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Epidemiological Investigation on Aino Virus and Abortions in Cattle and Small Ruminants in Türkiye

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Abstract: Due to climate change, different arboviral infections have begun to appear in Türkiye as well as all over the world. Aino virus (AINOV) is a vector-borne agent that is known to cause abortion in cattle and small ruminants. But there is no information about the role of the AINOV in abortion cases in farm animals in Türkiye. Therefore, the aim of this study was to investigate the presence of AINOV in bovine, ovine, and caprine foetuses. A total of 277 bovine foetuses, 823 ovine foetuses, and 75 caprine foetuses from different herds and flocks in the Aegean, Mediterranean, and Central Anatolian regions of Türkiye were analysed from 2014 to 2017. Molecular diagnosis was performed using a one-step real-time reverse transcription polymerase chain reaction (RT-PCR). AINOV was not detected in any of the foetal tissue samples. Despite this study being the fist long-term study to examine the role of AINOV in abortions in small ruminants and cattle, the results of the study are restricted to only three regions of Türkiye. The absence of AINOV detection in this study does not mean there is no circulation in Türkiye; it could be because of methodological limitations or the regions being studied. Türkiye has seven different geographical regions, and climate conditions vary in these geographical regions. The distribution area of potential vectors is influenced by climatic conditions. Therefore, conducting studies is crucial to figure out the role of AINOV in abortion cases of small ruminants and cattle in other regions of the country.

1. Introduction

Aino virus (AINOV) is a vector-borne agent belonging to the Simbu group of the *Orthobunyavirus* genus within the *Peribunyaviridae* family (ICTV, 2023). The transmission of AINOV occurs through haematophagous arthropod vectors, such as *Culicoides* biting midges and mosquitoes (Yanase et al., 2005), and wild ruminants and livestock, such as goats, sheep, cattle, pigs, and horses, can be infected by AINOV (Cho et al., 2009; Yeh et al., 2021; Hwang et al., 2022).

Adult ruminants are usually asymptomatic or self-limiting to AINOV infection, but it can cause abortion, stillbirth, and congenital malformations. The type of abnormality could be linked to the time of infection. Tsuda et al. (2004) reported that infection during 132 to 156 days of gestation in cattle, frequently a result of the viral infection in the skeletal muscles, axial skeleton, and central nervous system, can cause arthrogryposis-hydranencephaly syndrome. However, Uchinuno et al. (1998) reported that an aborted foetus of gestation day 187 had no congenital malformations. AINOV is considered endemic to Southeast Asia, Australia, Japan, and Korea (Cybinski and George, 1978; Fukuyoshi et al., 1981; Hwang et al., 2022). There is no evidence of AINOV circulation in Türkiye, but there is an absence of studies and diagnostic techniques to support surveillance for the virus's introduction. Moreover, Schmallenberg virus (SBV) and akabane virus (AKAV), which are members of the Simbu serogroup viruses with teratogenic potential, have been detected in ruminants in Türkiye (Yilmaz et al., 2014; Şevik, 2017). The Simbu serogroup viruses have been reported to have antigenic relationships. It has been reported that the S segment of AKAV has a 73.5% similarity with the AINOV (Akashi et al., 1997), whereas the M segment of SBV has a 71% similarity with the AINOV (Hoffmann et al., 2012). The genetic, epidemiological, and clinical similarities between SBV, AKAV, and AINOV may obscure the consideration of AINOV in Türkiye.

The early detection of emerging vector-borne diseases, including AINOV, is essential for minimizing their impact on animal welfare, and the economic costs of outbreaks. The disease's nonspecific clinical signs make it difficult to differentiate from common enzootic diseases or environmental factors (dos Santos et al., 2005). However, considering the potential circulation of other viruses within the Simbu serogroup is crucial due to the current emergence and reemergence of arboviruses in Türkiye. Therefore, priority should be given to monitoring and reporting specific pathogens (Ergünay et al., 2020). Given climate change around the world, the presence of other simbu viruses (SBV and AKAV) and potential vectors, and a high density of susceptible animals, the probability of different arboviruses being found in Türkiye increases. For example, climate change led to the first appearance of lumpy skin disease, an arboviral disease that had not been seen before, in Türkiye in 2013. Furthermore, presence of *C. imicola*, *C. schultzei complex*, *C. longipennis* and *C. circumscriptus*, which play a role in AKAV transmission, and *C. obsoletus*, *C. scoticus*, *C. pulicaris* and *C. punctatus*, which play a role in SBV transmission, has been reported in Türkiye (Şevik, 2017; Dağalp et al., 2021; Deniz et al., 2023). However, there is no information about the role of AINOV in abortion cases in farm animals in Türkiye. Furthermore, abortions with or without congenital malformations can be caused by AINOV infection (Uchinuno et al., 1998; Tsuda et al., 2004; De Regge, 2017). Abortion cases with congenital malformations were the main focus of most studies examining the relationship between AINOV and abortion (Noda et al., 1998; Fukutomi et al., 2000; Tsuda et al., 2004). However, information about the role of AINOV in abortions without congenital malformations is lacking. Therefore, the aim of this study was to investigate the presence of AINOV in bovine, ovine, and caprine foetuses without congenital malformations in Türkiye.

2. Materials and Methods

2.1. Study location

The Veterinary Control Institute (Konya, Türkiye) received aborted foetuses from 277 cattle, 823 sheep, and 75 goats from different flocks and herds in the Aegean (Afyonkarahisar Province), Mediterranean (Burdur, Isparta, and Antalya Provinces), and Central Anatolian (Karaman, Niğde, Konya, and Aksaray Provinces) regions of Türkiye from 2014 to 2017 (Figure 1). The Aegean region has climatic conditions that include cold winters and hot summers, whereas the climatic conditions in the Mediterranean region are characterised by hot and dry summers and rainy and warm winters. The climate of Central Anatolia is characterized by snowy and cold winters and hot summers. The provinces studied have an altitude range of between 20 meters and 1229 meters. The average temperatures in the Aegean, Mediterranean, and Central Anatolian regions are 15°C, 19°C, and 16°C, respectively. The rural economic development of the studied regions is crucially dependent on the breeding of cattle and sheep.

Figure 1. The location of the provinces in Türkiye where the current study was done is surrounded by blue

2.2. Abortion samples

The General Directorate of Food and Control approved this research on 27.12.2017 with the reference number E.3335546. The distribution of samples by province and animal species is shown in Figure 2. The selection criteria for the samples was that each foetus should come from a different flock/herd in a different province and should not be rotten. The foetal brain stems were not obtained in this study, so foetal tissue samples (such as the spleen, kidney, liver, and lung) were utilised to detect AINOV. During necropsy, tissue samples were collected aseptically with sterile instruments to prevent contamination. Sterile tubes labelled with the animal's ID number were used to store foetal tissue samples from each animal at -20°C until nucleic acid extraction. The tissue samples from each foetus were examined within 3 days after they arrived at the laboratory.

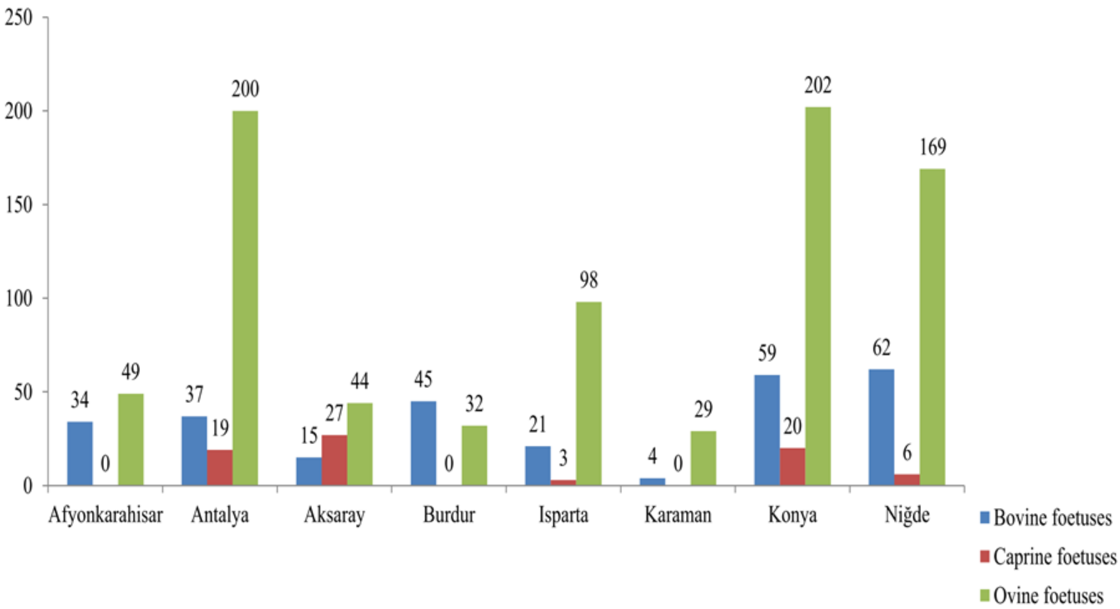

Figure 2. The distribution of bovine, caprine, and ovine foetuses at the provincial level

2.3. Viral RNA extraction

The foetal tissue samples of each foetus were pooled and homogenised with a tissue homogeniser (TissueRuptor, Qiagen, Germany) in 2 mL phosphate-buffered saline. The homogenised samples were centrifuged at 12,000 x g for 10 minutes at 4°C, and viral RNA was extracted from the supernatants by using a commercial kit (QIAamp Cador Pathogen Mini Kit, Qiagen, Germany). The extraction procedure was carried out according to the manufacturer's instructions, and viral RNA, extracted from foetal tissue samples, was stored at -85°C. Nuclease-free water was used as a control sample during extraction to confirm that cross-contamination was not present.

2.4. One-step real-time RT-PCR assay for the detection of AINOV

The partial sequence of the S segment of AINOV was amplified by one-step real-time RT-PCR using the probe (FAM-CGTCTCTCAGGATATCTAGCAA-MGB), primers AINOV-5' (GACACTGCCCTCACTCTCCAT) and AINOV-3' (TTTACATTGGTCTGCAACCCATT) as described by Stram et al. (2004). One-step real-time RT-PCR mix was prepared, which contained 12.5 µl of master mix (QuantiFast Probe RT-PCR Master Mix, Qiagen, Germany), 0.5 µl of RT-PCR Enzyme Mix, 5 µl of extracted RNA, 0.4 µM of each AINOV primer, 0.2 µM of the AINOV probe, and water to a final volume of 25 µl. The reaction was performed in the Rotor-Gene Q Instrument (Qiagen, Germany), and amplification conditions were 50°C for 20 min, 95°C for 5 min, and then 45 cycles of 95°C for 15 s and 60°C for 30 s. Ct value equal or lower than 35 was considered as positive (Stram et al., 2004). The positive control, a set of synthetic oligonucleotides described by Yanase et al. (2010), and the negative control (nuclease-free water) were used to ensure the reliability of the results.

3. Results

The ages of the examined bovine, ovine, and caprine foetuses varied between 2 to 8 months, 2 to 5 months, and 2 to 5 months, respectively. Congenital malformations were not observed in the examined foetuses. Furthermore, AINOV-specific RNA was not detected in any of the foetuses by the one-step real-time RT-PCR assay (Figure 3).

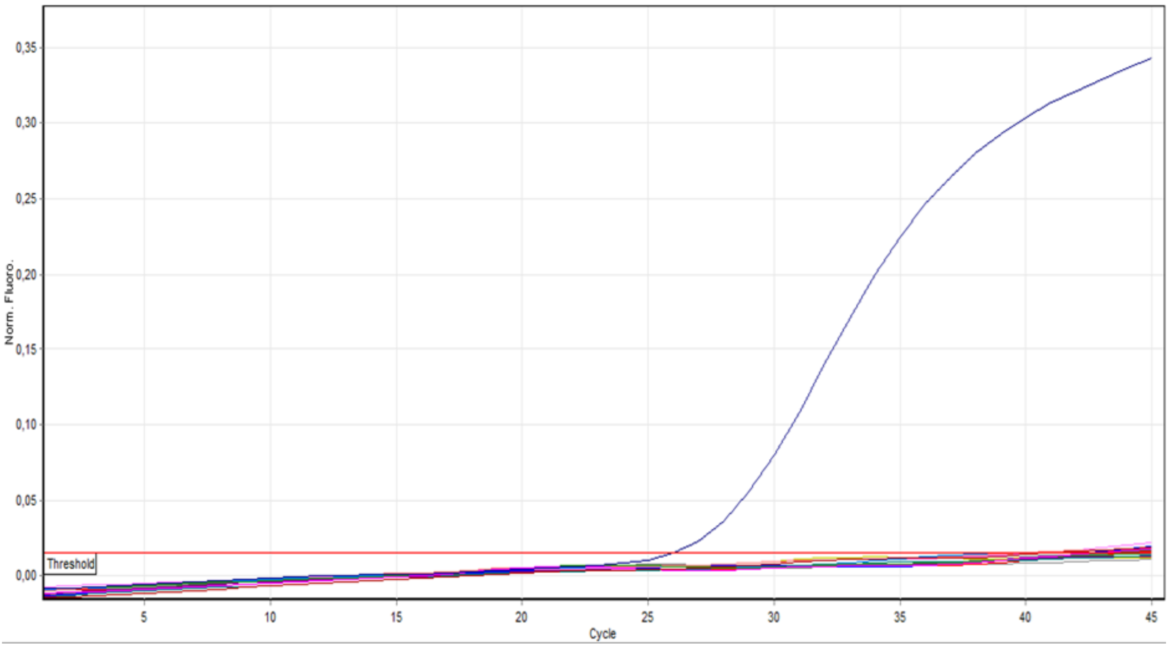

Figure 3. A one-step real-time RT-PCR assay that uses Segment 1 of AINOV, with blue line indicating positive control and red line indicating negative control

4. Discussion

The incidence of emerging viral diseases globally has increased significantly over the past decade. Of these emerging viral diseases, Simbu serogroup viruses, which are transmitted between vertebrate hosts by insects and other arthropods, have seen the greatest global spread in recent times (Mansfield et al., 2024; Logiudice et al., 2025). Simbu serogroup viruses, especially AKAV, AINOV, and SBV, have significant economic effects on the livestock industry worldwide due to abortions, stillbirths, and congenital abnormalities (Inaba et al., 1975; Tsuda et al., 2004; Wernike et al., 2015). In Türkiye, previous arboviral studies have reported the detection of Simbu serogroup viruses, including AKAV and SBV, in bovine and sheep foetuses and *Culicoides* biting midges (Yilmaz et al., 2014; Şevik, 2017; Dogan et al., 2022). However, there has been no information on the prevalence and reproductive impact of AINOV in the country's ruminant population. Therefore, this study aimed to investigate the presence of AINOV in bovine, ovine, and caprine foetuses. To the best of my knowledge, this is the first long-term study that investigated the role of AINOV in abortions in cattle, sheep, and goats in Türkiye.

Molecular diagnostic methods are reported to be rapid and reliable, and they have high sensitivity and specificity for detecting AINOV (Stram et al., 2004). Additionally, World Organisation for Animal Health (WOAH) recommended the use of molecular diagnostic methods to diagnose AINOV infection (WOAH, 2023). Therefore, in this study, a one-step real-time RT-PCR assay was used to detect AINOV in aborted foetuses.

Infection of AINOV in cattle, sheep, and goats can lead to abortions, stillbirths, and congenital abnormalities characterised by cerebellar hypoplasia, arthrogryposis, and hydranencephaly (Tsuda et al., 2004). Furthermore, AINOV infection can cause abortions without congenital malformations (Uchinuno et al., 1998; De Regge, 2017). Therefore, during this study, all foetuses that did not have congenital malformations were examined for AINOV infection.

In this study, AINOV was not detected in any of the examined foetuses. In accordance with the results of this study, previous studies from Japan (Matsumori et al., 2018; Yoshizawa et al., 2022) and South Korea (Byeon et al., 2021) could not detect AINOV in bovine and caprine foetuses. However, different studies from Japan have detected AINOV-specific RNA in aborted foetuses (Fukutomi et al., 2000; Tsuda et al., 2004). Possible explanations for this discrepancy may be the sample type (aborted foetuses without congenital malformations in this study vs. only foetuses with congenital malformations in Fukutomi et al. (2000) and Tsuda et al. (2004)), the diagnostic technique used (one-step real-time RT-PCR in this study vs. virus isolation and serum neutralization test in Fukutomi et al. (2000) and Tsuda et al. (2004)), and the presence of *Culicoides* species which are capable of transmitting AINOV. *Culicoides* biting midges are believed to be the vectors for AINOV transmission to animals, and it has been reported that *Culicoides brevitarsis* and *Culicoides wadii* are major vectors of AINOV (Weir, 2003). *Culicoides* species that play a major role in the transmission of two other important Simbu serogroup viruses, AKAV and SBV, have been identified in Türkiye (Dağalp et al., 2021; Muz et al., 2023), but the presence of *Culicoides brevitarsis* and *Culicoides wadii* has not yet been reported in Türkiye. This could explain why AINOV was not detected in this study.

It has been reported that the brain stem material is the most suitable tissue for detecting AINOV (WOAH, 2023). Unfortunately, most of the brain stems of foetuses were not obtained in this study, and foetal tissue samples (such as the spleen, kidney, liver, and lung) were used for the detection of AINOV. This may be the explanation for why AINOV was not detected in the aborted foetuses. Additionally, the foetuses in this study had different gestational ages. Most of the bovine tissue samples were obtained from foetuses with a gestational age of 5 to 8 months, whereas ovine and caprine tissue samples were obtained from foetuses with a gestational age of 3 to 5 months. Immune responses start developing in the bovine foetus between days 125 and 150 of pregnancy (Mee et al., 2023), whereas in the ovine and caprine foetuses, it develops after day 70 of pregnancy (Lopez et al., 2012). Therefore, could not detection of AINOV in aborted foetuses in this study may be due to virus clearance by the foetal immune response (WOAH, 2023).

This study has some limitations. First, this study did not use brainstem materials to detect AINOV, which could have caused the virus to not be detected in the samples. Second, concerns regarding the geographical variation and the representativeness of the obtained data for all regions are being expressed as a result of the study conducted in three different geographical regions of Türkiye. Türkiye has seven different geographical regions, and climate conditions vary in these geographical regions. The distribution area of potential vectors is influenced by climatic conditions. Türkiye's Aegean and Mediterranean coastal regions and the Southeastern Anatolia region have suitable conditions for the spread of vectors due to their climatic conditions (Şevik, 2017; Dağalp et al., 2021). The spread of new arboviral infections to Türkiye is at risk due to climate change, particularly through the Eastern Mediterranean and Middle Eastern countries.

5. Conclusion

In conclusion, this study found no evidence of AINOV in the aborted foetuses, suggesting that there was no AINOV circulation in the study area. Nonetheless, the findings of this study are confined to the specific regions examined and do not reflect the entire country, and brainstem materials were not used in this study. The study results could have been impacted by these factors. A variety of factors can drive the emergence of new diseases in areas where they were previously absent, including the effects of climate change, the disruptions to ecosystems, and international trade (Church, 2004). Given the disease is vector-borne, potential vectors are abundant in the country, which could allow the establishment of Simbu serogroup viruses, and abortions with or without congenital malformations have been observed; further epidemiological studies are needed.

Declaration of Author Contributions

M.Ş. was responsible for designing the study, performing analyses, evaluating results, writing the original draft, and editing the final draft of the manuscript.

Declaration of Conflicts of Interest

The author does not have any conflicts of interest and the study does not receive financial support from anyone or organization.

Ethical approval

HADYEK approval is not necessary for this study as it falls within the category of "clinical applications for diagnosis and treatment" according to the Regulation on the Principles and Procedures of Animal Experiments (Article 8, Clause 1). The General Directorate of Food and Control approved this research on 27.12.2017 with the reference number E.3335546, and foetal samples were collected during the necropsy.

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