

*Case report*

## CASE STUDY: SALMONELLA – ASSOCIATED ABORTIONS IN CHINCHILLAS – INSIGHTS FROM MICROBIOLOGICAL AND NECROPSY FINDINGS

Miloš VUČIĆEVIĆ<sup>1</sup> , Ana PEŠIĆ<sup>1</sup> , Milan ANIČIĆ<sup>2\*</sup> , Isidora PROŠIĆ<sup>3</sup> ,  
Nataša GALIĆ<sup>4</sup> , Darko MARINKOVIĆ<sup>2</sup> , Dejan KRNJAIĆ<sup>3</sup> 

<sup>1</sup>University of Belgrade, Faculty of Veterinary Medicine, Department of Equine, Small Animal, Poultry and Wild Animal Diseases, Bulevar oslobođenja 18, 11000, Belgrade, Serbia; <sup>2</sup>University of Belgrade, Faculty of Veterinary Medicine, Department of Pathology, Bulevar oslobođenja 18, 11000 Belgrade, Serbia; <sup>3</sup>University of Belgrade, Faculty of Veterinary Medicine, Department of Microbiology and Immunology, Bulevar oslobođenja 18, 11000, Belgrade, Serbia; <sup>4</sup>Institute of Public Health of Serbia “Dr Milan Jovanović Batut”, dr Subotića starijeg 5, 11000, Belgrade, Serbia;

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*Salmonella* spp. infections pose a significant threat to both animal and public health, with various serotypes affecting a wide range of hosts. This case report investigates an outbreak of salmonellosis on a chinchilla farm in Serbia, focusing on the clinical, pathological, and microbiological aspects of the infection. Two chinchilla cadavers were brought to the Faculty of Veterinary Medicine, University of Belgrade. The animals on the farm exhibited anorexia, apathy, diarrhea, and abortions before succumbing to the infection. Pathohistological findings revealed severe necrohaemorrhagic metritis and placentitis, along with multifocal randomly dispersed areas of necrosis in the liver. Conventional microbiological analysis revealed the presence of *Salmonella* Typhimurium which was confirmed by serological typing. Antimicrobial susceptibility analysis showed that bacteria were susceptible to all tested antibiotics. The outbreak could maybe attributed to inadequate husbandry practices and stress, particularly among gravid females. This report underscores the importance of proper husbandry, judicious antibiotic use based on susceptibility testing and implementation of preventive measures to mitigate the recurrence and spread of salmonellosis in chinchilla populations, both in farm and pet settings.

**Keywords:** abortions, chinchillas, outbreak, *Salmonella* Typhimurium

### INTRODUCTION

A wide range of domestic and non-domestic animals can be affected by salmonellosis, including mammals, birds and reptiles. There are reported occurrences of salmonellosis in both farm raised and pet chinchillas. Most of the cases of salmonella infection on

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\*Corresponding author: e-mail: anicicm@vet.bg.ac.rs

farms are caused by *S. enterica* subsp. *enterica* serotypes Enteritidis or Typhimurium [1-4]. There are also reported cases of salmonellosis in farm chinchillas caused by a variety of serotypes: *S. enterica* subsp. *enterica* serotype Pullorum, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *enterica* serotype Dublin and *S. enterica* subsp. *salamae* serotype Sofia [3,5-7]. Although salmonellosis is a common occurrence in farm-raised chinchillas, there are relatively few published articles on this subject [4].

This paper describes the outbreak of salmonellosis on one chinchilla farm in Serbia. It contains description of symptoms in living individuals, macroscopic and histopathological findings, microbiological examinations, treatment and resolution of the infection.

## CASE PRESENTATION

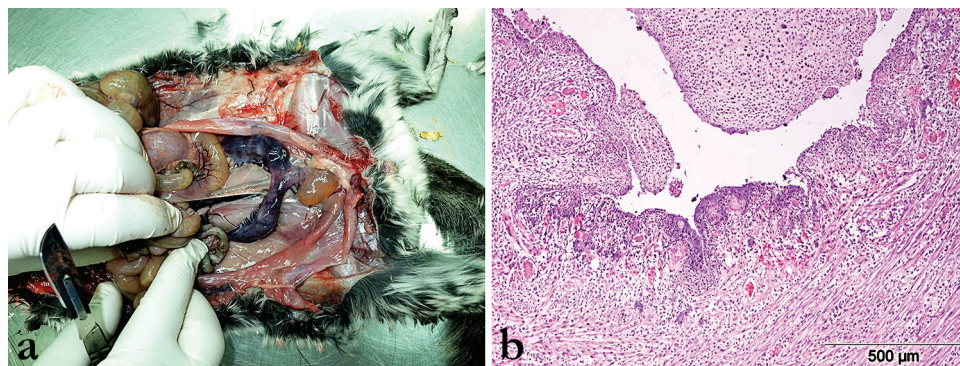
Two chinchilla cadavers from a chinchilla farm in Serbia were brought to the Faculty of Veterinary Medicine, University of Belgrade in June 2022. There were approximately 1000 animals on the farm that were kept in a battery system in groups of 6 (5 females and 1 male). Symptoms included anorexia, apathy, diarrhea, abortion in later stages of pregnancy culminating in death. There had been about two deaths per day, in the previous two months. Almost all fatalities occurred in pregnant females. Roughly five percent of the fatalities were males and younglings. The owner treated chinchillas with sulfadimidine via drinking water, but no improvement had been observed. On the farm, salmonellosis had been confirmed the year before, and fluoroquinolone medication was successful, although the specific serotype of Salmonella was not identified. Following the treatment, thorough disinfection, pest control and rodent eradication measures were implemented in the facility to ensure a comprehensive and effective approach to preventing the recurrence of the infection. The corpses were sent to the Department of Pathology for necropsy.

### Pathological findings

The carcasses were fresh and in fair body condition. A small amount of dark red discharge was noted from the vulva, leading to subsequent staining of the fur in the perineal region. Internal examination revealed an enlarged, purple-bluish uterus filled with dark red, amorphous, friable content. The liver appeared enlarged, pale, and friable. The lungs were diffusely red, non-collapsed, with dark red patchy areas in one animal. Additionally, petechial hemorrhages were observed on the stomach mucosa of one of the animals. Other findings during examination were unremarkable.

Tissue samples were collected and fixed in 10% buffered formalin. After processing in an automated tissue processor, the samples were embedded in paraffin blocks, cut into 4 µm thick sections and stained with hematoxylin and eosin for microscopic examination. Microscopic examination of the uterus revealed severe, multifocal to diffuse necrohaemorrhagic metritis and placentitis, featuring numerous intracellular

and free bacteria. Inflammatory infiltrates consisted mainly of macrophages, with fewer neutrophils and lymphocytes. The uterine lumen contained necrotic remnants of fetal tissues, likely representing approximately one-third of the way through pregnancy. Many endometrial blood vessels were filled with fibrin thrombi, some of which contained bacterial colonies. The liver displayed multifocal random areas of necrosis and mild centrilobular vacuolation. Occasional mild mononuclear periportal hepatitis was also observed. Small foci of hemorrhage were present in the brain, heart, and kidneys, along with the lungs exhibiting hyperemia and edema. Large areas of hemorrhage, accompanied by fibrin deposits, were identified in the spleen. Lymphohistiocytic enteritis was also noted. These histological findings were suggestive of septicemia (Figure 1).



**Figure 1.** Chinchilla, uterus. **A)** Macroscopic appearance; **B)** Photomicrograph of necrohemorrhagic endometritis with myriad bacterial colonies, mixed inflammatory infiltrate (histiocytes, neutrophils, lymphocytes) and placental and fetal tissue remnants in the lumen (HE,  $\times 100$ ).

### Microbiological findings

Lung, liver, spleen, kidney, uterus and bowel samples from the chinchillas were taken at necropsy and sent for microbiological examination to the bacteriology laboratory at the Department for Microbiology, Faculty of Veterinary Medicine, University of Belgrade. Tissue samples were inoculated onto blood agar plates (Becton Dickinson, USA), Mac Conkey agar plates (Becton Dickinson, USA) and Sabouraud agar plates (HiMedia, India) and incubated under aerobic conditions. In addition, samples were inoculated on CL Broth (Chopped Liver Broth, HiMedia, India) and blood agar under anaerobic condition using GasPak Anaerobe Container System (Becton Dickinson, USA). The selective media for fungi, as well as plates incubated under strictly anaerobic conditions, remained sterile. Isolation of *Salmonella* spp. was performed according to the ISO standard SRPS EN ISO 6579-1:2017/A1:2020 [8]. The suspected *Salmonella* spp. colonies observed on the agar plates were confirmed using biochemical methods and serological typing. The biochemical tests showed results characteristic of *Salmonella* spp.: positive reactions for glucose fermentation, H<sub>2</sub>S production, lysine decarboxylation, methyl red reaction, and citrate test, and negative

reactions for indole production and Voges-Proskauer test. Serological typing of the isolates was performed at the National Reference Laboratory for *Salmonella*, *Shigella*, *Vibrio cholera* and *Yersinia enterocolitica*, Institute of Public Health of Serbia “Dr Milan Jovanovic Batut”, Belgrade, Serbia. The isolate was identified as *S. enterica* serovar Typhimurium (4,12 : i : 1,2). The antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Becton Dickinson, USA) using the disk diffusion method in accordance with guidelines issued by the Clinical Laboratory Standard Institute (CLSI, document M100, Performance Standards for Antimicrobial Susceptibility Testing, 28th ed.) [9]. The fifteen tested antibiotics (Becton Dickinson, USA) were: amoxicillin/clavulanic acid (20/10 µg), ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), amikacin (30 µg) gentamicin (10 µg), meropenem (10 µg), nalidixic acid (30 µg) tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tigecycline (15 µg) and colistin (10 µg). All the results were compared to the CLSI clinical break points and no antimicrobial resistance was found. Minimum inhibitory concentration (MIC) values were obtained at the National reference laboratory for *Salmonella*, *Shigella*, *Vibrio cholera*, *Yersinia enterocolitica* in Belgrade, Republic of Serbia with the broth microdilution method using a commercial test SENSITITRE PANEL Salmonella/E. coli EUVSEC3 (Thermo Scientific, USA) and the results were interpreted according to the Commission Implementing Decision 2020/1729/EU (CID) [10]. The results are shown in Table 1.

**Table 1.** MIC values for the isolate of *S. enterica* serovar Typhimurium

Antimicrobial agents/ MIC interval of concentration (mg/L)	Read MIC value (mg/L)	Interpretation of AMR (*ECOFF)
Ampicillin / 1 – 32	≤ 1	WT
Meropenem / 0,03 – 16	= 8	WT
Ciprofloxacin / 0,015 – 8	= 0.03	WT
Amikacin / 4 – 128	≤ 4	WT
Gentamicin / 0,5 – 16	≤ 0.5	WT
Tigecycline / 0,25 – 8	≤ 0.25	WT
Ceftazidime / 0,25 – 8	= 0.5	WT
Cefotaxime / 0,25 – 4	≤ 0.25	WT
Chloramphenicol / 8 – 64	≤ 8	WT
Colistin / 1 – 16	≤ 1	WT
Nalidixic acid / 4 – 64	≤ 4	WT
Tetracycline / 2 – 32	≤ 2	WT
Trimethoprim / 0,25 – 16	≤ 0.25	WT
Sulfamethoxazole / 8 – 512	= 16	WT

\*Epidemiological cut-off value; **WT** – wild-type – susceptible

## DISCUSSION

This study highlights the occurrence of abortions and deaths in farm-raised adult chinchillas due to *S. enterica* serovar Typhimurium infection. Although chinchillas are commonly kept as pets, reproductive infections are primarily a concern in breeding conditions. There were no previous reports about salmonellosis in chinchillas in Serbia, which underscores the significance of this finding.

Salmonellosis is known to affect chinchillas, particularly under stressful conditions. Factors such as pregnancy, overcrowding, and inadequate housing contribute to the susceptibility of chinchillas to this disease [11]. Our findings suggest that there was probable subclinical infection which exacerbated under stress conditions, such as pregnancy. It implies that chinchillas can be possible carriers of this bacterium and a source of infection for humans. The absence of regulations for chinchilla farming and reliance on self-diagnosis by farm owners often exacerbate the problem, as proper veterinary consultation and effective preventive measures are not consistently implemented. This report indicates that eradication of *Salmonella* spp. from the premises is difficult, highlighting the need for closer collaboration between farm owners and veterinary services to prevent and manage outbreaks effectively.

The frequency of salmonellosis in chinchillas, particularly *S. Typhimurium*, remains underreported, as farm owners rarely seek veterinary assistance or invest in comprehensive diagnostic testing. According to Santos et al. [6], *S. Typhimurium* infections in chinchillas are rare and sparsely documented in the literature, indicating a lack of data in this area.

In conclusion, while salmonellosis can occur even in well-maintained facilities, it is essential to address predisposing factors and improve veterinary oversight to reduce the risk of outbreaks in chinchilla farms. Further research and more systematic reporting are needed to better understand the prevalence and impact of salmonellosis in chinchillas.

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### Ethical statement

The research has been approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (approval No. 01-01/2024).

### Authors' contributions

MV, DM, and DK contributed to the design and conception of the study. MV and AP collected samples. MV, AP, MA, IP and DM wrote the manuscript. DK, NG and IP performed the microbiology analyses. DM and MA performed the necropsy and

pathohistology. DM and DK critically revised the manuscript and made substantial contribution to interpretation of data. All authors read and approved the final manuscript.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### ORCID iDs


Miloš Vučićević  <https://orcid.org/0000-0002-3208-147X>


Ana Pešić  <https://orcid.org/0009-0003-5423-534X>

Milan Aničić  <https://orcid.org/0000-0002-3953-2501>

Isidora Prošić  <https://orcid.org/0000-0002-5363-0650>

Nataša Galić  <https://orcid.org/0000-0002-5282-8649>

Darko Marinković  <https://orcid.org/0000-0002-5456-1343>

Dejan Krnjić  <https://orcid.org/0000-0003-3817-0438>

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## **MIKROBIOLOŠKI I PATOMORFOLOŠKI NALAZ KOD ABORTUSA ČINČILA UZROKOVANIH *SALMONELLA* *TYPHIMURIUM* – PRIKAZ SLUČAJA**

Miloš VUČIĆEVIĆ, Ana PEŠIĆ, Milan ANIČIĆ, Isidora PROŠIĆ, Nataša GALIĆ,  
Darko MARINKOVIĆ, Dejan KRNJAIĆ

Rod *Salmonella* čini veliki broj serovarijeteta koji mogu uzrokovati oboljenje različitih domaćina i predstavlja značajnu pretnju po zdravlje ljudi i životinja. Ovaj prikaz slučaja pruža uvid u izbijanje salmoneloze na farmi činčila u Srbiji, fokusirajući se na kliničke, patološke i mikrobiološke aspekte oboljenja. Dve činčile koje su ispoljavale simptome u vidu anoreksije, apatije, dijareje i pobačaja su nakon smrti transportovane na Fakultet veterinarske medicine Univerziteta u Beogradu. Patohistološki nalaz pokazao je, između ostalog, teži oblik nekrohemoragičnog metritisa i placentitisa, kao i nasumična multifokalna područja nekroze u jetri. Konvencionalna mikrobiološka analiza otkrila je prisustvo *Salmonella* Typhimurium, što je potvrđeno serološkim tipiziranjem izolata. Testiranjem minimalnih inhibitornih koncentracija u cilju ispitivanja antimikrobne osetljivosti dokazano je da je izolovani soj osetljiv na sve testirane antibiotike. Postoji mogućnost da su do izbijanja zaraze na farmi činčila doveli neadekvatna uzgojna praksa i stres, posebno kod gravidnih ženki. Ovaj prikaz slučaja naglašava važnost preventivnih mera, pravilnog uzgoja životinja kao i racionalne upotrebe antibiotika koja se zasniva na terapiji propisanoj na osnovu testiranja osetljivosti bakterija na antibiotike. Navedeni postupci sprovode se u cilju smanjenja izbijanja i širenja salmoneloze u populacijama činčila, kako na farmama, tako i kod životinja koje se čuvaju kao kućni ljubimci.