

SEASONAL DYNAMICS OF THE PRESENCE OF CULICOIDES SPP. IN SERBIA IN THE PERIOD 2015-2016

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Abstract

Genus *Culicoides spp.* includes small insects 0.5-2 mm in length, usually grey or black and at first glance very similar to mosquitoes. They are strictly hematophagous, feeding by attacking hosts outdoors and indoors (although they are less susceptible to the stationing like *Aedes* mosquitoes do). The sting is very painful at the injection site and often followed by hypersensibilisation with the consequent formation of allergic dermatitis. In addition, the insects carry and transmit a multitude of diseases, often of a zoonotic character, and therefore are of great epidemiological importance. In our country, continuous monitoring of *Culicoides spp.* has been carried out and seasonal dynamics of their appearance in the period 2015-2016 is presented in this article. During October 2015, the presence of *Culicoides spp.* was confirmed in 10.00% of samples; in November, their presence was not established, whereas in December, 2.35% of samples proved positive for the presence of *Culicoides spp.* During 2016, from January to March, no *Culicoides spp.* were found in any of the examined samples. During April, their prevalence was 9.63%, in May - 6.74%, in June - 3.70%, in July - 15.78%, in August - 18.07%, in September - 27.27%, and in October - 45.65%. In Serbia, the dominant *Culicoides spp.* species are *Obsoletus* complex and *Pulicaris* complex established in 57.21% and 33.37% of samples, respectively. Other species are present in lesser extent. In *Obsoletus* complex, the dominant species was *Culicoides obsoletus/scoticus*. The percentage of *Culicoides obsoletus /scoticus* males in samples was 25.52%. Non-pigmented (young)

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females were present in 66.06% of samples; females who took blood in 7.55% and 0.87% were gravid females. In *Pulicaris* complex, the dominant species was *Culicoides pulicaris*. Males of *Culicoides pulicaris* were found in 19.23% of samples, non-pigmented (young) females in 70.96%, females who took blood in 9.08% while 0.73% were gravid females.

Key words: *Culicoides* spp., Seasonal dynamics, Serbia

SEZONSKA DINAMIKA PRISUSTVA CULICOIDES SPP. U SRBIJI U PERIODU 2015-2016. GODINE

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Kratak sadržaj

Rod *Culicoides* spp. su mali insekti veličine 0,5-2 mm, većinom sivkaste ili crne boje i na prvi pogled veoma slični komarcima. Oni su striktno hematofage. Ubod je veoma bolan, a mesta uboda su često hipersenzibilisana uz posledično nastajanje alergijskih dermatitisa. Uz to oni prenose mnoštvo oboljenja često zoonotskog karaktera tako da su od izuzetnog epidemiološkog značaja. U našoj zemlji se vrši stalni monitoring *Culicoides*-a i ovde dajemo prikaz sezonske dinamike njihovog pojavljivanja u periodu 2015-2016.godine. Tokom 2015. godine u oktobru je njihovo prisustvo ustanovljeno u 10,00% uzoraka, u novembru ih nije bilo, a tokom decembra su bili prisutni u 2,35%. Tokom 2016. godine tokom januara, februara i marta nisu nađeni ni u jednom prispelom uzorku. Tokom aprila meseca njihova prevalencija je iznosila 9,63%, u maju je bila 6,74%, u junu 3,70%, u julu 15,78%, u avgustu 18,07%, u septembru 27,27% i u oktobru 45,65%. U Srbiji su dominantne vrste *Culicoides* spp. iz *Obsoletus* kompleksa i ustanovljeni su u 57,21%. *Culicoides* spp. iz *Pulicaris* kompleksa ustanovljeni su 33,37% dok su ostale vrste manje zastupljene. Kod *Culicoides* spp. iz *Obsoletus* kompleksa dominantna vrsta je bila *Culicoides*

obsoletus/scoticus. Mužjaci su nađeni u 25,52%, nepigmentisane (mlade) ženke u 66,06%, ženke koje su uzele krv u 7,55%, a 0,87% su bile gravidne ženke. Kod *Culicoides* spp. iz Pulicaris kompleksa dominantna vrsta je bila *Culicoides pulicaris*. Mužjaci su nađeni u 19,23%, nepigmentisane (mlade) ženke u 70,96%, ženke koje su uzele krv u 9,08%, a 0,73% su bile gravidne ženke.

Ključne reči: *Culicoides* spp., sezonska dinamika, Srbija

INTRODUCTION

Culicoides spp. are small insects whose females sting and suck blood (Blackwell, 2009; Pavlović et al., 2009). The first report of these insects dates back from 1731, when the priest, naturalist and philosopher William Derham (1657-1735) described their biological cycle and gave details of their stings (Pavlović, 2016a). *Culicoides* spp. currently contains 1343 existant and 44 extinct species, representing the largest genus of the *Ceratopogonidae* and comprising 21.5% of all Ceratopogonid species (Borkent, 2014).

Epidemiological importance of *Culicoides* spp. was described in 1944 by Rene du Toit from ARC - Onderstepoort Veterinary Institute, who believed that these insects can play an important role in the transmission and spread of viruses that cause animal diseases such as bluetongue and acute allergic dermatitis in horses (Meiswinkel et al. 2008). Later, the genus received considerable attention through the role of several species as biological vectors of pathogens of medical and veterinary importance. In addition to several nematode and protozoan species, over 50 arboviruses have been isolated from species of *Culicoides* and their role in the transmission of veterinary and human pathogens has been reviewed (Blackwell, 2001; Pavlović et al., 2002; Borkent, 2004).

Genus *Culicoides* has not been investigated in Serbia, thus, there were conflicting opinions about its presence in our region. It was only with the emergence of Bluetongue disease in 2006, when research of these insects begin to gain importance, leading to the first survey aimed at determining the presence and extent of these insects. The studies carried out in Serbia during 2006-2007 confirmed the presence of these insects in our region. Later studies conducted from 2011 to 2012 enabled the determination of *Culicoides* types in Serbia (Rajković et al., 2009; Pavlović et al, 2009, 2014; Pavlović, 2016b). Finally, the research performed in 2014, after the re-emergence of Bluetongue disease, revealed that this type of insects became widespread and covers the entire area

of Serbia as was expected after the results of previous investigation. Since then, continuous epidemiological monitoring of these insects in the entire territory of Serbia has been performed with an aim of establishing their biodiversity, spread, abundance and seasonal dynamics.

In our paper, we presented results of examination of biodiversity and seasonal dynamics of *Culicoides spp.* in Serbia in the period 2015-2016.

MATERIAL AND METHODS

Based on the instructions of Veterinary Directorate on performing entomological and virological tests for the monitoring and surveillance of Bluetongue disease (BTD) in the Republic of Serbia No. 323-02-7461/2015-05 dated 14/09/2015 in the period from 01/10/2015 to 30/09/2016 entomological tests were carried out in order to control Bluetongue disease.

In the period from 01/10/2015 to 31/10/2016, a total of 775 entomological check-ups were made. *Culicoides spp.* samples were collected from the epizootiological areas such as Belgrade - 36 samples, Šabac - 61, Zaječar and Jagodina - 66, Požarevac - 96, Kraljevo - 112, and Niš - 227 samples. In Vojvodina Province, samples of *Culicoides spp.* were collected from several epizootical areas, i.e., Novi Sad -29 samples, Zrenjanin - 8, Sombor - 24, Pančevo - 45 and Subotica - 71.

Determination of *Culicoides spp.* insects was made by morphometric method recommended by the Italian National Reference Centre for Exotic Diseases (National Reference Centre for the study of Exotic Animal Diseases (CESME) Reference Laboratory for Bluetongue OIE, Istituto Sperimentale Zooprofilattico dell'Abruzzo e del Molise "G. Caporale" (IZSAM) from Teramo, Italy. Species definition of *Culicoides spp.* has traditionally been based on the morphology of adult insects. Adult individuals of *Culicoides spp.* are notable for their characteristic wing pigmentation pattern and distribution of wing microtrichia, which in certain species can be used as the principle diagnostic feature. In practice, however, the requirement is that specimens should be slide mounted, image-captured, measured and analysed which is time consuming and therefore the use of morphometrics for identification purposes in high-throughput systems such as surveillance programs is recommended (Weeks et al., 1999).

RESULTS

Of the total number of insect samples, the presence of *Culicoides spp.* was established in 11.22% (87/775). In the epizootiological area of Belgrade, the

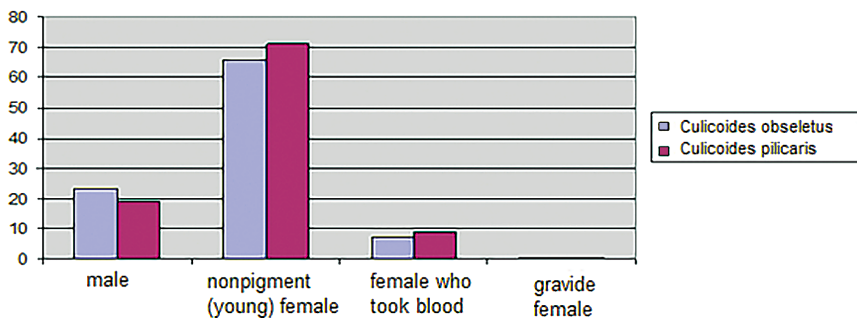
presence of *Culicoides spp.* was established in 8.33% (3/36) samples, Požarevac 1.04% (1/96), Kraljevo 2.67% (3/112), Jagodina 6.06% (4/66), Niš 11.89% (27/227), Zaječar 15.15% (10/66) and Šabac 31.14% (19/61).

In the area of Vojvodina, in the epizootical area of Novi Sad, the presence *Culicoides spp.* was established in 13.79% (04/29) of samples, Pancevo 13.3% (6/45), Subotica 11.26% (8/71) and Sombor 8.33% (2/24). In the epizootiological area of Zrenjanin, the presence of *Culicoides spp.* was not established (0/8).

During this research, *Culicoides spp.* from Obsoletus complex were detected in 57.21% of the total catch and the dominant species was *Culicoides obsoletus/scoticus*. Of the total population, male individuals made 25.52%, non-pigmented (young) females 66.06%; females who took the blood 7.55% and 0.87% were gravid females (Figure 1).

Pulicaris complex was established in 33.37% of total catch and the dominant species was *Culicoides pulicaris*. Males made 19.23%, non-pigmented (young) females 70.96%, females who took the blood 9.8% of the population, whereas 0.73% were gravid females (Figure 1).

Figure 1. The relationship between gender and stages of female *C. obsoletus/scoticus* and *C. Pulicaris*

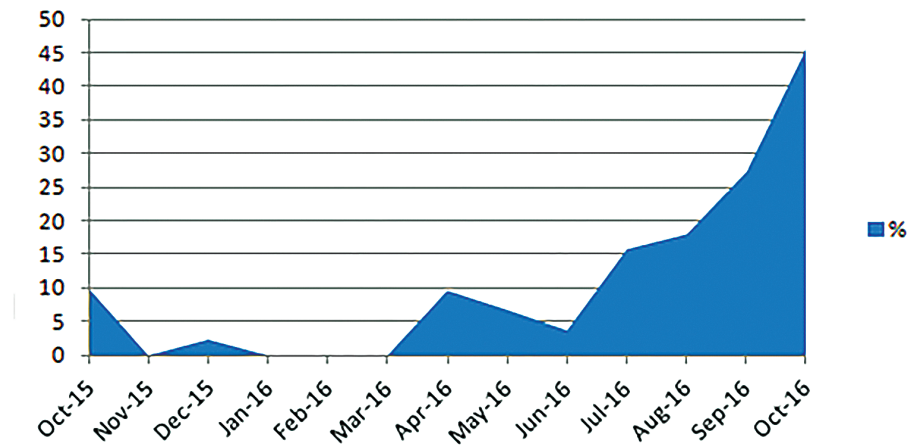


The ratio of gender and stages of female *Culicoides spp.* from Obsoletus complex (*Culicoides obsoletus/scoticus*) and Pulicaris complex (*Culicoides pulicaris*) are shown in Figure 1. Other types of *Culicoides* are set up in less than 10% of the examined samples (9.42%).

In this paper, seasonal dynamics of the presence of *Culicoides spp.* is monitored during the one-year period. During 2015, in October, their presence is established in 10.00% (4/40) samples, in November there were not any positive findings, and in December the insects were present in 2.35% (2/85) of samples.

During 2016, in January, February and March, *Culicoides spp.* were detected in neither of samples. During April, insects were present in 9.63% (8/83), in May in 6.74% (6/89), in June in 3.70% (2/54), in July in 15.78% (9/57), in August in 18.07% (15/83), in September in 27.27% (18/66), and in October in 45.65% (21/46) of samples. Results seasonal dynamics of occurrence *Culicoides spp.* are shown in Figure 2.

Figure 2. Seasonal dynamic of *Culicoides spp.* in the period October 2015-October 2016



DISCUSSION

Genus *Culicoides spp.* belongs to the class *Insecta*, order *Diptera*, family *Ceratopogonidae*, subfamily *Ceratopogoninae* and genus *Culicoides*. These are small insects the size of 0.5-2 mm, usually grey or black and at first glance very similar to mosquitoes. Nevertheless, they are morphologically different from mosquitoes and *Simulidae* by long antennae with 13-14 segments and Palpae, which have 3-4 segments. The dorsal side of the insect has a protrusion similar to *Simulidae*, width of the body and mild elongation which are clear morphological characteristics but which can also cause confusion in determination (Rawlings, 1996). The most obvious difference from the genera *Aedes*, *Culex* and *Phlebotomus* is in the wings with characteristically spotted pattern (Pavlović, 2009).

Systematics and taxonomy of this genus is still confusing and there are many subgenera and species which are not in the most accurate taxonomic sites (Blackwell, 2009). The current sub-generic classification of *Culicoides* consists of 31 subgenera containing 63% of existent species, 38 unplaced groups of species containing 24% of existent species and a further 13% of existent species that are not placed into any of these groupings by now (Blackwell, 2009; Bosnić, 2011; Pavlović, 2016a).

The entire genus *Culicoides* are strictly hematophagous and attack their hosts outdoors and indoors (although they are less susceptible to the stationing like *Aedes* mosquitoes are). They use an attractant to locate the host. One of the most important characteristics of hosts is carbon dioxide emission. As vertebrates breathe, the air of carbon dioxide is released and stimulates female *Culicoides* to fly upwind to the source of carbon dioxide. They are most active at sunset and in case of strong infestation and favourable weather conditions they attack even during the day. Female *Culicoides* feed on a wide range of hosts including reptiles, mammals, birds, humans, and even blood from fed mosquitoes. Southern *Culicoides spp.* prefer to feed on the blood of some animals species, mostly in Europe, they are known for their habit biting humans (Blackwell, 2001). They pose a serious threat to humans in certain parts of the world due to their ability to transmit deadly human diseases and some researchers tend to consider them cause of two of the ten biblical plagues of ancient Egypt.

Studies of the ecology of adult *Culicoides* is primarily focused on two areas: seasonal occurrence and feeding pattern. Many species reach their peak population in spring and summer months in moderate temperature regions and some species occur constantly during the year. Some species have two population peaks so that the first peak population is in the spring followed by a second peak in the autumn (Conte et al, 2007; De Liberato et al., 2010). Primary ecological factors affecting the *Culicoides* include rain, temperature and relative humidity, insolation, vegetation composition and pedological soil composition. This is why in many parts of the world insects of this genus occur seasonally. The air temperature in part affects the seasonal fluctuation of the population of some species of *Culicoides* and rain or other sources of water are crucial for the development of immature stages (Ducheyne et al., 2006, Hendrickx et al., 2006).

It is believed that rain is the most influential factor for the occurrence *Culicoides*, which are vector of BTD virus. For example, in Australia, it has been observed that the vector of BTD virus is prevalent usually in border areas where the levels of precipitation during rainy seasons are over 700 to 800 mm

(Meiswinkel et al., 2008; Pavlović et al., 2016). The same is observed in Europe where similar climatic conditions exist - in some parts of Italy and the Mediterranean (Conte et al, 2007; De Liberato et al., 2010).

The influence of climatic conditions is also observed in the Western Balkans (Croatia, Serbia, and Bulgaria) during the outbreak of the BTM in recent years (Bosnić, 2011; Maksimović-Zorić et al., 2016; Pavlović et al., 2016b). Unlike Mediterranean conditions, where *C. immicola* is most present and abundant species, continental climate contributed to the prevalence of species of *Culicoides obsoletus* Complex followed by *Pulicaris* Complex (Ducheyne et al., 2006; Hendrickx et al, 2006).

CONCLUSION

The *Culicoides* spp. are present in Serbia and they occur regularly throughout entire territory with high prevalence from June to October. The most abundant species is *C. obsoletus/scoticus*. The global environmental factors play a key role in expanding and changing biodiversity of insects of the genus *Culicoides* and therefore, given the exceptional vector potential of these small but dangerous insects, the epidemiological situation in the world has to be monitored.

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