RESEARCH ARTICLE

Diversity, Abundance and Host Preference of *Culicoides* (Diptera: Ceratopogonidae), Potential Vectors and Nuisance Insect in Zoo Park Košice, Slovakia

Authors

Zuzana Kasičová[1], Petronela Komorová[1], Patrik Pastorek[2], Andrea Schreiberová[1], Miroslava Friedman[2], Andrea Kimáková[1], Alica Kočišová[1*]

Affiliations

[1] Department of Epizootiology, Parasitology and Protection of One Health, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic;

[2] Zoological Garden Košice, Široká 31, 040 06 Košice – Kavečany, Slovak Republic

[*] Corresponding author:

Alica Kočišová, Department of Epizootiology, Parasitology and Protection of One Health, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic; e-mail: alica.kocisova@uvlf.sk

Abstract

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) were captured in the course of three seasons (2017–2020) in the Zoo Košice. The total number of captured individuals was 5,667 biting midges, and 12 species of *Culicoides* were identified, while the most frequently captured and the most populous species were *C. obsoletus/C. scoticus*, *C. furcillatus*, and *C. festivipennis*. The most frequent hosts on which the biting midges were blood-feeding were humans (*Homo sapiens*), Burchell's zebra (*Equus quagga burchellii)*, and domestic cow (*Bos taurus*). This is the first study to confirm a wide range of biting midges species in a zoo which are capable of using not only animals as their hosts, but also humans, in whom a bite may induce an allergic reaction. More than 80% of the fauna of biting midges in the Zoo Košice represented *Culicoides obsoletus*/*C. scoticus*, which are in the Central Europe regarded as the potential vectors of arboviruses, to which primarily ruminants are highly susceptible.

Keywords: biting midges, species composition, blood feeding, exotic animals

1. Introduction

Ecosystems in zoological gardens provide a favourable environment for various pathogens to spread, as a variety of exotic and domestic animals live on typically a small territory.[1] Biting midges belong to the important vectors that participate in the active biological transmission of a wide range of globally significant arboviruses, including the Bluetongue Virus (BTV) and Schmallenberg Virus (SBV), as well as parasites, such as *Onchocerca*, *Haemosporidium* and other.[2-4] Onchocerciasis is induced by more than 20 species of *Onchocerca* spp., while their microfilariae are transmitted by black flies (*Simulium* spp.) or biting midges (*Culicoides* spp.), depending on a species, with a varied range of definitive hosts, including domestic and wild ungulates, carnivores, and humans.[5] In recent years, there have been recurrent cases of primarily human onchocerciasis induced by the zoonotic species *Onchocerca cervicalis* and *O. gutturosa*, while the disease was clinically manifested by dermatitis, ocular involvement, as well as epilepsy.[6] Microfilariae of these worms are transmitted by biting midges of *Culicoides nubeculosus*, *C. variipennis*, *C. kingi* and other *Culicoides* spp.[7-9] Besides the fact that biting midges are important vectors of pathogens, their saliva, which penetrates into a host during the blood sucking, is an important allergen that induces dermatitis.[10-12] Females of these diptera feed on blood of a wide range of vertebrae. They parasitise mainly on mammals and birds, and some of them also on the blood of reptiles and amphibians.[13] In zoological gardens, their bites affect the overall health and welfare of animals, caretaking staff and visitors. From the epizootiological and epidemiological points of view, the transmission of pathogens is limited mainly by the specificity of vectors and the number of hosts. Due to the fact that zoological gardens comprise a variety of animal species, *Culicoides* biting midges may use several different types of potential hosts at one place, and this improves the conditions for the transmission of pathogens among animals bred in captivity.[14] Moreover, this also increases a risk of infection in humans. The purpose of this study is to present the current situation regarding the species composition, abundance and host preference of biting midges in the Zoo Košice.

2. Methods

2.1. Trapping

Zoo Košice (48°47'16.32"S, 21°12'18.59"V) is the second largest zoo in the Central Europe; it extends over the area of 289 ha in the natural environment of the Čierna Hora Mountains near Košice, in the Kavečany Municipal Region, at an altitude of 381 meters above mean sea level. In the period from 2017 to 2020, in the seasons from April to November, the monitoring of the fauna of biting midges was carried out. Biting midges were trapped using the CDC 1212 light traps (John Hock Company, USA) (Fig. 1). The light traps were installed in the evenings and collected next mornings.

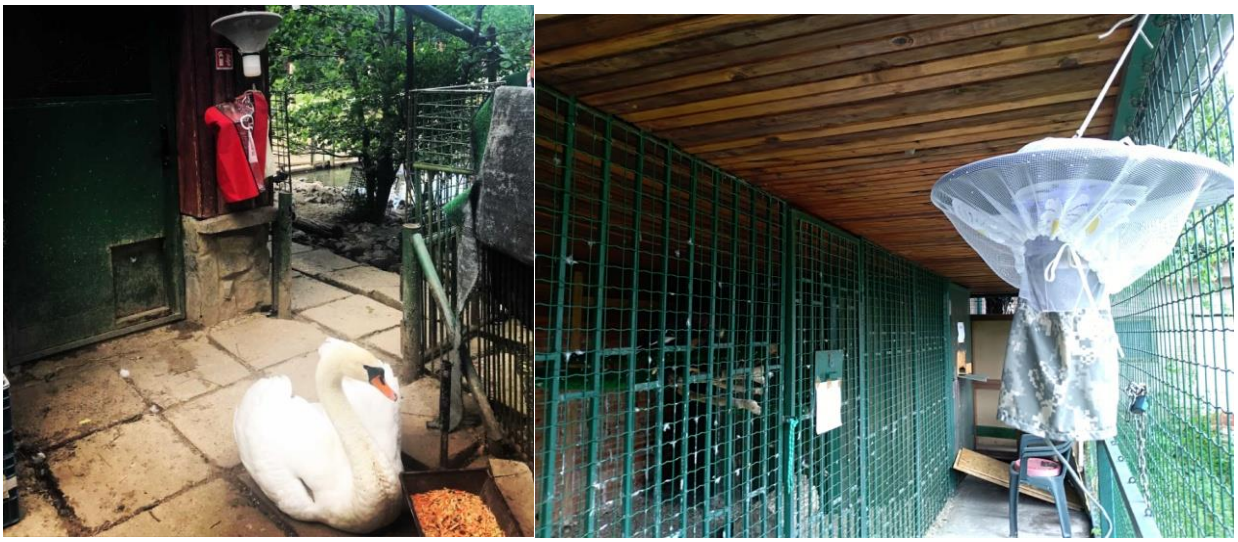

Fig. 1. Traps in aviaries

Trap A was installed near perissodactyls, in particular: water buffalo (*Byvol arni*), Przewalski's horse (*Equus ferus przewalskii*), Chapman's zebra (*Equus quaqqa chapmanni*), and blue wildebeest (*Connochaetes taurinus taurinus*).

Trap B was installed near aviaries with the following birds: great white pelican (*Pelecanus onocrotalus*), Dalmatian pelican (*Pelecanus crispus*), greylag goose (*Anser anser*), Muscovy duck (*Cairina moschata*), swan goose (*Anser cygnoides*), black swan (*Cygnus atratus*), mute swan (*Cygnus olor*), wood duck (*Aix sponsa*), red-crested pochard (*Netta rufina*), mandarin duck (*Aix galericulata*), mallard (*Anas platyrhynchos*), Andean condor (*Vultur gryphus*), and Steller's sea eagle (*Haliaeetus pelagicus*) (Fig. 2).

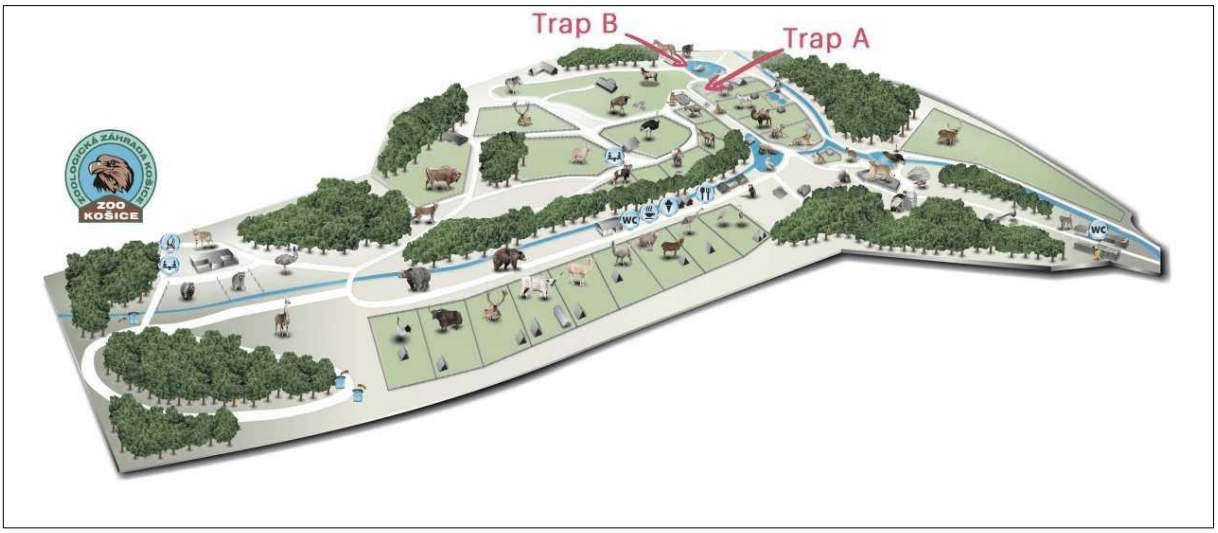

Figure 2. Map depicting individual animals and locations where the traps were installed.

2.2. Temperature and humidity data

Climate data – minimum, maximum and average ambient temperatures, relative humidity and mean wind speed were identified using digital data loggers, and the measurements were carried out using an anemometer.

2.3. Identification of *Culicoides*

The trapped biting midges were preserved in 70% ethanol. The species diagnostics of biting midges was carried out under the Zeiss Stemi DV4 binocular microscope. In the first step, biting midges were categorised on the basis of wing morphology, using a fast identification key.[15] They were divided into following complexes: Obsoletus, Pulicaris, Nubeculosus and other *Culicoides* spp. The species were identified on the basis of the morphological features typical for individual species, i.e. wing spots, a chest colour, a shape of 3rd phalanx of the palps, and the ratio of the length of 10th to the length of 11th segment of the antenna, using the available identification keys.[16,17] The species which were not identifiable on the basis of those morphological features were processed into permanent slides and they were subsequently identified on the basis of the following features: the number and shapes of spermathecae, shapes of antenna segments and the presence of sensilla on individual segments, size and shape of the 3rd palpal segment and shapes of sensilla, the number of thorns on the second pair of legs, etc. The females of individual species of biting midges were then divided into 4 groups on the basis of their abdomen pigmentation.[18]

2.4. Blood-meal molecular analysis

Following the identification of the species of biting midges, the selected females with abdomens filled with blood were subjected to DNA isolation using the Qiagen blood & tissues kit (QIAGEN, GmbH., Hilden, Germany). DNA extraction was carried out using entire biting midge individuals. The host blood detection was carried out while applying the PCR method, while focussing on a portion of the sequence of the MT-CYB gene using the cyt bb 1 (5´-CCA TCM AAC ATY TCA DCA TGA TGA AA-3´) and cyt bb 2 (5´-GCH CCT CAG AAT GAY ATT TGK CCT CA-3´) primers.[19]

3. Results

In the course of four seasons in years 2017 through 2020, 37 cycles of trapping the *Culicoides* biting midges were performed, while the total number of trapped individuals was 6,905 biting midges. The data on the species composition and their abundance are listed in Table 1. The trapped biting midges belonged to the subgenera *Avaritia*, *Culicoides*, *Sensiculicoides*, *Pontoculicoides*, *Silvaticulicoides*, *Beltranmyia* and *Monoculicoides*. The most frequent species were *C. obsoletus/C. scoticus*, in particular 5,556 individuals (80.46%), which are described as the potential vectors of SVB and BTV, followed by the species: *C. furcillatus* (645 pc; 11.81%); *C. festivipennis* (279 pc; 4.04%); *C. pulicaris* (202 pc; 2.92%); and *C. lupicaris* (103 pc; 1.49%). Other detected species of biting midges included *C. punctatus*, *C. dewulfi*, *C. riethi*, *C. tauricus/C. slovacus*, *C. kibunensis*, *C. subfascipennis*, *C. circumscriptus* *C. nubeculosus* and *C. stigma.*

The detection of host blood was carried out using 16 specimens of biting midges of species *C. furcillatus*, *C. festivipennis* and *C. obsoletus*. The resultant finding was that their hosts were a Burchell's zebra (*Equus quagga burchellii*) and a domestic cow (*Bos taurus*); however, the most frequent hosts were humans (*Homo sapiens*) (Table 2).

Table 1. Species composition of *Culicoide*s biting midges in years 2017–2020.

Species	Monitored years				Total number	%
	2017	2018	2019	2020		
C. obsoletus/C. scoticus	1,020	1,406	930	2,200	5,556	80.46
C. dewulfi	2	-	-	-	2	0.02
C. furcilatus	-	20	618	7	645	9.32
C. festivipennis	-	24	154	101	279	4.04
C. pulicaris	97	48	24	33	202	2.92
C. lupicaris	20	56	23	4	103	1.49
C. punctatus	-	15	2	-	17	0.24
C. riethi	-	-	2	-	2	0.03
C. kibunensis	16	-	-	-	16	0.23
C. tauricus/C. slovacus	4	1	-	-	5	0.07
C. subfascipennis	-	1	-	-	1	0.01
C. circumscriptus	22	-	1	-	23	0.33
C. stigma	-	-	1	-	1	0.01
C. nubeculosus	-	-	-	1	1	0.01
Culicoides spp. damaged (unidentified)	57	-	-	-	57	0.82
Total	1,238	1,571	1,755	2,341	6,905	100

Table 2. Evaluation of host blood in biting midges in Zoo Košice.

Biting midge species	Host blood after sequencing	Sequences of a portion of the MT-CYB gene	Sequence length	Identity with NCBI	Comparison with NCBI
C. furcillatus	*Equus quagga burchellii*	catgatgaaactttggctccctcctaggaatctgcctaatcctccaaatcctaacaggcctattcctagccatacactacacatcagacacaacaactgccttctcgtctgtcacccatatctgccgagacgttaactacggatgaatcattcgctacctccatgccaacggagcatccatatttttcatctgcctcttcatccacgtaggacgtggcctctactatggctcttacacattcctagaaacatgaaacattggaattatcctacttctcacagtaatagccacagcattcataggctatgtcctaccatgaggccaaat	328	99.7%	NC_044858.1
C. festivipennis	*Homo sapiens*	aatgatccgtaatataggcctcgcccgatgtgtaggaagaggcagataaagaatattgaggcgccattggcgtgaaggtagcggatgattcagccataatttacgtctcgagtgatgtgggcgattgatgaaaaggcggttgaggcgtctggtgagtagtgtatggctaggaatagtcctgt	182	100%	MK617272.1

C. festivipennis	*Bos taurus*	aacatctcagcatgatgaaatttcggttccctcctggg aatctgcctgatcctacaaatcctcacaggcc tattcctagcaatacactacacatccgacacaacaac agcattctcctctgttacccatatctgccgaga cgtgaactacggctgaatcatccgatacatacacgca aacggagcttcaatgtttttatctgcttatat atgcacgtaggacgaggcttatattacgggtcttaca cttttctagaaacatgaaatattggagtaatcc ttctgctcacagtaatagccacagcatttataggatac	318	99.69%	MN148732.1
C. festivipennis	*Homo sapiens*	tccatccaacatctcagcatgatgaaacttcggctcac tccttggcgcctgcctgatcctccaaatcacc acaggactattcctagccatacactactcaccagacg cctcaaccgccttttcatcaatcgcccacatca ctcgagacgtaaattatggctgaatcatccgctacctt cacgccaatggcgcctcaatattctttatctg cctcttcctacacatcgggcgaggcctatattacggat catttctctactcagaaacctgaaacatcggc attatcctcctgcttgcaactatagcaacagccttcata ggctatgtcctcccgtgagg	339	99.7%	MK617272.1
C. obsoletus	*Homo sapiens*	ccgccttttcatcaatcgcccacatcactcgagacgta aattatggctgaatcatccgctaccttcacgc caatggcgcctcaatattctttatctgcctcttcctacac atcgggcgaggcctatattacggatcattt ctctactcagaaacctgaaacatcggcattatcctcct gcttgcaactatagcaacagccttcataggct atgtcctcccgtgaggccaaatatc	235	100%	MW172501.1

4. Discussion

The entomological research in Zoo Košice was carried out in the course of four seasons in years 2017 through 2020. The total number of trapped biting midges was 6,905 individuals, and they belonged to 14 different species. The individual monitored seasons differed in the species composition; whereas the differences were observed not only in the frequency of occurrence, but also in the percentages of individual species. The study was carried out because the zoos with a variety of avian and mammal hosts represent ideal environments for the nuisance and potential spread of pathogens in animals and humans. The study results obtained over the last ten years indicate that approximately 25 pathogens were transmitted by blood-feeding arthropods in zoos.[20]

First cycles of trapping *Culicoides* biting midges were carried out in 2017. These small haematophagous Diptera are known to feed on blood primarily of mammals and birds.[21] That is why the light traps were installed mostly near the aviaries and enclosures for ungulates, which provide ideal conditions for the development of larvae.[22] In a sufficiently warm and humid environment with a sufficient amount of organically decomposing materials, the larvae of biting midges complete their development faster, and thus the number of generations per year is higher.[23] In the first monitored year 2017, the traps were installed primarily near ungulates, as well as carnivores and bears. 7 trapping cycles were carried out, and 1,238 biting midges of six species were collected; however, these females did not have any pigment or host blood in their abdomens. In two cases, the traps were not installed safely enough and they were torn down and broken. This was one of the reasons why in the next seasons the traps were installed mainly in aviaries. In the following year 2018, the total number of trapped biting midges was 1,571 individuals of 8 species (Table 1). A

burgundy-red pigment was detected in the abdomens of females trapped in aviaries, in particular in 19 females of *C. festivipennis* (n=3) and *C. obsoletus/C. scoticus* (n=3); 13 females of *C. festivipennis* were gravid and with visible eggs.[18] From the epizootiological point of view, there was a high risk of pathogen transmission particularly by these females, because they had sucked blood from hosts that might have been the sources of viruses or parasites. The females of *Culicoides festivipennis* biting midges are the vectors of avian haemosporidia, as was also confirmed by the studies carried out in the Czech Republic, Germany, and Bulgaria.[24-27]

Trap B was used to capture biting midges near ungulates, where 8 species of biting midges were identified, in particular: *C. obsoletus/scoticus*, *C. lupicaris*, *C. pulicaris*, *C. festivipennis*, *C. furcillatus*, *C. punctatus*, *C. tauricus/C. slovacus* and *C. subfascipennis.* In season 2018, there was the highest activity of biting midges in the enclosures for perissodactyls, in particular Champan's zebra and blue wildebeest. At an average temperature of 20.1 °C and relative humidity of 60.6 %, 680 biting midges of the *Obsoletus* complex were captured. These females were present in all physiological stages, i.e. nulliparous (n=101), parous (n=572), engorged (n=4) and gravid (n=3). Biting midges of the *Obsoletus* complex, primarily *C. obsoletus/C. scoticus*, belong to the most frequent species in the Palaearctic realm; the same result was presented in the study performed within the project of monitoring *Culicoides* in a zoo in South Carolina.[28]

Due to the fact that the frequency of occurrence of biting midges in avian biotypes was low in the first two years of the research (2017 and 2018), such biotypes with a lower number of individuals were excluded from the study in the following seasons. Similarly, as for monitoring ungulates, the traps were not installed in the enclosures for buffalos. The second season was accompanied by certain technical problems with the power supply for traps and fluorescent tubes, and this was probably manifested by a lower number of biting midges collected in traps.

In the following year 2019, 10 species of biting midges were captured (most of them in June), in particular the following 8 species: *C. obsoletus/C. scoticus*, *C. furcillatus*, *C. festivipennis*, *C. riethi*, *C. pulicaris*, *C. punctatus*, *C. lupicaris* and *C. stigma*. In June, the average temperature was 20.5 °C and relative humidity was 74.2%. Also, the number of *C. festivipennis* biting midges trapped near birds was higher than in 2018.

In the following season of 2020, the number of trapped biting midges was 2,341 and they belonged to 6 species. The most frequent species were *C. obsoletus/C. scoticus* (n=2,200), representing almost 94% of the biting midges captured that year. Extraordinarily high temperatures and air humidity in November, when the average daily temperature in aviaries was 11.9 °C and the relative humidity was 95.2%, were manifested by an increased activity of biting midges. In November 2020, 17 females of *C. obsoletus/C. scoticus* were captured, including 16 females with pigmented abdomens. Biting midges of the *Pulicari*s group, which belong to the second most frequent in the Palaearctic realm, represented 322 individuals (4.6%) captured over the monitored years. Three different species were detected, in particular *C. pulicaris*, *C. lupicaris*, and *C. punctatus*. The fauna of biting midges of this complex that live in Slovakia also includes the *C. newsteadi* and *C. bysta* species; however, these species have not yet been captured. Similar results were also presented in the study by England et al. (2020); in two zoos in the United Kingdom they captured biting midges of the *Pulicaris* complex, in particular *C. pulicaris* (69.9%) and *C. punctatus* (8.1%). As was the case in our surveillance, a dominant species of biting midges in the UK were *C. obsoletus/C. scoticus* (69.9%).[29,30]

In addition to animal hosts, *Culicoides* biting midges also parasitise on humans. Year 2019 exhibited the highest diversity of the species composition of biting midges. It is an interesting fact that particularly in 2019 the Zoo Košice observed the highest visit rate – 254,528 visitors, i.e. in 30,054 visitors more than in the previous year. In this regard, the investigation of the host blood detected in the captured biting midges revealed that human blood was present in three specimens (Table 2), representing 18.8%. This represents a potential risk of the transmission of pathogens to humans, especially while bearing in mind that in 2020 the presence of *C. nubeculosus,* i.e. the most important vector of zoonotic microfilariae *Onchocerca cervicalis* and *O. gutturosa*, was confirmed for the first time in the Zoo Košice.[6]

So far, 40 studies (23 females and 17 males, aged from 2 to 78 years were infected) have been conducted on epidemiological, clinical, diagnostic, pathological, and therapeutic aspects of *Onchocerca* infection with *Onchocerca* species infecting animals have been found in human tissues as reviewed by Beaver et al.[31], Orihel and Eberhard[33], Pampiglione et al.[34], Wright et al.[37], Sallo et al.[35], Koehsler et al.[32], Wesołowska et al.[36]. Zoonotic onchocerciasis is characterized by lesions observed as subcutaneous nodules developed around adult worms in various parts of the body causing inflammation of musculature and, in some cases, penetrated the eye. The most prevalent species infect humans *Onchocerca lupi* was identified, followed by *O. dewittei japonica*, *O. jakutensis*, *O. gutturosa*, and *O. cervicalis*.[38] In Europe, cases of human orchocerciasis have been reported in Albania, Switzerland, Hungary, Austria and Poland.[34-36] The interest in biting midges has enormously increased over the last two decades, mainly due to the *Bluetongue* and *Schmallenberg* virus transmission to ruminants with the possibility of transmission these *Culicoides*-borne arboviruses to susceptible zoo animals.[39] These results demonstrate that zoos can be foci of biting midge-borne pathogens to animals and humans, indicating the need for preventative and mitigating measures would need to be taken.

Conclusions

To our knowledge, this is the first study to directly confirm blood-feeding of *Culicoides* on zoo animals in Slovakia and shows that they are able to utilise of animals as well as human host species. The above results show that in the urban environment in localities with higher attendance of people and animal husbandry, we confirmed the occurrence of potential *Culicoides* vectors. If zoonotic pathogens infect animals inhabiting the same areas as people do, there is a risk that they become infected too. The results of this study may be used in future as an important indicator when modelling the transmission of pathogens and designing veterinary emergency plans in zoo parks.

Acknowledgement

The research was carried out within the execution of the VEGA 1/0043/19 project.

References

1. Swanson AD, Kapaldo NO, Maki E, Carpenter JW, Conhstaedt LW. Diversity and abudance of nonculicid biting flies (Diptera) in a zoo enviroment. *Journal of the American mosquito control association*. 2018; 34(4): 265–271.
2. Mellor PS, Boorman J. Multiplication of bluetongue virus in *Culicoides nubeculosus* (Meigen) simultaneously infected with the virus and the microfilariae of *Onchocerca cervicalis* (Railliet & Henry). *Annals of Tropical Medicine & Parasitology*. 1980; 74(4): 463–469. doi: 10.1080/00034983.1980.11687368
3. Dohnal J, Blinn J, Vahl G, Schulz-key H. Distribution of microfilariae of *Onchocerca lienalis* and *Onchocerca gutturosa* in the Skin of cattle in Germany and their development in *Simulium ornatum* and *Culicoides nubeculosus* following artificial infestation. *Veterinary Parasitology*. 1990; 36(3-4): 325–332. doi: 10.1016/0304-4017(90)90044-C
4. Wang R, Yu ZC, Luo XP, Chen LJ, Shao GY, Yang XY. Survey of infection and determination of the transmission vector of *Onchocerca fasciata* in camels (*Camelus bactrianus*) in Inner Mongolia, China. *Medical and Veterinary Entomology*. 2019; 34: 59–68.
5. Cambra-Pellejà M, Gandasegui J, Balaña-Fouce R, Muñoz J, Martínez-Valladares M. Zoonotic Implications of Onchocerca Species on Human Health. *Pathogens*. 2020; 9: 761. doi:10.3390/pathogens9090761
6. Gumisiriza N, Mubiru F, Siewe Fodjo JN, Mbonye Kayitale M, Hotterbeekx A, Idro R, Makumbi I, Lakwo T, Opar B, Kaducu J, et al. Prevalence and incidence of nodding syndrome and other forms of epilepsy in onchocerciasis-endemic areas in northern Uganda after the implementation of onchocerciasis control measures. *Infect. Dis. Poverty*. 2020; 9: 12. doi: 10.1186/s40249-020-0628-3
7. Mellor PS. Studies on *Onchocerca cervicalis* Railliet and Henry 1910: I. *Onchocerca cervicalis* in British horses. *J. Helminthol*. 1973; 47: 97–110.
8. Foil LD, Kleir TR, Miller RI, Foil CS, French DD, Smith JN. Seasonal changes in density and tissue distribution of *Onchocerca cervicalis* microfilariae in ponies and related changes in *Culicoides variipennis* populations in Louisiana. *J. Parasitol*. 1987; 73: 320–326.
9. El Sinnary K, Hussein HS. *Culicoides kingi*, Austen: A vector of *Onchocerca gutturosa* (Neumann, 1910) in the Sudan. *Ann. Trop. Med. Parasitol*. 1980; 74: 655–656.
10. Scott DM, Griffin WHCE. Skin immune system and allergic skin diseases. In: Saunders W. (Ed.), *Small Animal Dermatology*. 2001; 543–666.
11. Schaffartzika A, Hamzab E, Jandab J, Crameria R, Martib E, Rhyner C. Equine insect bite hypersensitivity: What do we know? *Veterinary Immunology and Immunopathology*. 2012; 147: 113– 126.
12. Crilly JP, Nuttall T, del Pozo J, Hopker A, Tomlinson M, Sargison N. Hypersensitivity to *Culicoides* midges causing seasonal dermatitis in sheep. *Veterinary Parasitology: Regional Studies and Reports*. 2016; 3–4: 53–56. doi: 10.1016/j.vprsr.2016.06.001
13. Slama D, Haouas N, Mezhoud H, Babba H, Chaker E. Blood meal analysis of *Culicoides* (Diptera: Ceratopogonidae) in central Tunisia. *PLoS One*. 2015; 10 (3): doi.org/10.1371/journal.pone.0120528.
14. Keesing F, Holt RD, Ostfeld RS. Effects of species diversity on disease risk. *Ecol Lett*. 2006; 9: 485–498.
15. Goffredo M, Meiswinkel R. Entomological surveillance of bluetongue in Italy: methods of capture, catch analysis

and identification of *Culicoides* biting midges. *Vet. Ital.* 2004; 40: 260–265.
16. Delécolle J.-C.: Nouvelle contribution à l'étude systematique et iconographique des espèces du genre *Culicoides* (Diptera: Ceratopogonidae) du nord-est de la France. Université Louis Pasteur de Strasbourg. 1985.
17. Mathieu B, Cetre-Sossah C, Garros C, Chavernac D, Balenghien T, Carpenter S, Setier-Rio ML, Vignes-Lebbe R, Ung V, Candolfi E, Delécolle JC. Development and validation of IIKC: an interactive identification key for *Culicoides* (Diptera: Ceratopogonidae) females from the Western Palaearctic region. *Parasites & Vectors*, 2012; 5, 137.
18. Dyce AL. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogidae) without dissection. *Journal of the Australian Entomology Society*. 1969; 8:11–15.
19. Rádrová J, Seblová V, Votýpka J. Feeding behavior and spatial distribution of *Culex* mosquitoes (Diptera: Culicidae) in wetland areas of the Czech Republic. *Journal Medical Entomology*. 2013; 50, 1097–104.
20. Adler PH, Tuten HC, Nelder MP. Arthropods of medico veterinary importance in zoos. *Annu Rev Entomol*. 2011; 56:123–142.
21. Puente MJ, Ruiz S, Soriguer R, Figuerola J. Effect of blood meal digestion and DNA extraction protocol on the success of blood meal source determination in the malaria vector *Anopheles atroparvus*. *Malaria journal*. 2013; 12(1): 109.
22. Sandres CJ, Shortall CR, Gubbins S, Burgin L, Gloster J, Harrington R, Reynolds D, Mellor P, Carpenter S. Influence of season and meteorological parameters on flight activity of *Culicoides* biting midges. *Journal of Applied Ecology*. 2011; 48: 1355–1364.
23. Bursell E. Enviromental aspects: Humidity. Rockstein, ed. *Physiology of the Insecta*. Academic Press, New York. 1964; 324–358.
24. Valkiúnas G. Avian malaria parasites and other haemosporidia. *CRC PRESS*. 2004; 946 pp. ISBN: 9780415300971
25. Synek P, Munciinger P, Albrecht T, Votýpka J. Avian haemosporidians in haematophagous insects in the Czech Republic. *Parasitol Res*. 2013; 112: 839–845.
26. Alarcon S D, Havelka P, Pineda E, Segelbacher G, Schaefer M. Urban forests as hubs for novel zoonosis: blood meal analysis, seasonal variation in *Culicoides* (Diptera: Ceratopogonidae) vectors, and avian haemosporidians. *Parasitology*. 2013; 140 (14): 1799–810. doi: 10.1017/S0031182013001285.
27. Bobeva A, Zehtindjiev P, Ilieva M, Dimitrov D, Mathis A, Bensch S. Host preferences of ornithophilic biting midges of the genus *Culicoides* in the Eastern Balkans. *Medical and Veterinary Entomology*. 2015; 29, 290–296.
28. Nelder PM, Swanson AD, Adler PH, Grogan LW. Biting midges of the genus *Culicoides* in South Carolina zoos. *J Insect Sci*. 2010; 10, 55. doi: 10.1673/031.010.5501
29. Sarvašová A, Kočišová A, Candolfi E, Mathieu B. Description of *Culicoides* (*Culicoides*) *bysta* n. sp., a new member of the Pulicaris group (Diptera: Ceratopogonidae) from Slovakia. *Parasites & Vectors*. 2017; 10, 279.
30. Englad EM, Pearce-Kelly P, Brugman AV, King S, Gubbins S, Sach F, Sanders JCH, Masters JN, Denison E, Carpenter S. *Culicoides* species composition and molecular identification of host blood meals at two zoos in the UK. *Parasites & Vectors*. 2020; 13, 139. doi.org/10.1186/s13071-020-04018-0
31. Beaver PC, Horner GS, Bilos JZ. Zoonotic onchocercosis in a resident of

Illinois and observations on the identification of *Onchocerca* species. *Am J Trop Med Hyg.* 1974; 23, 595–607.

32. Koehsler M, Soleiman A, Aspöck H, Auer H, Walochnik J. *Onchocerca jakutensis* filariasis in humans. *Emerg Infect Dis*. 2007; 13,1749–52.
33. Orihel TC, Eberhard ML. Zoonotic filariasis. *Clin. Microbiol. Rev.* 1998; 11, 366–381.
34. Pampiglione S, Vakalis N, Lyssimachou A, Kouppari G, Orithel TC. Subconjunctival zoonotic *Onchocerca* in an Albanian man. *Ann Trop Med Parasitol*. 2001; 95, 827–832.
35. Sallo F, Eberhard ML, Fok E, Baska F, Hatvani I. Zoonotic intravitreal *Onchocerca* in Hungary. *Ophthalmology*. 2005; 112, 502–504.
36. Wesołowska M, Zając‐Pytrus H, Masny A, Pytrus W, Knysz B, Golab E, Sałamatin R. *Onchocerca jakutensis* ocular infection in Poland: a new vector‐borne human health risk? *Parasites & Vectors*. 2020; 13, 61. doi.org/10.1186/s13071-020-3925-6
37. Wright RW, McLean M, Neafie RC, Markman AW. Zoonotic onchocerciasis of the shoulder: A case report. *The Journal of Bone and Joint Surgery*. 2002; 84, 627-629.
38. Cambra-Pellejà M, Gandasegui J, Balaña-Fouce R, Muñoz J, Martínez-Valladares M. Zoonotic implications of *Onchocerca* species on human health. *Pathogens.* 2020; 9, 761. doi:10.3390/pathogens9090761
39. Stavrou A, Daly JM, Maddison B, Gough K, Tarlinton R. How is Europe positioned for a re-emergence of Schmallenberg virus? *Veterinary Journal*. 2017; 230, 45–51.

