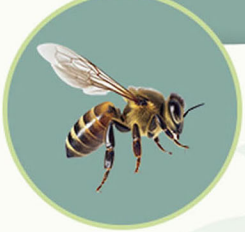




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## An outbreak of Akabane disease in a cattle herd on the Mughan plain, Iran

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Article Info	Abstract
<b>Article history:</b> Received: 26 September 2023 Accepted: 05 February 2024 Available online: 15 June 2024	<p>In November 2021, an investigation was conducted into an outbreak of abortion, stillbirth, and the birth of calves with congenital abnormalities (arthrogryposis and hydranencephaly) at a dairy farm in Dasht-e-Mughan city, Ardabil province. A total of 70 cows experienced these issues. To determine the cause of the outbreak, post-mortem brain tissue samples were collected from two calves affected by hydranencephaly, which occurred shortly after their birth. Polymerase chain reaction (PCR) testing was conducted for multiple viruses, including bovine viral diarrhea (BVD), border disease, Akabane, Schmallenberg, and bluetongue viruses (BTVs). The samples were positive only for Akabane virus. Serum samples were collected from a group of 60 cattle, consisting of 45 adult cows and 15 younger calves aged between 8 to 10 months. These samples were analyzed to detect the presence of antibodies against the Akabane and Schmallenberg viruses. Both of these viruses are known to be responsible for causing abortion, stillbirth, and congenital abnormalities in calves. Among 45 cows that tested by competitive enzyme-linked immunosorbent assay (cELISA), 26.66% and 33.33% exhibited antibodies against Akabane and Schmallenberg viruses, respectively. Notably, 20.00% of cows co-exhibited antibodies for both viruses. Despite PCR evidence implicating Akabane virus as the principal etiology of clinical signs observed in the affected herd, the high co-seropositivity to Schmallenberg virus, warrants a thorough investigation into potential viral interactions. Further research is required to determine the source of the virus and their transmission routes. This information could facilitate the refinement of disease control strategies and improving the management of reproductive challenges in such affected herds.</p>
<b>Keywords:</b> Akabane virus Cattle cELISA RT-PCR Schmallenberg	

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### Introduction

Research has shown that viruses are responsible for most cases of congenital malformations in calves.<sup>1</sup> Specifically, if a virus enters the uterus during the 60 to 180-day period of pregnancy and infects the embryo, it can result in congenital malformations.<sup>2</sup> It is important to identify the specific viruses responsible for congenital abnormalities in cows so that appropriate measures can be taken to mitigate their spread. The *Bunyaviridae* family, specifically the *Simbu* group viruses, are known to cause congenital malformations in cows.<sup>2</sup> The four teratogenic viruses within this group, including Akabane virus, Aino virus, Cache Valley virus, and Schmallenberg virus, have been linked to neurological disorders such as hydranencephaly, porencephaly, hydrocephalus, and cerebellar hypoplasia.<sup>3-5</sup> In addition to the *Bunyaviridae*

family, other viruses such as Orbiviruses (bluetongue) and Pestiviruses (bovine viral diarrhea [BVD]) and border disease have also been known to cause congenital malformations in cows, highlighting the importance of disease surveillance and control measures to prevent the spread of these viruses within herds.<sup>2</sup> In the case of Akabane and Schmallenberg viruses, skeletal disorders such as arthrogryposis may also be present in conjunction with brain lesions, making them typical teratogenic viruses.<sup>4</sup> Definitive diagnosis requires laboratory testing since clinical symptoms alone are not sufficient. These viruses are arbovirus infections that affect bovine cattle, sheep, and goats. When these diseases spread, they can result in significant economic losses for farmers and livestock producers due to a reduction in milk production, as well as an increased incidence of abortion and stillbirth among infected animals.<sup>4</sup> Although there are similarities between

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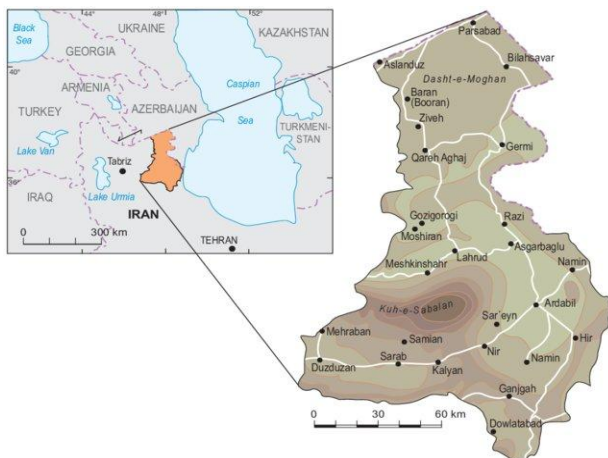


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the clinical symptoms and pathological changes associated with both viruses, there are also notable differences. For instance, Schmallenberg is more severe in sheep and goats than in cattle.<sup>6</sup> The duration of viremia is typically shorter for both Akabane and Schmallenberg viruses. Akabane virus viremia lasts between 1 - 4 days, while Schmallenberg virus viremia lasts between 1 - 9 days.<sup>6</sup> It is worth noting that Akabane virus has been known to cause encephalitis in adult cattle, while such cases have not yet been reported for Schmallenberg disease.<sup>6</sup>

## Materials and Methods

**Historical background and study area.** In November 2021, an outbreak of abortion, stillbirth, the expulsion of mummified fetuses and the birth of calves with congenital abnormalities occurred in a dairy farm of 23,000 Holstein cows in Parsabad city, located in the Mughan plain of Ardabil province in northwest Iran. The Mughan plain is bordered by the Aras River to the northwest and the Republic of Azerbaijan to the northeast (Fig. 1). A consistent vaccination protocol was in place within the herd, protecting against common threats such as brucellosis, foot-and-mouth disease, black leg, and anthrax. During a farm visit, a concerning cluster of reproductive problems affecting 70 pregnant cows was observed. These problems included abortions, stillbirths, and the expulsion of mummified fetuses, all occurring within a 2-month period (Fig. 2). Additionally, some newborn calves suffered from conditions such as exophthalmos and domed skull (Fig. 3), hydranencephaly (Fig. 4), and arthrogyrosis. Some calves were born with behavioural disorders, as evidenced by their disinterest in drinking milk. However, when their mouths were placed in the milk bucket, they began to drink as much as possible. The calves that survived after birth lived for varying periods, depending on the severity of the brain damage they had sustained. Farmers faced challenges in caring for



**Fig. 1.** The geographical location of Dasht-e-Mughan where Akabane disease occurred.

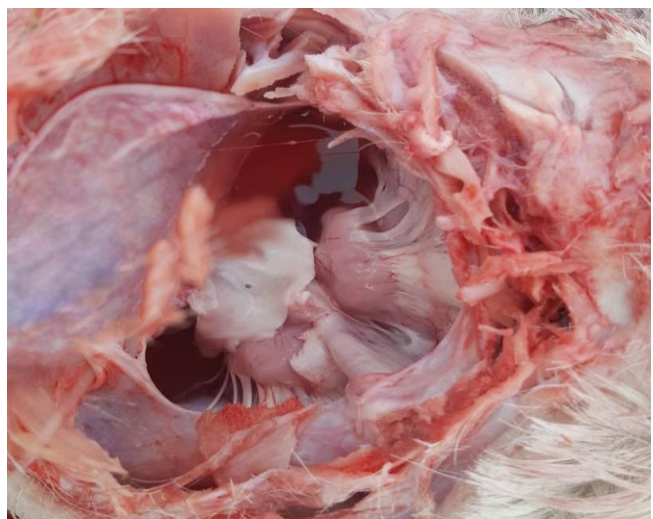
them, and those that lived for an extended period were ultimately slaughtered. Over time, the severity of the disease diminished, leading to sporadic occurrences of these disorders until September.



**Fig. 2.** Aborted mummy fetuses at 6- and 7-months gestational age exhibiting a characteristic domed skull morphology, which is a common symptom of infection with the Akabane virus.



**Fig. 3.** The figure shows a calf that was born with exophthalmia and a domed skull as a result of exposure to the Akabane virus.



**Fig. 4.** It shows the brain after removing the dorsal aspect of the skull. In this case, the hemispheres of the brain are completely absent due to hydranencephaly which can occur in fetuses infected with the virus between 79 and 104 days of gestation.

According to information provided by local veterinarians, several cases of congenital abnormalities were observed in cows in the surrounding area of Parsabad, in addition to the affected cattle farm. Additionally, 2 months before the outbreak of this disease in the Parsabad cattle farm, a significant incidence of abortion occurred in a sheep farm located 186 km away in Meshginshahr city. The aborted fetuses that were aborted during this period exhibited skeletal malformations, including scoliosis, torticollis, arthrogryposis, and other congenital abnormalities. Unfortunately, no diagnostic tests were carried out during that period.

**Necropsy examination.** In two of the dead calves, the top of their skulls were removed. Upon pushing aside, the dura, a substantial amount of fluid drained out, revealing the remnants of their brains (Fig. 4).

**Sampling, RNA extraction and reverse transcription polymerase chain reaction (RT-PCR).** The testing procedure involved three main steps: sampling, RNA extraction, and RT-PCR. Once the skull was removed, two samples were obtained from the brain tissue. Subsequently, the samples underwent processing in a laboratory, where RNA extraction was conducted using the Qiagen RT-PCR kit (Hilden, Germany) designed to detect Akabane, BVD, and border disease, bluetongue as well as Schmallenberg.

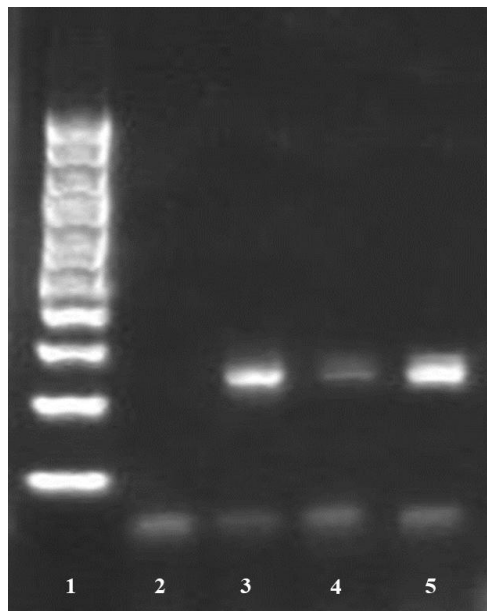
**Antibody detection.** During May 2022, blood samples were collected from 45 cows that had experienced abortions, along with an additional 15 calves aged 8 to 10 months old. Using clot activator vacuum tubes. The samples were then centrifuged and stored at  $-20.00^{\circ}\text{C}$  until testing. The presence of antibodies against Akabane and Schmallenberg viruses was assessed using commercially available competitive enzyme-linked immunosorbent assay (cELISA) kits (IDVet Innovative Diagnostics, Montpellier, France), following the manufacturer's recommended procedures. This methodology effectively identifies the presence or absence of antibodies against these viruses in the serum samples.

## Results

The serological analysis of serum samples from 45 cows using the cELISA assay revealed the presence of Akabane virus-specific antibodies in 12 (26.66%) cows and Schmallenberg virus-specific anti-bodies in 15 (33.33%) cows. Antibodies against both viruses were found to co-occur in 9 (20.00%) cows. Importantly, none of the 15 calves exhibited antibodies against either virus. Based on the RT-PCR test results of the brain samples from the two calves, they were only positive for Akabane virus. There was no evidence of BVD, border disease, Schmallenberg, or bluetongue viruses in the samples. The gel electrophoresis RT-PCR assay for Akabane virus involves five wells, as shown in Figure 5.

Each well contains a specific sample or control, which is subjected to electrophoresis and PCR amplification to detect the presence of Akabane virus RNA. The resulting PCR products were then separated by gel electrophoresis to determine the size and quantity of the amplified fragments.

Affected calves born with congenital defects, survived for varying durations, depending on the extent of brain damage. Those that lived longer were eventually slaughtered due to the challenges faced by farmers in nursing them.



**Fig. 5.** The gel electrophoresis RT-PCR assay for Akabane virus. Lane 1 contains a ladder with 100 bp fragments to help determine the size of the amplified products. Lane 2 is the negative control, which contains no template DNA and serves as a reference for detecting any contamination during the experiment. Lane 3 is the positive control, which contains known quantities of the target template DNA and serves as a reference for detecting any variations in the reaction conditions. Lanes 4 and 5 contain the positive samples that are being tested for the presence of Akabane virus. These samples are compared to the controls to determine if they contain the target DNA fragment.

## Discussion

In ruminants, several viruses can cause congenital neurological abnormalities.<sup>2</sup> The *Simbu* group, which includes Akabane and Schmallenberg viruses, is considered one of the most important teratogenic virus groups.<sup>3</sup> Infection with these viruses can result in conditions such as abortion, stillbirth, congenital abnormalities, and neurological disorders in affected animals.<sup>7,8</sup> Akabane virus has been isolated from sheep, goats, and cattle in several countries including Japan, Korea, Taiwan, Australia, Israel, and Turkey.<sup>9-14</sup> Serological tests have also detected the virus in African countries,

Bahrain, Cyprus, Indonesia, India, Iran, Kuwait, Malaysia, Nepal, Oman, Pakistan, the Philippines, Saudi Arabia, Singapore, Taiwan, Thailand, Yemen and Iran.<sup>15-18</sup> Significant congenital neurological abnormalities were observed in the recent Parsabad cattle farm outbreak. RT-PCR testing only detected Akabane virus among potential causes like BVD, border disease, Schmallenberg, and bluetongue viruses in tested calves. Interestingly, ELISA tests revealed Akabane and Schmallenberg antibodies in adult cows, suggesting past exposure to both viruses. Notably, all 8-month-old calves tested negative for antibodies against Akabane and Schmallenberg viruses. The negative ELISA test results in calves older than 8 months in our study could be attributed to two factors. Firstly, the small sample size used in this study might have limited our ability to detect antibodies in the calves. Secondly, the timing of their birth, which occurred after October when insects were inactive in the area, may have played a role. It is possible that the lack of active insect vectors during the period of the calves' early life prevented exposure to the viruses and subsequent antibody production. Furthermore, at the time of blood sampling in May, it appears that the antibodies transferred from the colostrum in the studied calves had likely diminished or disappeared. According to recent research, calves possess maternal immunity against Schmallenberg virus until they reach the age of 5 - 6 months.<sup>19</sup> Similarly, for Akabane disease, this immunity persists until the calves are approximately 6 - 7 months old.<sup>20</sup>

In the present study, it was found that out of 45 cows tested for Akabane and Schmallenberg viruses using ELISA, 36 were positive for either one or both viruses. All the cows that had positive results in this study suffered from adverse reproductive outcomes such as abortions, stillbirths, delivery of mummified fetuses, or gave birth to calves with congenital defects like hydranencephaly, arthrogryposis, and exophthalmia. The positive blood ELISA results for Schmallenberg virus in the studied cows, along with the negative PCR results for the brains of their calves, may suggest that the virus infection occurred before pregnancy. It is important to note that the viremic period for Schmallenberg virus is short (indicated by the negative PCR), but the antibody titre in the blood remains high for an extended period (indicated by the positive ELISA).<sup>21-23</sup> As a result, cows exposed to the virus develop long-lasting immunity and may still have detectable antibody in their blood up to 6 years after initial exposure. This type of cattle is known to have long-lasting immunity against viral infections, which prevents them from suffering from complications even when infected during pregnancy. The timing of infection typically corresponds with the peak of vector activity of.<sup>9</sup> For example, cows in Mughan plain were diagnosed with the disease between November and January, which suggests that they may have been exposed to the virus between April and May, during

the height of insect and mosquito activity. The Akabane virus typically does not harm the embryo before the formation of the placenta.<sup>24</sup> If a mother becomes infected during this time, which is at the beginning of the embryo's formation, the fetus will remain unaffected. In cows, cotyledons begin to develop after the 30<sup>th</sup> day of pregnancy and become visible around the 70<sup>th</sup> day of pregnancy.<sup>24</sup> However, if a cow is infected with the Akabane virus, it can cause significant damage to the calf between the third and sixth month of pregnancy.<sup>9</sup>

The Mughan epidemic revealed various forms of the disease, including abortion, mummification, hydranencephaly, arthrogryposis, and the birth of calves with exophthalmia and confusion no response to environmental stimuli. In this outbreak, the birth of lethargic and limp calves that could not stand after birth and staggered while walking was seen frequently. This is probably due to a flaccid paralysis.<sup>25</sup> Akabane virus may contribute to mild to moderate flaccid paralysis in some cases. This likely involves spinal cord involvement either through inflammatory processes or direct viral neurotropism. These distinct forms are differentiated based on the month in which the mother exposed to the virus during her pregnancy. In a herd, abortion is one of the most notable signs of Akabane virus outbreak and typically occurs after the fourth month of pregnancy.<sup>9</sup> Hydranencephaly is one of the various forms of the present outbreaks. Studies indicate that fetus up to 4 months of age can contract hydranencephaly, while those infected between days 105 and 150 of gestation are likely to display arthrogryposis of multiple joints and limbs.<sup>25</sup> It appears that the development of the immune system in the calf fetus plays a crucial role in minimizing the damage caused by the Akabane virus.<sup>8</sup> The range of clinical forms seen in the Mughan plain epidemic may be attributed to cows' exposure to the virus during various stages of pregnancy.

There was limited information as regards the origins of Akabane and Schmallenberg in this outbreak. The viruses are known to circulate among ruminants and monogastric animals in the Middle East, including in neighboring countries such as Turkey, Bahrain, Iraq, Russia, Pakistan, and others.<sup>10,17,26,27</sup> These viruses can be spread through the movement of infected animals or through the transportation of infected culicoides midges over long distances, hundreds or even thousands of kilometers by wind.<sup>28-31</sup> Therefore, further research is needed to fully understand the spread and the mode of transmission of these viruses in the Mughan plain area. The Mughan plain, where the disease occurred, does not share a common border with Turkey. However, it does share a border with the Republic of Azerbaijan, which neighbours Turkey. Additionally, if infected livestock are transported to other parts of the country, this could lead to further spread of the disease in different areas. At present, no nationwide or

regional serosurveillance has been conducted in Iran to investigate the prevalence of the Akabane virus. While there is limited research on the matter, some studies have suggested that antibodies against the Akabane virus can be found in certain regions. Various serological studies conducted across Iran have identified the presence of these antibodies in both cattle and sheep populations.<sup>32,33</sup> Recently, there has been a report of Akabane disease in a cattle farm near Tehran. This incident involved several instances of abortion, stillbirth, and the birth of deformed calves due to congenital malformations.<sup>34</sup> This suggests that the disease has spread from border areas into more central parts of the country. It is important for farmers and veterinary authorities to implement necessary measures to control the spread of the disease and prevent further outbreaks.

Currently, there have been no cases of Schmallenberg disease reported in Iran based on virus isolation or PCR testing. However, studies conducted using ELISA, have reported antibody-positive cattle and horses in the northern and north-eastern parts of Iran.<sup>18, 35</sup>

The Mughan plain outbreak revealed not only Schmallenberg virus seropositivity in some cattle but also a concerning number of animals harboring antibodies against both Akabane and Schmallenberg viruses. While seropositivity does not necessarily indicate an active infection, it does signal past circulation of the virus in the herd. The co-occurrence of Akabane and Schmallenberg viruses, both known teratogens in calves, raises concerns about their potential combined effects and cumulative impact on reproductive health in affected herds. Further research is crucial to understand their complex interactions and implement effective preventive measures.

The disease can be effectively managed through a comprehensive approach that includes mosquito control measures and regular vaccination. One crucial step in controlling the spread of the disease is addressing the mosquito population. This can be achieved through initiatives such as eliminating stagnant water sources where mosquitoes breed, using insect repellents, and employing mosquito nets or screens to prevent bites.

A recent outbreak of Akabane disease in a cattle farm on the Mughan plain marks the first report of the disease in the region. Additionally, ELISA tests revealed some cows within the herd had antibodies for Schmallenberg virus, signalling previous exposure to the pathogen. The concurrent presence of seropositivity raises concerns about the possibility of the virus spreading across regions. As livestock transfers from the Mughan plain hold a high risk of carrying these viruses to other areas of the country, there is a potential threat to both animal health and the economic stability of the livestock sector. To prevent disease dissemination and minimize financial losses, it is crucial to prioritize the vaccination of susceptible animals.

## Acknowledgments

We would like to express our gratitude to the veterinarians and animal husbandry personnel of Dasht-e-Mughan for their invaluable assistance and support in making this study possible. Thank you to all those who contributed to this project.

## Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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