

DOI 10.7251/VETJEN2401038S

UDK 616.988-036.22:595.42

Original Scientific Paper

CULICOIDES AS VECTORS OF ORBIVIRUSES, CAUSATIVE AGENTS OF PARTICULARLY DANGEROUS INFECTIOUS DISEASES OF IMPORTANCE FOR VETERINARY MEDICINE

Nataša STEVIĆ*, Drago NEDIĆ, Sonja RADOJIČIĆ

University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia

* Corresponding author: Nataša Stević, natasas@vet.bg.ac.rs

Summary

In the past, the worldwide distribution of bluetongue virus was limited to areas between approximately 35 degrees south and 40 degrees north latitude, but recently it has dramatically expanded in Europe to 58 degrees north latitude. The virus is thought to have been introduced into central and western Europe by the transport of infected animals or by wind-borne transmission of infected *Culicoides*, as well as by unknown routes of spread. Nowadays, European countries are experiencing regular outbreaks of bluetongue caused by different serotypes. Climate change is expected to affect the distribution and range of many vector species due to global warming and altered precipitation patterns. Climate change modifies long-term weather and climate patterns that affect the behavior of arthropod vectors. Increased average winter air temperatures allow arthropods to survive and extend their active period. An example of this is the emergence of bluetongue virus serotype 3 in the summer of 2023 in the Netherlands, the spread of the virus and its re-emergence in 2024. In the last 25 years, there has also been an increase in the number of horse sickness serotypes circulating at the northern border of the virus in sub-Saharan Africa. The current situation of equine sickness resembles the distribution of bluetongue virus before the sudden expansion into northern Europe in 2006. The wind-borne transmission of infected *Culicoides* is particularly important from a control perspective, as it makes the restriction of movement of susceptible animals significantly less effective in preventing the spread of the disease. Currently, the most important role of *Culicoides* from a public health perspective is their ability as biological vectors to transmit *Oropouche* virus, the causative agent of Oropouche fever in humans.

Key words: climate change, culicoids, orbiviruses, ruminants, vectors.

INTRODUCTION

During the past three decades, an increasing number of arbovirus infections has been seen. This has been attributed to climate change, mainly caused by human activities, which favors the emergence and spread of arthropods to new territories, as well as the

resistance and transmission of viruses. Diseases caused by arboviruses that can endanger a country are a threat to human and animal health, with potentially devastating social and economic consequences, and urbanization, globalization and climate change in recent years have led to the expansion of arboviruses. The global distribution of bluetongue and epizootic hemorrhagic disease in the world has changed significantly in recent years, especially due to the introduction of multiple serotypes of bluetongue virus into Europe. The unexpected emergence of bluetongue or epizootic hemorrhagic disease is related to the potential consequences of climate change on the geographical distribution and severity of vector-borne diseases. Bluetongue and epizootic hemorrhagic disease viruses infect domestic and wild ruminants and cause clinically almost identical changes. Symptoms include swelling and cyanosis of the tongue, coronitis of the hoof, bleeding and death. Cattle are considered reservoirs for bluetongue and epizootic hemorrhagic disease viruses, because infected animals, although usually asymptomatic, are viremic. The enormous genetic diversity of these orbiviruses can lead to the emergence of new serotypes with unique biological properties, as well as the possibility of not only horizontal but also vertical transmission.

Different species of Culicoides are the primary vectors of arboviruses in different parts of the world. Hematophagous insects of the genus Culicoides are distributed throughout the world and in some regions can reach high numbers seasonally, which affects livestock farming, agriculture, forestry and tourism in many parts of the world, leading to great economic losses. Most species of Culicidae are hematophagous, feeding on all vertebrates, including humans. In addition to being unpleasant to bite, Culicidae can transmit viruses, protozoa and nematodes during feeding, with some viruses causing high morbidity and mortality in ruminants, horses and humans. Their greatest importance for veterinary medicine is reflected in the fact that they transmit numerous pathogens of domestic and wild animals, especially orbiviruses, which cause non-contagious diseases of ruminants. They are primary vectors of Orbiviruses (bluetongue virus, epizootic haemorrhagic disease virus and African horse sickness virus), *Orthobunyaviruses*, *Oropouche*, *Akabane* and *Schmallenberg* viruses.

There are several routes of introduction of arboviruses that carry Culicidae to new territories, such as wind-borne transmission of infected Culicidae, import of viremic animals, use of unregistered attenuated vaccines (emergence of bluetongue virus serotypes 6 and 11 in Europe). The routes of introduction of bluetongue virus serotypes 8 and 25 are currently unknown. It is assumed that serotype 8 was introduced by the transport of infected Culicidae, animals and humans, or even by the import of cut flowers. Transport by ship is also potentially possible. However, it should be emphasized that Culicidae eggs are very sensitive to desiccation, so their transport is not a likely route of introduction of the virus into a country. The presence of the low-virulent serotype 25 of the bluetongue virus (*Toggenburg* virus) was "incidentally"

detected during routine surveillance of the highly pathogenic serotype 8 in Switzerland (Carpenter et al., 2013).

Vector-borne viruses have mechanisms for “overwintering” during periods of low temperatures to survive during the winter months when vectors are inactive. Mechanisms for virus survival in Europe include overwintering in “long-lived” insects, prolonged viremia in ruminants, and vertical transmission between ruminants and vectors. It should also be noted that climate change affects vectors, their competence, virus transmission capacity, geographic distribution, extrinsic incubation period, duration of activity (late autumn, early spring), and also the virus genome (Hudson et al., 2023).

Culicoides

Insects of the family *Ceratopogonidae* are distributed throughout the world. There are 6,206 species of *Ceratopogonidae* in the world. Within the family *Ceratopogonidae*, there are four genera that feed on the blood of vertebrates (*Culicoides*, *Leptoconops*, *Lasiohelea* and *Austroconops*), of which the genus *Culicoides*, with 1,347 described species, is of greatest importance when it comes to infectious diseases. To date, over 50 arboviruses have been isolated from these insects, 45% of which have not been detected in other arthropods (Mellor et al., 2000). These insects are potential vectors of infectious disease agents, the most important of which are bluetongue virus, epizootic hemorrhagic disease virus, Schmallenberg virus, African horse sickness virus, as well as *Oropouche* virus, the only known virus transmitted by *Culicoides* to humans. *Culicidae* are some of the smallest hematophagous insects, rarely exceeding three millimeters in length. The life cycle consists of an egg, four larval stages, a pupa, and an adult. The larvae are very diverse in habit, behavior, and ecology, and develop in moist habitats. Adults can also be important pollinators. Females require a protein diet to produce eggs. They are active at dusk and dawn. They are present in most parts of the world, with the exception of Antarctica and New Zealand. For the development of eggs, larvae, and pupae, they require moisture-rich habitats, such as moist, organically enriched soil, which is often found around farms, and can also be found in places such as tree cracks, rotting vegetation, pond edges, wet soils, and certain types of herbivorous dung. Due to their size (1-2.5 mm), under favorable conditions, *Culicoids* can easily be carried by winds over long distances. *Culicoids* have also been shown to be present at high altitudes. Epizootic haemorrhagic disease virus serotype 8 is thought to have been introduced into Italy from Tunisia in this way, by wind-borne vectors, which is the same route of transmission as bluetongue virus serotype 3 in 2017. Research on the spread of *Culicoids* by wind has shown that the high-risk zone of wind-borne vector spread from the sites of the first outbreaks of Epizootic haemorrhagic disease virus serotype 8 in France remains largely confined to the south-west of the country, but with possible penetration northwards to the Gironde region or eastwards along the Pyrenees mountains. In addition, the spread of the vector towards northern

Spain is possible (Bibard et al., 2024b). While climate change may affect the future spread of infected vectors by wind, the transport of infected animals is a more likely route of introduction of orbiviruses into a country and region that has disease-free status. The emergence of African horse sickness in the Middle East and Southwest Asia from 1959 to 1962 and in North Africa and Spain in 1965 was due to the movement of nomadic tribes (Carpenter et al., 2017). The import of zebras from Namibia into Spain in 1987 also introduced serotype 4 of the African horse sickness virus, and in 2020, serotype 1 was introduced into Thailand through the import of equidae.

The ability of hematophagous insects to be vectors of pathogens is innate and genetically determined. It is a consequence of the evolutionary adaptation of the pathogen during which it acquired the ability to evade the immune defenses of the arthropod, infect the vector, multiply and develop in its cells and organs, reach the salivary glands and transmit to the susceptible species during feeding of the vector. Four criteria must be met to determine the vectorial ability of arthropods for different pathogens: confirmation of the virus in vectors caught in the field that have not taken a blood meal, demonstrate the ability to become infected after feeding on an infected host or blood containing the pathogen, establish the ability to transmit the pathogen to the host by biting, and determine the abundance and association of vectors in the field with appropriate susceptible hosts (Ruder et al., 2015).

Culicoids are biological vectors of orbiviruses, which means that after ingestion, the virus infects the midgut cells of these insects and multiplies there, travels to the body cavity of the insect, and then infects the salivary glands, where it also multiplies. Only then can the virus be transmitted to a new host. The time that elapses from the ingestion of the virus until it reaches the salivary glands of the vector is called the extrinsic incubation period, and its duration depends on the activity of the viral RNA polymerase, which, in turn, depends on the ambient temperature. At 15°C, the extrinsic incubation period lasts several weeks, while at 30°C it lasts only a few days (Wittmann et al., 2002). After feeding on the Culicoids and taking a meal of infectious blood, the bluetongue virus enters the midgut, multiplies and can infect other organs. At 25°C, the virus from the midgut reaches the salivary glands within two days of infection. After entry into the acinar cells of the salivary glands, the virus replicates again, and then the virus particles are released into the terminal acini from where they can be transmitted in the saliva during the vector bite. In contrast to mammalian cells, which are severely damaged by the process of virus exit by exocytosis, virus release from insect cells occurs exclusively by membrane budding (Kampen and Werner, 2023). The time required for the vector to digest a blood meal also decreases at higher temperatures, increasing the frequency of feeding. At temperatures below 12°C, viral replication ceases completely, although the virus can survive in infected Culicoides and resume replication if the temperature increases (Wellby et al., 1996). At such low temperatures, vector activity also decreases. As a result, virus transmission is almost completely interrupted for several months of the year due to cold weather. However, epizootics

often continue after periods far longer than the typical lifespan of the adult vector or the normal infectivity period of the host. During the winter months, arthropods are inactive, and Culicoides are thought to overwinter in the larval stage. During the winter in Europe, after the first frosts, adult Culicoides are present in small numbers or completely absent. A study in Germany showed that Culicoides of the *Obsoletus* group are present almost all year round inside stables, indicating a high risk of virus transmission to ruminants in closed environments. In this study, the *Obsoletus* group was active both indoors and outdoors during all months of the year. Some species of the *Pulicaris* complex were active from March, but were also present during the cold months until early December. Therefore, the results show that there are periods of low vector activity during the year, but not without vectors (Groschupp et al., 2023). The resistance of *C. sonorensis* to temperatures of -20°C and significant desiccation in the egg stage may indicate resistance to climate change in the future.

Studies indicate that the same species of Culicoides are involved in the transmission of bluetongue, epizootic haemorrhagic disease and African horse sickness. Of all Culicoides species, *Culicoides imicola* is the most important vector worldwide. It is present throughout Africa, the Near and Middle East, parts of South and Southeast Asia and southern Europe. The most important vector globally, *C. imicola*, has so far been discovered only in Mediterranean countries, and the northernmost part of Europe where it is present is southern Switzerland (Groschupp et al., 2023). Also, another important vector in Africa, south of the Sahara, is *C. bolitinos*, a species related to the *Imicola* complex. In North America, the main vector is *C. sonorensis* and is the best studied Ceratopogonid species. In Central and South America, it is *C. insignis*, and in Australia, *C. brevitarsis* (Winder, 1978). At the end of the twentieth century, it was assumed that the native Culicoidea species that are widespread and numerous throughout northern Europe, *C. obsoletus* and *C. pulicaris*, could also be competent vectors. This was definitively established in 2006, when bluetongue disease broke out in central and northern Europe and when the presence of *C. imicola* was not detected, but only the presence of *C. obsoletus* and *C. pulicaris* (Kampen and Werner, 2023). Until the 2006 outbreak of bluetongue in central and northern Europe, the virus was mainly found in Africa, Asia and the southern part of Europe, where it was primarily transmitted by the species *C. imicola*. The outbreak of bluetongue disease in central and northern Europe, where *C. imicola* is not present, suggests that local Culicoidea species are also competent biological vectors (Meiswinkel et al., 2007). Four members of the *Obsoletus* group of the genus *Culicoides* (*Culicoides obsoletus sensu stricto*, *Culicoides scoticus*, *Culicoides dewulf*, *Culicoides chiopterus*) and three members of the *Pulicaris* complex (*Culicoides pulicaris sensu stricto*, *Culicoides punctatus*, *Culicoides impunctatus*) were considered to have a potential role as vectors (Nolan et al., 2007; Savini et al., 2005; Torina et al., 2004; Caracappa et al., 2003). There is little information on the diversity of Culicoidea species and their host populations in Eastern Europe. A study conducted in Romania revealed for the first time the presence of the species *C.*

griseidorsum, *C. puncticollis* and *C. submaritimus*, and it was also proven that *Culicoides* feed mainly on ruminants, which is in agreement with other studies from Europe. Cattle could play an important role in the multiplication and spread of pathogens between wild and domestic ruminants (Tomazatos et al., 2020).

Orbiviruses

Orbiviruses are members of the *Reoviridae* family, which includes 14 genera. There are currently 22 recognized species and at least 160 different serotypes in the genus *Orbivirus*, distributed worldwide, predominantly in tropical and subtropical regions, including Europe, Asia, and Africa (Attoui and Mohd Jaafar, 2015). Orbiviruses infect vertebrates and invertebrates, plants, and fungi. Due to their impact on livestock, the viruses that cause bluetongue, epizootic hemorrhagic disease, and African horse sickness are of great economic importance globally (Hudson et al., 2023). These ten-segmented, double-stranded RNA viruses are non-enveloped. Orbiviruses are transmitted by various arthropod vectors such as mosquitoes, flies, and ticks, and members of the genus *Culicoides* transmit most orbiviruses that cause major economic losses in domestic animals. Due to the segmented structure of the virus, in cases of co-infection, gene reassortment or segment exchange between orbivirus species may occur, which inevitably affects susceptible hosts, virulence, immune evasion, and antiviral resistance. Orbivirus gene reassortment plays an important role in the emergence of diversity and evolution of this group of viruses. An example of this is the recently discovered new strain of Tibetan orbivirus in China, which was found to be the result of natural reassortment (Ren et al., 2021). Diseases caused by orbiviruses occur seasonally, mainly in late summer, in subtropical and temperate regions, which is related to external temperature, vector activity and virus replication (Guthrie and Weyer, 2015). The population of Culicidae increases during rainy periods (Aklilu et al., 2014). The epizootiology of orbiviruses is changing under the influence of climate change that affects viruses, vectors, but also susceptible animals. Climate change can potentially worsen the negative global economic impact of orbiviruses. Climate change, i.e. an increase in average air temperature, can lead to increased virus replication, and thus the emergence of new mutations. The spread of vectors to new territories and the infection of vectors with different serotypes will also increase the degree of virus reassortment.

Blue tongue disease

The most important disease in Europe transmitted by Culicidae is bluetongue. Bluetongue is a non-contagious disease of ruminants (cattle, sheep, deer, goats and camels), with sheep being the most severely affected (Maclachlan et al., 2009). The virus belongs to the genus *Orbivirus*, family *Reoviridae*. So far, 36 serotypes of the virus have been described, which differ genetically and in virulence (Ries et al., 2021). The clinical picture varies from mild to severe. In cattle, the disease usually proceeds

subclinically, but this species represents a reservoir of the virus because it develops long-term viremia (MacLachlan et al., 2009). The virus is transmitted by Culicidae, with the exception of a small number of atypical serotypes that can be transmitted by direct contact between ruminants: some strains of this virus can be transmitted directly from animal to animal, transplacentally, iatrogenically and orally, and even mechanically, by flies carrying the virus on their mouth. These routes may be important during the “overwintering” period of the virus (Wilson et al., 2008). There is little evidence for vertical transmission of bluetongue virus from one generation of Culicidae to the next, and it is thought that virus particles are not transferred to eggs, which precludes transovarial transmission (Nunamaker et al., 1990). In ruminants, bluetongue virus may persist over the winter by establishing a chronic or latent infection or by transplacental transmission from infected dams to offspring and giving birth to infected young in the spring, which has been linked to serotype 8 (Mansfield et al., 2024). Bluetongue is characterized by fever, circulatory disorders, lip hyperemia, petechiae, ulcerations of the nasal and oral mucosa, increased salivation, head edema, difficulty breathing, cyanosis of the tongue, and lameness, and abortion is possible. The changes may be more pronounced in young animals (Stevanović and Nedić, 2019; MacLachlan et al., 2009). The economic impact, in addition to direct losses in the form of mortality and abortion, also includes restrictions on international trade in animals and animal products. Vaccines are available, but are used restrictively except in outbreak situations. Morbidity and mortality, trade restrictions, and vaccination are significant costs and have a major economic impact on farmers and the livestock industry (Đurić et al., 2022; Đurić et al., 2021; Gethmann et al., 2020).

Bluetongue was first described in the early 20th century in southern Africa. By the mid-20th century, the disease had spread to North America, and the causative agent was identified in 1952 in the USA (McKercher et al., 1953). In the late 1950s, bluetongue was reported in southern Asia (Pakistan, India, China, Indonesia, Malaysia, Japan) (Prasad et al., 2009; Daniels et al., 2009). From Asia, wind-borne infected vectors were introduced to Australia, and the first cases of the disease were detected in 1975 (Firth et al., 2017). In Europe, the first cases of the disease appeared in 1924, but major epizootics occurred in Cyprus (1943) and the Iberian Peninsula (1956) (Gambles, 1949; Manso-Ribeiro et al., 1957). The distribution of the virus has changed significantly in the last three decades, as reflected by the appearance of the virus in regions where it was previously absent, as well as the emergence of new serotypes in enzootic areas (Mansfield et al., 2024). During the first half of the twentieth century, bluetongue virus was present in Africa and Cyprus, and in 1951 it was confirmed in Israel; in 1961 in Asia, and then, in 1975 in Australia (Wilson and Mellor, 2009). In Europe, in 1956 and 1960, a major epizootic caused by serotype 10 occurred in Spain and Portugal, killing almost 180,000 animals (Manso-Ribeiro et al., 1957). By the end of the twentieth century, bluetongue had spread and was reported worldwide between approximately 40 degrees north and 35 degrees south latitude (Wilson and Mellor, 2009). Serotype 9

was discovered in Greece in 1998, from where it spread further north and west. Until 1998, bluetongue occurred sporadically in Europe, with each epizootic being associated with a single serotype of the virus. However, since 1998, the disease has spread northwards and into the Mediterranean region, and then into Europe, extending 800 km northwards (Purse et al., 2005). The spread of the virus to new territories is a consequence of climate change that has favoured the survival of the virus during the winter and the Culicoidea, the spread of *C. imicola* northwards, but also the transmission of the virus by native vector species. The activity of the virus in Europe before 2001 was apparently limited to the known range of *C. imicola*, and its spread northwards, where *C. imicola* is not present, represents a turning point in the epizootiology of bluetongue in Europe and confirmation that virus transmission by local Culicoidea species is possible. In the Republic of Serbia, the virus was first isolated in 2001 and the results were confirmed in the World Organisation for Animal Health reference laboratory in Pirbright, United Kingdom, where it was determined to be serotype 9 (Djuricic et al., 2004a), and the presence of the disease has also been confirmed in other Balkan countries (Stevanović et al., 2018; Djuricic et al., 2004b). In August 2006, the bluetongue virus crossed 50° north latitude for the first time, and serotype 8 appeared in the Netherlands, Belgium, Germany, France and Luxembourg (Kampen and Werner, 2023). Analysis of the genome sequence of the serotype 8 virus indicated a high similarity to a strain of this serotype previously isolated from sub-Saharan Africa (Maan et al., 2008). Interestingly, the summer and autumn of 2006 were the warmest since official temperature records began, and the average air temperature in Europe in July was the warmest on record (Purse et al., 2008). In 2007, the Friedrich Loeffler Institute confirmed that the virus had successfully overwintered in western Europe. Since 2006, bluetongue has spread further into northwestern Europe, occupying areas as far north as 58 degrees north latitude (Wilson and Mellor, 2009). Thanks to a mandatory vaccination program against bluetongue, serotype 8 was eradicated from Europe in 2011, but in 2015, it reappeared in France, this time with reduced virulence, in 2017 in Switzerland, and in 2018 in Germany, for the first time in nine years (Niedbalski and Fitzner, 2020). The presence of serotype 4 was proven in 2014 in Spain, Portugal, Croatia, Greece, Bulgaria, Romania, Slovenia, and a few years later, a genetically modified serotype 4 was discovered in Italy and France. Serotypes 1, 2 and 16 have also been detected in the Mediterranean region. Serotype 3 was present only in Sicily and Sardinia, but after a decade of bluetongue-free status, the presence of this serotype was confirmed in the Netherlands in 2023, when clinical symptoms and deaths occurred in sheep and cattle. The origin and route of introduction of the virus are unknown, as is the competent species of Culicidae as a vector (Holwerda et al., 2023). In Italy, small ruminants were affected, while the disease in cattle was mainly subclinical. However, in the Netherlands, the disease spread rapidly and the same serotype 3 caused a severe clinical picture and high mortality in affected sheep and cattle.

There are several possible routes of introduction of bluetongue into the country: import of live animals through legal or illegal trade, import of reproductive material, movement of wild animals, wind-borne vectors, introduction of vectors by means of transport or use of attenuated vaccines. The route of introduction of the virus into the Netherlands is still unclear, but it is thought that serotype 3 was introduced into Italy by infected vectors carried by the wind from Tunisia (Holwerda et al., 2023). Under favourable meteorological conditions, *Culicoides* can be carried by air currents over distances of up to 700 km over sea and 500 km over land (Eagles et al., 2014; García-Lastra et al., 2012). In their study, Bibard et al. (2024a), assessed the risk of wind-borne introduction of bluetongue virus (serotype 3) from Sardinia to continental Europe. The risk of vector spread from Sardinia was estimated to be limited to the Mediterranean region, mainly potentially affecting the southwestern area of the Italian peninsula, Sicily, Malta and Corsica, and even northern and central Italy, the Balearic Islands and continental France and Spain.

Epizootic hemorrhagic disease

Epizootic hemorrhagic disease is a disease of domestic and wild ruminants caused by the epizootic hemorrhagic disease virus belonging to the genus *Orbivirus*. Epizootic hemorrhagic disease was first described in 1955 in New Jersey, USA, when a large number of white-tailed deer were affected, and the virus causing the disease was isolated in the same year (Maclachlan et al., 2015). Over the following decades, the virus spread to other continents (Jiménez-Cabello et al., 2023). Epizootic hemorrhagic disease virus, related to bluetongue virus, has a double-stranded RNA with ten segments, is non-enveloped and 80 nm in size. To date, seven serotypes of the virus have been identified (1, 2, 4-8), and there are two more, potentially new serotypes discovered in South Africa and China (Yang et al., 2020). The disease occurs seasonally, from mid-summer to late autumn, in enzootic areas every 2 to 3 years, and epizootics occur every 8 to 10 years. Differences in these cycles can be explained by the combined effects of population immunity, animal transport and fluctuations in the vector population (Becker et al., 2024). The prevalence depends on the presence and abundance of *Culicidae*, the immunity and susceptibility of susceptible individuals. In enzootic areas, it causes significant morbidity and mortality in deer, which are highly susceptible, and losses can be up to 90%. Although cattle are somewhat less susceptible and the disease usually has a subacute course and a milder form, losses occur in the dairy industry, and in the last two decades there has been an increasing number of clinical cases of the disease in this species of animals (Jiménez-Cabello et al., 2023). The disease occurs in different forms depending on the serotype of the virus: in white-tailed deer it can occur as asymptomatic, with mild symptoms, up to severe hemorrhagic disease and death (Avelino de Souza Santos et al., 2023). The most common symptoms of epizootic hemorrhagic disease are: fever, weakness, lack of appetite, ulcerations of the oral mucosa, increased salivation, difficulty swallowing,

rash on the udder, bleeding, redness of the hoof skin, difficulty breathing, swelling of the tongue, exophthalmos, tachycardia, tachypnea, enlarged lymph nodes, pulmonary edema, lameness, difficulty moving and standing, and sudden deaths within 36 hours are possible, which occurs especially in deer. In wild ruminants, facial swelling, bloody diarrhea and hematuria, dehydration, and conjunctival hyperemia may also occur. Major epizootics occurred in Japan in 1959 and 1997 (Ohashi et al., 1999), but in recent years there has been an increasing number of cases in the USA, Asia, the Middle East, North Africa, and Mediterranean countries (Jiménez-Cabello et al., 2023). Although the disease has mainly occurred in wild ruminants in the USA (serotypes 1, 2, and 6), the disease has also occurred in cattle in Asia (serotype 2 in Japan), the Middle East (serotype 7 in Israel, 6 in Turkey and Oman), and North Africa (serotype 6 in Tunisia, Morocco, and Algeria). Serotype 8 was originally identified in Australia in 1982 and has not been reported in any country since then until late 2021 when clinical cases appeared in cattle in Tunisia (Sghaier et al., 2023). The disease first appeared in Europe in Sardinia and southern Spain in late 2022 and, despite a ban on animal movements and increased surveillance, in France in September 2023. The serotype of the virus from Italy is genetically identical to the serotype originating in Tunisia. This similarity of serotypes suggests that the virus was transmitted from North Africa to Southern Europe (Lorusso et al., 2023). Vaccines against serotypes 1, 2 and 6 are available in some parts of the world (Kampen and Werner, 2023). The lack of a vaccine in Europe poses an additional threat to countries free of this disease.

African horse sickness

African horse sickness is a viral disease of equidae that circulates among horses, donkeys, zebras and mules in enzootic areas of sub-Saharan Africa (Mellor and Hamblin, 2004). The causative agent of African horse sickness is *Orbivirus*, a non-enveloped virus of approximately 70 nm in size that possesses segmented double-stranded RNA. To date, there are nine serotypes of African horse sickness virus (Drolet et al., 2022). Only equidae are susceptible to African horse sickness. The disease is usually subclinical in zebras and African donkeys, with zebras and other wild ungulates considered natural reservoirs of the virus (Zientara et al., 2015). In horses, the disease can manifest in three different forms, with a high mortality rate of up to 90%. African horse sickness is manifested by fever, edema, sweating, difficulty breathing, coughing fits, frothy nasal discharge, swelling in the head, bleeding, and colic (Carpenter et al., 2017). African horse sickness occurs enzootically in sub-Saharan Africa, but the disease has also occurred in North Africa, the Middle East, India, and southern Europe. The largest recorded epizootics occurred in South Africa (17th century), when 70,000 animals died, and in the Middle East (1959-1961), when 300,000 infected animals died (Carpenter et al., 2017; Mellor and Hamblin, 2004). The first cases of African horse sickness in Southeast Asia, in Thailand, were reported in 2020 (Bunpapong et al.,

2021). Attenuated vaccines are used to control the disease (Kampen and Werner, 2023), which would not be an acceptable scenario in the event of an outbreak in Europe.

CONCLUSION

After the introduction of infectious Culicoids into a new territory, it is necessary for the vectors to adapt and then transmit the virus to a susceptible animal species by biting, or for the imported viremic animals to come into contact with a sufficiently large population of indigenous Culicoids to make virus transmission possible. Climate change will lead to the expansion of diseases caused by orbiviruses and their occurrence in countries that are free from the disease in such a way that the increase in average air temperatures will affect the expansion of the vector's range, prolonging the period of their activity, shortening the extrinsic incubation period and facilitating the overwintering of the virus. This is exactly the scenario that was recorded in 2006, when the highest air temperatures since the beginning of systematic temperature recording were measured and when there was a sudden spread of bluetongue disease. From a prevention and control perspective, it is very important to educate and train veterinarians and farmers about the clinical manifestations of diseases caused by orbiviruses, especially those that are notifiable and have not appeared for a long time, as many veterinarians may not have had the opportunity to see clinical symptoms in their practice. Given that there is no adequate therapy, and often no effective vaccine, efforts should be done to develop organizational, structural and vector control measures. Control of arboviruses transmitted by Culicoides depends on several factors such as the economic impact of the disease, the possibility of the arbovirus surviving in a certain territory, the existence of an effective vaccine, and may include euthanasia, bans on the movement and trade of animals, control of the number of arthropods, as well as reducing contacts of susceptible animals with biological vectors.

Acknowledgment

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract No. 451-03-66/2024-03/200143).

Conflict of interest statement: The authors declare that there is no conflict of interest.

REFERENCES

- Aklilu N., Batten C., Gelaye E., Jenberie S., Ayelet G., Wilson A., Belay A., Asfaw Y., Oura C., Maan S., Bachanek-Bankowska K., Mertens P. P. C. (2014): African Horse Sickness Outbreaks Caused by Multiple Virus Types in Ethiopia. *Transbound Emerg Dis*, 61:185-192.
- Attoui H., Mohd Jaafar F. (2015): Zoonotic and emerging orbivirus infections. *Rev Sci Tech*, 34:353-361.
-

- Avelino de Souza Santos M., Rojas Gonzales J., Swanenburg M., Vidal G., Evans D., Horigan V., Betts J., La Ragione R., Horton D., Dórea F. (2023): Epizootic Hemorrhagic Disease (EHD) – Systematic Literature Review report. *EFSA supporting publication*, 20(11):8027E, 43,
- Becker M., Gentry G., Husseneder C., Foil L. (2024): Seven-year prospective study on yearly incidence of Orbivirus infection of captive white-tailed deer and potential Culicoides vectors. *J Med Entomol.* 13;61(2):465-472.
- Bibard A., Martinetti D., Giraud A., Picado A., Chalvet-Monfray K., Porphyre T. (2024a): Quantitative risk assessment for the introduction of bluetongue virus into mainland Europe by long-distance wind dispersal of Culicoides spp.: A case study from Sardinia. *Risk Anal.*, 1-20.
- Bibard A., Martinetti D., Picado A., Chalvet-Monfray K., Porphyre T. (2024b): Assessing the Risk of Windborne Dispersal of *Culicoides* Midges in Emerging Epizootic Hemorrhagic Disease Virus Outbreaks in France, *Transboundary and Emerging Diseases*, 1-11.
- Bunpapong N., Charoenkul K., Nasamran C., Chamsai E., Udom K., Boonyapisitsopa S., Tantilertcharoen R., Kesdangsakon-wut S., Techakriengkrai N., Suradhat S., Thanawongnuwech R., Amonsin A. (2021): African horse sickness virus serotype 1 on horse farm, Thailand, 2020. *Emerg. Infect. Dis.*, 27(8):2208-2211.
- Caracappa S., Torina A., Guercio A., Vitale F., Calabrò A., Purpari G., Ferrantelli V., Vitale M., Mellor P. S. (2003): Identification of a novel bluetongue virus vector species of Culicoides in Sicily. *Vet Rec.*, 153(3):71-4.
- Carpenter S., Groschup M. H., Garros C., Felipe-Bauer M. L., Purse B. V. (2013): Culicoides biting midges, arboviruses and public health in Europe. *Antiviral Res.*, 100(1):102-13.
- Carpenter S., Mellor P. S., Fall A. G., Garros C., Venter G. J. (2017): African horse sickness virus: History, transmission, and current status. *Annu. Rev. Entomol.*, 62:343-358.
- Daniels P., Lunt R., Prichard I. (2009): Bluetongue viruses in Australasia and East Asia. In Bluetongue. Eds. P. Mellor, M. Baylis, P. Mertens, Elsevier Academic Press, 223-234.
- Djuricic B., Jermolenko G., Milosević B., Radojčić S., Debeljak Z., Tomić A. (2004a): The first bluetongue virus isolation in Yugoslavia. *Vet Ital.*, 40(4):581-2.
- Djuricic B., Nedić D., Lausević D., Pavlović M. (2004b): The epizootiological occurrence of bluetongue in the central Balkans. *Vet Ital.*, 40(3):105-7.
- Drolet B. S., McGregor B. L., Cohnstaedt L. W., Wilson W. C., McVey D. S. (2022): Veterinary microbiology. In 63. Reoviridae. Eds. D.S. McVey, M. Kennedy, M. M. Chengappa, R. Wilkes, Hoboken (NJ): John Wiley & Sons, Inc., 679-692.
- Đurić S., Vejnović B., Janjić J., Teodorović R., Nikolić A., Nedić D., Mirilović M. (2021): "Cost-benefit" analysis of bluetongue disease in domestic ruminants in
-

- the Republic of Serbia. In 32. Conference of Serbian Veterinarians, Proceedings, 64-72.
- Đurić S., Vejnović B., Nedić D., Teodorović R., Janjić J., Nikolić A., Mirilović M. (2022): Analysis of costs and benefits of the model for the control and eradication of bluetongue in the Republic of Serbia. In 27. Anniversary Annual Counselling of Doctors of Veterinary Medicine of Republic of Srpska (Bosnia and Heryegovina) with international participation, Proceedings, 115-118.
- Eagles D., Melville L., Weir R., Davis S., Bellis G., Zalucki M. P., Walker P. J., Durr P. A. (2014): Long-distance aerial dispersal modelling of *Culicoides* biting midges: case studies of incursions into Australia. *BMC Vet Res*, 10(135).
- Firth C., Blasdell K. R., Amos-Ritchie R., Sendow I., Agnihotri K., Boyle D. B., Daniels P., Kirkland P. D., Walker P. J. (2017): Genomic analysis of bluetongue virus episystems in Australia and Indonesia. *Vet. Res.*, 48(1):82.
- Gambles R. M. (1949): Bluetongue of sheep in Cyprus. *J. Comp. Pathol.*, 59:176-190.
- García-Lastra, R., Leginagoikoa I., Plazaola J. M., Ocabo B., Aduriz G., Nunes T., Juste R. A. (2012): Bluetongue virus serotype 1 outbreak in the Basque Country (Northern Spain) 2007-2008. Data support a primary vector windborne transport. *PLoS ONE*, 7(3):e34421.
- Gethmann J., Probst C., Conraths F. J. (2020): Economic impact of a bluetongue serotype 8 epidemic in Germany. *Front. Vet. Sci.*, 7:65.
- Groschupp S., Kampen H., Werner D. (2023): Occurrence of putative *Culicoides* biting midge vectors (Diptera: Ceratopogonidae) inside and outside barns in Germany and factors influencing their activity. *Parasit Vectors*, 31;16(1):307.
- Guthrie A. J., Weyer C. T. (2015): African Horse sickness, Chapter 34. In Robinson's current therapy in equine medicine, 7th edition. Eds. K. A. Sprayberry, N. E. Robinson, Elsevier.
- Holwerda M., Santman-Berends I. M. G. A., Harders F., Engelsma M., Vloet R. P. M., Dijkstra E., van Gennip R. G. P., Mars M. H., Spierenburg M., Roos L., van den Brom R., van Rijn P. A. (2023): Emergence of Bluetongue Virus Serotype 3, the Netherlands, September 2023. *Emerg Infect Dis.*, 30(8):1552-1561.
- Hudson A. R., McGregor B. L., Shults P., England M., Silbernagel C., Mayo C., Carpenter M., Sherman T. J., Cohnstaedt L. W. (2023): *Culicoides*-borne Orbivirus epidemiology in a changing climate. *J Med Entomol.*, 14;60(6):1221-1229.
- Jiménez-Cabello L., Utrilla-Trigo S., Lorenzo G., Ortego J., Calvo-Pinilla E. (2023): Epizootic Hemorrhagic Disease Virus: Current Knowledge and Emerging Perspectives. *Microorganisms*, 11(5):1339.
- Kampen H., Werner D. (2023): Biting Midges (Diptera: Ceratopogonidae) as Vectors of Viruses. *Microorganisms*, 11(11):2706.
- Lorusso A., Cappai S., Loi F., Pinna L., Ruii A., Puggioni G., Guercio A., Purpari G., Vicari D., Sghaier S., Zientara S., Spedicato M., Hammami S., Ben Hassine T.,

- Portanti O., Breard E., Sailleu C., Ancora M., Di Sabatino D., Morelli D., Calistri P., Savini G. (2023): Epizootic Hemorrhagic Disease Virus Serotype 8, Italy, 2022. *Emerg Infect Dis.*, 29(5):1063-1065.
- Maan S., Maan N. S., Ross-Smith N., Batten C. A., Shaw A. E., Anthony S. J., Samuel A. R., Darpel K. E., Veronesi E., Oura C. A., Singh K. P., Nomikou K., Portgieter A. C., Attoui H., van Rooij E., van Rijn P., De Clercq K., Vandenbussche F., Zientara S., Breard E., Sailleu C., Beer M., Hoffman B., Mellor P. S., Mertens P. P. (2008): Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. *Virology*, 377:308-318.
- MacLachlan N. J., Drew C. P., Darpel K. E., Worwa G. (2009): The pathology and pathogenesis of bluetongue. *J. Comp. Pathol.*, 141:1-16.
- MacLachlan N. J., Zientara S., Savini G., Daniels P. W. (2015): Epizootic haemorrhagic disease. *Rev. Sci. Tech.*, 34:341-351.
- Mansfield K. L., Schilling M., Sanders C., Holding M., Johnson N. (2024): Arthropod-Borne Viruses of Human and Animal Importance: Overwintering in Temperate Regions of Europe during an Era of Climate Change. *Microorganisms*, 12:1307.
- Manso-Ribeiro J., Rosa-Azevedo J., Noronha F., Braco-Forte-Junior M., Vasco-Fernandez M. (1957): Fievre catarrhale du mouton (bluetongue). *Bull. Off. Int. Epiz.*, 48:350-367.
- McKercher D. G., McGowan B., Howarth J. A., Saito J. K. (1953): A preliminary report on the isolation and identification of the bluetongue virus from sheep in California. *J. Am. Vet. Med. Assoc.*, 122:300-301.
- Meiswinkel R., van Rijn P., Leijts P., Goffredo M. (2007): Potential new Culicoides vector of bluetongue virus in northern Europe. *Vet Rec.*, 161:564-5.
- Mellor P. S., Boorman J., Baylis M. (2000): Culicoides biting midges: their role as arbovirus vectors. *Annu Rev Entomol.*; 45:307-40.
- Mellor P. S., Hamblin C. (2004): African horse sickness. *Vet. Res.*, 35:445-66.
- Niedbalski W. I., Fitzner A. (2020): Recent changes in the global distribution of arboviral infections in ruminants. *Med. Weter.*, 76(2):71-76.
- Nolan D. V., Carpenter S., Barber J., Mellor P. S., Dallas J. F., Mordue Luntz A. J., Pierniey S. B. (2007): Rapid diagnostic PCR assays for members of the Culicoides obsoletus and Culicoides pulicaris species complexes, implicated vectors of bluetongue virus in Europe. *Vet Microbiol.*, 124(1-2):82-94.
- Nunamaker R. A., Sieburth P. J., Dean V. C., Wigginton J. G., Nunamake, C. E., Mechem J. O. (1990): Absence of transovarial transmission of bluetongue virus in culicoides variipennis immunogold labeling of bluetongue virus-antigen in developing oocytes from Culicoides variipennis (Coquillett). *Comp. Biochem. Physiol. A Comp. Physiol.*, 96(1):19-31.
- Ohashi S., Yoshida K., Watanabe Y., Tsuda T. (1999): Identification and PCR restriction fragment length polymorphism analysis of a variant of the Ibaraki virus from
-

- naturally infected cattle and aborted fetuses in Japan. *J. Clin. Microbiol.*, 37:3800-3803.
- Prasad G., Sreenivasulu D., Singh K. P., Mertens P. P. C., Maan S. (2009): Bluetongue in the Indian subcontinent. In Bluetongue. Eds. P. Mellor, M. Baylis, P. Mertens, Elsevier Academic Press, 167-195.
- Purse B. V., Brown H. E., Harrup L., Mertens P. P. C., Rogers D. J. (2008): Invasion of bluetongue and other orbivirus infections into Europe: the role of biological and climatic processes. *OIE Rev Sci Tech.*, 27(2):427-442.
- Purse B., 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. *Nat. Rev. Microbiol.*, 3:171-181.
- Ren N., Wang X., Liang M., Tian S., Ochieng C., Zhao L., Huang D., Xia Q., Yuan Z., Xia H. (2021): Characterization of a novel reassortment Tibet orbivirus isolated from *Culicoides* spp. in Yunnan, PR China. *J Gen Virol.*, 102(9):1-10.
- Ries C., Vögtlin A., Hüsey D., Jandt T., Gobet H., Hilbe M., Burgener C., Schweizer L., Häfliger-Speiser S., Beer M., Hoffmann B. (2021): Putative novel atypical BTV serotype '36' identified in small ruminants in Switzerland. *Viruses*, 13(5):721.
- Ruder M. G., Lysyk T. J., Stallknecht D. E., Foil L. D., Johnson D. J., Chase C. C., Dargatz D. A., Gibbs E. J. (2015): Transmission and epidemiology of bluetongue and epizootic hemorrhagic disease in North America: current perspectives, research gaps, and future directions. *Vector Borne Zoonotic Dis.*, 15(6):348-363.
- Savini G., Goffredo M., Monaco F., Di Gennaro A., Cafiero M. A., Baldi L., de Santis P., Meiswinkel R., Caporale V. (2005): Bluetongue virus isolations from midges belonging to the *Obsoletus* Complex (*Culicoides*, Diptera: Ceratopogonidae) in Italy. *Vet Rec.*, 157:133-9.
- Sghaier S., Silleanu C., Marcacci M., Thabet S., Curini V., Ben Hassine T., Teodori L., Portanti O., Hammami S., Jurisic L., Spedicato M., Postic L., Gazani I., Ben Osman R., Zientara S., Bréard E., Calistri P., Richt J. A., Holmes E. C., Savini G., Di Ciallonardo F., Lorusso A. (2023): Epizootic haemorrhagic disease virus serotype 8 in Tunisia, 2021. *Viruses*, 15(1).
- Stevanović O., Nedić D. (2019): Clinical presentation of bluetongue and the malignant form of contagious ecthyma in sheep: Description of cases. *Veterinarski Glasnik*, 73(1):57-63.
- Stevanović O., Šević K., Marić J., Krneta D., Kasagić D., Nedić D. N., Sladojević Ž. (2018): Epidemiology of bluetongue disease in sheep: results of systematic surveillance in the Republic of Srpska. In 23. Anniversary Annual Counselling of Doctors of Veterinary Medicine of Republic of Srpska (Bosnia and Heryegovina) with international participation, Proceedings, 53-54.
- Tomazatos A., Jöst H., Schulze J., Spinu M., Schmidt-Chanasit J., Cadar D., Luhken R. (2020): Blood-meal analysis of *Culicoides* (Diptera: Ceratopogonidae)

- reveals a broad host range and new species records for Romania. *Parasites Vectors*, 13(79):2-12.
- Torina A., Caracappa S., Mellor P. S., Baylis M., Purse B. V. (2004): Spatial distribution of bluetongue virus and its *Culicoides* vectors in Sicily. *Med Vet Entomol.*, 18:81-9.
- Wellby M. P., Baylis M., Mellor P. S. (1996): Effect of temperature on survival and rate of virogenesis of African horse sickness virus in *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) and its significance in relation to the epidemiology of the disease. *Bull. Entomol. Res.*, 86:715-720.
- Wilson A. J., Mellor P. S. (2009): Bluetongue in Europe: past, present and future. *Philos Trans R Soc Lond B Biol Sci.*, 364(1530):2669-81.
- Wilson A., Darpe K., Mellor P. S. (2008): Where does bluetongue virus sleep in the winter? *PLoS Biol.*, 6(8):e210.
- Winder, J. A. (1978): Cocoa flower Diptera: Their identity, pollinating activity and breeding sites. *Int. J. Pest. Manag.*, 24:5-18.
- Wittmann E. J., Mellor P. S., Baylis M. (2002). Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. *Med. Vet. Entomol.*, 16:147-156.
- Yang H., Li Z., Wang J., Li Z., Yang Z., Liao D., Zhu J., Li H. (2020): Novel Serotype of Epizootic Hemorrhagic Disease Virus, China. *Emerg. Infect. Dis.*, 26:3081-3083.
- Zientara S., Weyer C. T., Lecollinet S. (2015): African horse sickness. *Rev. Sci. Tech.*, 34:315-327.
-