



OCCURRENCE, ACTIVITY AND CONTROL OPTIONS AGAINST BITING MIDGES (DIPTERA: *CULICOIDES*) IN HORSES

Richard Pavlík, Alica Kočíšová, Andrea Schreiberová, Zuzana Kasičová, Nikola Janošková

Department of Epizootiology, Parasitology and Protection of One Health, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

alica.kocisova@uvlf.sk

ABSTRACT

Biting midges are insects which annoy horses as they cause discomfort and by the sucking of blood, they cause painful lesions that, in certain cases, can trigger acute allergic reactions. They also act as potential vectors of many pathogens of bacterial, viral and parasitic origin. The aim of this study was to investigate seasonal dynamics, abundance and species composition of biting midges, and implementing protection options at the Equestrian centre of the University of Veterinary Medicine and Pharmacy (UVMP) in Košice in the years 2021 and 2022. During this time period (of 2021 and 2022), we captured 4797 biting midges. Our results showed that in 2021 the most frequently captured species were *C. obsoletus/C. scoticus* (prevalence 86.6 %), followed by *C. punctatus* and *C. pulicaris* (prevalence 5.37 % and 3.86 %, resp.). In addition, *C. lupicaris*, *C. newsteadi*, *C. furcillatus*, *C. festivipennis*, *C. slovacus*, *C. tauricus*, *C. clastrieri*, *C. dewulfi*, *C. fagineus* and *C. circumscriptus* were identified as the minority species. In this year, the highest abundance of these insects (1758 biting midges) was observed at the end of June. In 2022, we detected a clear dominance of *C. obsoletus/C. scoticus* (prevalence 97.0 %) and the highest number

of biting midges (655) was captured in mid-May). In this year, the minority species included, *C. pulicaris*, *C. punctatus* and *C. lupicaris*. The identification of the host blood showed that man (*Homo sapiens*) was the dominant host, namely of 80 % of the examined biting midges of species *C. obsoletus/C. scoticus*, *C. dewulfi*, *C. punctatus* and *C. festivipennis*. In one female of species *C. fagineus* we identified the host blood from horses (*Equus caballus*). In 2022, we detected the blood of a domestic rabbit (*Oryctolagus cuniculus*) in the species of *C. obsoletus/C. scoticus*.

Key words: *Culicoides*; host preference; prevalence; species composition

INTRODUCTION

Biting midges are tiny, blood-sucking insects that annoy not only animals, but also humans. Raids of their large swarms irritate people involved in outdoor activities, affect productivity and work usability of farm and pet animals and induce various forms of hypersensitive responses, particularly in horses. Equine Insect Bite Hypersensitivity (IBH) is the most frequent allergic skin disease in horses

[17] manifested as chronic recurrent seasonal allergic dermatitis. The sensitive reaction of horses is the response to proteins found in the saliva of the biting midges that are considered strong allergens. The animals react to them by increased production of serum IgE antibodies as a result of hypersensitive type I and IV reactions [20]. This involves a hereditary disease that appears usually in the second and third year of life and tends to grow worse with the increasing age of the affected individuals. It was confirmed that particularly the saliva of *Culicoides pulicaris* [21] is considered the causative agent of the above mentioned allergic dermatitis although other biting midge species parasitise on horses, such as *C. obsoletus*/*C. scoticus*, *C. lupicaris*, *C. punctatus* and other [19]. Horses affected by IBH show seasonal manifestations with gradual progression from spring to autumn when the *Culicoides* are active, and usually regress during the winter period, however, clinical signs can persist in winter in serious chronic cases. The initial clinical signs can be characterised as numerous papillae, hyperaesthesia and sensitisation of skin with intense pruritus resulting in local loss of hair and excoriations evident along the dorsal medial line, at the basis of the mane and tail [4] and, occasionally, beside ears [11]. However, the most important reason for permanent studies of biting midges, their species composition, occurrence and activity is their ability to transmit pathogens that induce many diseases included in the list of diseases defined by the World Organisation for Animal Health (WOAH) that result in serious economic losses related to disturbances of the health state, emergency measures and restrictions concerning the international trade of animals and animal products [22].

The aim of this study was to investigate the seasonal dynamics, abundance, species composition of biting midges and the potential prevention options that are comprehensively affected by many biotic and abiotic factors. The study was carried out at the Equestrian centre of the University of Veterinary Medicine and Pharmacy (UVMP) in Košice in the years 2021 and 2022, where equine patients with signs of IBH occurred recently.

MATERIALS AND METHODS

The entomological investigations were carried out in the period of May to October of the years 2021 and 2022 in the Equestrian centre of UVMP in Košice. This is a

specialised facility of the University of Veterinary Medicine and Pharmacy in Košice with the capacity of housing approximately 60 horses. In addition to the housing area, there is available also an indoor riding arena with special surface, covered and outdoor sand riding area, as well as grass runs for horses, grass parkour and social facilities for personnel.

Capture of the biting midges

Biting midges were collected using miniature light traps CDC 1212 (John W. Hock, USA) with UV light that were situated at two sites, inside the house for horses and outside at the runs. The light was turned on at dusk (between 6:00 and 7:00 p.m.) when the activity of biting midges gradually increases. The catch was collected early in the morning (between 6:00 and 7:00 a.m) on the following day.

Storage, processing and conservation of samples

In the laboratory, the biting midges were separated from other insects, rinsed in 50 % ethanol, conserved in the vessel containing 70 % ethanol and placed in refrigerated apparatus prepared for determination of species.

Morphological diagnosis of biting midges

Using a binocular magnifying lens ZEISS-STEVIDV-4 and an entomological tweezers we sorted the biting midges on a Petri dish into basic complexes and determined the species on the basic typical morphological characteristics according to the morphological key [14]. Subsequently, the females were divided to four groups according to their physiological state: nulliparous (female that never sucked blood – has empty abdomen); parous (female that sucked blood – blood fragments in abdomen are visible under binocular lens); engorged (visibly filled-up abdomen that acquires a more oval shape); gravid (engorged abdomen full of eggs) [9]. Such separation of females is necessary as for detection of host blood and pathogens the biting midges with fresh blood in the abdomen are used.

PCR species diagnosis of biting midges and the host blood

In the cases of morphological similarity of species, we used a molecular diagnostic method for their identification. Genomic DNA was isolated employing the commercial kit Dneasy® Blood and Tissue Kit (QIAGEN, GmbH,

Hilden, Germany). It was isolated from the female that previously sucked blood (engorged) according to the protocol in the information leaflet of the kit manufacturer. DNA samples were stored in a freezer at -20 °C. The concentration of the obtained DNA was measured by means of a Qubit 4 Fluorometer (Thermo Fisher Scientific, USA) and diluted to the final concentration of 20–50 ng.µl⁻¹.

Fragment of mitochondrial gene of cytochrome b (cyt b) of size approximately 350 bp was used to identify the host blood. The total volume of each sample destined for PCR analysis reached 47 µl and contained the following: 21 µl Mastermix Emerald AmpGT, 21 µl nuclease free water, 1 µl primer cyt bb1, 1 µl primer cyt bb2 a 3 µl DNA. The PCR reaction took place in Mastercycler nexus X2 (Eppendorf, Germany), using universal primers for vertebrates, namely forward primer cyt bb1 (5'-CCA TCM AAC ATY TCA DCA TGAAA-3') and reverse one cyt bb2 (5'-GCH CCT CAG AAT GAY) ATT TKG CCT CA-3'). The amplification process consisted of the initial denaturation step at 94 °C for 5 min, followed by 35x (94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min) and extension at 72 °C for 7 min [18]. Altogether 24 samples were subjected to isolation and PCR analysis for identification of host blood.

Visualisation, purification and sequencing of PCR products for identification of biting midge species, diagnosis of host blood and pathogen species are identical. Electrophoresis was performed in agarose gel (1 %) stained with GoodView TM Nucleic Acid Stain into which

we loaded 5 µl of the PCR product and visualisation took place under UV light. The positive PCR products were sent to a qualified workplace in the Czech Republic for purification and sequencing. The sequencing was performed in both directions with the same primers as those used for PCR analysis.

The result of sequencing is a symbolic linear code referred to as the section that represents arrangement of the sequenced molecule. Immediately after analysis of chromatograms by means of software MEGA X [12], we conducted nucleotide sequence alignment by means of software GeneTool Lite 1.0 (BioTools Inc., California, USA).

RESULTS

Altogether, we captured 4797 biting midges. The most numerous were the species *C. obsoletus/C. scoticus* with a prevalence of 86.6 % in 2021 and 97 % in 2022. Minority species included *C. punctatus*, *C. pulicaris*, *C. lupicaris*, *C. newsteadi*, *C. furcillatus*, *C. festivipennis*, *C. slovacus/C. tauricus*, *C. clastieri*, *C. dewulfi*, *C. fagineus* and *C. circumscriptus* (Table 1). In 2021, the highest abundance of biting midges was recorded at the end of June (1758) (Fig. 1), while in 2022, the highest number of them were captured in mid-May (655) (Fig. 2).

Table 1. Species composition of biting midges captured in the Equestrian centre of UVMP in 2021–2022

Species	2021	2022	Total	Prevalence 2021	Prevalence 2022
<i>C. obsoletus/C. scoticus</i>	3116	1165	4281	86.6 %	97.0 %
<i>C. lupicaris</i>	28	1	29	0.78 %	0.08 %
<i>C. newsteadi</i>	25	0	25	0.70 %	0.00 %
<i>C. pulicaris</i>	139	22	161	3.86 %	0.02 %
<i>C. punctatus</i>	193	12	205	5.37 %	0.01 %
<i>C. furcillatus</i>	61	0	61	1.70 %	0.00 %
<i>C. festivipennis</i>	3	0	3	0.08 %	0.00 %
<i>C. clastieri</i>	3	0	3	0.08 %	0.00 %
<i>C. dewulfi</i>	1	0	1	0.03 %	0.00 %
<i>C. tauricus/C. slovacus</i>	21	0	21	0.58 %	0.00 %
<i>C. fagineus</i>	2	0	2	0.06 %	0.00 %
<i>C. circumscriptus</i>	1	0	1	0.03 %	0.00 %
<i>Culicoides</i> spp.	4	0	4	0.11 %	0.00 %
Total	3597	1200	4797	100 %	100 %

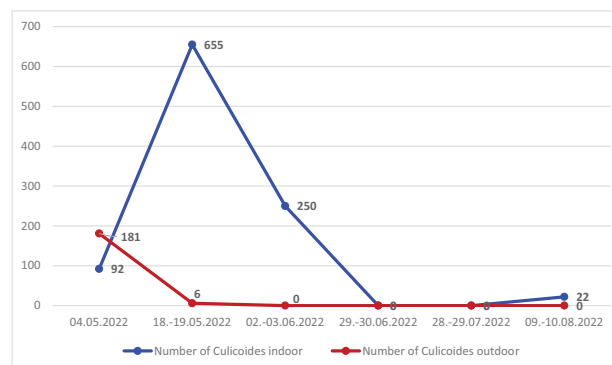
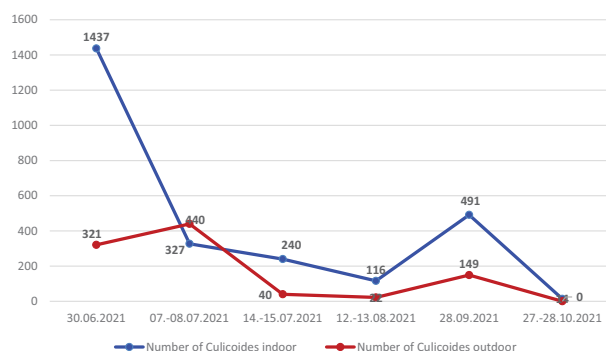


Table 2. Identification of host blood in the captured biting midges

Species	Host	Sequence of cytb host gene
<i>C. fagineus</i>	<i>Equus caballus</i>	catgatgaaacttcggctcctctctaggaatctgcctaatctctcaaatcttaacaggcctattcctagc catacactacatcagatacgacaactgcctttctatccgtcactcacatctgcgcgagacgttaactac ggatgaattatcgctaccctccatgccaaaggagcatcaatattttatctgcctcttcttacagtag gacggcgctctactacggctcttacacattcttagagacatgaaacattggaatcatctactttcac agttatagctacagcattcatgggctatgtcctaccatgaggccaaatac
<i>C. obsoletus/C. scoticus</i>	<i>Homo sapiens</i>	tcagcatgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactattcc tagccatgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcgagacgtaaa ttatggctgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctcttctacac atcgggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcattatcctctgc ttgcaactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. dewulfi</i>	<i>Homo sapiens</i>	tcagcatgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactattcc tagccatgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcgagacgtaaa ttatggatgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctcttctacac atcgggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcattatcctctgc ttgcaactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. punctatus</i>	<i>Homo sapiens</i>	tcagcatgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactattcc tagccatgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcgagacgtaaa ttatggctgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctcttctacac atcgggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcattatcctctgc ttgcaactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. punctatus</i>	<i>Homo sapiens</i>	tcagcatgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactattcc tagccatgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcgagacgtaaa ttatggctgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctcttctacac atcgggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcattatcctctgc ttgcaactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. festivipennis</i>	<i>Homo sapiens</i>	tcagcatgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactattcc tagccatgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcgagacgtaaa ttatggctgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctcttctacac atcgggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcattatcctctgc ttgcaactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. obsoletus/C. scoticus</i>	<i>Homo sapiens</i>	tcagcatgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactat- tcttagccatgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcga- gacgtaaattatggctgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctct- tctacacatcgggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcatt- tatcctctgccttgcaactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. tauricus/C. slovacus</i>	<i>Homo sapiens</i>	tcagcatgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactattcc tagccatgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcgagacgtaaa ttatggctgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctcttctacac atcgggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcattatcctctgc ttgcaactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. obsoletus/C. scoticus</i>	<i>Homo sapiens</i>	catgatgaaacttcggctcactccttggcgctgcctgatctctcaaatcaccacaggactattcctagc catgcactactaccagacgctcaacgcctttcatcaatcgcccacatcactcgagacgtaaattat ggctgaatcatcgctaccttcacgccaatggcgctcaaatattctttatcgctcttctacacatcg ggcgaggcctatattacggatcatttctctactcagaacactgaaacatcggcattatcctctgcctgc aactatagcaacagcctcataggctatgtcctcccgtagg
<i>C. obsoletus/C. scoticus</i>	<i>Oryctolagus cu- niculus</i>	tgatgaaacttggctctctactaggcctgtgccttaataattcaatttactggcctattcttagcca tacactacacctctgacacaacaacagcattctcatcagtaaccctatttggcgagatgttaactatgg ctgactattcggatacctccacgctaacggagcatctatatattcttattgctctcatcacacgtaggc cgcggaattactatggatcacacatacctagagacctgaaacattggcatcatcctctattcgcag taatagccacagcattatgggttatgtctcccatgagg

Identification of host blood in biting midges in our study showed that the dominant host of these insects were humans (*Homo sapiens*), namely in as many as 80 % of the investigated biting midges, specifically in *C. obsoletus/C. scoticus*, *C. dewulfi*, *C. punctatus* and *C. festivipennis*. In a female of *C. fagineus*, we identified blood from a horse (*Equus caballus*). In 2022 we identified blood of a domestic rabbit (*Oryctolagus cuniculus*) in *C. obsoletus/C. scoticus* (Table 2).

DISCUSSION

In Slovakia, biting midges show a pronounced seasonal activity. Our study was conducted in the years 2021 to 2022, when we captured altogether 4797 biting midges of 14 species, the dominant species being *C. obsoletus/C. scoticus* with prevalence of 86.6 % in 2021 and 97 % in 2022. The total number captured in 2021 was 3597, most of them (73 %) captured inside in the house for horses. In 2022, the catch totalled 1200 biting midges and again most of them (85 %) in the interior. Interesting were the differences in abundance and species composition determined in the same place in the period of 2018 to 2020 [10] and the results obtained in this study. In the years 2018 to 2020 there were captured as many as 35 463 biting midges of 17 species. The highest abundance and species diversity was observed in 2019. Of the minority species there were detected *C. bysta*, *C. picturatus*, *C. clastieri*, *C. dewulfi* and *C. deltus* [10]. The differences in an abundance and species composition were probably related to the construction changes in the past two years. Both the internal and external facilities underwent reconstruction that resulted in the elimination of biotopes suitable for propagation of larvae of biting midges requiring an abundance of decomposing organic matter and humid environment. The highest abundance of biting midges was recorded in species of subgenus *Avaritia* that includes biting midges *C. obsoletus*, *C. scoticus* and *C. dewulfi*. These species also show morphological similarity. According to Z i t r a et al. [24] morphological diagnosis of their females is impossible, thus it is necessary to use molecular methods for better identification of the species. The mentioned species are the main viral vectors in the palearctic realm and at the same time belong among the most numerous in Europe [3]. Their high prevalence was recorded also in Germany [2], Ireland [7], the Netherlands, Italy and Sweden [15].

Identification of host blood is inevitable for the control of spreading of new species of biting midges and the associated transmission of pathogens. Sucking blood of host by the insect depends on its ability to remain active when the suitable hosts are available. Blood of the host parasitized by the vector affects its intestinal microbiota. During blood sucking the microbicidal effector molecules such as antimicrobial peptides are expressed [16]. Last but not least, the blood is a source of proteins necessary for the females before laying the eggs. Some studies implied that the selection of a suitable host for biting midges *Culicoides* is limited by the availability of hosts in their surroundings [23]. On the other hand, anthropogenic activities provide suitable opportunities for the transmission of novel pathogens among people. In the study by C a l v o at al. [5], conducted in the National park Picos de Europa in northern Spain, the scientists focused on identification of host blood in biting midges and ascertained that 95.8 % of host blood originated from mammals and 6.00 % from the domestic fowl (*Gallus gallus*). The most frequent hosts were sheep (87.7 %), people (6.5 %), cattle (3.7 %) and small rodents *Microtus savii* (2.1 %). This study indicated that ruminants were the preferred source of blood subsistence for *Culicoides* in the investigated territory. However, there were observed many situations in which the biting midges exhibited opportunistic behaviour related to the abundance of specific potential hosts as the sources of blood. In the case of low abundance of the preferred hosts, the biting midges are capable of adapting. This can explain the fact that in 80 % of samples examined in this study, we detected in human blood, namely in those from *C. obsoletus/C. scoticus*, *C. dewulfi*, *C. punctatus*, *C. Taurica's/C. slovacus* and *C. festiveness*. In only one sample obtained from *C. fagineus*, we identified horse (*Equus caballus*) as the source of blood. Rabbit blood (*Oryctolagus cuniculus*) was detected in *C. obsoletus/C. scoticus*.

The above statements indicates that the necessity of protecting people and other animals from midge bites. From the entomological aspect, we could suggest the use of insecticide or repellents to protect susceptible animals from bites, however, one study conducted in Switzerland showed that the common insecticides and repellents failed to produce the required effect [13]. With respect to protection and well-being of animals, they should be kept in housings protected against insects (e.g. by treatment of windows and doors by contact insecticides) and regular use

of light traps. Regrettably, this provokes some criticism as some of such measures appear to be impractical, expensive and financially infeasible and have none or only minimal effect. For people temporarily exposed to biting midges the use of repellents appears to be a suitable measure of protection against insect bite; N,N-diethyl-m-toluamid (DEET) is considered the gold standard for this purpose [8]. With respect to protection of animals, Carpenter et al. [6] recommended to disrupt the development of biting midges by elimination of suitable biotopes for larvae which was supported also by our study that showed improvement after reconstruction of the Equestrian centre. In horses it is possible to use allergen-specific immunotherapy (allergen-SIT), consisting of subcutaneous administration of an allergen extract from biting midges. Although the mechanism by which the allergen-SIT mediates its anti-inflammatory effects has not yet been completely explained, various studies revealed that allergen-SIT affects the response of APC, cells, T cells, B cells and effector cells [1]. Allergen-SIT results in early induction of Treg cells suppressing Th2-Th1 cells and other effector cells which can result in early protection against midge baits.

CONCLUSIONS

There are many reasons that justify the need for entomological monitoring of species composition, abundance, seasonal activity and host preference of *Culicoides* species. Global changes (anthropogenic, climatic and social) that occurred in the past two decades resulted in spreading and emergence of new serious diseases of various aetiology. Biting midges are also involved in transmission of their causative agents. The methods of molecular biology allowed us to determine the host activity of biting midges in the Equestrian centre of the UVMP in Košice and to characterise biological specialties of parasite-host relationships that are affected by local adaptation to the available hosts as well as the environmental factors.

ACKNOWLEDGEMENT

The research was carried out within the VEGA Project No. 1/0043/19.

REFERENCES

1. Akdis, C. A., Akdis, M., 2011: Mechanisms of allergen-specific immunotherapy. *J. Allergy Clin. Immunol.*, 127, 1, 18–27. DOI: 10.1016/j.jaci.2010.11.030.
2. Ayllón, T., Nijhof, A. M., Weiher, W., Clausen P.-H., 2014: Feeding behaviour of *Culicoides* spp. (Diptera: Ceratopogonidae) on cattle and sheep in northeast Germany. *Parasit. Vect.*, 7, 1, 34. DOI: 10.1186/1756-3305-7-34.
3. Bartsch, S., Bauer, B., Wiemann, A., Clausen, P. H., Steuber, S., 2009: Feeding patterns of biting midges of the *Culicoides obsoletus* and *Culicoides pulicaris* groups on selected farms in Brandenburg, Germany. *Parasitol. Res.*, 105, 2, 373–80. DOI: 10.1007/s00436-009-1408-y.
4. Broström, H., Larsson, Å., Troedsson, M., 1987: Allergic dermatitis (sweet itch) of Icelandic horses in Sweden: An epidemiological study. *Equine Vet. J.*, 19, 229–236.
5. Calvo, J., Berzal, B., Calvete, C., Miranda, M., Estrada, R., Lucientes, J., 2012: Host feeding patterns of *Culicoides* species (Diptera: Ceratopogonidae) within the Picos de Europa National Park in northern Spain. *Bull. Entomol. Res.*, 102, 6, 692–697.
6. Carpenter, S., Szmargd, C., Barber, J., Labuschagne, K., Gubbins, S., Mellor, P., 2008: An assessment of *Culicoides* surveillance techniques in northern Europe: Have we underestimated a potential bluetongue virus vector? *J. Appl. Ecol.*, 45, 1237–1245.
7. Collins, Á. B., Mee, J. F., Doherty, M. L., Barrett, D. J., England, M. E., 2018: *Culicoides* species composition and abundance on Irish cattle farms: Implications for arboviral disease transmission. *Parasit. Vect.*, 11, 1, 472. DOI: 10.1186/s13071-018-3010-6.
8. Corbel, V., Stankiewicz, M., Pennetier, C., Fournier, D., Stojan, J., Girard, E., et al., 2009: Evidence for inhibition of cholinesterases in insect and mammalian nervous systems by the insect repellent deet. *BMC Biol.*, 7, 1, 47. DOI: 10.1186/1741-7007-7-47.
9. Dyce, A. L., 1969: The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogidae) without dissection. *J. Austral. Entomol. Soc.*, 8, 11–15.
10. Kasičová, Z., 2022. *Epizootological Potential and Ecology of Biting Midges (Culicoides) in Slovakia* (In Slovak). Dissertation thesis. UVLF Košice, 121 pp.
11. Kleider, N., Lees, M. J., 1984: *Culicoides* hypersensitivity in the horse: 15 cases in southwestern British Columbia. *Can. Vet. J.*, 25, 26–32.

12. Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018: MEGA X: Molecular evolutionary genetic analysis across computing platforms. *Mol. Biol. Evol.*, 35, 6, 1547–1549. DOI: 10.1093/molbev/msy096.
13. Lincoln, V. J., Page, P. C., Koppa, C., Mathis, A., von Niederhäusernd, R., Burger, D., Herholz, C., 2015: Protection of horses against *Culicoides* biting midges in different housing systems in Switzerland. *Vet. Parasitol.*, 210, 3–4, 206–214. DOI: 10.1016/j.vetpar.2015.04.006.
14. Mathieu, B., Cêtre-Sossah, C., Garros, C., Chavernac, D., Balenghien, T., Carpenter, S., et al., 2012: Development and validation of IIC: An interactive identification key for *Culicoides* (Diptera: Ceratopogonidae) females from the Western Palearctic region. *Parasit. Vect.*, 5, 137.
15. Möhlmann, T. W. R., Wennergren, U., Tälle, M., Favia, G., Damiani, C., Bracchetti, L., et al., 2018: Community analysis of the abundance and diversity of biting midge species (Diptera: Ceratopogonidae) in three European countries at different latitudes. *Parasit. Vect.*, 11, 217. DOI: 10.1186/s13071-018-2792-x.
16. Nayduch, D., Lee, M. B., Saski, C. A.: 2014: Gene discovery and differential expression analysis of humoral immune response elements in female *Culicoides sonorensis* (Diptera: Ceratopogonidae). *Parasit. Vect.*, 7, 388. DOI: 10.1186/1756-3305-7-388.
17. Pilsworth, R. C., Knottenbelt, D. C., 2004: Equine insect hypersensitivity. *Equine Vet. Educ.*, 16, 324–325.
18. Radrova, J., Seblova, V., Votypka, J., 2013: Feeding behaviour and spatial distribution of *Culex* mosquitoes (Diptera: Culicidae) in wetland areas of the Czech Republic. *J. Med. Entomol.*, 50, 5, 1097–1104. DOI: 10.1603/me13029.
19. Sarvašová, A., 2014: *Biodiversity, Competence and Epidemiological Role of Blood-Sucking Diptera Species (Diptera: Ceratopogonidae) in Eastern Slovakia* (In Slovak). Dissertation thesis. UVLF Košice, 205 pp.
20. Schaffartzik, A., Hamza, E., Janda, J., Cramer, R., Marti, E., Rhyner, C., 2012: Equine insect bite hypersensitivity: What do we know? *Vet. Immunol. Immunopathol.*, 147, 3–4, 113–126. DOI: 10.1016/j.vetimm.2012.03.017.
21. Van der Rijt, R., van den Boom, R., Jongema, Y., van Oldrutenborgh-Oosterbaan, M. M., 2008: *Culicoides* species attracted to horses with and without insect hypersensitivity. *Vet. J.*, 178, 91–97. DOI: 10.1016/j.tvjl.2007.07.005.
22. Venter, G. J., Boikanyo, S. N. B., de Beer, C. J., 2019: The influence of temperature and humidity on the flight activity of *Culicoides imicola* both under laboratory and field conditions. *Parasit. Vect.*, 12, 4. DOI: 10.1186/s13071-018-3272-z.
23. Videvall, E., Bensch, S., Ander, M., Chirico, J., Sigvald, R., Ignell, R., 2013: Molecular identification of blood-meals and species composition in *Culicoides* biting midges. *Med. Vet. Entomol.*, 27, 1, 104–112. DOI: 10.1111/j.1365-2915.2012.01038.x.
24. Zittra, C., Wöss, G., Van der Vloet, L., Bakran-Lebl, K., Shahi Barogh, B., Sehnal, P., Fuehrer, H. P., 2020: Barcoding of the genus *Culicoides* (Diptera: Ceratopogonidae) in Austria – an update of the species inventory including the first records of three species in Austria. *Pathogens*, 23, 9, 5, 406. DOI: 10.3390/pathogens9050406.

Received July 10, 2023

Accepted August 17, 2023