Dicle Üniversitesi Veteriner Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/duvetfd
Araştırma Makalesi/Research Article
ISSN:1307-9972 Dicle Üniv Vet Fak Derg 2024;17(1):41-45 e-ISSN:1308-0679
DOI: 10.47027/duvetfd.1427384

Prevalence of *Salmonella* spp. in Neonatal Calves with Acute Diarrhoea: A Study in Şanlıurfa Province, Southeastern Anatolia, Türkiye

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Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
29.01.2024	13.05.2024	30.06.2024

Abstract

Diarrhoea is one of the most important health problems in neonatal calves. Salmonellosis, one of the causes of diarrhoea in neonatal calves, continues to be one of the major health problems worldwide. Prevalence data on enteropathogens can provide basic information for control and prevention strategies. The aim of this study was to determine the prevalence of *Salmonella* spp. in neonatal calves with acute diarrhoea in Şanlıurfa province located in the Southeastern Anatolia Region of Türkiye. The animal material of the study consisted of 100 neonatal calves with acute diarrhoea aged 1-28 days. The presence of *Salmonella* spp. was determined by PCR method from rectal fecal samples taken from the calves included in the study. As a result of the study, 5% prevalence of *Salmonella* spp. was determined. This study showed that there is a potential risk of zoonotic *Salmonella* spp. infection in neonatal calves with acute diarrhoea in the region and an original contribution was made to determine the prevalence of *Salmonella* spp. in Türkiye. It was emphasized that treatment for the causative agent and necessary protective measures should be taken in the region. In addition, further studies with multifocal serotyping in human and animal populations living in this region and other regions of Türkiye are needed in terms of the epidemiology of zoonotic *Salmonella* spp.

Key Words: Calf, PCR, prevalence, *Salmonella*, Türkiye

Akut İshalli Neonatal Buzağılarda *Salmonella* spp. Prevalansı: Şanlıurfa İli, Güneydoğu Anadolu, Türkiye'de Bir Araştırma

Öz

İshal, neonatal buzağıların en önemli sağlık problemlerinden birisi olarak görülmektedir. Neonatal buzağılarda ishal nedenlerinden biri olan Salmonellozis, dünya çapında önemli sağlık problemlerinden biri olmaya devam etmektedir. Enteropatojenlere ilişkin prevalans verileri, kontrol ve koruma stratejileri için temel bilgi sağlayabilmektedir. Bu araştırmanın amacı, Tükiye'nin Güneydoğu Anadolu Bölgesinde yer alan Şanlıurfa ili yöresindeki akut ishalli neonatal buzağılarda *Salmonella* spp.'nin seroprevalansının belirlenmesidir. Araştırmanın hayvan materyalini 100 adet 1-28 günlük yaşta bulunan akut ishalli neonatal buzağı oluşturmuştur. Araştırmaya dahil edilen buzağılardan alınan rektal dışkı örneklerinden PZR yöntemi ile *Salmonella* spp. etken varlığı belirlenmiştir. Araştırma sonucunda %5 *Salmonella* spp. prevalansı tespit edilmiştir. Bu araştırma, bölgede bulunan akut ishalli neonatal buzağılarda potansiyel olarak zoonotik *Salmonella* spp. enfeksiyonu riskinin varlığı göstermiştir ve Türkiye'nin *Salmonella* spp. prevalansının belirlenmesine yönelik özgün bir katkı yapılmıştır. Bölgede etkene yönelik tedavinin ve gerekli koruma tedbirlerin alınması gerektiği vurgulanmıştır. Ayrıca, *Salmonella* spp.'nin zoonotik epidemiyolojisi açısından bu bölgede ve Türkiye'nin diğer bölgelerinde yaşayan insan ve hayvan popülasyonlarında çok odaklı serotipleme ile daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Buzağı, prevalans, PZR, *Salmonella*, Türkiye

INTRODUCTION

Neonatal calf diarrhoea is a gastrointestinal disease that can be fatal due to acidosis and hypovolemia in neonatal calves. It stands out as one of the most critical issues, leading to economic and production losses (1-3). Neonatal calf diarrhoea mortality, contributing significantly to high mortality rates, is reported to be 14% in calves younger than 3 weeks and 23% in calves older than 3 weeks in the United Nations (4).

Neonatal calf diarrhoea is a disease caused by infectious agents and non-infectious factors (managerial, host factor, nutritional, and environmental factors) (5,6), the prominent etiological factors are bacterial (*Cl. perfringens, E. coli*), viral (*Coronavirus, Rotavirus*) and parasitic (*Cryptosporidium parvum, Eimeria* spp.*, Giardia* spp.) (7). Apart from the prominent infectious causes, *Salmonella* spp., *Adenovirus*, Bovine Viral Diarrhoea Virus, *Torovirus*, *Calicivirus*, *Nebovirus*, *Norovirus* and *Candida* spp. are also reported to cause neonatal calf diarrhoea (1,2,8,9).

Salmonella spp. are gram-negative aerobic bacteria belonging to the *Enterobacteriaceae* family (10). Possessing zoonotic importance, *Salmonella* spp. can infect cattle of any age, with a higher frequency reported in calves aged 4-28 days (11). Calves suffering from salmonellosis exhibit hyperthermia, weakness, anorexia, and diarrhoea containing blood and mucus (12,13). Globally, salmonellosis in calves remains one of the most significant health problems, primarily due to calf mortality, weakness or lack of development, and the risk of transmission to humans (10,14).

Prevalence data on enteropathogens are crucial for understanding the interactions between etiological agents, hosts, and environmental factors. These data also provide essential information for developing control and protection strategies. Hence, prevalence studies are highly significant for comprehending the dynamics of pathogens in calves. Prevalence data of *Salmonella* spp. in Türkiye is not sufficiently available in calves. Although there are prevalence studies in Türkiye (15-17), these reports often cover the entire country, and it is noteworthy that the number of calves used in the studies may be insufficient to represent the entire country. Given Türkiye's diverse geographical and environmental conditions, it is crucial to determine the prevalence in different regions. Therefore, the aim of this study was to determine the prevalence of *Salmonella* spp. in neonatal calves with acute diarrhoea in Şanlıurfa province, located in the Southeastern Anatolia Region of Türkiye.

MATERIAL AND METHODS

Ethics Committee Approval

This study received approval from the Harran University Animal Experiments Local Ethics Committee, Session No. 2022/002, Decision No. 01-13.

Animal Material and Sample Collection

The study included 100 Holstein and Simmental breed calves in the neonatal period (1-28 days of age) brought to Harran University Faculty of Veterinary Medicine, Animal Hospital, Department of Internal Medicine, due to diarrhoea complaint from August 2022 to June 2023. Fecal samples, obtained through rectal massage and/or spontaneous defecation, were subjected to PCR analysis for detecting *Salmonella* spp.

Inclusion/Exclusion Criteria

Calves that had previously undergone medical interventions for diarrhoea (such as fluid therapy, antibiotics, vitamin-mineral supplements, and positive inotrope applications; epinephrine, digoxin, dopamine, dobutamine, etc.), which could potentially impact etiological determinations, were excluded from the study.

Reference Strain, Bacterial Isolation, and Genus Level Identification of *Salmonella* spp.

Salmonella Typhimurium ATCC 14028 strain, sourced from Harran University Faculty of Veterinary Medicine, Department of Microbiology, Diagnostic Laboratory, was utilized as the reference strain in this study's analyses.

Fecal samples, collected under aseptic conditions and stored at -80 °C until analyses, underwent thawing for *Salmonella* spp. detection in the genus level. For pre-enrichment, a 25 g sample was homogenized in 225 ml Buffered Peptone Water and incubated at 37°C for 18-20 hours in aerobic conditions. Subsequently, 100 µl of these homogenates were inoculated into tubes containing 10 ml Tetrathionate Brilliant Green Broth and incubated at 42°C for 24 hours for selective enrichment under aerobic conditions. Post-incubation, 250 µl of all cultures were transferred to sterile, RNase and DNase-free microcentrifuge tubes.

The boiling method was used for DNA isolation. DNAs obtained from the boiling method were used in PCRs for the identification of *Salmonella* agents at the genus level. For this purpose, the method used by Rahn et al. (18) was modified and used in this study. Thus, the genus-specific primers 139 (5'- GTG AAA TTA TCG CCA CGT TCG GGC AA-3') and 141 (5'- TCA TCG CAC CGT CAA AGG AAC C-3'), which form a 284 bp PCR band specific for the *invA* gene region of Salmonella bacteria, were used in the PCRs of Cohen et al. (19) primers Fim1A (5'- CCT TTC TTC TCC ATC GTC CTG AA -3') and Fim2A (5'- TGG TGT TAT CTG CCT GAC CA -3') primers, which generate an 85 bp PCR band specific to the *fimA* gene region of *Salmonella* bacteria, were used together and as a result of the PCR analysis. Positivity for *Salmonella* spp. was determined by the simultaneous detection of 284 bp and 85 bp PCR products.

RESULTS

According to the results of PCR analyses (Figure 1), positivity for *Salmonella* spp. was determined in 5 (5%) out of 100 neonatal calves with acute diarrhoea. Demographic information of calves with salmonellosis is summarized in Table 1.

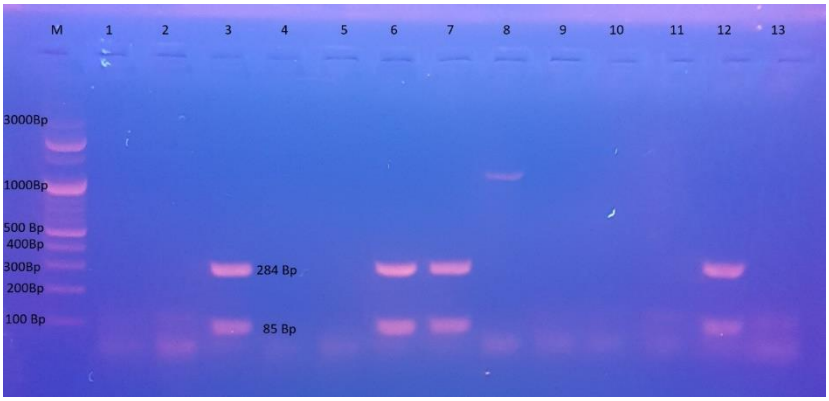

Figure 1. M: 100 bp Marker, Line 3: Positive Control (*Salmonella* Typhimurium ATCC 14028), Line 5: Negative control (water), Line 6: Case no 1, Line 7: Case no 2, Line 12: Case no 3, Line 1, 2, 4, 8, 9, 10, 11 and 13: Some of the negative samples included in the study.

Table 1. Demographic information of positive cases in the study

Case Number	Sex (M/F)*	Age (Day)	Breed (H/S)**
1	F	3	S
2	M	25	H
3	F	10	H
4	F	20	H
5	F	7	H

*M: Male, F: Female, **H: Holstein, S: Simmental

When the diversity of farms where seropositive cases were brought was evaluated, it was determined that Cases 3 and 5 were brought to the hospital from the same farm at different times, and the other positive cases were brought to the hospital from different farms in Şanlıurfa province. The mean age of the cases was determined 13 days. Out of 5 cases, 4 were female and 1 was male, 4 were Holstein and 1 was Simmental breed.

DISCUSSION AND CONCLUSION

Diarrhoea stands out as a major health concern in neonatal calves, with implications for economic losses (2,3). Rapid identification of the etiology of calf diarrhoea is crucial during this period to mitigate both direct and indirect economic losses through effective treatment strategies (20). The findings of our study revealed a 5% prevalence of *Salmonella* spp. in neonatal calves with acute diarrhoea in Şanlıurfa province, Türkiye. This result holds significance in understanding the etiological factors contributing to diarrhoea in calves within this region, recognizing potential zoonotic conditions, and formulating effective control strategies.

Salmonellosis in cattle is associated with significant production loss and potential transmission to other hosts (12,13). Prevalence rates may vary based on factors such as the animals' age, study design, diagnostic methods, geographical location, climate, and management practices (11,21-23). Salmonellosis may occur at any age in calves. However, the highest prevalence of *Salmonella* spp. is reported at the age between 2 - 28 days (11). Therefore, young animals may be considered as the major source of infection. In the present study, a total of 100 calves 1-28 days old dairy calves were enrolled.

Global prevalence rates of *Salmonella* spp. in calves vary widely, ranging from 2% to 33%. Noteworthy rates include 4.09% to 18.1% in Egypt (24-26), 2% in Mozambique (27), 10.6% in India (28), 23.8% in Australia (29), 8.8% to 33% in Bangladesh (30,31), 16.4% in Brazil (32), 8.33% in Nigeria (33), 3.8% in Uruguay (34), and 3.2% to 7.5% in Türkiye (15-17). However, there is a lack of data on the prevalence and spatial distribution of salmonellosis in calves in the Southeastern region of Türkiye. Moreover, the findings suggest that the sample size of calves in the studies conducted in Türkiye may not adequately represent the broader population of the country, compared with the other studies reported in the paragraph.

There is only one regional study conducted in Türkiye. İstanbul is a city in northwest Türkiye, where the prevalence of *Salmonella* spp. in diarrheic calves was determined as 4.7% (16). When compared with the results of our study, it is seen that similar results may be obtained in different regions of the country. While the results of this study are comparable to ours, it's essential to note that the number of cases (n=21) in the İstanbul study is considerably lower than those in prevalence studies conducted in other countries. This difference may be attributed to the study's aim, which included the prevalence of environmental samples alongside calves with diarrhoea.

Other studies conducted in Türkiye were carried out by evaluating samples collected from various regions of the country (15,17). In a study conducted from 869 fecal samples (437 calves with diarrhoea, 287 cows, 100 buffaloes, and 45 camels) collected from 13 provinces and 25 farms in Türkiye, a prevalence of 7.5% was found in calves (15). The results of the aforementioned study and the prevalence result of our study are similar. The prevalence result of the aforementioned study may be considered to show the prevalence across Türkiye. However, there are various deficiencies in this study because the percentage of calf distribution and calf age information in the provinces where samples were collected were not given. It should be remembered that prevalence rates may vary according to the age of the animals, design of the study, diagnostic techniques, geographical location, climatological conditions, and management practices (11,21-23). The cases included in our study were calves with acute diarrhoea aged 1-28 days. Since the main aim of this study, which determined the prevalence across Türkiye, was to determine the *Salmonella* serotypes and antibiotic susceptibility of the agents in Türkiye, it is natural that there are limitations.

In another study conducted in Türkiye, samples were collected from various regions of the country and various animal species (chicken, lamb, sheep, calf, goose, turkey, starling, gull, parrot, cow, turtle, quail, and fertilizer) with various sampling methods (42.5% drag swabs, 25.1% dust swabs, 21.8% environmental samples, 4.1% organ, 4% fecal, 2.2% slaughterhouse and 0.1% organic fertilizer) between 2015-2020. A prevalence of 3.2% was found as a result of samples collected from 34 calves (17). The prevalence result of the aforementioned study is similar to the prevalence result of our study. However, when the number of calves included in the aforementioned study was compared with the number of calves in other countries, it was observed that the number was quite low. In addition, there is no information on the age of the calves and the country region of containment included in the aforementioned study. The reason for this is thought to be that the aim of the study was to determine the distribution of *Salmonella* serotypes of animal origin in Türkiye.

The present study was conducted in Şanlıurfa, a province of Southeastern Türkiye and the overall prevalence from 1-28 days aged, a total of 100 acute diarrheic neonatal calves was 5%. There are some limitations in this research. In this study, only calves with acute diarrhoea were used and healthy calves were not included. It should be kept in mind that *Salmonella* spp. can be found as asymptomatic carriers in calves (35). Therefore, the presence of *Salmonella* spp. in healthy calves is possible. However, it is not known how the

inclusion of healthy calves in the study will change the prevalence of the study. Another limitation is that the determination of *Salmonella* spp. serotypes was not included in the study. In the studies conducted in Türkiye, the most common serotypes were 57.58% *S.* Kentucky (15), 4.7% *S.* Kottbus (16), and 52.9% *S.* Montevideo (17). The higher prevalence of different serotypes in different studies in Türkiye has been interpreted by us as the prevalence of serotypes in the country varies according to regions. In this study, *Salmonella* spp. serotypes were not investigated. It was aimed to determine the prevalence of *Salmonella* spp. that the prevalence information was unknown in the region. However, by adding the determination of serotypes to the study, it would have been possible to determine the prevalence rates of zoonotic potential and host-specific serotypes with the determination of serotype distribution in the region. Therefore, it is thought that there is a need for more regional prevalence studies in terms of *Salmonella* spp. in Türkiye and it is significant to determine the prevalence of serotypes while carrying out these studies. In this way, zoonotic risk and host-specific serotype distribution in the region can be determined and appropriate treatment and control strategies can be developed.

In conclusion, the present study was conducted in Şanlıurfa, a province of Southeastern Türkiye and the prevalence from 1-28 days aged, a total of 100 acute diarrheic neonatal calves was 5%. The presence of the potentially zoonotic risk of *Salmonella* infection in acute diarrheic neonatal calves indicated the importance of treatment and necessary preventative measures. Further studies with multilocus serotyping in human and animal populations living in this region and other regions of Türkiye are warranted regarding the epidemiology of zoonotic *Salmonella* spp.

ACKNOWLEDGMENT

This research has been supported within the content of the project no 22093 by Harran University Scientific Research Projects Department.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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