Original Article

Outbreak of Bluetongue Disease in Zel Sheep Flocks in Mazandaran Province, Iran

Hossein Esmaeili[1*], Seyed Mehdi Joghataei[1], Mohammadreza Ghorani[2]

1. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Use your device to scan and read the article online

How to Cite This Article Esmaeili, H., Joghataei, S. M., & Ghorani, M. (2026). Outbreak of Bluetongue Disease in Zel Sheep Flocks in Mazandaran Province, Iran. *Iranian Journal of Veterinary Medicine, 20*(1), 53-66. http://dx.doi.org/10.32598/ijvm.20.1.1005651

http://dx.doi.org/10.32598/ijvm.20.1.1005651

ABSTRACT

Background: Bluetongue (BT) is a significant arthropod-borne viral disease affecting sheep, with varying clinical manifestations depending on the strain of the virus, the host breed, their immune status, and environmental conditions. While endemic in Iran, few studies have focused on outbreaks, particularly in native breeds.

Objectives: This study aims to provide comprehensive data on the clinical presentation, post-mortem findings, and molecular diagnosis of a BT outbreak in Zel sheep flocks in Mazandaran Province, Iran, to better understand the virus's impact and precognition.

Methods: During the 2018 outbreak, clinical and post-mortem evaluations were conducted on 12 flocks (1800 sheep). Samples, including blood and tissue from both live and dead sheep, were collected and analyzed using reverse transcription polymerase chain reaction (RT-PCR) and nested PCR to confirm BT virus (BTV) presence.

Results: Clinical, necropsy observations, and molecular analysis confirmed BTV infection in both live and dead sheep. The morbidity rate was approximately 13.33%, with a mortality rate of 2.11% across the entire population and 15.83% among the infected sheep. The most common clinical signs observed were high fever (80%), swelling and edema of the lips and face (96.25%), and redness of the buccal and nasal mucosa (87.5%). Nasal discharge and frothy, blood-stained salivation were present in 15% of the infected sheep, while tongue erosion and ulcers affected 22.9%. Necropsies revealed 100% mucosal lesions, hemorrhagic pulmonary artery lesions in 81.6%, and systemic congestion of major organs in 50% of cases. Serous effusions were found in 42%, indicating severe systemic involvement in the dead sheep. These findings align with reports from other regions experiencing BTV outbreaks and suggest that climatic conditions and vector proliferation contributed to the spread of the disease.

Conclusion: This study reaffirms the enzootic nature of BT in Iran and stresses the critical role of clinical, necropsy, and molecular diagnostics in managing BT outbreaks. To minimize future outbreaks and economic losses from BTV's effects on sheep health in the regions, adopting preventive measures such as vaccination programs, serotype monitoring, enhanced surveillance, vector control, and regulation of livestock movement is recommended.

Keywords: Bluetongue virus (BTV), Mazandaran Province, Outbreak, Vector-borne disease, Zel sheep

Article info:
Received: 10 Oct 2024
Accepted: 19 Nov 2024
Publish: 01 Jan 2026

*** Corresponding Author:**
Hossein Esmaeili, Associate Professor.
Address: *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+98 (21) 61117051*
E-mail: *hesmaeli@ut.ac.ir*

Introduction

Bluetongue (BT) is a noncontagious and vector-borne disease caused by the BT virus (BTV), which belongs to the genus *Orbivirus* within the Sedoreoviridae family. BTV primarily affects domestic and wild ruminants, with sheep being the most susceptible species, often displaying severe clinical signs (Constable et al., 2017; Gestier et al., 2023; MacLachlan & Dubovi, 2016; Maclachlan et al., 2015; Sadri, 2012). BT is a remarkable disease affecting sheep, and depending on the virus strain, environmental conditions, the host's breed, and immune status, infected sheep may remain asymptomatic or display a range of clinical signs, from mild to severe, particularly during outbreaks (Jahanroshan et al., 2023; Kirkland et al., 2024; Sailleau et al., 2017). The disease is caused by vascular damage similar to that seen in human hemorrhagic viral fevers, characterized by tissue infarction, hemorrhage, vascular leakage, edema, and hypovolemic shock (Ferrer et al., 2024; Maclachlan et al., 2015). The virus is transmitted by biting midges of the genus *Culicoides*, making its distribution largely dependent on the presence and abundance of these vectors. With over 29 known serotypes, BTV poses substantial economic losses and remarkable challenges for control and prevention, particularly in regions where multiple serotypes co-circulate (Constable et al., 2017; Ralitsa & Gergana, 2023; Subhadra et al., 2023).

In Iran, BT is recognized as an enzootic disease, and numerous seroprevalence studies have been carried out in different regions to assess its presence (Hassani & Madadgar, 2020; Jahanroshan et al., 2023; Khezri & Azimi, 2012; Momta et al., 2011). Iran's diverse climate and variety of sheep breeds (Esmaeili & Joghataei, 2024; Esmaeili et al., 2022; Ghorani & Esmaeili, 2022), each with differing susceptibilities to BTV infection, play a significant role in the epidemiology of BT. Understanding these susceptibilities is crucial for management strategies in high-risk areas. The climatic variability across different regions of Iran provides a conducive environment for the proliferation of *Culicoides* midges (Paquette et al., 2023), the primary vectors responsible for transmitting the BTV (Constable et al., 2017; Subhadra et al., 2023). Regions with warmer, humid climates, such as the northern provinces near the Caspian Sea, are particularly susceptible to higher vector densities, leading to increased transmission rates (Caminade et al., 2019). However, comprehensive studies focusing on clinical observations, necropsy findings, and molecular diagnosis of BTV in Iran are limited. This lack of research is crucial, given the need to distinguish BT from other diseases with similar signs (Jahanroshan et al., 2023).

This study seeks to deliver in-depth information on the clinical signs, post-mortem observations, and molecular diagnosis of a BT outbreak in Zel sheep flocks in Mazandaran Province, Iran. By offering detailed insights into the nature of BTV infections in this specific region and specific breed, the research aims to enhance the understanding of how BTV affects local sheep populations. These findings will be instrumental in shaping future surveillance strategies, guiding control measures, and improving response protocols to mitigate the impact of BT in Mazandaran and other similar regions. Moreover, the data generated from this study will aid veterinarians, researchers, and policymakers in making informed decisions to protect livestock health, ensuring better preparedness for potential future outbreaks.

Materials and Methods

Study area

The study was conducted in 2018 in Mazandaran Province, situated in the northern region of Iran, along the southern shoreline of the Caspian Sea. Mazandaran covers an area of approximately 23842 km^2. It is geographically characterized by a diverse landscape that includes coastal plains, high mountains, and forested regions located at latitudes of 35.6° to 36.7° N and longitudes of 50.6° to 54.8° E.

Mazandaran Province enjoys a moderate, subtropical climate with an average temperature of 25 °C in summer and around 8 °C in winter, and it receives significant rainfall ranging from 700 to 1300 mm annually. The province's climate is shaped by various geographical factors, including its latitude, the Alborz range, elevation from sea level, proximity to the Caspian Sea, the southern barren regions of Turkmenistan, local and regional air currents, and the diverse vegetation cover. These diverse climatic zones illustrate the complex environmental conditions of Mazandaran, which are heavily influenced by the province's topography and geographical positioning, shaping its weather patterns and ecosystems (Goli et al., 2024). Mazandaran is one of Iran's prominent agricultural hubs, known for its fertile land and high productivity. Livestock farming, including sheep rearing, is a vital economic activity in the province, supporting the livelihoods of many rural communities (Chenari et al., 2024; Rashidimehr et al., 2024). The Zel sheep, a local breed well-adapted to the region's environmental conditions, is extensively farmed in Mazandaran. Zel sheep are known for their hardiness and ability to thrive in the varied topography and climate of the province. They are primarily raised for meat production, with wool and milk as secondary products.

Flocks and animals

The study was conducted on 12 flocks of Zel sheep distributed across different rural locations in Mazandaran Province, Iran. Each of these flocks varied in size, containing between 50 and 200 sheep. The study involved 1800 sheep from these flocks, encompassing a range of ages and including both male and female animals. The flocks showed a typical demographic distribution, with lambs, ewes, and rams present. The flocks were not vaccinated against BT.

Case definition, clinical, and necropsy examination

The outbreak, which occurred in June 2018, was initially identified due to the occurrence of deaths in sheep and the presence of clinical signs indicative of BT disease. The affected sheep exhibited a febrile disease accompanied by oral inflammation, leading to a suspicion of BT. The severity and specificity of these observations prompted further investigations to confirm the presence of BT disease.

During the outbreak, sheep in the affected flocks were carefully monitored for related signs. Veterinary teams conducted comprehensive physical examinations of each animal, assessing overall health as well as specific indicators of BT. Detailed records of the clinical signs observed and the history of BT disease among the affected sheep were maintained based on information gathered from interviews with ranchers and the attending veterinarian. Body temperature was taken to confirm the presence of fever, and any lesions or swelling were evaluated visually and through palpation. In cases where sheep showed severe signs or a rapid deterioration in health, more intensive examinations were carried out to determine the severity of the disease's impact. Additionally, sheep that died from the disease underwent necropsy, with thorough observations and detailed notes taken. The necropsies involved careful documentation of any gross pathological changes observed.

A systematic approach to clinical examination was adopted to differentiate BT from other similar diseases presenting with overlapping clinical signs, such as foot-and-mouth disease (FMD) or peste des petits ruminants (PPR). This approach included ruling out other possible causes of observed signs through differential diagnosis based on the clinical history, signs, and the absence of vesicular lesions typical of other diseases.

Sampling

Blood and tissue samples, such as tongue scraping samples, were collected from symptomatic sheep to confirm the diagnosis of BT disease and distinguish it from other conditions with similar signs. Blood samples were drawn via jugular venipuncture using vacuum tubes. These blood samples were immediately stored in cooled containers and transported to the laboratory. Tissue samples were obtained from sheep that had succumbed to the disease during necropsy, focusing on organs and tissues typically affected by BT, such as the tongue, lungs, spleen, lymph nodes, and mucosal membranes. The samples were immediately placed in sterile, labeled containers and transported to the laboratory.

Laboratory diagnosis

Sample preparation

One gram of biopsy blood and tissue samples was processed by grinding them in approximately 5 mL of phosphate-buffered saline (PBS) containing antibiotics (penicillin and streptomycin) using a sterile glass tissue grinder. The mixture was subjected to two freeze-thaw cycles to disrupt cells and release viral particles, followed by clarification through centrifugation at 4000 rpm for 45 minutes. The supernatant was then aliquoted and stored at -20 ˚C until further analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from the prepared samples using the SinaPure™ viral kit, per the manufacturer's instructions (SinaClon Company, Iran). The extracted RNA was then reverse-transcribed into complementary DNA (cDNA) using the first-strand cDNA synthesis kit, also provided by SinaClon Company, Iran.

Oligonucleotide primers

Two sets of primers were utilized to amplify the BTV *S7* gene. The primary primer pairs used were SZ1 (5'-GTAAAAATCTATAGAGATG-3') and SZ2 (5'-GTAAGTGTAATCTAAGAGA-3'), and SA1 (5'-TTAAAAAATCGTTCAAGATG-3') and SA2 (5'-GTAAGTTTAAATCGCAAGACG-3'), designed to amplify the entire length of the *S7* gene (1156 bp). For nested PCR, internal primers IntS7F (5'-ACAACTGATGCTGCGAATGA-3') and IntS7R (5'-AACCCACACCCGTGCTAAGTGG-3') were used, which target an internal segment of the *S7* gene, yielding a 770 bp product (Anthony et al., 2007; Maan et al., 2016). All primers were synthesized commercially by CinnaGen Co., Iran.

Reverse transcription polymerase chain reaction (RT-PCR) protocol

The one-step RT-PCR kit (QIAGEN® one-step RT-PCR Kit, USA) was employed to detect the *S7 BTV* gene in blood samples. The RT-PCR master mix was prepared with the following components: 10 µL of 5× Qiagen RT-PCR buffer, 2 µL of dNTPs mixture (0.2 mM each), 0.5 µL of each primer (20 pmol) from the four primers (SZ1, SZ2, SA1, SA2), 2 µL of Qiagen Enzyme Mix, and 28 µL of RNase-free water. To this mix, 6 µL of denatured RNA was added. The thermal cycling conditions were set to an initial reverse transcription at 45 ˚C for 30 min, followed by activation at 95 ˚C for 15 min. The process continued with 40 cycles of denaturation at 95 ˚C for 1 min, annealing at 45 ˚C for 1 min, and extension at 72 ˚C for 2 min, with a final extension at 72 ˚C for 10 min.

RNA extracted from the reference BTV1 strain (RSAv-vvv/01), obtained from the Institute of Animal Health, Pirbright, UK, was a positive control for assay controls. At the same time, sterile distilled water was used as the negative control.

Nested PCR protocol

The products from the initial RT-PCR amplification were used as templates for the Nested PCR. The master mix for nested PCR consisted of 5 µL of 10x PCR buffer, 1 µL of dNTPs (10 mM), 1 µL of $MgCl_2$ (50 mM), 1 µL of each internal primer (IntS7F and IntS7R, 20 pmol each), 0.5 µL of Taq polymerase (2.5 U), and 35 µL of RNase-free water. Finally, 5 µL of the RT-PCR product was added as the template. The thermal cycling conditions for nested PCR started with an initial denaturation at 95 ˚C for 1 min, followed by 30 cycles of 95 ˚C for 1 min, 59 ˚C for 1 min, and 72 ˚C for 1 min, concluding with a final extension at 72 ˚C for 10 min.

Molecular investigation of FMD and PPR

To rule out the possibility of FMD in the affected sheep, a molecular investigation was conducted following the method described by Mahravani et al. The 3D gene segment of the FMD virus was examined in the collected samples using the real-time RT-PCR method (Mahravani et al., 2012). Similarly, to exclude the potential presence of PPR, a molecular diagnostic test was performed based on the technique used by Alwan et al. The samples were analyzed using the one-step RT-PCR method, explicitly targeting the sheep PPR virus's nucleoprotein (*N*) gene (Alwan & Al Saad, 2023).

Gel electrophoresis and visualization

The PCR products were resolved by electrophoresis on a 1.2% agarose gel and stained with ethidium bromide (1 µg/mL) for 20 minutes. The stained gels were visualized and analyzed using a Gel documentation system (Bio Doc-It Imaging System, UK), allowing the detection and verification of specific amplified products indicative of BTV infection.

Data collection and analysis

The data collected from clinical observations, necropsy findings, and molecular investigations were analyzed using descriptive statistics to determine the prevalence and distribution of various clinical signs and lesions associated with BT disease. Percentages were calculated to express the proportion of affected animals showing specific clinical signs and necropsy lesions among the total number of sheep examined.

Results

Clinical findings

During the outbreak, out of 1800 sheep observed across the 12 affected flocks, 240 showed clinical signs of BT disease, indicating a morbidity rate of 13.33%. Among the clinically affected sheep, 38 succumbed to the disease, resulting in a mortality rate of 2.11% for the total population and 15.83% among those showing clinical signs. Clinical observations, necropsy findings, and PCR analysis confirmed the presence of BTV infection. The goats in the 12 flocks affected by the outbreak showed no signs of the disease and demonstrated complete resistance to the infection.

This study collected 10 blood and tissue samples from lesions from infected animals, along with two necropsy samples from dead animals in each herd. A total of 120 blood and lesion samples, along with 24 necropsy samples, were collected from the herds. Molecular testing through RT-PCR demonstrated that all infected animals, both those that provided samples and those that had died, tested positive for BTV, confirming the presence of the infection. Notably, 12 samples that initially returned negative results in the RT-PCR assay showed positive results upon re-testing with Nested PCR, thereby confirming the infection.

The molecular investigation conducted to rule out FMD revealed no evidence of FMD virus involvement. Using the real-time PCR method, there was no detec-

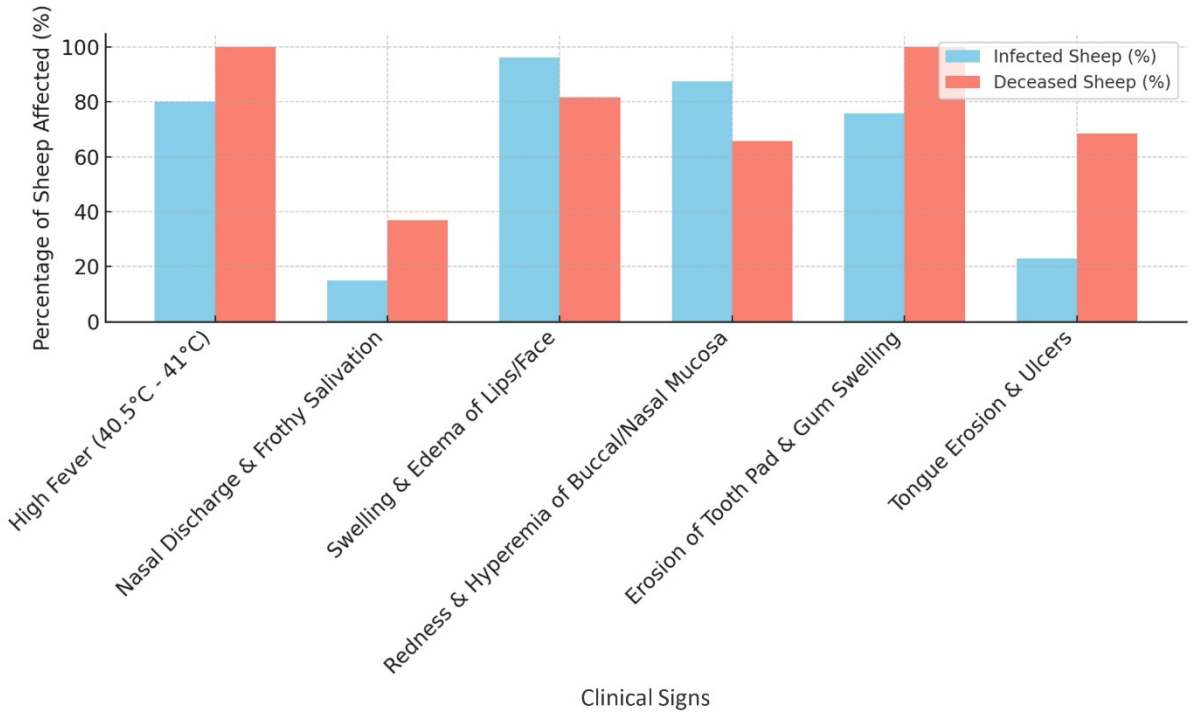

Figure 1. The percentage of sheep showing various clinical signs

tion of FMD virus RNA in any sample, confirming the absence of FMD cases in the tested sheep during this outbreak. Additionally, the RT-PCR method was employed to check for PPR by targeting the *N* gene of the PPR virus. No band indicating the presence of the 333 bp *N*-gene was observed, further confirming the absence of PPR in the affected sheep. These results helped to exclude FMD and PPR as potential causes of the observed clinical signs.

Infected sheep exhibited several notable clinical signs (Figure 1), with the onset typically marked by high fever. Nasal discharge and frothy, blood-stained salivation were observed (Figure 2a), indicating significant mucosal irritation. Swelling and edema of the lips and face were widespread, often concentrated around the eyes and ears, with some sheep showing drooping ears. Redness and hyperemia of the buccal and nasal mucosa, accompanied by ulcers on the lips, were commonly seen, along with erosion of the dental pad (Figure 2d) and swelling of the gums. Tongue erosion and ulcers were also present in some cases, particularly in deceased animals, where the lesions appeared more frequently. Lenticular necrotic ulcers were noted on the tongue, though swelling and purplish discoloration of the tongue were less common.

The most prominent signs in deceased sheep included high fever, erosion of the dental pad, and swelling of the gums, followed by pronounced swelling and edema of the lips and face. In contrast, nasal discharge and frothy salivation were observed less frequently in deceased sheep. Among those that recovered, the most common signs were swelling and edema of the lips and face, followed by redness of the buccal and nasal mucosa, with tongue erosion and ulcers being relatively rare, indicating that these signs were more associated with fatal cases.

Death typically occurs about six days after the onset of clinical signs, often resulting from respiratory complications or severe systemic collapse. The convalescence period was prolonged for the sheep that survived the initial acute phase, frequently taking several months to recover fully. During this time, partial or complete loss of fleece was common, affecting up to 20% of the surviving sheep, which led to significant financial losses for the farmers.

Post-mortem findings

Necropsies performed on all 38 dead sheep revealed various gross lesions characteristic of a BT outbreak. Mucosal lesions were present in 100% of the cases, primarily manifesting as cyanotic or hemorrhagic changes in the oral mucosa. These lesions were accompanied by erosions and ulcers affecting the tongue (Figure 2c), dental pad, and, in 47% of the cases (18 out of 38), the rumen. A hallmark of BT infection, hemorrhagic lesions at the base of the pulmonary artery (Figure 2b) were identi-

Figure 2. Clinical and necropsy findings in sheep infected with BT virus

a) Excoriation of the buccal mucosa leads to saliva stained with blood from the mouth, b) A hemorrhagic lesion was observed at the base of the pulmonary artery (black arrow), a typical finding in BT-affected sheep, c) Cyanosis, mucosal necrosis, and ulceration of the tongue are indicative of mucosal involvement in BT disease, d) Erosion and ulceration in the dental pad in a BT-affected sheep

fied in 81.6% of the dead sheep (31 out of 38). Systemic congestion of major organs, including the heart, lungs, liver, and kidneys, was observed in 50% of the cases (19 out of 38), further demonstrating the widespread impact of the infection. Additionally, serous effusions were present in 42% of the dead animals (16 out of 38), indicating fluid accumulation in the pleural, pericardial, and peritoneal cavities, which is consistent with severe systemic involvement often seen in BT fatalities.

Discussion

The current documented findings from the 2018 BT outbreak in Mazandaran Province, Iran, reveal remarkable clinical, post-mortem, and molecular evidence consistent with BTV infections and outbreaks in sheep flocks, mirroring observations reported in other studies worldwide. Vector dynamics and climate change influence the spread of BTV, necessitating continuous monitoring and research to understand its progression and effects on livestock health. This study also made a valuable contribution by enhancing awareness and preparedness (Kopanke et al., 2022; Subhadra et al., 2023). While significant progress has been made in understanding BTV, the complexity of its clinical presentation and the emergence of atypical strains highlight the ongoing challenges in differential diagnosis, necessitating further research and improved diagnostic tools (Constable et al., 2017; Darpel et al., 2012). The findings of the current study show clinical manifestations that align with those reported in numerous studies discussed below (Saminathan et al., 2020). For example, the work by Maclachlan et al. observed similar signs during the BT outbreaks in various geographic regions, indicating shared pathological mechanisms in BTV infections (Maclachlan et al., 2009).

In West Asia, particularly in Iran, research on BT outbreaks has been limited, especially concerning specific sheep breeds. The majority of existing studies have primarily focused on seroprevalence. There has been considerably less emphasis on clinical observations and necropsy findings during these outbreaks. This lack of detailed clinical and pathological information is concerning, given the critical need for differential diagnosis between BT and other similar diseases, such as FMD, contagious ecthyma, PPR, and sheep pox. Understanding these clinical and necropsy manifestations is crucial for accurately diagnosing and managing BT outbreaks, improving disease control, and protecting sheep populations (Jahanroshan et al., 2023; Liu et al., 2021; Ralitsa & Gergana, 2023; Subhadra et al., 2023). It is also worth noting that one study pointed out that atypical BTV strains show different immunological responses than classical strains, which makes diagnosis more challenging (Ries et al., 2022).

The clinical disease manifests when new BTV strains or susceptible non-native species are introduced into enzootic areas (Constable et al., 2017; Jahanroshan et al., 2023). Immunity against BTV infection is specific to serotypes (Maclachlan et al., 2015; Wilson et al., 2008). In instances where BT disease first emerges in a flock, the incidence rate typically ranges from 50% to 75%, with a mortality rate between 20% and 50% (Hassani & Madadgar, 2020; Maclachlan et al., 2015). The morbidity and mortality rates observed in the current study support the idea that, since BT has not been eradicated in Iran and the region is enzootic, livestock may have partial immune memory. Consequently, the severity of the disease is significantly lower in such enzootic areas (Constable et al., 2017; Maclachlan et al., 2015). The widespread nature of this outbreak could likely be due to the introduction of a new and more virulent strain. Notably, outbreaks have been reported even among vaccinated animals. Additionally, sheep in these flocks were routinely exposed to natural environmental conditions that facilitate the breeding of *Culicoides* midges, the primary vectors of BTV transmission (Constable et al., 2017; Pugh, 2012).

The climate is key to BTV transmission, as *Culicoides* midges thrive in warm, humid conditions. Rainfall is the main factor supporting midge populations, while cold winters or dry summers can reduce their numbers and lower disease transmission risk. The humid climate and frequent rainfall in Mazandaran pose a significant infection risk, as adult midges and larvae thrive in temperatures above 13 ˚C (55 ˚F), with optimal activity between 18 ˚C and 30 ˚C (64 ˚F to 86 ˚F). (Constable et al., 2017; Hassani & Madadgar, 2020; Maclachlan & Mayo, 2013; Zhong et al., 2024). Under favorable environmental conditions, *Culicoides* populations can increase rapidly, and adult midges can be transported by the wind over several kilometers in a single night, facilitating the swift spread of the diseases they carry. In recent years, climate change and global warming have extended the active period of biting midges, lengthening the time frame for BTV transmission. These climatic shifts have led to frequent and widespread BT outbreaks globally (Caminade et al., 2019; Leta et al., 2019; Purse et al., 2005; Sadri, 2012). Data from the Iran Meteorological Organization indicates that while there are both increasing and decreasing trends in annual rainfall across different regions, most studied stations have reported rising temperatures in recent years (IRIMO, 2025). Such climatic changes can impact the density of vector populations, thereby influencing the prevalence of BT disease in Iran (Hassani & Madadgar, 2020).

The results of the current study are consistent with those observed by Jahanroshan et al. (2023) in Qazvin City, Iran, demonstrating the presence of BTV within sheep populations across the country. Comparable clinical signs, including fever, bloody nasal and oral discharges, as well as swelling and edema of the lips, were frequently observed, with Jahanroshan et al. reporting a 72% morbidity rate in a flock of Lacaune sheep imported from France. The lack of signs like stomatitis, coronitis, and lameness in the current study highlights the diverse manifestations of BTV infection across different environments and sheep breeds (probably between the Zel and Lacaune). A fever with temperatures frequently surpassing 40 ˚C was also observed, consistent with findings from the current investigation. While Jahanroshan et al. reported a case fatality rate of 7% and a mortality rate of 9.7%, the present study recorded a lower overall mortality rate of 2.11% among the total population but a higher case fatality rate of 15.83% among those showing clinical signs. This lower mortality rate can mainly be attributed to the enzootic nature of the region and the native adaptation of the Zel breed. In contrast, despite being vaccinated four months prior, Lacaune sheep experienced higher mortality (Jahanroshan et al., 2023).

The findings of the current study parallel those of Gestier et al. (2023), who reported the emergence of BTV serotype 16 in New South Wales (NSW), Australia. Similar to the clinical manifestations observed in our study, Gestier et al. documented fever, nasal discharge, nasal edema, and oral hyperemia in experimentally infected sheep. Both studies reported remarkable vascular involvement, including hemorrhages at the base of the

pulmonary artery, highlighting common pathological features associated with BTV infections across different serotypes and geographic regions. Additionally, Gestier et al. noted moderate disease severity in sheep. At the same time, our study observed more severe clinical outcomes, such as a higher case fatality rate, which could be attributed to differences in serotype virulence or sheep breed susceptibility (Gestier et al., 2023). In a separate study, MacLachlan et al. observed comparable incidence and mortality rates during BT outbreaks in Europe and Africa, highlighting the worldwide risk this virus poses to sheep populations (Maclachlan et al., 2009).

Vascular involvement, including hemorrhages around the base of the pulmonary artery (a hallmark and nearly pathognomonic lesion for BTV), suggests that the increased vascular permeability and edema seen in severe BT cases caused by virulent virus strains may result from indirect effects on endothelial cells via soluble mediators produced during BTV infection (DeMaula et al., 2002; Drew et al., 2010; M. Saminathan et al., 2020). Mediators like tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and interferon (IFN)-β can cause tissue damage, increased vascular permeability, shock, and death, similar to viral hemorrhagic fevers. Tissue factors, complement fragments, and viral proteins may also contribute (Flórez-Álvarez et al., 2022; Marty et al., 2006). Given the similar pathological patterns observed in the present investigation and other reported outbreaks, it is evident that increased vascular permeability, likely mediated by host immune responses and inflammatory cytokines, plays a significant role in disease progression and severity.

The observations in the current study are consistent with those reported by Elbers et al. during the 2006 BTV serotype 8 (BTV-8) outbreak in North-West Europe, which affected countries including the Netherlands, Belgium, Germany, Luxembourg, and France. Elbers et al. documented that clinical disease was more pronounced in sheep than in cattle. The present study's findings of fever, salivation, facial and mandibular edema, oral mucosal erosions, mucosal ulceration, swelling of the face, and hemorrhagic lesions in sheep closely align with the clinical signs reported during the European BT outbreak (Elbers et al., 2008). The clinical findings in the present study are similar to those reported by van den Brink et al. during the 2023 BTV-3 outbreak in the Netherlands, where sheep showed some of the most severe signs and experienced the highest mortality rates compared to other affected species. The morbidity and mortality rates observed in the present study correspond with those of van den Brink et al., who noted a case fatality rate of 74.8% among sheep, underscoring the substantial impact of BTV on sheep populations (van den Brink et al., 2024). The findings from Pérez et al.'s study of BTV in a Rambouillet sheep flock in southern California align with the observations from the current study. In both studies, affected sheep exhibited clinical signs such as nasal discharge, labored breathing, and frothy discharge from the mouth. Additionally, both studies reported post-mortem findings of severe pulmonary edema, necro-ulcerative lesions in the oral cavity and gastrointestinal tract, and hemorrhages at the base of the pulmonary artery, which are hallmark features of BT infection. The mortality rates observed were also consistent, with Pérez et al. reporting less than 10% mortality, similar to the mortality patterns seen in the current study (Pérez et al., 2023).

The clinical findings in the present investigation are consistent with those reported by Kirkland et al. during the 2023 BTV-16 outbreak in New South Wales, Australia. Both studies observed similar signs in affected sheep, including facial swelling, swelling of the lips and face, and nasal discharge. Additionally, reduced mobility tolerance and lethargy were noted in both outbreaks, highlighting the impact of BTV on the overall health and mobility of infected animals. Kirkland et al. also reported encrustations on the nasal plane and variable congestion of the pulmonary artery, which align with the mucosal erosions and hemorrhagic lesions documented in the present study. The Kirkland et al. study's morbidity rate was relatively high (20%) (Kirkland et al., 2024).

Necropsy findings, such as hemorrhagic lesions at the base of the pulmonary artery, mucosal ulceration, and edema of the head and neck, were consistent with the findings of Savini et al. (2011), who documented these lesions as characteristic features of BTV infection in sheep (Savini et al., 2008). The high prevalence of mucosal lesions (79%) observed in this study is in agreement with the work of Darpel et al. (2007), which highlighted mucosal damage as a hallmark of BT disease, often leading to severe oral ulcerations and secondary bacterial infections (Darpel et al., 2007; Darpel et al., 2012; Maclachlan et al., 2009).

The findings from the present investigation closely align with those reported by Maclachlan et al., who experimentally infected Merino sheep with a virulent BTV. In both studies, sheep showed severe clinical signs such as oral mucosal ulceration and pulmonary artery hemorrhage. Necropsy also revealed similar findings. These consistent observations across different studies and strains of BTV highlight the typical pathological features of BT infection, characterized by increased vascular permeability and extensive tissue damage (Maclachlan et al., 2008).

The observation that goats in the 12 flocks affected by the BT outbreak showed no signs of the disease and demonstrated complete resistance can be attributed to several factors related to the pathogenesis of the BTV and species-specific responses. Goats, unlike sheep, often exhibit minimal to no clinical signs when infected with BTV, as seen in various outbreaks (Backx et al., 2007). The findings from Mahmoud and Khafagi's study, which demonstrated a higher seroprevalence of BTV infection in sheep (17.5%) compared to goats (14.7%) (Mahmoud & Khafagi, 2014), support the idea that goats might have a more robust immune response, effectively neutralizing the virus without showing clinical signs (Caporale et al., 2014; Esmaeili et al., 2024; Milovanović et al., 2023). These findings are consistent with previous studies, which suggest that inherent differences in immune response and endothelial cell susceptibility could explain why goats tend to remain unaffected by the disease. At the same time, sheep are more prone to it (Esmaeili et al., 2025; Mura et al., 2013; Sánchez-Cordón et al., 2013). Studies show that while BTV can infect goats, the clinical outcomes vary significantly, often resulting in asymptomatic infections (Schulz et al., 2018). The study by Bréard et al. found that goats inoculated with BTV-27 variants exhibited low antibody levels despite being seropositive and did not show obvious clinical signs, which aligns with the idea that goats may possess a robust immune response that controls the virus without manifesting signs (Bréard et al., 2018). In contrast, while goats may show resistance, the presence of the virus in asymptomatic hosts raises concerns about potential silent transmission, which could complicate control measures in affected regions.

Goats generally do not exhibit clinical signs of BTV infection, a phenomenon observed across various studies. This lack of signs is consistent with historical data and recent experimental findings. In an experimental infection study of BTV-8, while sheep displayed severe clinical signs such as fever and oral lesions, goats showed minimal to no signs (Backx et al., 2007). Experimental infections with BTV-27 in goats also resulted in no observable clinical signs despite the presence of viral RNA and antibodies (Bréard et al., 2018). A broader epizootiologic study indicated that while BTV was isolated from goats, the incidence of clinical signs was significantly lower compared to sheep and cattle (Osburn et al., 1981). These findings underscore the importance of continuous surveillance and further research into the epidemiology of BTV in small ruminants, particularly in goats.

In this study, the combination of clinical observations, necropsy findings, and PCR methodologies confirmed BTV infection within the flocks. This comprehensive diagnostic approach aligns with numerous other studies; however, the significant role of nested PCR is often overlooked. Various RT-PCR methods have been employed for diagnosing BTV, typically targeting conserved genes across all serotypes (Shoshtari et al., 2011). These genes encode core proteins such as VP7, VP1 (L1), VP3 (L3), and NS1 (M6), as well as nonstructural proteins like NS3/NS3A (NS10). Yin et al. (2010) successfully utilized RT-PCR to detect the *NS1* gene across all 24 BTV serotypes in China. Similarly, Vandenbussche et al. applied multiplex RT-PCR as a routine diagnostic tool, demonstrating its high sensitivity by detecting multiple genes simultaneously in BTV (Momta et al., 2011; Vandenbussche et al., 2010).

Among the genes mentioned, the *S7* segment is widely regarded as an optimal target for virus detection due to its stability across different BTV serotypes and topotypes and its high sensitivity in PCR. The *S7* segment shows 93-97% nucleotide sequence identity among different BTV serotypes, indicating its conserved nature (Anthony et al., 2007; Carpenter et al., 2024; Kovi et al., 2006; Momta et al., 2011; Yang et al., 2015). In the present investigation, the *S7* segment was employed as a marker to identify infected samples. Given the limited information on prevalent BTV strains in Iran, a duplex RT-PCR approach was adopted using two supplementary primer pairs targeting the 3' and 5' ends to ensure comprehensive detection across serotypes. Recent findings suggest that noncoding regions of the *S7* segment are consistent among serotypes 7, 10, and 19, with minor variations across others (Anthony et al., 2007; Carpenter et al., 2024; Razmaraii et al., 2008). Using two primer pairs allows for the identification of all BTV serotypes regardless of their specific type.

RT-PCR and Nested PCR for molecular confirmation of BTV infection proved highly effective, with positive results in all symptomatic and dead animals. Likewise, in a study conducted in Iran by Jahanroshan et al., BTV was identified in all samples collected from sheep showing clinical signs of the disease (Jahanroshan et al., 2023). Similar molecular diagnostic approaches have been widely endorsed in studies by Momta et al. (2011), Khezri et al. (2012), and Shaw et al. (2013), who found RT-PCR to be a reliable and sensitive method for detecting BTV, particularly when complemented with Nested PCR for cases initially testing negative (Khezri & Azimi, 2012; Momta et al., 2011; Shaw et al., 2007). In this study, 12 negative samples in RT-PCR showed

positive results in nested PCR, underscoring the importance of using a combination of molecular techniques for accurate diagnosis. This finding is corroborated by the research of Maan et al. (2012), which emphasized the utility of nested PCR in enhancing diagnostic sensitivity for BTV detection, especially in low viral load scenarios (Maan et al., 2007).

Additionally, one-step RT-PCR in the present study offers the advantages of reducing assay time and minimizing the risk of false positives by eliminating additional steps during cDNA synthesis and reducing the number of pipetting actions (Gasparini et al., 2021; Mahravani et al., 2012; Rocchigiani et al., 2020; Shoshtari et al., 2011). In the current study, samples were first tested with RT-PCR, followed by nested PCR to confirm results and improve virus detection sensitivity. This method, known for its simplicity, speed, and high sensitivity, can detect as few as ten copies of the target gene, comparable to real-time PCR (Kala et al., 2022; Momta et al., 2011). Numerous studies have shown that nested PCR is 10 to 100 times more sensitive than conventional PCR, making it invaluable when RNA concentrations are below 100 femtograms (fg). It has been demonstrated that Nested PCR can detect even 0.1 fg of the BTV genome, equivalent to five BTV genome molecules in cell culture (Kala et al., 2022; Rocchigiani et al., 2020; van Rijn & Boonstra, 2021).

As previously noted, immunity against BTV infection is serotype-specific, making vaccination efforts more challenging in enzootic regions where multiple BTV serotypes are prevalent. A major complication is that some live attenuated BTV vaccines may cause more disease than many wild-type viruses (Maclachlan et al., 2015; Wilson et al., 2008). While BT is not a zoonotic disease, infections in carnivores are well-documented, raising concerns about the potential for BTV to 'jump' between species. A more detailed understanding of the environmental and human-related factors that contribute to the emergence of BTV infections is essential for predicting the future occurrence and spread of the disease, as well as for its effective control (Constable et al., 2017; Maclachlan & Mayo, 2013). Control of BT is approached using either preventive (prophylactic) measures or therapeutic methods. Treating ruminants affected by BT is often ineffective and logistically difficult during outbreaks, as it primarily involves nonspecific supportive and nursing care. Preventing BT and BTV infection can be achieved by protecting animals from insect bites or through prophylactic vaccination. Eliminating *Culicoides* midges from the environment is generally impractical, especially in extensive pastoral areas. However, keeping sheep in protected shelters during periods of high midge activity (such as dusk and early evening) can help reduce exposure, particularly for vector species that predominantly feed outdoors (exophagy). This strategy is less effective for midge species that feed indoors (endophagy). For precious animals, housing them in fully insect-proof enclosures can prevent contact with vector midges during outbreaks, and applying repellents can further reduce the risk of vector attacks (Ralitsa & Gergana, 2023; Zientara & Sánchez-Vizcaíno, 2013).

Conclusion

The present study underscores the presence of BTV in 12 Iranian sheep flocks, reaffirming the enzootic nature of the disease in Iran. The current findings highlight the persistent threat BTV poses to livestock health in Iran and emphasize the urgent need for a more comprehensive and structured approach to managing the disease. Currently, the region remains vulnerable to recurring outbreaks without dedicated control measures, such as targeted vaccination programs or systematic monitoring of serotypes. The presented results strongly suggest that proactive strategies must be implemented, including improved surveillance, vector control targeting *Culicoides* midges, and movement regulation of livestock to mitigate the impact of BTV and protect the health of susceptible sheep breeds in Iran.

Ethical Considerations

Compliance with ethical guidelines

During the outbreak, livestock ranchers and owners from the affected flocks were fully informed about the study's objectives and procedures. Written consent was obtained, and necessary measures, including sampling and examinations, were conducted with their permission.

Funding

This study was conducted with financial support from the University of Tehran, Iran (Grant No.: 28786).

Authors' contributions

Project administration, supervision, and visualization: Hossein Esmaeili; Validation: Hossein Esmaeili and Seyed Mehdi Joghataei; Writing: Seyed Mehdi Joghatai; Investigation, data curation, formal analysis, and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors want to express their sincere gratitude to the ranchers for their invaluable cooperation during the sampling process, which made this study possible. The authors also extend their heartfelt thanks to the laboratory team at the Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, for their support and assistance in carrying out the laboratory analyses.

References

Alwan, G. F., & Al Saad, K. M. (2023). Investigation of the clinical and diagnostic aspects of Peste des Petits Ruminants (PPR) in sheep from the Southern Region of Iraq. *Archives of Razi Institute, 78*(2), 561–570. [DOI:10.22092/ARI.2022.359291.2396] [PMID]

Anthony, S., Jones, H., Darpel, K. E., Elliott, H., Maan, S., & Samuel, A., et al. (2007). A duplex RT-PCR assay for detection of genome segment 7 (VP7 gene) from 24 BTV serotypes. *Journal of Virological Methods, 141*(2), 188–197. [DOI:10.1016/j.jviromet.2006.12.013] [PMID]

Backx, A., Heutink, C. G., van Rooij, E. M., & van Rijn, P. A. (2007). Clinical signs of bluetongue virus serotype 8 infection in sheep and goats. *The Veterinary Record, 161*(17), 591–592. [DOI:10.1136/vr.161.17.591] [PMID]

Bréard, E., Schulz, C., Sailleau, C., Bernelin-Cottet, C., Viarouge, C., & Vitour, D., et al. (2018). Bluetongue virus serotype 27: Experimental infection of goats, sheep and cattle with three BTV-27 variants reveal atypical characteristics and likely direct contact transmission BTV-27 between goats. *Transboundary and Emerging Diseases, 65*(2), e251–e263. [DOI:10.1111/tbed.12780] [PMID]

Caminade, C., McIntyre, K. M., & Jones, A. E. (2019). Impact of recent and future climate change on vector-borne diseases. *Annals of the New York Academy of Sciences, 1436*(1), 157–173. [DOI:10.1111/nyas.13950] [PMID]

Caporale, M., Di Gialleonorado, L., Janowicz, A., Wilkie, G., Shaw, A., & Savini, G., et al. (2014). Virus and host factors affecting the clinical outcome of bluetongue virus infection. *Journal of Virology, 88*(18), 10399–10411. [DOI:10.1128/JVI.01641-14] [PMID]

Carpenter, M., Kopanke, J., Lee, J., Rodgers, C., Reed, K., & Sherman, T. J., et al. (2024). Assessing reassortment between bluetongue virus serotypes 10 and 17 at different coinfection ratios in Culicoides sonorenesis. *Viruses, 16*(2), 240. [DOI:10.3390/v16020240] [PMID]

Chenari, H. M., Emami, S. F., Agha, F. N. J., & Khodarahmi, R. (2024). Assessing the environmental capacity of the Southern Caspian Sea for the development of agritourism with emphasis on rural areas of Guilan, Mazandaran and Golestan Provinces of Iran. *Journal of Agricultural Sciences–Sri Lanka, 19*(2). [Link]

Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. E. (2017). *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats* (11 ed.). Philadelphia: Saunders Ltd. [Link]

Darpel, K. E., Batten, C. A., Veronesi, E., Shaw, A. E., Anthony, S., & Bachanek-Bankowska, K., et al. (2007). Clinical signs and pathology shown by British sheep and cattle infected with bluetongue virus serotype 8 derived from the 2006 outbreak in northern Europe. *The Veterinary Record, 161*(8), 253–261. [DOI:10.1136/vr.161.8.253] [PMID]

Darpel, K. E., Monaghan, P., Simpson, J., Anthony, S. J., Veronesi, E., & Brooks, H. W., et al. (2012). Involvement of the skin during bluetongue virus infection and replication in the ruminant host. *Veterinary Research, 43*(1), 40. [DOI:10.1186/1297-9716-43-40] [PMID]

DeMaula, C. D., Leutenegger, C. M., Bonneau, K. R., & MacLachlan, N. J. (2002). The role of endothelial cell-derived inflammatory and vasoactive mediators in the pathogenesis of bluetongue. *Virology, 296*(2), 330–337. [DOI:10.1006/viro.2002.1476] [PMID]

Drew, C. P., Heller, M. C., Mayo, C., Watson, J. L., & Maclachlan, N. J. (2010). Bluetongue virus infection activates bovine monocyte-derived macrophages and pulmonary artery endothelial cells. *Veterinary Immunology and Immunopathology, 136*(3-4), 292–296. [DOI:10.1016/j.vetimm.2010.03.006] [PMID]

Elbers, A. R., Backx, A., Meroc, E., Gerbier, G., Staubach, C., & Hendrickx, G., et al. (2008). Field observations during the bluetongue serotype 8 epidemic in 2006. I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium, France and the Netherlands. *Preventive Veterinary Medicine, 87*(1-2), 21–30. [DOI:10.1016/j.prevetmed.2008.06.004] [PMID]

Esmaeili, H., Baghal Arani, E., Mokhber Dezfouli, M., Joghataei, M., & Ganjkhanlou, M. (2024). Study of the bacterial and nutritional causes of diarrhea in Alpine and Saanen kids. *Journal of Medical Bacteriology, 12*(1), 1-8. [DOI:10.18502/jmb.v12i1.15014]

Esmaeili, H., Ghorani, M., Hamidiya, Z., Joghataei, S. M., Villanueva-Saz, S., & Lacasta, D. (2025). Causes of abortion in Iranian goat herds and associated risk factors. *Preventive Veterinary Medicine, 234,* 106381. [DOI:10.1016/j.prevetmed.2024.106381] [PMID]

Esmaeili, H., & Joghataei, S. M. (2024). Meningoencephalitic Listeriosis in Iranian Sheep and Goats. *Journal of Medical Bacteriology, 12*(2). [DOI:10.18502/jmb.v12i2.15619]

Esmaeili, H., Shakeri, A. P., Rad, Z. N., Arani, E. B., Villanueva-Saz, S., & Ruiz, H., et al. (2022). Causes of abortion in Iranian sheep flocks and associated risk factors. *Veterinary Research Communications, 46*(4), 1227–1238. [DOI:10.1007/s11259-022-09986-5] [PMID]

Ferrer, L. M., Esmaeili, H., Lacasta, D., & Ramos, J. J. (2024). *Atlas of sheep and goat diseases*. Yorkshire: Dr.Herriot. [Link]

Flórez-Álvarez, L., de Souza, E. E., Botosso, V. F., de Oliveira, D. B. L., Ho, P. L., & Taborda, C. P., et al. (2022). Hemorrhagic fever viruses: Pathogenesis, therapeutics, and emerging and re-emerging potential. *Frontiers in Microbiology, 13,* 1040093. [DOI:10.3389/fmicb.2022.1040093] [PMID]

Gasparini, M., Laguardia-Nascimento, M., Sales, É. B., Oliveira, A. G. G., Lobato, Z. I. P., & Camargos, M. F., et al. (2021). Study of molecular diagnosis and viremia of bluetongue virus in sheep and cattle. *Brazilian Journal of Microbiology: [Publication of the Brazilian Society for Microbiology], 52*(3), 1623–1626. [DOI:10.1007/s42770-021-00518-y] [PMID]

Gestier, S., Finlaison, D. S., Parrish, K., & Kirkland, P. D. (2023). The potential for bluetongue virus serotype 16 to cause disease in sheep in New South Wales, Australia. *Australian Veterinary Journal, 101*(12), 510–521. [DOI:10.1111/avj.13288] [PMID]

Ghorani, M., & Esmaeili, H. (2022). Comparison of susceptibility of different goat breeds to live attenuated goatpox vaccine. *Small Ruminant Research, 212*, 106721. [DOI:10.1016/j.smallrumres.2022.106721]

Goli, I., Kriaučiūnienė, Z., Zhang, R., Bijani, M., Kabir Koohi, P., & Rostamkalaei, S. A., et al. (2024). Contributions of climate smart agriculture toward climate change adaptation and food security: The case of Mazandaran Province, Iran. *Trends in Food Science & Technology, 152*, 104653. [DOI:10.1016/j.tifs.2024.104653]

Hassani, M., & Madadgar, O. (2020). Serological evidence of bluetongue in Iran: A meta-analysis study. *Veterinary Sciences: Research and Reviews, 7*(1), 1-13. [DOI:10.17582/journal.vsrr/2021/7.1.1.13]

Jahanroshan, N., Dalir-Naghadeh, B., Lotfollahzadeh, S., Abdollahi, M., Azarmi, S., & Moosakhani, F. (2023). Bluetongue outbreak in a sheep flock from Iran. *Veterinary Medicine and Science, 9*(6), 2791–2795. [DOI:10.1002/vms3.1288] [PMID]

Islamic Republic of Iran Meteorological Organization (IRIMO). (2025). Iran current weather. Tehran: IRIMO. [Link]

Kala, J., Chauhan, H., Patel, A., Sharma, K., Patel, S., Shrimali, M., & Mohapatra, S., et al. (2022). Molecular detection and quantification of bluetongue virus in small ruminant and Culicoides vectors in Gujarat, India. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases, 43*(1), 12-21. [DOI:10.5958/0974-0147.2022.00002.2]

Khezri, M., & Azimi, S. (2012). Investigation of bluetongue virus in Kurdish sheep in Kurdistan province of Iran. *African Journal of Microbiology Research, 6*(35), 6496-6501. [Link]

Kirkland, P. D., Finlaison, D. S., Biddle, A., Parsons, M., Austin, H., & Boland, S., et al. (2024). Bluetongue disease in sheep in New South Wales - April 2023. *Australian Veterinary Journal, 102*(1-2), 26–29. [DOI:10.1111/avj.13292] [PMID]

Kopanke, J., Carpenter, M., Lee, J., Reed, K., Rodgers, C., & Burton, M., et al. (2022). Bluetongue research at a crossroads: Modern genomics tools can pave the way to new insights. *Annual Review of Animal Biosciences, 10*, 303–324. [DOI:10.1146/annurev-animal-051721-023724] [PMID]

Kovi, R. C., Dahiya, S., Prasad, G., & Minakshi. (2006). Nucleotide sequence analysis of VP7 gene of Indian isolates of bluetongue virus vis-à-vis other serotypes from different parts of the world. DNA Sequence: *The Journal of DNA Sequencing and Mapping, 17*(3), 187–198. [DOI:10.1080/10425170600807264] [PMID]

Leta, S., Fetene, E., Mulatu, T., Amenu, K., Jaleta, M. B., & Beyene, T. J., et al. (2019). Modeling the global distribution of Culicoides imicola: An Ensemble approach. *Scientific Reports, 9*(1), 14187. [DOI:10.1038/s41598-019-50765-1] [PMID]

Liu, F., Gong, Q. L., Zhang, R., Chen, Z. Y., Wang, Q., & Sun, Y. H., et al. (2021). Prevalence and risk factors of bluetongue virus infection in sheep and goats in China: A systematic review and meta-analysis. *Microbial Pathogenesis, 161*(Pt A), 105170. [DOI:10.1016/j.micpath.2021.105170] [PMID]

Maan, S., Maan, N. S., Samuel, A. R., Rao, S., Attoui, H., & Mertens, P. P. C. (2007). Analysis and phylogenetic comparisons of full-length VP2 genes of the 24 bluetongue virus serotypes. *The Journal of General Virology, 88*(Pt 2), 621–630. [DOI:10.1099/vir.0.82456-0] [PMID]

Maan, S., Maan, N. S., Belaganahalli, M. N., Potgieter, A. C., Kumar, V., & Batra, K., et al. (2016). Development and evaluation of real time RT-PCR assays for detection and typing of bluetongue virus. *Plos One, 11*(9), e0163014. [DOI:10.1371/journal.pone.0163014] [PMID]

MacLachlan, N. J., Crafford, J. E., Vernau, W., Gardner, I. A., Goddard, A., & Guthrie, A. J., et al. (2008). Experimental reproduction of severe bluetongue in sheep. *Veterinary Pathology, 45*(3), 310–315. [DOI:10.1354/vp.45-3-310] [PMID]

Maclachlan, N. J., Drew, C. P., Darpel, K. E., & Worwa, G. (2009). The pathology and pathogenesis of bluetongue. *Journal of Comparative Pathology, 141*(1), 1–16. [DOI:10.1016/j.jcpa.2009.04.003] [PMID]

MacLachlan, N. J., & Dubovi, E. J. (2016). *Fenner's Veterinary Virology*. Massachusetts: Academic Press. [DOI:10.1016/C2013-0-06921-6]

Maclachlan, N. J., & Mayo, C. E. (2013). Potential strategies for control of bluetongue, a globally emerging, Culicoides-transmitted viral disease of ruminant livestock and wildlife. *Antiviral Research, 99*(2), 79–90. [DOI:10.1016/j.antiviral.2013.04.021] [PMID]

Maclachlan, N. J., Mayo, C. E., Daniels, P. W., Savini, G., Zientara, S., & Gibbs, E. P. (2015). Bluetongue. *Revue Scientifique et Technique (International Office of Epizootics), 34*(2), 329–340. [DOI:10.20506/rst.34.2.2360] [PMID]

Mahmoud, M., & Khafagi, M. H. (2014). Seroprevalence of bluetongue in sheep and goats in Egypt. *Veterinary World, 7*(4), 205-208. [DOI:10.14202/vetworld.2014.205-208]

Mahravani, H., Azimi, M., Ahmadi, A., & Jeirani, F. (2012). Rapid and accurate diagnosis of Foot-and-mouth disease virus by real-time PCR in field samples. *Archives of Razi Institute, 67*(2), 101-107. [DOI:10.22092/ari.2016.103893]

Marty, A. M., Jahrling, P. B., & Geisbert, T. W. (2006). Viral hemorrhagic fevers. *Clinics in Laboratory Medicine, 26*(2), 345–viii. [DOI:10.1016/j.cll.2006.05.0011] [PMID]

Milovanović, M., Dietze, K., Wernery, U., & Hoffmann, B. (2023). Investigation of potency and safety of live-attenuated peste des petits ruminant virus vaccine in goats by detection of cellular and humoral immune response. *Viruses, 15*(6), 1325. [DOI:10.3390/v15061325] [PMID]

Momta, H., Nejat, S., Souod, N., Momeni, M., & Safari, S. (2011). Comparisons of competitive enzyme-linked immunosorbent assay and one step RT-PCR tests for the detection of bluetongue virus in south west of Iran. *African Journal of Biotechnology, 10*(36), 6857-6862. [Link]

Mura, E., Suman, M., Montelli, S., Peruffo, A., Cozzi, B., & Farina, V. (2013). Characterization of an established endothelial cell line from primary cultures of fetal sheep hypothalamus. *Research in Veterinary Science, 94*(3), 388–393. [DOI:10.1016/j.rvsc.2012.10.022] [PMID]

Osburn, B. I., McGowan, B., Heron, B., Loomis, E., Bushnell, R., & Stott, J., et al. (1981). Epizootiologic study of bluetongue: Virologic and serologic results. *American Journal of Veterinary Research, 42*(5), 884-887. [DOI:10.2460/ajvr.1981.42.05.884] [PMID]

Paquette, S. J., Simon, A. Y., Xiii, A., Kobinger, G. P., & Shahhosseini, N. (2023). Medically significant vector-borne viral diseases in Iran. *Microorganisms, 11*(12), 3006. [DOI:10.3390/microorganisms11123006] [PMID]

Pérez, E., Asín, J., & Uzal, F. A. (2023). An outbreak of bluetongue in sheep. *Brazilian Journal of Veterinary Pathology, 16*(2), 148-151. [DOI:10.24070/bjvp.1983-0246.v16i2p148-151]

Pugh, D. G., & Baird, A. N. (2012). *Sheep & Goat Medicine*. United Amsterdam: Elsevier Health Sciences. [DOI:10.1016/C2009-0-60474-8]

Purse, B. V., Mellor, P. S., Rogers, D. J., Samuel, A. R., Mertens, P. P., & Baylis, M. (2005). Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews. Microbiology, 3*(2), 171–181. [DOI:10.1038/nrmicro1090] [PMID]

Ralitsa, R., & Gergana, B. (2023). Overview of bluetongue outbreaks in europe and disease control in bulgaria in the period 2011-2021. *AgroLife Scientific Journal, 12*(1), 166-173. [DOI:10.17930/AGL2023119]

Rashidimehr, A., Nayebzadeh, H., Amini Farsani, Z., & Hataminia, M. (2024). Studying the factors affecting the prevalence of Platyhelminthes in slaughtered ruminants in Lorestan province, Iran (2012-2022). *Journal of Zoonotic Diseases, 8*(3), 547-565. [DOI:10.22034/jzd.2024.18099]

Razmaraii, N., Pourbakhsh, S., Keyvanfar, H., & Azimi, S. (2008). S7 gene Characterization of Bluetongue Viruses in Iran. *Archives of Razi Institute, 63*(1), 15-21. [DOI:10.22092/ari.2008.103747]

Ries, C., Beer, M., & Hoffmann, B. (2022). Bluetongue virus infection of goats: Re-emerged European serotype 8 vs. two atypical serotypes. *Viruses, 14*(5), 1034. [DOI:10.3390/v14051034] [PMID]

Rocchigiani, A. M., Tilocca, M. G., Portanti, O., Vodret, B., Bechere, R., & Di Domenico, M., et al. (2020). Development of a digital RT-PCR method for absolute quantification of bluetongue virus in field samples. *Frontiers in Veterinary Science, 7*, 170. [DOI:10.3389/fvets.2020.00170] [PMID]

Sadri, R. (2012). Seasonal effects on the prevalence of bluetongue in small ruminants in west Azarbaijan, Iran. *Iranian Journal of Veterinary Medicine, 6*(1), 19-22. [DOI:10.22059/ijvm.2012.24620]

Sailleau, C., Bréard, E., Viarouge, C., Vitour, D., Romey, A., & Garnier, A., et al. (2017). Re-emergence of bluetongue virus serotype 8 in France, 2015. *Transboundary and Emerging Diseases, 64*(3), 998–1000. [DOI:10.1111/tbed.12453] [PMID]

Saminathan, M., Singh, K. P., Khorajiya, J. H., Dinesh, M., Vineetha, S., & Maity, M., et al. (2020). An updated review on bluetongue virus: epidemiology, pathobiology, and advances in diagnosis and control with special reference to India. *The Veterinary Quarterly, 40*(1), 258–321. [DOI:10.1080/01652176.2020.1831708] [PMID]

Saminathan, M., Singh, K. P., Vineetha, S., Maity, M., Biswas, S. K., & Manjunathareddy, G. B., et al. (2020). Virological, immunological and pathological findings of transplacentally transmitted bluetongue virus serotype 1 in IFNAR1-blocked mice during early and mid gestation. *Scientific Reports, 10*(1), 2164. [DOI:10.1038/s41598-020-58268-0] [PMID]

Sánchez-Cordón, P. J., Pedrera, M., Risalde, M. A., Molina, V., Rodríguez-Sánchez, B., & Núñez, A., et al. (2013). Potential role of proinflammatory cytokines in the pathogenetic mechanisms of vascular lesions in goats naturally infected with bluetongue virus serotype 1. *Transboundary and Emerging Diseases, 60*(3), 252–262. [DOI:10.1111/j.1865-1682.2012.01343.x] [PMID]

Savini, G., MacLachlan, N. J., Sanchez-Vizcaino, J. M., & Zientara, S. (2008). Vaccines against bluetongue in Europe. *Comparative Immunology, Microbiology and Infectious Diseases, 31*(2-3), 101–120. [DOI:10.1016/j.cimid.2007.07.006] [PMID]

Schulz, C., Sailleau, C., Bréard, E., Flannery, J., Viarouge, C., & Zientara, S., et al. (2018). Experimental infection of sheep, goats and cattle with a bluetongue virus serotype 4 field strain from Bulgaria, 2014. *Transboundary and Emerging Diseases, 65*(2), e243–e250. [DOI:10.1111/tbed.12746] [PMID]

Shaw, A. E., Monaghan, P., Alpar, H. O., Anthony, S., Darpel, K. E., & Batten, C. A., et al. (2007). Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segment 1. *Journal of Virological Methods, 145*(2), 115–126. [DOI:10.1016/j.jviromet.2007.05.014] [PMID]

Shoshtari, A., Jeirani, F., Mahravani, H., & Azimi, S. M. (2011). Appling real time RT-PCR for bluetongue virus detection in Iran. *Archives of Razi Institute, 66*(2), 75-80. [DOI:10.22092/ari.2016.103868]

Subhadra, S., Sreenivasulu, D., Pattnaik, R., Panda, B. K., & Kumar, S. (2023). Bluetongue virus: Past, present, and future scope. *Journal of Infection in Developing Countries, 17*(2), 147–156. [DOI:10.3855/jidc.16947] [PMID]

van den Brink, K. M. J. A., Santman-Berends, I. M. G. A., Harkema, L., Scherpenzeel, C. G. M., Dijkstra, E., & Bisschop, P. I. H., et al. (2024). Bluetongue virus serotype 3 in ruminants in the Netherlands: Clinical signs, seroprevalence and pathological findings. *The Veterinary Record, 195*(4), e4533. [DOI:10.1002/vetr.4533] [PMID]

van Rijn, P. A., & Boonstra, J. (2021). Critical parameters of real time reverse transcription polymerase chain reaction (RT-PCR) diagnostics: Sensitivity and specificity for bluetongue virus. *Journal of Virological Methods, 295*, 114211. [DOI:10.1016/j.jviromet.2021.114211] [PMID]

Vandenbussche, F., Vandemeulebroucke, E., & De Clercq, K. (2010). Simultaneous detection of bluetongue virus RNA, internal control GAPDH mRNA, and external control synthetic RNA by multiplex real-time PCR. *Methods in Molecular Biology (Clifton, N.J.), 630,* 97–108. [DOI:10.1007/978-1-60761-629-0_7] [PMID]

Wilson, A., Darpel, K., & Mellor, P. S. (2008). Where does bluetongue virus sleep in the winter? *PLoS Biology, 6*(8), e210. [DOI:10.1371/journal.pbio.0060210] [PMID]

Yang, Z., Li, G., Hou, Y., Yao, X., Ren, R., & Ya, H., et al. (2015). Artificial synthesis of conserved segment s gene fragment of rift valley fever virus and preliminary study of its reverse transcription loop-mediated isothermal amplification detection method. *Tropical Journal of Pharmaceutical Research, 14*(12), 2193-2200. [DOI:10.4314/tjpr.v14i12.6]

Yin, H. Q., Zhang, H., Shi, L. J., Yang, S., Zhang, G. P., & Wang, S. Q., et al. (2010). Detection and quantitation of bluetongue virus serotypes by a TaqMan probe-based real-time RT-PCR and differentiation from epizootic hemorrhagic disease virus. *Journal of Virological Methods, 168*(1-2), 237–241. [DOI:10.1016/j.jviromet.2010.04.018] [PMID]

Zhong, J., Shu, E., Zhang, S., Yang, Q., Chen, Q., & Niu, B. (2024). Prediction and transmission analysis of bluetongue disease in China. *Preventive Veterinary Medicine, 230,* 106290. [DOI:10.1016/j.prevetmed.2024.106290] [PMID]

Zientara, S., & Sánchez-Vizcaíno, J. M. (2013). Control of bluetongue in Europe. *Veterinary Microbiology, 165*(1-2), 33–37. [DOI:10.1016/j.vetmic.2013.01.010] [PMID]