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Meningoencephalitic Listeriosis in Iranian Sheep and Goats

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ABSTRACT

Background: Meningoencephalitic listeriosis is a significant disease affecting goats and sheep, with potential implications for public health. This study aimed to investigate and report the occurrence, clinical manifestation, laboratory findings, and further understanding of neurolisteriosis in the goat and sheep populations in Tehran, Qazvin, and Kurdistan provinces of Iran.

Methods: From 2018 to 2021, a program was implemented to monitor multiple flocks for meningoencephalitic listeriosis. Clinical manifestations of animals that showed suspected neurological signs of listeriosis (19 animals) were recorded, and necropsies were performed. Sampling of the brain tissue of infected animals was done. The specimens underwent a 'cold-enrichment' procedure, and subsequent culture and biochemical tests were conducted to confirm the presence of *Listeria monocytogenes*.

Results: According to the clinical and bacteriological findings, 19 animals were diagnosed with neurolisteriosis. The affected animals exhibited various signs of the disease, including head deviation, head tilt, head pressing, mouthfuls, walking in circles, drooping of the ear, and unilateral hypalgesia. *L. monocytogenes* was isolated from brain samples of 11 animals.

Conclusion: The study revealed potential indications of meningoencephalitic listeriosis in certain breeds of goats and sheep. Recognizing these typical signs can contribute to early detection, diagnosis, and proper management of listeriosis cases, improving welfare and health outcomes for affected animals. The results underscore the importance of strengthened disease surveillance, prevention, and control strategies.

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Introduction

Meningoencephalitic listeriosis, also known as neurolisteriosis or circling disease, is a neurologic disease of ruminants, particularly small ruminants such as goats and sheep (1-3). This disease is caused by *Listeria monocytogenes* or rarely by *Listeria ivanoii* (4, 5). Although *L. monocytogenes* causes several other disorders in ruminants, including abortion with placentitis in the last trimester, stillbirth, septicemia, mastitis, and possibly ophthalmitis, these are usually not associated with meningoencephalitis and only one manifestation of the disease is observed (1, 3, 6, 7).

The Gram-positive mentioned above bacterium is ubiquitous in the environment of farm animals and can be shed in feces and many body fluids by both affected animals and seemingly healthy carrier animals (2, 5-7). After entering the body, *L. monocytogenes* moves to the brain stem through the axons of the cranial nerves and causes meningoencephalitis (8). Numerous small abscesses and inflammation in the brain cause multiple areas of necrosis and vasculitis in the blood vessels. As a result, various signs of dysfunction in the brainstem occur, which are multifocal and asymmetrically distributed (9, 10).

In a 2011 study by Sakhaee et al., listeriosis was diagnosed in a one-month-old male goat in a herd of 20 sheep and goats in the Kerman province, Iran. This kid exhibited signs indicative of meningoencephalitic Listeriosis, such as severe depression, mild epistotonus, lack of coordination, head deviation, circular walking patterns, dyspnea, ataxia, high body temperature, and anorexia (11). In a study by Oevermann et al., brain lesions in a small ruminant population in Switzerland were examined. The study revealed that encephalitic listeriosis was the dominant cause of central nervous system (CNS) lesions in goats and sheep. The prevalence of encephalitic listeriosis was unexpectedly high compared to other confirmed cases of CNS lesions in the same population. This study highlights the importance of active surveillance and thorough investigation of CNS lesions in small ruminants to assess the prevalence and impact of listeriosis accurately. By recognizing the true extent of listeriosis cases, appropriate measures can be taken to improve disease management and control strategies (12).

Meningoencephalitic listeriosis can be a health concern in small ruminants and endangers animal welfare in addition to economic losses. Even though few studies have been conducted in Iran on the epidemiology of outbreaks, case reports, and transmission through milk and other traditional dairy products or meat of small ruminants, To date, there has been no investigation specifically focusing on cases of meningoencephalitic listeriosis in goat and sheep herds across multiple provinces. In addition, studies on the prevalence of listeriosis in developing countries are necessary to identify the exact status of the disease worldwide (3, 13). Neurological listeriosis cases in Iran have been sparsely reported. In order to address this research gap and gain a more comprehensive understanding of this particular form of the disease, the current study focuses on investigating the occurrence, clinical features, and laboratory findings related to meningoencephalitic listeriosis in goats and sheep from Tehran, Qazvin, and Kurdistan provinces.

Materials and Methods

Animals

During the period from 2018 to 2021, several sheep and goat herds (with different breeds) in Tehran, Qazvin, and Kurdistan provinces were monitored for the incidence of listeriosis. There was no particular problem regarding the spread of the disease in the flocks under investigation. Foot-and-mouth disease and Enterotoxemia vaccines had been injected for flocks according to the instructions of the veterinary organization. In general, three ewes and two rams of the Île-de-France breed, five Alpine breed goats, six Shall

breed sheep, and three Kurdish breed ewes showed possible signs of listeriosis. The affected animals were above the age of two years.

Clinical and Necropsy Examinations

The affected goats and sheep were carefully observed and examined to document their clinical signs. In cases where animals had died or were slaughtered, necropsies were conducted following standard procedures. Additionally, cerebrospinal fluid (CSF) samples were collected from four animals and examined for fibrin clots, characteristic features of listeriosis. Given the signs consistent with the neurological form of listeriosis, small tissue samples from the medulla and pons of the brain were collected to isolate the causative organism (14). These sample collection methods were employed to investigate the presence of *L. monocytogenes*, the bacteria responsible for listeriosis. The isolation of the organism from the collected tissues would provide definitive evidence linking the observed clinical signs to the presence of *L. monocytogenes* infection.

Bacteriology Investigations

To isolate and confirm the presence of *L. monocytogenes*, the obtained brain tissue samples underwent a 'cold-enrichment' procedure. The tissue pieces were homogenized, and a 10% suspension was prepared in a nutrient broth. This suspension was then refrigerated at 4 °C. Twice four days apart, the suspension was inoculated onto blood agar and *Listeria* selective agar (14, 15). To further confirm the presence of *L. monocytogenes*, motility, hemolysis, and biochemical tests such as the esculin test, methyl red, Voges–Proskauer, indole, citrate, and catalase test were conducted. In addition to these tests, the hanging-drop method was employed to culture a fresh broth sample, which was incubated at 25 °C for 2 to 4 hours.

Result

Clinical and Necropsy Findings

Affected animals showed depression, lagging behind the flock, poor appetite, and reduced milk production. Fever was observed in all animals. In addition to the mentioned signs, incoordination, head deviation, sometimes with head tilt (Figure 1a), head pressing, walking in circles, and mouthfuls (Figure 1b) were observed. With the passage of time and the progression of signs, unilateral facial hypalgesia and unilateral facial paralysis were observed (Figure 1c). Facial hypalgesia was detected by applying pressure, and facial paralysis was manifested by drooping of the ear, paralysis of the lips, and ptosis on the same side of the face as the hypalgesia. Paresis of the jaw muscle, with poor tone or dropped jaw, was observed, in which case prehension and mastication were slow. Loss of lip and cheek muscle tone was best detected by observation of drooling of saliva from the ipsilateral side of the mouth and by palpation of the lips and nostrils.

Animals with advanced disease signs stood for a while with drooling saliva and food hanging. Finally, following the paralysis of the body, the grounding occurred, and death occurred due to dehydration and starvation. The position of the heads was different; in three cases, the head to one side with the poll-nose relationship undisturbed without rotation, and in five cases, the head tilt was visible. Animals were not able to actively correct the head deviation. Unilateral blindness was observed in seven animals. Ataxia was evident, and goats and sheep leaned against the examiner or fence. Mild meningeal congestion (in three animals), hyperemia of the meninges (in one animal), and opacity of the meninges (in five animals) were observed as gross changes in the brains of animals. In other animals, necropsy did not show significant gross lesions. A fibrin clot was observed in the CSF sample of 3 animals after 30 minutes. The clinical signs observed confirmed

the these small animals being affected by nervous listeriosis.

Bacteriological Confirmation

L. monocytogenes was detected in brain tissue samples obtained from 11 animals. After 24 hours of incubation onto a blood agar medium, tiny translucent colonies measuring approximately 1 to 2 mm in diameter were observed. These colonies displayed well-defined edges and subsequently changed in color to a grayish-white shade within the following 24-hour period. After subculturing the sample onto *Listeria* selective agar and subsequent aerobic incubation for 24 hours, small colonies with a grayish-yellow appearance and a diameter of approximately 1-2 millimeters were identified. Microscopically, organisms had coccobacillary and Gram-positive morphology. The isolates exhibited hemolytic activity and generated a narrow zone of hemolysis on sheep blood agar. All isolates hydrolyzed esculin broth and were positive for catalase test, methyl red, and Voges–Proskauer, but indole and citrate were negative. 'tumbling motility' was observed in the hanging-drop test. The results confirmed the presence of *L. monocytogenes* in the brain tissue of sheep and goats suffering from encephalitic Listeriosis.

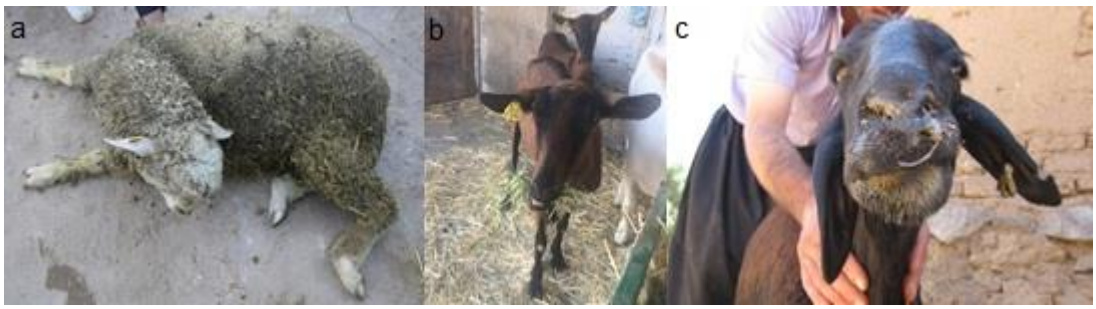

Figure 1. **a**: Unilateral head deviation in a sheep with meningoencephalitic listeriosis, **b**: Sign of "mouthfuls" in an Alpine goat suffering from meningoencephalitic listeriosis, **c**: an animal with meningoencephalitic listeriosis manifests unilateral facial hypalgesia and unilateral facial paralysis.

Discussion

In the current investigation, 19 goats and sheep were diagnosed with meningoencephalitis caused by listeriosis based on clinical and bacteriological findings. It is worth noting that the septicemic form of listeriosis is more commonly observed in young lambs and goat kids under five weeks of age. In contrast, the meningoencephalitis form is typically seen in older animals (3). This study revealed a higher incidence of meningo-encephalitic listeriosis in older animals, all over two years of age. The cases of meningo-encephalitis observed in Tehran, Qazvin, and Kurdistan provinces were characterized by their sporadic nature. These cases occurred in isolated incidents rather than being widespread or occurring in clusters. Listeriosis can manifest as sporadic or epidemic cases globally, with outbreaks leading to significant and severe damage (3). The observed clinical signs in this study were consistent with previous reports of meningoencephalitic listeriosis. Notably, the nervous and ocular signs were predominantly unilateral, which can be attributed to the unilateral spread of infection along the trigeminal nerve (16).

The presence of a fibrin clot in the CSF sample, indicating the presence of an inflammatory lesion in the CNS (17), further confirmed the diagnosis of meningoencephalitis. Overall, this investigation provides valuable insights into the occurrence and characteristics of meningoencephalitic listeriosis in goats and sheep in Iran, contributing to our understanding of this disease.

In a 2020 report, a case of meningoencephalitic listeriosis was diagnosed in an 18-month-old ram in Tehran city, Tehran province. The observed clinical signs included head deviation and neck rotation to the left, unilateral blindness, walking in circles, and convulsions. These clinical signs are consistent with the findings of the present study. Similar to the current study, *L. monocytogenes* was isolated from the brain tissue of the ram using the same isolation method (17). Isolation and identification of the causative organism through bacterial cultures are essential for the definitive diagnosis of listeriosis (16). In the present study, isolating *L. monocytogenes* from the brain tissue through culture in conjunction with clinical observations was a crucial and definitive outcome (16, 18). In another study conducted in 2020, a neurological listeriosis outbreak was reported in the Indian state of Punjab, involving 90 sheep and 16 goats. Clinical examination revealed fever, circling movement, unilateral blindness, facial paralysis with hanging of the jaw, drooling of saliva, and dropping of the tongue. Encephalitic listeriosis was confirmed in this study (18).

A study investigated the occurrence, pathology, and bacteriology of meningoencephalitic listeriosis in goats and sheep in northern Oman from 2017 to 2019. Similar to the present study, no specific necropsy signs were observed in all animals, and brain tissue samples were taken. The researchers documented clinical signs such as fever, loss of appetite, neck deviation, and circling. Microscopic examination revealed the presence of microabscesses, hemorrhages, and perivascular cuffs in the hindbrain. Additionally, positive immunostaining of intracellular *L. monocytogenes* antigens was observed in the mononuclear and polymorphonuclear cells of microabscesses and perivascular cuffs. The study emphasized the potential public health threat posed by *L. monocytogenes* (19). Headley et al. conducted a study in 2013 where they reported on the clinical, pathological, and molecular observations of encephalitic listeriosis in a male mixed-breed goat and a 3-year-old Texel sheep from northern Paraná, Brazil. The clinical signs observed in these cases were highly similar to those observed in the present study involving meningoencephalitis. Both animals exhibited signs such as circling, lateral tongue protrusion, head tilt, convulsions, ataxia, motor incoordination, and lateral recumbency. During necropsy, no significant gross lesions were observed in either animal. However, histopathological studies confirmed the presence of meningoencephalitis (7).

In the study by Barman et al., which took place from November 2016 to October 2017, nine suspected cases of listeriosis in goats with neurological signs were documented in peri-urban areas of Assam, India. Through bacteriological tests and clinical investigations, five positive isolates of *L. monocytogenes* were obtained from the suspected samples. The observed clinical signs in the affected goats, such as walking in circles, head tilt, fever, and terminal recumbency, indicated meningoencephalitis, an inflammation of the brain and meninges commonly caused by *L. monocytogenes* infection. The isolation and identification methods used in the study by Barman et al. were consistent with those employed in the current study, and the results obtained align with their findings. Additionally, Barman et al. also found positive cases of nervous listeriosis in older animals (20). Isolating *L. monocytogenes* from brain tissue is challenging, just as many studies have been unable to isolate the pathogen from all infected animals with manifestations of meningoencephalitis. Furthermore, the limited presence of *L. monocytogenes* in brain tissue decreases the chances of successful isolation.

According to the study conducted by Dreyer et al. in 2015, *L. monocytogenes* was successfully isolated solely from the brain samples of three out of seven cases of meningoencephalitis form of listeriosis in adult sheep (21).

Given the unique environmental conditions, climate, and breed diversity in the Provinces of Iran, it is essential to research the occurrence of meningoencephalitis caused by *Listeria* infection. While extensive studies have been conducted on *Listeria* infection in regions like Europe, North America, and Australia, understanding the clinical and laboratory characteristics of the disease in Iran will provide valuable insights due to its specific context. Various factors such as temperature, humidity, flock size, management practices, feeding regimens, and other infectious agents can significantly influence the frequency and severity of meningoencephalitis. Investigating these factors in the Provinces of Iran will contribute to a deeper understanding of the disease and aid in developing targeted prevention and control strategies tailored to this region. *L. monocytogenes* is a zoonotic food-borne pathogen with significant health and economic risks. In recent years, there has been an increase in reported outbreaks and sporadic cases associated with the consumption of contaminated meat and meat products containing *L. monocytogenes*, particularly in developing countries (16, 22). This matter highlights the importance of investigating the occurrence of this common disease in both humans and animals, not only in Some provinces of Iran but throughout Iran. By studying the specific occurrences and risk factors of meningoencephalitis caused by *Listeria* infection, we can contribute to the overall understanding of the disease and take appropriate measures to prevent its transmission and mitigate its impact on human and animal health (22).

The control of listeriosis poses challenges due to the widespread presence of *Listeria* bacteria in the environment, the absence of a straightforward identification method, and the limited understanding of risk factors apart from silage (3). Given the significance of *L. monocytogenes* infections in food animal species and their potential risks to public health (20), it is crucial to comprehend the incidence rate and factors influencing the emergence, clinical signs, and spread of meningoencephalitic listeriosis in Iran. This understanding is essential for the development of effective prevention and control strategies. By investigating the prevalence of *Listeria* infections and the clinical manifestations associated with meningoencephalitic listeriosis, this study aims to provide valuable insights into the epidemiology of the disease in goats and sheep populations in the region. By shedding light on the local epidemiological picture and the challenges involved, we can work towards improving disease surveillance, prevention, and control measures, ultimately safeguarding the health and welfare of goats and sheep in Iran. In developing countries, factors such as lack of awareness, diagnosis, and robust surveillance systems contribute to listeriosis outbreaks. Addressing these factors is crucial for mitigating the impact of the disease and implementing effective control measures (23). By conducting this study and raising awareness about listeriosis in Iran, we can improve the knowledge and practices surrounding the disease, thus minimizing its occurrence and impact on animal and human health.

Conclusion

Meningoencephalitic listeriosis poses remarable challenges when it comes to diagnosing it. The current study serves as an prognosis to clinicians, presenting crucial information regarding the clinical manifestations, necropsy findings, and laboratory diagnosis of this disease. Accurate and timely diagnosis of this disease plays a vital role in effectively managing it within breeding flocks. It enables the implementation of preventive measures and contributes to the overall improvement of animal health and welfare.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Morin DE. Brainstem and cranial nerve abnormalities: listeriosis, otitis media/interna, and pituitary abscess syndrome. *Vet Clin N Am Food Anim Pract* 2004; **20**(2):243-73.
2. Kennedy S, Passler T, Stockler J, et al. Risk factors associated with outcome in goats with encephalitic listeriosis: A retrospective study of 36 cases from 2008 to 2021. *JVIM* 2023; **37**(3):1271-7.
3. Saminathan M, Rana R, Ramakrishnan MA, et al. Prevalence, diagnosis, management and control of important diseases of ruminants with special reference to Indian scenario. *J Exp Biol Agric Sci* 2016; **4**(3S):3338-67.
4. Zundel E, Bernard S. *Listeria monocytogenes* translocates throughout the digestive tract in asymptomatic sheep. *J Med Microbiol* 2006; **55**(12):1717-23.
5. Ryser ET, Marth EH. *Listeria*, Listeriosis, and Food Safety. 3 ed: CRC Press; 2007. 873 p.
6. Rodriguez C, Taminiau B, García-Fuentes E, et al. *Listeria monocytogenes* dissemination in farming and primary production: Sources, shedding and control measures. *Food Control* 2021; **120**:107540.
7. Headley SA, Bodnar L, Fritzen JT, et al. Histopathological and molecular characterization of encephalitic listeriosis in small ruminants from northern Paraná, Brazil. *Braz J Microbiol* 2013; **44**:889-96.
8. Henke D, Rupp S, Gaschen V, et al. *Listeria monocytogenes* spreads within the brain by actin-based intra-axonal migration. *Infection and immunity* 2015; **83**(6):2409-19.
9. Oevermann A, Di Palma S, Doherr MG, et al. Neuropathogenesis of naturally occurring encephalitis caused by *Listeria monocytogenes* in ruminants. *Brain pathology* 2010; **20**(2):378-90.
10. Dhama K, Karthik K, Tiwari R, et al. Listeriosis in animals, its public health significance (food-borne zoonosis) and advances in diagnosis and control: a comprehensive review. *Veterinary Quarterly* 2015; **35**(4):211-35.
11. Sakhaee E, Pour RG, Azari O. A Case report of listeric septicemia in goat. *JUCMS* 2011:59.
12. Oevermann A, Botteron C, Seuberlich T, et al. Neuropathological survey of fallen stock: active surveillance reveals high prevalence of encephalitic listeriosis in small ruminants. *Vet Microbiol* 2008; **130**(3-4):320-9.
13. de Noordhout CM, Devleesschauwer B, Angulo FJ, et al. The global burden of listeriosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2014; **14**(11):1073-82.
14. Fentahun T, Fresebehat A. Listeriosis in small ruminants: a review. *Adv Biol Res* 2012; **6**(6):202-9.
15. Markey B, Leonard F, Archambault M, et al. Clinical veterinary microbiology e-book: Elsevier Health Sciences; 2013.
16. Brugère-Picoux J. Ovine listeriosis. *Small Ruminant Research* 2008; **76**(1-2):12-20.
17. Sadeghian Chaleshtori S, Abdollahi M, Shokrpoor S, et al. Case report of concurrent

occurrence of coenurosis and listerial encephalitis in a sheep. *Vet Clin Pathol* 2020; **14**(55):301-14.
18. Mahajan V, Bal MS, Filia G, et al. Diagnosis of encephalitic listerial outbreak in sheep immune-histopathological study. *IJCMAS* 2020; **9**:3235-8.
19. Ali H, Tohamy HG, Al-Hattali R, et al. Encephalitic listeriosis in small ruminants in Oman: pathophysiology, antimicrobial sensitivity, and molecular characterization. *Pak Vet J* 2024; **44**(1):87-92.
20. Barman NN, Nath AJ, Doley S, et al. Listeriosis in a peri-urban area: Cultural and molecular characterization of *Listeria monocytogenes* isolated from encephalitic goats. *Vet World* 2020; **13**(9):1743.
21. Dreyer M, Thomann A, Böttcher S, et al. Outbreak investigation identifies a single *Listeria monocytogenes* strain in sheep with different clinical manifestations, soil and water. *Vet Microbiol* 2015; **179**(1-2):69-75.
22. Matle I, Mbatha KR, Madoroba E. A review of *Listeria monocytogenes* from meat and meat products: epidemiology, virulence factors, antimicrobial resistance and diagnosis. *OJVR* 2020; **87**(1):1-20.
23. Malik S, Barbuddhe S, Chaudhari S. Listeric infections in humans and animals in the Indian subcontinent: a review. *Trop Anim Health Prod* 2002; **34**:359-81.