.5 McFarland standard corresponding to approximately 1.5 $\times 10^8$ CFU/mL. Tenfold serial dilutions were prepared to obtain final inoculum concentrations of 103 to 106 CFU/mL (**13**). Bacterial cell concentrations were verified using a QUANTOM Tx Microbial Cell Counter (Logos Biosystems, Anyang, Republic of Korea) according to the manufacturer's instructions. For enumeration, bacterial suspensions were stained using the QUANTOM Total Cell Staining Kit (Logos Biosystems, Korea). The kit included a membrane-permeable nucleic acid-binding fluorescent dye, after which the stained suspension was loaded onto QUANTOM M50 Cell Counting Slides and subjected to automated image-based analysis.

Experimental Animals and Ethical Approval

A total of 80 outbred white female laboratory mice (Mus musculus, CD-1, Swiss), aged 5-6 weeks, weighing 17-22 g were used in the experiment. Animals were maintained under standard conditions in the animal facility at 20-22°C and 40%- 50% relative humidity under a 14 h light/10 hours dark cycle, with free access to water and a standard pelleted diet ad libitum. All experimental procedures were approved by the Local Bioethics Committee of the Research Institute for Biological Safety Problems (Protocol No. 4, 15 November 2023) and were conducted in accordance with national regulations and internationally accepted guidelines for the care and use of laboratory animals.

Bacteriological Re-isolation and Molecular Confirmation

Samples of liver, spleen and kidneys were aseptically collected from all experimental animals, including animals that died during the observation period and surviving animals euthanized on day 14. Homogenates made from these samples were plated onto *Salmonella*-Shigella agar (Condalab, Madrid, Spain) and bismuth sulfite agar (Condalab, Madrid, Spain) for bacteriological re-isolation. Presumptive colonies were confirmed by real-time PCR using Applied Biosystems (USA) and VetMAX *S. enterica* spp. Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The assay based on duplex TaqMan chemistry, enabling simultaneous detection of *S. enterica* DNA and an internal positive control within a single reaction well. The proprietary target gene and primer/probe sequences were not disclosed by the manufacturer.

Each reaction was performed in a total volume of 25 µL, consisting of 20 µL of reaction mixture and 5 µL of the extracted DNA template. Amplification was carried out under the following conditions: 50 °C for 2 min (UNG activation), 95 °C for 10 min (initial denaturation), followed by 45 cycles of 95 °C for 15 s (denaturation) and 60 °C for 1 min (combined annealing/extension and fluorescence

acquisition). Fluorescence was measured at 60 °C. According to the manufacturer's interpretation criteria, samples with a Ct value < 45 in the *Salmonella*-specific channel were considered positive, whereas samples with Ct ≥ 45 were considered negative.

Determination of ID_{50} and LD_{50}

The median infectious dose (ID_{50}) and median lethal dose (LD_{50}) were estimated using the Reed-Muench method (**12**). ID_{50} was calculated from the proportion of infected animals in each dose group (10^3-10^6 CFU), with infection defined by re-isolation of *Salmonella* from internal organs followed by PCR confirmation. LD_{50} was determined from cumulative mortality recorded in each group during the 14-day observation period.

Statistical Analysis

Kaplan-Meier survival curves were constructed based on time-to-death data obtained at the highest challenge dose (10^6 CFU). Differences between survival curves were evaluated using the log-rank test. Differences in infection rates between isolates at each dose level were assessed using Fisher's exact test. Statistical significance was defined as a p-value ≤ 0.05. All statistical analyses were conducted using R software, version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characterization of Bacterial Inocula

Bacterial suspensions prepared from all four *S. enterica* isolates were analyzed successfully. The mean diameter of the detected cells was 2.1 µm. No substantial differences in cell distribution or concentration were observed among the isolates. Representative images generated by the QUANTOM Tx system are shown in **Figure 1**.

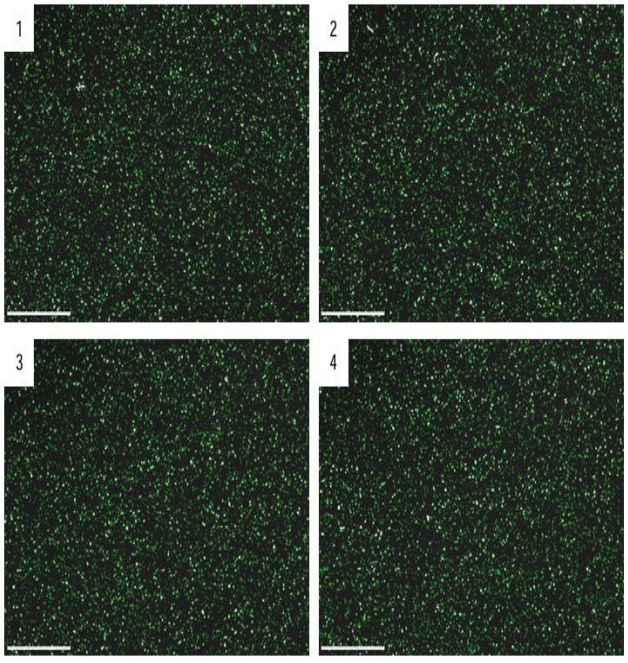

Figure 1. Representative QUANTOM Tx images and size distribution of detected bacterial cells from the four *S. enterica* isolates: (1) Kaz_6/2025, (2) Kaz_7/2025, (3) Kaz_8/2025 and (4) Kaz_9/2025

Table 1. Mortality of mice over a 14-day period after intraperitoneal infection with *S. enterica* isolates

Isolate	Dose (CFU)	No. of mice	Dead (n)	Survived (n)	Mortality (%)
Kaz_6/2025 (serovar Infantis)	1×10^3	5	0	5	0
	1×10^4	5	0	5	0
	1×10^5	5	1	4	20
	1×10^6	5	3	2	60
Kaz_7/2025 (serovar Infantis)	1×10^3	5	0	5	0
	1×10^4	5	0	5	0
	1×10^5	5	1	4	20
	1×10^6	5	4	1	80
Kaz_8/2025	1×10^3	5	0	5	0
	1×10^4	5	0	5	0
	1×10^5	5	1	4	20
	1×10^6	5	3	2	60
Kaz_9/2025	1×10^3	5	0	5	0
	1×10^4	5	0	5	0
	1×10^5	5	1	4	20
	1×10^6	5	3	2	60

Clinical Observations and Mortality

Following intraperitoneal challenge, the animals were monitored for 14 days. Mortality was dose-dependent and differed among the isolates (**Table 1**). Most deaths occurred within 18-72 h post-inoculation. Animals that succumbed to infection exhibited lethargy, ruffled fur, reduced mobility, and anorexia. No mortality or bacterial detection was observed in control animals throughout the 14-day observation period.

At the highest challenge dose (10^6 CFU), the isolates Kaz_6/2025 and Kaz_7/2025 caused the highest mortality (60% and 80%, respectively), whereas Kaz_8/2025 and Kaz_9/2025 caused 60% mortality. At 10^5 CFU, one death (20%) was recorded for each isolate, while no mortality was observed at 10^3 or 10^4 CFU for any strain. Mortality patterns are illustrated in **Figure 2.**

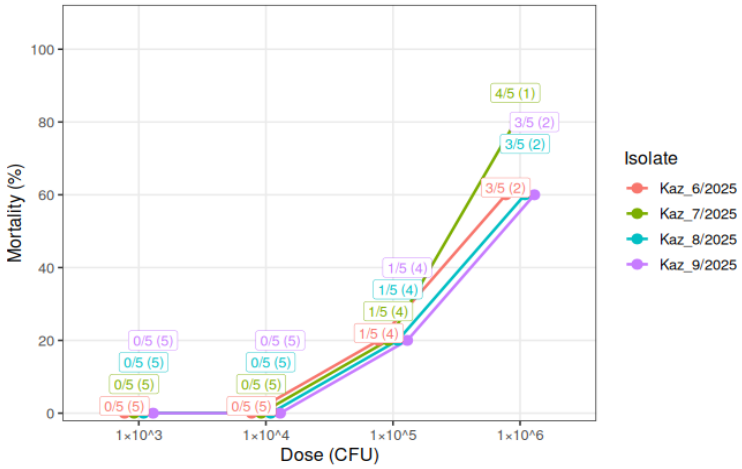

Figure 2. Dose-dependent mortality of mice following intraperitoneal challenge with *S. enterica* isolates

Table 2. Median lethal dose (LD_{50}) of *S. enterica* isolates in mice

Isolate	LD_{50} (CFU)	Log_{10} (LD_{50})
Kaz_6/2025 (serovar Infantis)	3.16×10^5	5.50
Kaz_7/2025 (serovar Infantis)	3.16×10^5	5.50
Kaz_8/2025	1.78×10^6	6.25
Kaz_9/2025	1.78×10^6	6.25

Determination of LD_{50} Values

The LD_{50} for each isolate is shown in **Table 2**. Isolates Kaz_6/2025 and Kaz_7/2025 exhibited the lowest LD_{50} values, indicating a higher lethality than the other isolates. In contrast, Kaz_8/2025 and Kaz_9/2025 showed the highest LD_{50} values, indicating the lowest lethality. No formal statistical comparison of LD_{50} estimates was performed, as these values were derived using Reed-Muench interpolation from group- level mortality data and were presented descriptively.

Infection Rates and Determination of ID_{50}

PCR analysis of organ samples demonstrated a clear dose-dependent increase in infection rates (**Table 3**). At doses of 10^5 and 10^6 CFU, all mice were PCR positive for all isolates. A total of 80 samples were analyzed; Representative real-time PCR amplification profiles are shown in **Figure 3**. At lower doses, infection rates varied among the isolates. However, differences in infection rates between the isolates at each dose level were not statistically significant (Fisher's exact test, $P > 0.05$). These trends are illustrated in **Figure 4**.

Table 3. Detection rates of *Salmonella enterica* in mice based on PCR analysis of organ samples

Isolate	Dose (CFU)	No. of mice	PCR positive (n)	PCR negative (n)	Infection rate (%)
Kaz_6/2025 (serovar Infantis)					
	1×10^3	5	3	2	60
	1×10^4	5	4	1	80
	1×10^5	5	5	0	100
	1×10^6	5	5	0	100
Kaz_7/2025 (serovar Infantis)					
	1×10^3	5	3	2	60
	1×10^4	5	4	1	80
	1×10^5	5	5	0	100
	1×10^6	5	5	0	100
Kaz_8/2025					
	1×10^3	5	3	2	60
	1×10^4	5	2	3	40
	1×10^5	5	5	0	100
	1×10^6	5	5	0	100
Kaz_9/2025					
	1×10^3	5	2	3	40
	1×10^4	5	3	2	60
	1×10^5	5	5	0	100

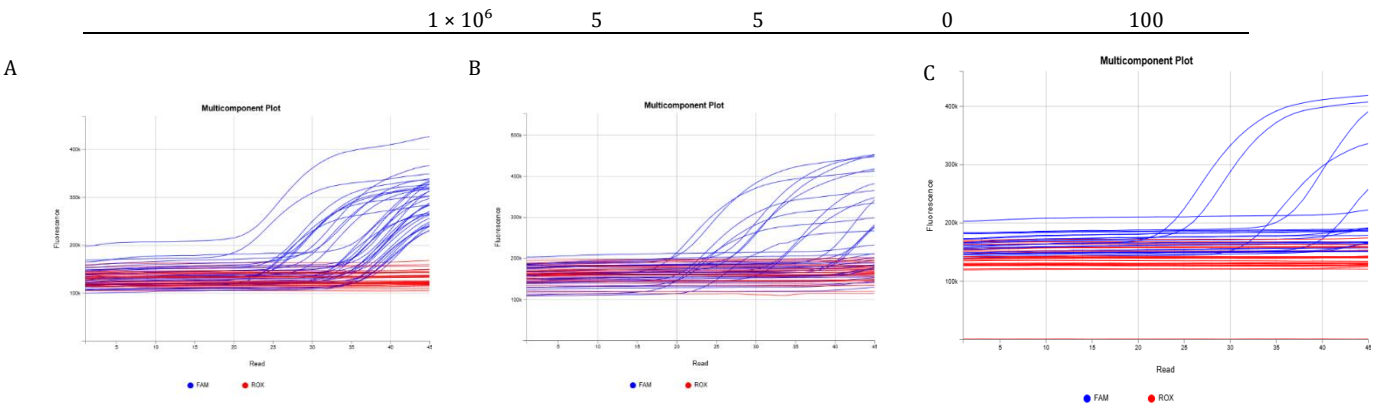

Figure 3. Representative multicomponent plots of the real-time PCR assay used for confirmation of *S. enterica* in organ samples. (A-C) Representative positive samples demonstrating characteristic amplification curves in the FAM channel. The ROX signal remained stable throughout the reaction, indicating proper assay performance. Samples with Ct values < 45 were considered positive according to the manufacturer's criteria

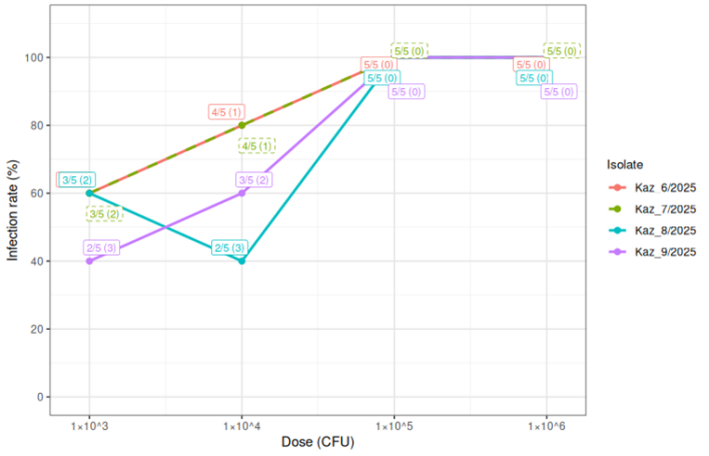

Figure 4. Dose-dependent infection rates of mice following intraperitoneal challenge with *Salmonella enterica* isolates

The lowest ID_{50} values were observed for Kaz_6/2025 and Kaz_7/, whereas the highest ID_{50} values were recorded for Kaz_8/and Kaz_9/2025 (Table 4).

Table 4. Median infectious dose (ID_{50}) of *Salmonella enterica* isolates

Isolate	ID_{50} (CFU)	Log_{10} (ID_{50})
Kaz_6/2025 (serovar Infantis)	1.78×10^3	3.25
Kaz_7/2025 (serovar Infantis)	1.78×10^3	3.25
Kaz_8/2025	1.47×10^4	4.17
Kaz_9/2025	6.81×10^3	3.83

Re-isolation from Internal Organs

Salmonella enterica was successfully re-isolated from the liver, spleen and kidneys of the infected mice, confirming systemic dissemination of the pathogen. No *Salmonella* was detected in organs from the control animals.

DISCUSSION

The present study provides a comparative in vivo assessment of the pathogenic potential of poultry-derived *S. enterica* isolates circulating in Kazakhstan using a standardized murine systemic infection model. The findings revealed clear strain-dependent differences in both infectivity and lethality, as demonstrated by variations in ID_{50} and LD_{50} values among the examined isolates. Notably, the *S.* Infantis isolates (Kaz_6/2025 and Kaz_7/2025) showed higher virulence than the other tested strains under identical experimental conditions.

The results of the present study indicated that the virulence of the tested *S. enterica* isolates was not strictly associated with serovar classification under the conditions of this experiment. Differences in pathogenicity were observed among the isolates, as reflected by variations in ID_{50} and LD_{50} values. These findings suggest phenotypic heterogeneity among the tested bacteria. Although the isolates were previously characterized in terms of antimicrobial resistance (9), the present study did not investigate specific virulence determinants, and therefore no direct conclusions regarding the molecular basis of virulence can be drawn. Nevertheless, previous studies have shown that *Salmonella* virulence may be influenced by

host adaptation, intracellular survival in macrophages, and type III secretion system effectors encoded by *Salmonella* pathogenicity islands (14–17). Importantly, the results of this study are consistent with our previously published genomic characterization of the same isolates (9), where genetic diversity among poultry-derived *S. enterica* strains circulating in Kazakhstan was revealed. Importantly, the results of this study are consistent with our previously published genomic characterization of the same isolates (**9**), where genetic diversity among poultry-derived *S. enterica* strains circulating in Kazakhstan were revealed. The data obtained for the virulence of the tested isolates of *S*. Infantis are consistent with the global significance of this serovar in poultry production systems. Indeed, previous studies have shown that contemporary strains of *S*. Infantis may carry large conjugative megaplasmids that are involved in antimicrobial resistance and colonization capacity (**18-20**). Although the present study did not investigate specific molecular determinants of virulence, the observed differences in ID_{50} and LD_{50} reflect phenotypic variability among the tested isolates.

An interesting observation was also made, where systemic infection was noted at bacterial doses that did not cause mortality. This indicates a certain level of dissociation between infectivity and lethality, suggesting that bacterial dissemination is possible even in the absence of lethal disease. Similar trends have also been noted in murine salmonellosis, where immune responses of the infected host may act to prevent disease progression, even when systemic colonization has occurred (**21,22**). This also emphasizes the importance of evaluating ID_{50} with LD_{50} for a more comprehensive assessment of pathogenic potential, particularly in the context of subclinical infection and epidemiological risk. The intraperitoneal infection route employed in the present study is an example of the systemic phase of the infectious process, bypassing the intestinal colonization phenomenon. This is not an accurate reflection of the natural process, where the infectious route is typically oral; however, it is an effective means of comparing the intrinsic systemic virulence potential of the field isolates (**23,24**).

The results of the current study indicate variability in systemic virulence among poultry-derived *S. enterica* isolates circulating in Kazakhstan, with the *S*. Infantis isolates showing comparatively higher virulence under the conditions of this study. The integration of genomic data, as generated in earlier studies, with experimental in vivo data provides a more complete understanding of biological and epidemiological properties of *S*. Infantis.

This research has demonstrated the existence of strain-dependent variability in the virulence of circulating poultry-derived *S. enterica* strains in Kazakhstan. The results have shown that S. Infantis strains are more infective and lethal compared to other tested strains. This points towards a stronger systemic pathogenic potential of these strains compared to the other tested strains. The difference in infectivity and lethality is a strong indicator of the importance of ID_{50} and LD_{50} testing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: A.T.T., B.A.Y., Z.S.K. and A.A.A.; Methodology: A.T.T., A.Z.Z., B.B.Y., A.R.A. and A.A.A.; Investigation: B.B.Y., N.S., S.E.A., M.K.S., E.B.S., A.M.A., K.K.T. and M.M.M.; Data Curation: E.B.S., A.M.A., K.K.T. and A.A.A.; Formal Analysis: A.R.A., A.A.K., K.D.Z. and A.A.A.; Resources: B.A.Y., Z.S.K., A.Z.Z., A.A.K. and K.D.Z.; Writing - Original Draft: A.A.A. and A.T.T.; Writing - Review & Editing: B.A.Y., Z.S.K., A.R.A., A.A.K., K.D.Z. and A.A.A.; Supervision: B.A.Y., Z.S.K. and K.D.Z.; Funding Acquisition: B.A.Y., A.A.K. and K.D.Z. All authors have read and approved the final version of the manuscript.

ARTIFICIAL INTELLIGENT DECLARATION

The authors declare that they are responsible for the accuracy and integrity of all content of the manuscript, including part generated by AI, and it is not used as a co-author.

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REFERENCES

1. World Health Organization. *Salmonella* (non-typhoidal) [Internet]. Geneva: World Health Organization; 2018 [cited 2026 Feb 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/*Salmonella*-(non-typhoidal)
2. Shaji S, Selvaraj RK, Shanmugasundaram R. *Salmonella* infection in poultry: a review on the pathogen and control strategies. Microorganisms. 2023;11(11):2814. 10.3390/microorganisms11112814
3. Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. Appl Environ Microbiol. 2011;77(13):4273-9. 10.1128/AEM.00598-11
4. Stecher B, Macpherson AJ, Hapfelmeier S, Kremer M, Stallmach T, Hardt WD. Comparison of *Salmonella enterica* serovar Typhimurium colitis in germfree mice and mice pretreated with streptomycin. Infect Immun. 2005;73(6):3228-41. 10.1128/IAI.73.6.3228-3241.2005
5. Salcedo SP, Noursadeghi M, Cohen J, Holden DW. Intracellular replication of *Salmonella* typhimurium strains in specific subsets of splenic macrophages in vivo. Cell Microbiol. 2001;3(9):587-97. 10.1046/j.1462-5822.2001.00137.x
6. Haraga A, Ohlson MB, Miller SI. *Salmonella*e interplay with host cells. Nat Rev Microbiol. 2008;6(1):53-66. 10.1038/nrmicro1788

7. Mendybayeva A, Abilova Z, Bulashev A, Rychshanova R. Prevalence and resistance to antibacterial agents in *Salmonella enterica* strains isolated from poultry products in Northern Kazakhstan. Vet World. 2023;16(3):657-67. 10.14202/vetworld.2023.657-667
8. Borovikov SN, Shirobokova DS, Akanova ZhZh, Dussenova GT, Syzdykova AS, Berezina E. Study of the distribution of *Salmonella* infection in poultry farms in the northern region of Kazakhstan. Cent Asian J Vet Sci. 2025;4(012):4-18. 10.51452/kazatuvc.2025.4(012).2063
9. Yespembetov B, Kirkimbayeva Z, Abdykalyk A, Akhmetova A, Shevtsov A, Syrym N, et al. Detection and preliminary genomic characterization of poultry-derived *Salmonella enterica* from southern Kazakhstan. Antibiotics (Basel). 2025;14(12):1195. 10.3390/antibiotics14121195
10. Dewaele I, Van Meirhaeghe H, Rasschaert G, Vanrobaeys M, De Graef E, Herman L, et al. Persistent *Salmonella* Enteritidis environmental contamination on layer farms in the context of an implemented national control program with obligatory vaccination. Poult Sci. 2012;91(2):282-91. 10.3382/ps.2011-01673
11. Monack DM, Bouley DM, Falkow S. *Salmonella* typhimurium persists within macrophages in the mesenteric lymph nodes of chronically infected Nramp1+/+ mice and can be reactivated by IFNgamma neutralization. J Exp Med. 2004;199(2):231–241.10.1084/jem.20031319.
12. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Epidemiol. 1938;27(3):493-7. 10.1093/oxfordjournals.aje.a118408
13. Walker GT, Gerner RR, Nuccio SP, Raffatellu M. Murine models of *Salmonella* infection. Curr Protoc. 2023;3(7):e824. 10.1002/cpz1.824
14. Bäumler AJ, Tsolis RM, Ficht TA, Adams LG. Evolution of host adaptation in *Salmonella enterica*. Infect Immun. 1998;66(10):4579-87. 10.1128/IAI.66.10.4579-4587.1998
15. Hensel M, Shea JE, Waterman SR, Mundy R, Nikolaus T, Banks G, et al. Genes encoding putative effector proteins of the type III secretion system of *Salmonella* pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. Mol Microbiol. 1998;30(1):163-74. 10.1046/j.1365-2958.1998.01047.x
16. Waterman SR, Holden DW. Functions and effectors of the *Salmonella* pathogenicity island 2 type III secretion system. Cell Microbiol. 2003;5(8):501-11. 10.1046/j.1462-5822.2003.00294.x
17. Hautefort I, Proença MJ, Hinton JCD. Single-copy green fluorescent protein gene fusions allow accurate measurement of *Salmonella* gene expression in vitro and during infection of mammalian cells. Appl Environ Microbiol. 2003;69(12):7480-91. 10.1128/AEM.69.12.7480-7491.2003
18. Lawley TD, Bouley DM, Hoy YE, Gerke C, Relman DA, Monack DM. Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. Infect Immun. 2008;76(1):403-16. 10.1128/IAI.01189-07
19. Aviv G, Tsyba K, Steck N, Salmon-Divon M, Cornelius A, Rahav G, et al. A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent *Salmonella enterica* serovar Infantis strain. Environ Microbiol. 2014;16(4):977-94. 10.1111/1462-2920.12351
20. Lee WWY, Mattock J, Greig DR, Langridge GC, Connerton PL, Woodward MJ, et al. Characterization of a pESI-like plasmid and analysis of multidrug-resistant *Salmonella enterica* Infantis isolates in England and Wales. Microb Genom. 2021;7(3):000658. 10.1099/mgen.0.000658
21. Brown AC, Chen JC, Watkins LKF, Campbell D, Folster JP, Tate H, et al. CTX-M-65 extended-spectrum beta-lactamase-producing *Salmonella enterica* serotype Infantis, United States. Emerg Infect Dis. 2018;24(12):2284-91. 10.3201/eid2412.180500
22. Mastroeni P, Grant A, Restif O, Maskell D. A dynamic view of the spread and intracellular distribution of *Salmonella enterica*. Nat Rev Microbiol. 2009;7(1):73-80. 10.1038/nrmicro2034
23. Vazquez-Torres A, Jones-Carson J, Bäumler AJ, Falkow S, Valdivia R, Brown W, et al. Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. Nature. 1999;401(6755):804-8. 10.1038/44593
24. Zhou A, Kang B, He H, Van der Verren SE, Li J, Zhang J, et al. Whole-genome comparative and pathogenicity analysis of *Salmonella* Rissen, an emerging zoonotic *Salmonella* serovar. G3 (Bethesda). 2020;10(7):2159-70. 10.1534/g3.120.401336