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OCCURRENCE OF BITING MIDGES (DIPTERA: *CULICOIDES*) ON DAIRY FARMS IN EASTERN SLOVAKIA IN RELATION TO ABIOTIC FACTORS

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ABSTRACT

Within the scope of our research, we have performed 59 trapping sessions and collected 15,756 biting midges from 20 species at four farms (Kluknava, Ostrov, Turňa nad Bodovou and Zemplínska Teplica), The most frequent types of captured insects were representatives of the *Avaritia subgenus*, *C. obsoletus/C. scoticus*, representing on average 85.1 % (13,295 individuals) of the fauna of the biting midges, with the exception of the farm in Ostrov where this group represented only 41.7 % of the fauna. At this particular farm, the most frequently trapped insects belonged to the *Culicoides* subgenus (54.1 %), in particular the *C. bysta, C. lupicaris, C. newsteadi, C. Pulicaris* and *C. Punctatus* species. During the trapping sessions, we monitored factors affecting the number of trapped biting midges, such as the temperature, relative air humidity and airflow rate: the air temperature during the trapping of the biting midges ranged from 9.8 to 26.2 °C; the relative air humidity ranged from 35.1 to 100 %; and the air flow rate ranged from no wind to a wind velocity of 8.2 m.s^{-1}. However, in the final evaluation, we failed to observe a statistically significant correlation between the air flow and the number of trapped biting midges. The largest amounts of biting midges were trapped at temperatures ranging from 15.8 to 24.6 °C and at a relative air humidity ranging from 54.2 % to 68.6 %. While monitoring the seasonal dynamics of the physiological conditions of biting midge females at the selected farms, we confirmed that during the period from June to August, the most frequently trapped females were parous (50.1 %; 7,826 individuals). In addition, nulliparous females comprised 43.8 % (6,842 individuals) and were continuously trapped throughout the season (April—November).

Key words: abundance; biting midges; relative humidity; species composition; temperature

INTRODUCTION

Biting midges (Diptera: *Culicoides*) are very small hematophagous diptera, which are important for mammals because they transmit pathogens, primarily viruses and parasites, especially microfilaria of the *Onchocerca* genus [22]. There are 1,347 species of biting midges identified so far, and they are categorised into 31 subgenera [5, 20]. Out of them, 589 species were identified in Europe and 64 particularly in Slovakia [37, 38]. Over the last 15 years, we have witnessed a significant increase in the interest in monitoring the occurrence and investigating the species composition of biting midges, primarily due to their ability to transmit the bluetongue virus (BTV) and the Schmallenberg virus (SBV) [1, 19, 39]. Extensive studies were launched in 2006, when BTV caused marked financial losses to breeders of ruminants in the Netherlands and Belgium, and later in Germany, Luxembourg and Northern France [7, 13, 35, 41]. Despite the fact that no primary focus of infection was confirmed in Slovakia at that time, a certain region of Slovakia was classified as a prohibited zone due to the presence of foci of infections in the neighbouring countries (Hungary and Czech Republic) [25]. This region has therefore become subject to extraordinary emergency measures ordered by law, as well as the requirement to perform quarantine examinations of animals and protect them, which had negative effects on the costs of breeding. Considering the epizootological aspect of the spread of this disease and the recurrent new foci of infection in Europe, in particular in Germany, Belgium and France [14, 16], it is necessary to monitor a relationship between a causal agent of the disease, a vector and a susceptible host. Obviously, the parasite-host relationships must be evaluated while monitoring local abiotic factors, such as a temperature, relative humidity and air flow, as they significantly affect the development and spread of biting midges, as well as the replication of viruses and the development of infective stages of parasites [9, 29, 40]. In addition to the factors listed above, the occurrence of biting midges is also undoubtedly affected by the presence of a sufficient number of hosts [33]. The available literature contains a number of references indicating that beef cattle in particular are very attractive for biting midges. This is primarily a result of a high production of carbon dioxide, acetone and other phenol derivatives, which are highly attractive for these Diptera [4, 24, 27].

Therefore, we have been conducting an entomological survey of the fauna of biting midges in cattle breeds for more than ten years, especially in eastern Slovakia.

MATERIALS AND METHODS

Field trapping

This entomological survey was carried out in 2016 (Kluknava and Zemplínska Teplica) and 2019 (Turňa nad Bodvou and Ostrov) (Figure 1).

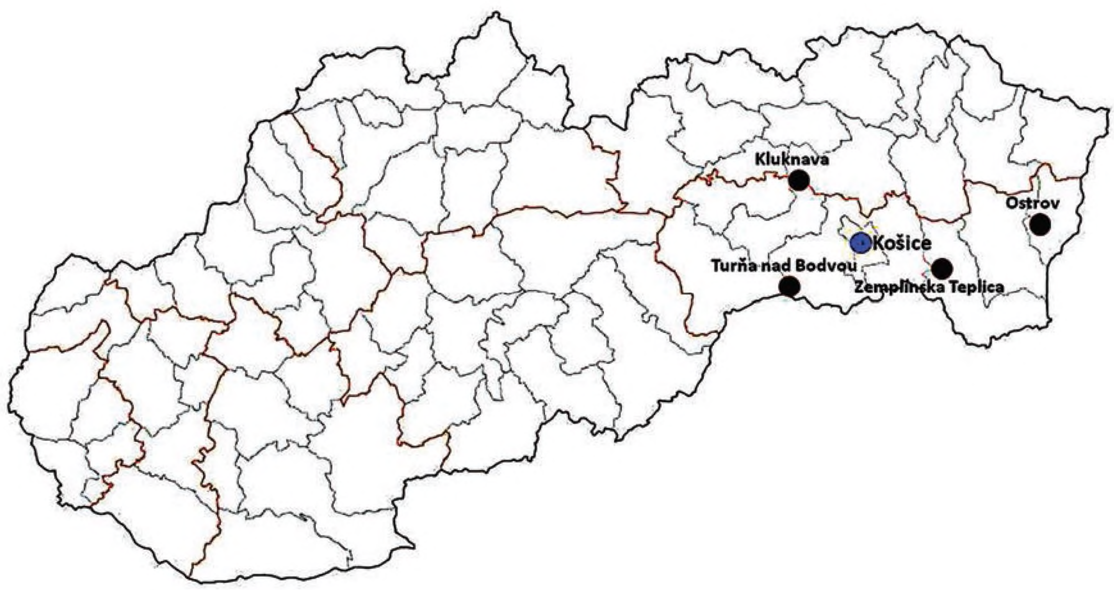

Fig. 1. Biting midges trapping sites in Slovakia

Kluknava (48.923475, 20.932527)

This is a village in the Gelnica District, located in the south-east part of the Spiš Region, in the Kluknava Valley; the pastures are located on the western hillsides of the Čierna Hora Hill on both banks of Dolinský Brook and on the northern hillsides of the Slovak Ore Mountains.

Ostrov (48.719501, 22.165222)

This village is located in the Sobrance District, the Zemplín Region. It lies in the north-east part of the Eastern Slovakia Lowland.

Turňa na Bodvou (48.61095, 20.87444)

This village is located in the Košice-vicinity District, the Abov Region, on a border between the Turnianska Hollow Basin (Slovenský kras) and the Košice Plane (Košická kotlina). North of the village, there is Turňa Castle Hill that belongs to the Zádielska Plateau.

Zemplínska Teplica (48.384596, 21.342568)

It is a village in the Trebišov District, located in the Southern Zemplín Region, near the eastern roots of the Slanské Mountains in the Podslanská Highlands.

Biting midges were collected using the CDC Miniature Light Trap 1212 (John Hook Company, USA). The collected insects were processed in a laboratory by applying a standard procedure [18], put in test tubes containing 70 % ethanol, and stored in a fridge at a temperature of 4—6 °C.

Morphological determination of biting midges

The primary morphological determination was carried out using the Zeiss-Stemi DV-4 microscope. Using the rapid diagnostic key [18], biting midges were divided into four complexes: *Obsoletus, Pulicaris, Nubeculosus* and the other *Culicoides* spp. Subsequently, the biting midges were identified based on typical morphological features visible under a binocular magnifier, such as wing spots, a chest colour, a shape of the 3rd segment of the palp, a ratio of the length of 10th segment to the length of the 11th segment of the antenna, etc. The morphological identification was carried out using available standard diagnostic keys [10, 17] and the interactive key [28].

Female age grading

Within the species determination, we divided female midges into four categories based on their abdominal pigmentation and their physiological condition [12]:

Nulliparous—young females which have never sucked blood and their abdomens were of a white or off-white colour;

Parous—older females which had at least once sucked blood of a host and their abdomens contained grains of the Burgundy Red Pigment, which were formed during the 1st gonotrophic cycle and persisted even after the oviposition;

Engorged—females with fresh host's blood of a deep red colour in their abdomens;

Gravid—females with abdomens filled with eggs.

Measurement of temperature and air humidity

A temperature and air humidity were measured using a digital datalogger Log 32. Dataloggers were launched at the same time as the light traps were installed. Following each trapping, a record was saved in the PDF version.

Statistical evaluation of the results

The statistical evaluation was carried out using the Pearson's correlation coefficient in order to evaluate the effects of a temperature and air humidity on the fauna of biting midges. The data were processed into a point chart.

Ethical statement

The Ethics Committee of the University of Veterinary Medicine and Pharmacy has approved the present research in accordance with applicable national and international animal welfare legislation. No animals were killed for the purpose of this study.

The authors declare no conflict of interest.

RESULTS

Within our research, we have performed 59 trappings and collected 15,756 biting midges of 20 species (99.1 %) (Table 1). The most frequently captured individuals were the representatives of the *Avaritia* subgenus, *C. obsoletus/C. scoticus*, representing 85.1 % (13,295 individuals) of the fauna of biting midges, except at the Ostrov farm where they amounted to only 41.7 % of the fauna. At this particular farm, the most frequently trapped midges belonged to the *Culicoides* subgenus (54.1 %), in particular *C. bysta, C. lupicaris, C. newsteadi, C. Pulicaris*, and *C. Punctatus*

Table 1. Species composition of biting midges collected in beef cattle during one season

Subgenus	Species	Biting midges trapping sites – beef cattle/dairy cows			
		Kluknava	Ostrov	Turňa nad Bodvou	Zemplínska Teplica
Avaritia	*C. chiopterus*	–	–	–	–
	C. dewulfi	–	–	–	–
	C. obsoletus/C. scoticus	2,484	1,131	4,551	5,129
Beltranmyia	*C. circumscriptus*	–	26	6	–
	C. manchuriensis	–	1	–	–
	C. salinarius	–	8	–	–
Culicoides	*C. bysta*	–	2	7	–
	C. lupicaris	3	92	65	31
	C. newsteadi	–	853	217	–
	C. pulicaris	8	14	70	3
	C. punctatus	–	505	70	12
Monoculicoides	*C. nubeculosus*	11	–	–	29
	C. riethi	–	–	32	–
	C. stigma	–	3	17	13
Pontoculicoides	*C. slovacus/C. tauricus*	–	6	3	–
Silvaticulicoides	*C. subfasciipennis*	–		1	–
Unclassified	*C. clastrieri*	–	1	–	–
	C. festivipennis	–	67	6	–
	C. furcilatus	–	1	125	–
	C. vexans	17	–	–	–
Total females		2,523	2,710	5,170	5,221
Including: Nulliparous		1,034	1,255	2,099	2,454
Parous		1,287	1,236	2,588	2,715
Engorged		151	113	154	17
Gravid		51	106	329	35
Unidentified		18	–	–	14
Males		3	2	31	1
Damaged		26	13	8	16
Number of trappings in a season		10	10	25	14
Total		**2,570**	**2,725**	**5,209**	**5,252**

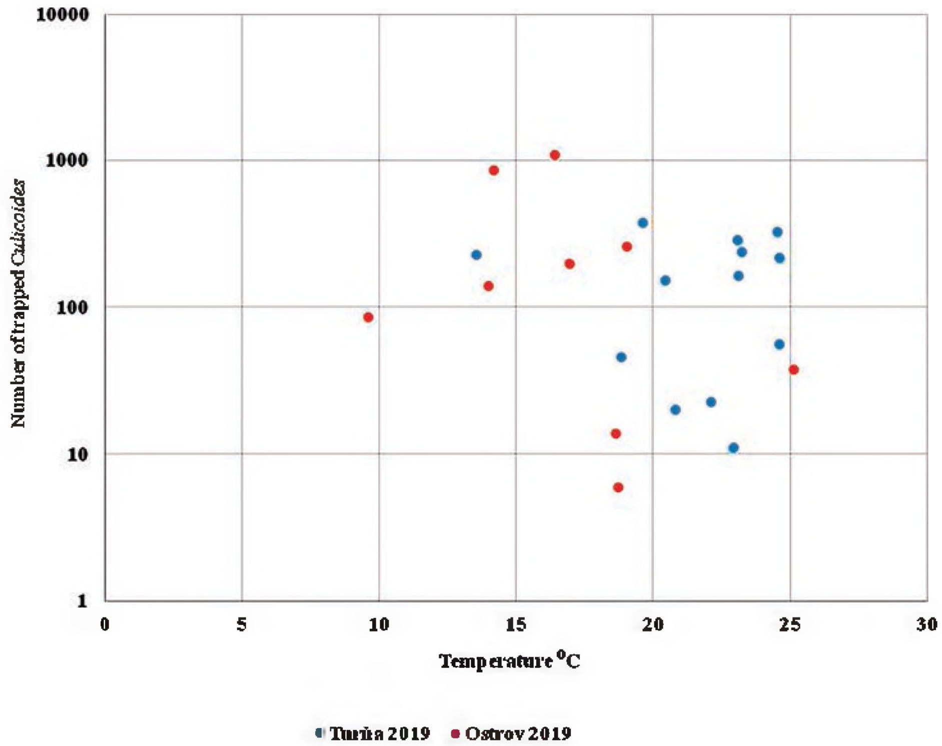

Fig. 2. Effect of temperature on the abundance of the trapped biting midges

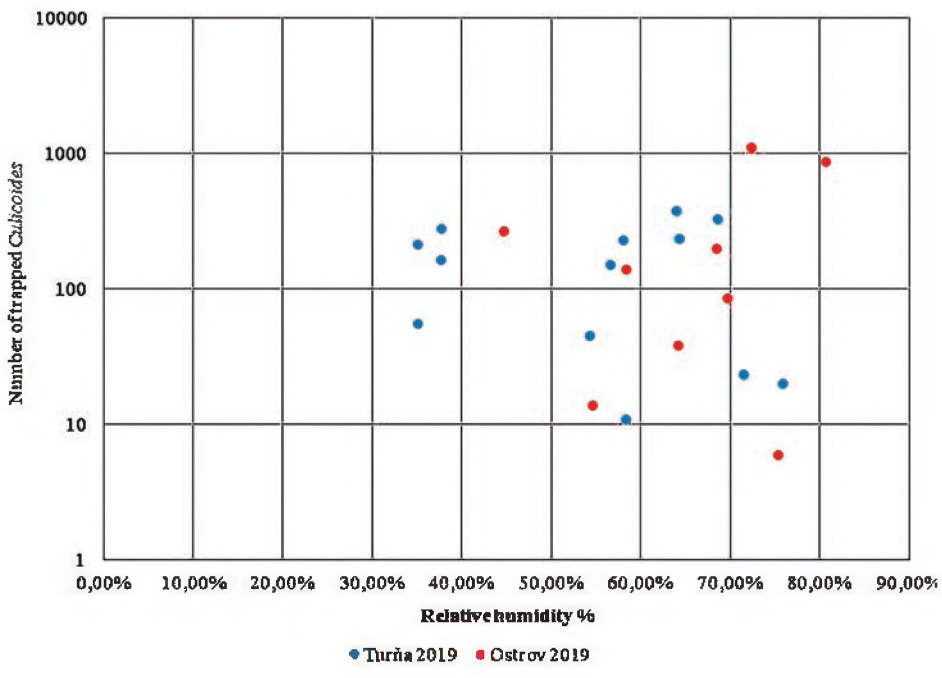

Fig. 3. Effects of humidity on the abundance of trapped biting midges

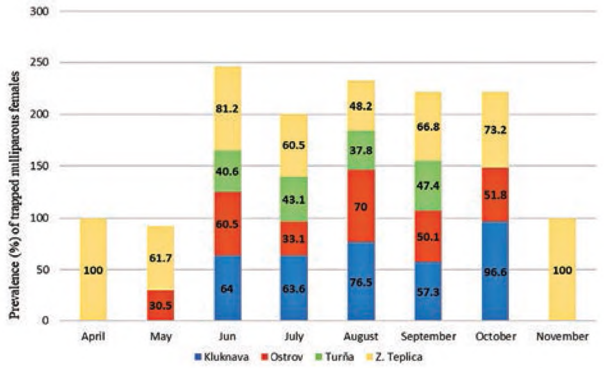

Fig. 4. Seasonal dynamics of the trapped nulliparous females

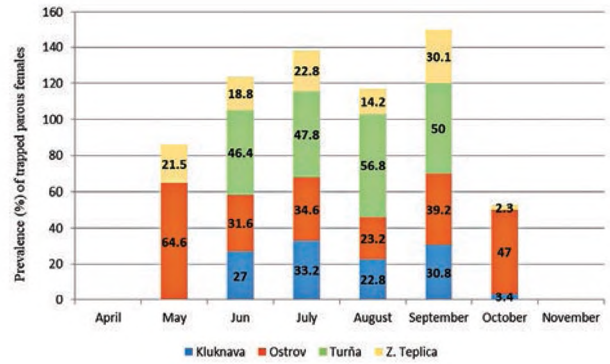

Fig. 5. Seasonal dynamics of the trapped parous females

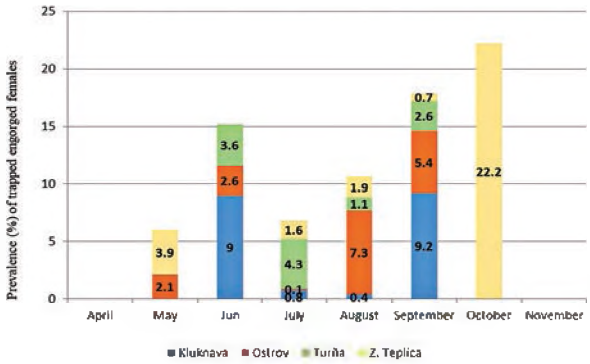

Fig. 6. Seasonal dynamics of the trapped engorged females

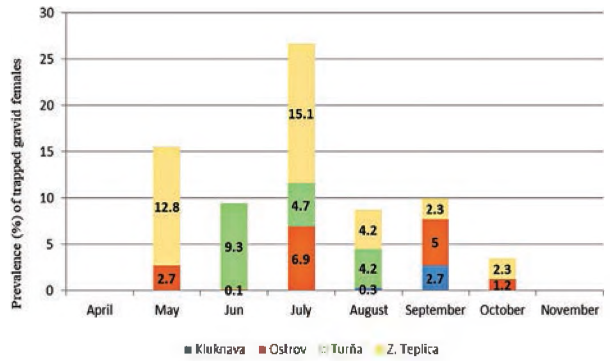

Fig. 7. Seasonal dynamics of the trapped gravid females

species. On the pastures of the farm in Kluknava, we have captured 5 species and a similarly low number of species, i. e. 8, were collected in the cattle-ranges at the farm in Zemplínska Teplica, although the amount of the collected biting midges was the highest at this particular farm (Table 1).

As for the species composition, 14 species at the farm in Ostrov and 13 species at the farm in Turňa nad Bodvou were identified. The factors which were monitored during the trappings included the temperature, relative air humidity and the air flow rate, as they affect the number of the trapped biting midges. During the trappings, a temperature ranged from 9.8 to 26.2 °C; relative air humidity ranged from 35.1 to 100 %; and the air flow rate ranged from no wind to a wind velocity of 8.2 m.s^{-1}. Intensive measurements of abiotic factors in a correlation to the number of trapped biting midges were carried out at the farm in Turňa nad Bodvou and at the farm in Ostrov. We found out that the optimal air velocity was 0.1—0.3 m.s^{-1} due to the number of midges caught; however, the final evaluation revealed that the air flow rate was not a statistically significant factor for changes in the number of the collected biting midges. The effects of a temperature and relative air humidity on the abundance of biting midges are presented in Figures 2 and 3.

The largest amounts of biting midges were trapped at temperatures ranging from 15.8 to 24.6 °C and at the relative air humidity ranging from 54.2 % to 68.6 %.

The investigation of the seasonal dynamics of the physiological condition of females of biting midges at the farms revealed that there are two peaks of their activity, in June and in August (Figures 3 through 7). The first nulliparous females were trapped at the farm in Zemplínska Teplica in April, but also as late as in early November. They were found in the traps throughout the entire collection season at every farm, and their occurrence reached peaks in May, June and September. These nulliparous females represented 43.8 % (6,842 individuals) of all the collected females. The most frequently trapped females were older females (parous)—they represented 50.1 % (7,826 individuals) and occurred at farms in the period from late June to September. Females with fresh host's blood in their abdomens (435 individuals) and gravid females (521 individuals) represented 2.8 % and 3.3 % of all females, respectively. Females of both groups were sporadically found in the collected material throughout the entire season, but primarily in August and September.

DISCUSSION

In epidemiological studies, it is necessary to perform the diagnostics of pathogen vectors. Our study provided information on the fauna of biting midges in eastern Slovakia at four farms where beef cattle were bred and where we confirmed the occurrence of 20 species of biting midges which were potential vectors of pathogens. The species with the highest occurrence were *C. obsoletus/C. scoticus*; they represented on average 85.1 % (13,295 individuals) of the fauna of biting midges and belong to the most frequent species in the Palaearctic region. These species are potential vectors of the catarrhal fever virus and the Schmallenberg virus in Europe, as well as filariae. Haematophagous females of biting midges parasitise on a wide range of vertebrates [27]. Some studies claim that the range of suitable hosts from which *Culicoides* biting midges may choose is limited by the availability of such hosts in their environment [33]. On the other hand, such an anthropogenic activity facilitates the spread of new pathogens among humans. The anthropogenic impact changes the landscape through various agricultural interventions, irrigation and production of manure and silage, where hatching sites for biting midges are formed. Moreover, climate changes that increase the frequency of storms and floods, as well as the sea level rise, increase the availability of brackish hatching sites used by, for example, *C. newstedi, C. halophilus, C. circumscriptus, C. salinarius* and *C. maritimus* [41]. Biting midges of *C. newsteadi* were the second most frequent species diagnosed at farms in Ostrov and in Turňa nad Bodvou. In Portugal, the *C. newsteadi* biting midges were observed mainly in autumn months [34]; however, at the Slovak farms, their presence was detected in June. During the following months, the abundance of *C. newsteadi* biting midges decreased. It is known that the ideal conditions for the development of these biting midges are in shallow brackish waters, bordered with decomposing plants [15]. As for abiotic factors, the occurrence of biting midges is most significantly affected by the ambient temperature, which affects the geographical spread of the species, the sizes of populations and their survival rates, as well as the number of generations per season. The development from an egg to an imago accelerates with higher temperatures; however, at a certain temperature, survival rates begin to decrease. A shadowed and cooler biotope prolongs the development from an egg to an adult [9], and this results in a lower number of generations in a year. On the other hand, at higher temperatures, the period of hatching from eggs to larvae accelerates and it leads to a higher number of generations in a season. Also, the ambient temperature regulates the duration of a gonotrophic cycle, and hence also of the sucking frequency; as a result, the probability of pathogen transmission increases too. A temperature affects also the replication of infectious agents in the bodies of biting midges [31].

Recently, the impact of higher temperatures has been monitored within the investigation into climate changes and global warming. An increase in the average annual temperature by 2 °C corresponds to a shift of the northern border of the insect-spread area by approximately 200 km [23]. The research conducted by B i s h o p et al. [3] indicated that an increase in temperature by 2 °C in winter prolonged the season of the presence of *C. brevitarsis* by 0.7 month per year. Another risk related to increased temperatures in winter months is that warmer winter makes it possible for adults, as well as pathogens they carry, to survive. Our observations indicated that the highest amounts of individuals were collected at temperatures ranging from 15.8 to 24.6 °C. Higher air humidity positively affects the activity of biting midges, while the optimal relative air humidity ranges from 80 to 90 % [30]. At low relative humidity and low temperatures, the survival rate of biting midges is low [43]. During our trappings, the optimal relative air humidity ranged from 54.2 % to 68.6 %, at which we observed the highest amount of trapped biting midges. The air flow facilitates active or passive motion of biting midges, which actively fly and search for a host in windless conditions, but in windy weather the intensity of this search for a host decreases. According to L e h a n e [26], the wind with a velocity of 1—2 m.s^{-1} may reduce their flying activity and the search for a host. W i t m a n, B a y l i s [42] stated that at wind velocities below 3 m.s^{-1}, biting midges actively fly, whereas when a wind velocity exceeds 3 m.s^{-1}, they only float [29], and at wind velocities above 11 m.s^{-1}, they stay hidden in the groundcover [42]. Biting midges move actively, but they are also passively moved by the wind for as far as 700 km [21]. The flying activity and dispersion of biting midges is one of the factors that condition the introduction and spread of pathogens, especially the viruses they transmit [36]. Biting midges which fly to higher altitudes may be easily carried away by the flowing air for dozens of kilometres; this explains the fast expansion of arboviruses many kilometres away from a source, while probably a single sucking by an infected biting midge is sufficient for the transmission of a virus to a susceptible host [2]. Most biting midges are active at dusk, and increased abundance is observed at the light intensity of 101—10,000 lux, which corresponds to dusk or cloudy weather [32]. *C. obsoletus* may also attack hosts during the day at the light intensity of 20,000 lux, optimally at ca 5,000 lux [17]. In our survey, especially at the farm in Ostrov, we diagnosed the biting midges primarily of the *Culicoides* genus (54.1 %), in particular the *C. bysta, C. lupicaris, C. newsteadi, C. pulicaris* and *C. punctatus* species. Similar results were reported by Colins et al. [8], who monitored the fauna of biting midges at farms in Ireland in 2014. These species are assumed to be potential vectors of the bluetongue virus [11]. The third most frequent species of biting midges observed in our study was *C. furcillatus* (2.34 %). Their presence was detected from June to August. This species has also been detected in years 2003—2006 at several farms in Switzerland, where mostly beef cattle and goats were bred [6].

CONCLUSIONS

With regard to the fact that biting midges cause enormous economic losses, the knowledge obtained during the entomological survey contributes to understanding the biology of biting midges, which will be beneficial in epizootology and in the implementation of preventive measures. However, climate changes bring constant changes in the spread of vectors and pathogens, and the present paper provides the latest information. The prospects presented by climatologists are alarming, and the current information does not necessarily have to be valid in a very near future. It is therefore necessary to conduct continuous investigation, not only in eastern Slovakia, but also elsewhere.

ACKNOWLEDGEMENTS

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