

## A Case Report of Hepatosplenomegaly Due to Chronic Salmonellosis in Holstein Calves

Alper ERTÜRK<sup>1\*</sup> , Selçuk ÜNGÜR<sup>2</sup> 

<sup>1</sup>Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Internal Medicine, Hatay, Türkiye

<sup>2</sup>Kızılışık Veterinary Clinic, Kayseri, Türkiye

\*Corresponding author: alper.erturk@mku.edu.tr

Received 04.10.2023

Accepted 21.11.2023

Published 31.12.2023

### Abstract

In this case report, our aim was to present the clinical, macroscopic, bacteriological, and antibiogram results of a chronic salmonellosis case in calves on a dairy farm. A total of 12 Holstein calves weaned at 75-90 days of age on a dairy farm with clinical signs were presented in the case report. The calves displayed intermittent fever, diarrhea, and pneumonia that did not respond to treatment. The most notable finding at necropsy was hepatosplenomegaly. The diagnosis of *Salmonella* spp. was confirmed from the samples taken from the calves, and antibiograms revealed the resistance to many antibiotics. The disease did not recur following attention to management practices and application of protective hyperimmune serum. The presented case report emphasizes that chronic *Salmonella* spp. infection may lead to severe losses in calves, highlighting pathological findings, antibiotic resistance, and preventive and management practices.

**Keywords:** Calf, chronic salmonellosis, hepatosplenomegaly.

### Holstein Buzağılarda Kronik Salmonellozis'e Bağlı Hepatosplenomegali Vaka Raporu

#### Öz

Bu vaka raporunda bir süt işletmesinde buzağılarda karşılaşılan kronik salmonellozis olgularının klinik, makroskobik, bakteriyolojik ve antibiyogram sonuçlarını sunmayı amaçladık. Süt işletmesinde 75-90 günlük yaş aralığında olan, süttten kesilmiş ve klinik bulgu gösteren toplam 12 adet Holstein ırkı buzağı vaka raporuna dahil edildi. Buzağılarda tedaviye yanıt vermeyen aralıklı ateş, ishal ve pnömoni bulguları mevcuttu. Yapılan nekropside en dikkat çekici bulgu hepatosplenomegali olarak belirlendi. Buzağılardan alınan örneklerde *Salmonella* spp. tespit edildi ve antibiyogramda birçok antibiyotiğe dirençli olduğu görüldü. Yönetim uygulamalarına dikkat edilmesi ve koruyucu hiperimmün serum uygulaması sonrası hastalık bir daha görülmedi. Sunulan vaka raporunda kronik *Salmonella* spp. enfeksiyonunun buzağılarda ciddi kayıplara neden olabileceğine ve aynı zamanda enfeksiyonun patolojik bulguları, antibiyotik direnci, koruyucu ve yönetim uygulamalarına dikkat çekilmiştir.

**Anahtar kelimeler:** Buzağı, kronik salmonellozis, hepatosplenomegali.



## Introduction

*Salmonella* (as genus) spp. is a Gram-negative bacterium commonly found in cattle. It causes enteric and septicemic infections in humans and many animal species (Mohler et al., 2009). Most of the *Salmonella* spp. important for veterinary medicine belong to the subspecies *Salmonella enterica* (*S. enterica*). Within this subspecies, *Salmonella typhimurium* (*S. typhimurium*) and *Salmonella dublin* (*S. dublin*) are the most common serotypes in cattle (Smith, 2009). The most common mode of transmission is the fecal-oral route. Salmonellosis causes fever, loss of appetite, bloody and mucous feces in calves (Wray & Davies, 2000). Salmonellosis is usually seen in subclinical and acute clinical forms and acute forms of salmonellosis are represented as the tip of the iceberg. Infection can be seen in epidemic as a result of management mistakes and exposure of calves to stress (Hadimli et al., 2011). Although pathologists usually associate salmonellosis with enteric lesions such as diphtheritic membranes (Wray & Davies, 2000), highly variable necropsy findings have been identified in various literature data (de Aguiar et al., 2021; Molossi et al., 2021; Casaux et al., 2023).

Acknowledging the limited quantity of case reports on *Salmonella* spp. in the literature, this case report presents the clinical, macroscopic, bacteriological and antibiogram results of a chronic *Salmonella* spp. outbreak observed in calves aged 75-90 days on a dairy cattle farm. The purpose of this case report is to aid veterinarians in the diagnosis, control and prevention of similar incidents.

## Case Description

On a farm in the Central Anatolian region, clinical signs were observed in 12 Holstein calves from a group of 60 weaned calves aged 75-90 days. The

calves on the farm received routine vaccinations against respiratory diseases with a double dose at 30 and 60 days of age. The calves on the farm were systematically weaned at the age of 60 days. After weaning, the calves are fed with a diet consisting of flaked maize, alfalfa and calf starter feed. Before weaning, the calves were fed with milk, alfalfa and calf starter feed. No clinical signs were observed in non-weaned calves. However, it was determined that all animals on the farm utilized the same water source.

Each of the 12 calves examined in the study exhibited clinical manifestations, including intermittent high fever, pneumonia, diarrhea, anorexia, and dehydration. Arthritis, fibrinous and hemorrhagic feces were detected in only two calves. The fecal samples from the 12 calves in the study demonstrated a watery and voluminous consistency (Figure 1). The feces of 8 of the 12 calves with diarrhea were analyzed for rotavirus, coronavirus, *Cryptosporidium parvum*, *Giardia* and *Escherichia coli* with the rapid antigen test kit (BoviD-5 Ag, Bionote, Inc. Korea) and no causative agent was detected. Blood samples taken from 12 calves showing clinical signs were polymerase chain reaction (PCR) tested for Bovine viral diarrhea virus in a private laboratory and negative results were obtained.



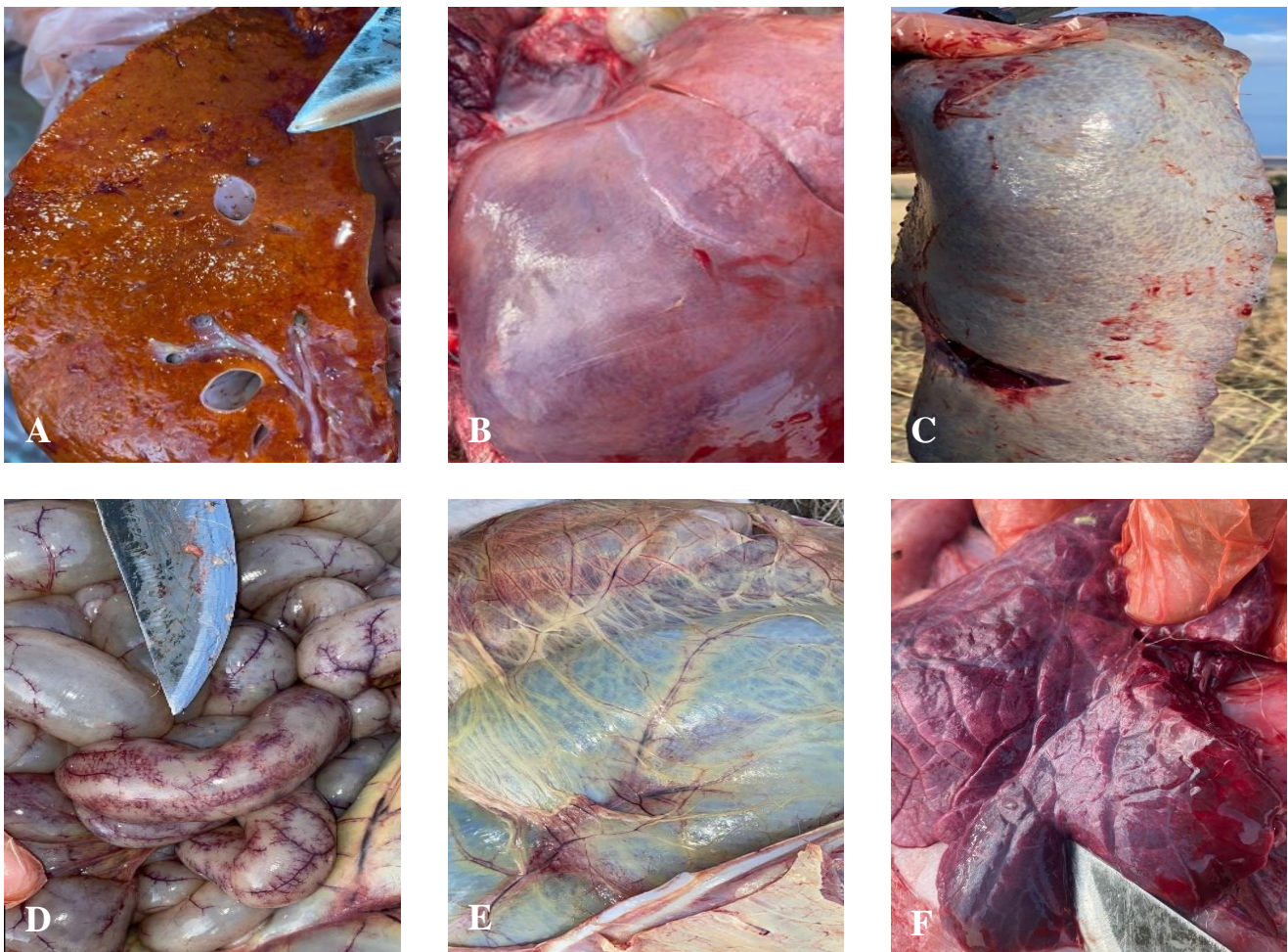
**Figure 1.** Diarrhea in a calf infected with *Salmonella* spp.

Sulfamethoxazole/trimethoprim was used to all calves with clinical signs by the farm veterinarian.

When there was no response to the treatment, florfenicol was applied. Meanwhile, the calves were also given supportive treatment with nonsteroidal anti-inflammatory drugs, including flunixin meglumine and meloxicam. Nine out of the 12 calves that showed clinical signs while treated died within seven days. Necropsy was not performed on these calves. The remaining three calves had no response to treatment, were cachectic, and died within 21-24 days. Necropsy of the three calves that died later was performed in detail by the farm veterinarian immediately after death. Samples taken from the internal organs (liver, spleen, lung, intestine) and fecal

samples taken from the intestinal contents of the calves were stored at +4 °C and sent to a specialized laboratory for cultivation.

In all calves necropsied under farm conditions, notable findings included pulmonary congestion, hyperemia in the heart and intestines, enlargement of mesenteric lymph nodes, thickening of intestinal walls, hepatosplenomegaly and a yellow-orange colored liver. Diffuse red consolidated areas were observed in the lungs. Examination of one calf revealed mild yellow discoloration of the omentum, serosal surfaces of the forestomachs and adipose tissue (Figure 2).



**Figure 2.** Macroscopic findings in calves with salmonellosis. A) The liver has light orange discoloration. B) Enlargement of the liver. C) Enlargement of the spleen is evident from the rounded borders and slight capsular tension. D) Intestinal hyperemia. E) The omentum, serosal surfaces of the forestomachs and adipose tissue are yellowed. F) The lung has a smooth, bright and reddened surface.

In the bacteriological evaluation, Gram-negative, lactose-negative, sucrose-glucose-negative, hydrogen sulphide-positive, lysine decarboxylation-positive, o-nitrophenyl-beta-D-galactoside-positive, urea-negative agents were identified as *Salmonella* spp. and isolated from all samples. Antibiogram results of the isolated *Salmonella* spp. are presented (Table). Bacteriological cultivation and antibiogram analyses were assessed in a specialized laboratory.

**Table.** Antibiogram results of the isolated *Salmonella* spp.

Antibiotics Used	Results
Amoxicillin-clavulanic acid	Resistant
Ampicillin	Resistant
Carbenicillin	Resistant
Cefoperazone	Resistant
Cephalexin	Resistant
Cloxacillin	Resistant
Erythromycine	Resistant
Florfenicol	Resistant
Lincomycin	Resistant
Oxytetracycline	Resistant
Penicillin G	Resistant
Spectinomycin	Resistant
Sulphamethoxazole/trimethoprim	Resistant
Cefquinome	Sensitive
Danofloxacin	Sensitive
Enrofloxacin	Sensitive
Doxycycline	Sensitive
Gentamicin	Sensitive
Kanamycin	Sensitive
Marbofloxacin	Sensitive
Neomycin	Sensitive

In the presented case report, the diagnosis of salmonellosis was confirmed based on clinical examination, macroscopic findings, and laboratory results. Following the diagnosis, detailed investigations at the farm revealed that the flaked maize was heavily contaminated with dog feces, suggesting that this was the probable source of the outbreak. All newborn calves in the herd were administered a protective dose of 10 mL subcutaneous antiserum (Multisera Combined, Atafen®) at one-week intervals, repeated three times. Additionally, stray dogs were removed from the farm. In the close follow-

up of the farm for one year, it was determined that the infection did not recur, and deaths related to this disease ceased.

## Discussion

Salmonellosis is a common infection in farms. *Salmonella* spp. typically affect cattle between one and three months of age, and this age group accounts for 80% of cattle cases. In the present case, the age range of the calves diagnosed with *Salmonella* spp. is consistent with the literature data (Hadimli et al., 2011; Pecoraro et al., 2017; Casaux et al., 2023). Salmonellosis can be diagnosed through clinical findings, macroscopic, microbiological and PCR methods (Costa et al., 2018). In addition to identifying the causative agent, clinical examination and macroscopic findings are important diagnostic methods for the disease (Pecoraro et al., 2017; Costa et al., 2018). The diversity of *Salmonella* spp. serotypes and the potential for different serovars to have distinct virulence factors require broad prophylactic strategies (Hadimli et al., 2011).

Clinical findings such as intermittent high fever, pneumonia, arthritis, diarrhea, etc. in calves are consistent with literature data (Hadimli et al., 2011; Pecoraro et al., 2017). Enteritis and respiratory clinical findings may help in the differential diagnosis of salmonellosis (Pecoraro et al., 2017). The high mortality observed in the presented case report is consistent with the literature data (Casaux et al., 2023).

*Salmonella* spp. observed in calves may partially benefit from antibiotic treatment (de Aguiar et al., 2021), but generally do not respond to antimicrobial agents due to resistant *Salmonella* spp. strains (Molossi et al., 2021). Especially in calves with septicemic, aggressive antibiotic treatment in the early period of infection and treatment according to the antibiogram result is recommended (Wray & Davies, 2000). In the presented case report, although antibiotic

treatment was applied to all sick calves, no response to treatment was obtained. It is stated that antibiotic-resistant *Salmonella* spp. species will cause severe epidemics in animals and humans due to unconscious antibiotic use (Varma et al., 2006). In the presented case report, *Salmonella* spp. resistant to many antibiotics were detected in the antibiogram analysis of the samples obtained from the farm. Even if animals infected with *Salmonella* spp. are treated, they remain as carriers and continue to be a source of infection. Therefore, the primary strategy for salmonellosis is vaccination and preventive measures (Mohler et al., 2009; Hadimli et al., 2011).

While *Salmonella* spp. is a major cause of diarrhea, serovar and host-associated factors can lead to severe systemic consequences. Hyperemia in the heart and intestine, enlarged mesenteric lymph nodes, thickening of the intestinal wall and hepatosplenomegaly observed at necropsy are consistent with the literature data (de Aguiar et al., 2021; Molossi et al., 2021; Casaux et al., 2023). At necropsy, hepatosplenomegaly was remarkable in the calves. The extraintestinal route of *Salmonella* spp. can cause of systemic inflammatory infections such as splenomegaly. Although *Salmonella* spp. is a mucosal pathogen, the main anatomical sites of bacterial replication are the spleen and liver (Jones & Falkow, 1996). In an experimental study, splenomegaly was determined in mice infected with *Salmonella* spp. It has been reported that increases in spleen cells, phagocytes and neutrophils may cause this condition and that these increases peak especially in the chronic phase of the disease. The spleen may enlarge more than ten times (Jackson et al., 2010; Rosche et al., 2015). In human medicine, hepatomegaly is reported in almost all cases infected with *Salmonella* spp. (Ramachandran et al., 1974). Hepatosplenomegaly may have been caused by the calves being in the chronic stage of the disease and the spread of infection by

haematogenous route. Mild jaundice detected in only one calf is consistent with the literature data (Molossi et al., 2021; Casaux et al., 2023).

The occurrence of the disease in weaned calves and the detection of feed contaminated with the feces of stray dogs; environmental factors, management errors and exposure of calves to stress are consistent with the increase in clinical cases (Mohler et al., 2009; Hadimli et al., 2011). The absence of clinical signs in dairy cows and suckling calves suggests that the source of infection is not dairy cows. All calves were fed from mixed milk in the tank. Additionally, the absence of animal entry from outside and the detection of feed contaminated with dog feces suggest that dogs are the source of infection. Wildlife is reported to play an important role in the transmission of *Salmonella* spp. infections (Evans & Davies, 1996).

## Conclusion

It was determined that intermittent fever, diarrhea, and pneumonia, which did not respond to antibiotics, could be observed in calves with chronic *Salmonella* spp. The most striking macroscopic finding was hepatosplenomegaly. It is emphasized that hyperimmune serum used for prophylaxis can stop the outbreak and the importance of management practices.

## Financial Support

This study did not receive a grant by any financial institution/sector.

## Ethical Statement

This study does not present any ethical concerns.

## Author Contributions

Investigation: A.E., S.Ü; Material and Methodology: A.E., S.Ü; Supervision: A.E.;

Visualization: A.E., S.Ü; Writing-Original Draft: A.E.; Writing – Review & Editing: A.E.

## Conflict of Interest

The authors declared that there is no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References

- Casaux, M. L., Neto, W. S., Schild, C. O., Costa, R. A., Macías-Rioseco, M., Caffarena, R. D., Silveira, C. S., Araoz, V., Diaz, B. D., 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. *Brazilian Journal of Microbiology*, *54*(1), 475-490. <https://doi.org/10.1007/s42770-022-00898-9>
- Costa, R. A., Casaux, M. L., Caffarena, R. D., Macias-Rioseco, M., Schild, C. O., Fraga, M., Riet-Correa, F., & Giannitti, F. (2018). Urocystitis and ureteritis in holstein calves with septicaemia caused by *Salmonella enterica* serotype Dublin. *Journal of Comparative Pathology*, *164*, 32-36. <https://doi.org/10.1016/j.jcpa.2018.08.005>
- de Aguiar, A., Di Santis, G. W., Müller, M. C., Baptista, A. A. S., dos Santos, B. Q., Lisboa, J. A. N., & Bracarense, A. P. F. R. L. (2021). Salmonellosis in calves by serovar Dublin in Paraná State, Brazil-clinicopathological aspects. *Acta Scientiae Veterinariae*, *49*. <https://doi.org/10.22456/1679-9216.113852>
- Evans, S. J., & Davies, R. H. (1996). Case control study of multiple-resistant *S. typhimurium* DT1 04 infection of cattle in Great Britain. *Veterinary Record*, *139*, 557-557. <https://doi.org/10.1002/vetr.00100054>
- Hadimli, H. H., Sayın, Z., & Erganiş, O. (2011). Buzağılarda *Salmonella* Dublin enfeksiyonu ve otojen aşı uygulaması ile kontrolü. *Eurasian Journal of Veterinary Sciences*, *27*(2), 93-98.
- Jackson, A., Nanton, M. R., O'Donnell, H., Akue, A. D., & McSorley, S. J. (2010). Innate immune activation during *Salmonella* infection initiates extramedullary erythropoiesis and splenomegaly. *The Journal of Immunology*, *185*(10), 6198-6204. <http://doi.org/10.4049/jimmunol.1001198>
- Jones, B. D., & Falkow, S. (1996). Salmonellosis: host immune responses and bacterial virulence determinants. *Annual Review of Immunology*, *14*(1), 533-561. <https://doi.org/10.1146/annurev.immunol.14.1.533>
- Mohler, V. L., Izzo, M. M., & House, J. K. (2009). Salmonella in calves. *Veterinary clinics of North America: Food animal practice*, *25*(1), 37-54. <https://doi.org/10.1016/j.cvfa.2008.10.009>
- Molossi, F. A., Cecco, B. S. D., Henker, L. C., Vargas, T. P., Lorenzetti, M. P., Bianchi, M. V., Lorenzo, C. D., Sonne, L., Driemeir, D., & Pavarini, S. P. (2021). Epidemiological and pathological aspects of salmonellosis in cattle in southern Brazil. *Ciência Rural*, *51*. <https://doi.org/10.1590/0103-8478cr20200459>
- Pecoraro, H. L., Thompson, B., & Duhamel, G. E. (2017). Histopathology case definition of naturally acquired *Salmonella enterica* serovar Dublin infection in young Holstein cattle in the northeastern United States. *Journal of Veterinary Diagnostic Investigation*, *29*(6), 860-864. <https://doi.org/10.1177/1040638717712757>
- Ramachandran, S., Godfrey, J. J., & Perera, M. V. (1974). Typhoid hepatitis. *Journal of the American Medical Association*, *230*(2), 236-240. doi:10.1001/jama.1974.03240020026016
- Rosche, K. L., Aljasham, A. T., Kipfer, J. N., Piatkowski, B. T., & Konjufca, V. (2015). Infection with *Salmonella enterica* serovar Typhimurium leads to increased proportions of F4/80+ red pulp macrophages and decreased proportions of B and T lymphocytes in the spleen. *PLoS One*, *10*(6), e0130092. <https://doi.org/10.1371/journal.pone.0130092>
- Smith, B. P. (2009). Salmonellosis in ruminants. In: Smith, B. P. (Ed.), *Large Animal Internal Medicine* (4<sup>th</sup> ed.).
- Varma, J. K., Marcus, R., Stenzel, S. A., Hanna, S. S., Gettner, S., Anderson, B. J., Hayes, T., Shiferaw, B., Crume, T. L., Joyce, K., Fullerton, K. E., Voetsch, A. C., & Angulo, F. J. (2006). Highly resistant *Salmonella* Newport- MDRampC transmitted through the domestic US food supply: a FoodNet casecontrol study of sporadic *Salmonella* Newport infections, 2002–2003. *The Journal of Infectious Diseases*, *194*(2), 222–230. <https://doi.org/10.1086/505084>
- Wray, C., & Davies, R. (2000). *Salmonella* infections in cattle. In: Wray, C., Wray, W. (Ed.), *Salmonella in domestic animals* (1<sup>st</sup> ed., pp. 169-190). New York, CABI Publishing.