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SEASONAL ABUNDANCE OF *CULICOIDES* BITING MIDGES (*DIPTERA, CERATOPOGONIDAE)* AND THE STRATEGY FOR THE DETERMINATION OF THE VECTOR-FREE PERIOD FOR BLUETONGUE IN ROMANIA

ABUNDENȚA SEZONIERĂ A INSECTELOR *CULICOIDES* (*DIPTERA*, *CERATOPOGONIDAE*) ȘI STRATEGIA PENTRU DETERMINAREA PERIOADEI FĂRĂ VECTORI PENTRU BOALA LIMBII ALBASTRE ÎN ROMÂNIA

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ABSTRACT | REZUMAT

Biting midges are small insects, belonging to the family *Ceratopogonidae*,genus *Culicoides* (Latreille,1809) Their epidemiological role in Romania is mainly related to the transmission of the Bluetongue virus, which causes a disease with particular impact on the receptive domestic and wild ruminants. Following the notification of the bluetongue disease for the first time in Romania, in 2014, measures were taken for the application of the relevant European legislation on disease control, which required, among other provisions, the implementation of an entomological surveillance programme for *Culicoides* insects in order to determine the seasonally vector-free period. Establishing the start and the end of this period, indicates the timeframe when the risk of Bluetongue virus transmission is significantly reduced, making trade of live animals possible under specific conditions. The strategy for the determination of the seasonally free period for *Culicoides* vectors in Romania evaluate two relevant parameters: the temperature and the number of captured insects. Based on this strategy, the seasonally vector-free period begins when the following conditions are met: 7 consecutive days with temperatures below 10°C (and a minimum of 3 nights with temperatures below 0°C within this period) and captures with less than 5 females of the complexes Obsoletus, Pulicaris and Nubeculosus. The end of the seasonally vector-free period is declared when for 7 consecutive days temperatures above 10°C are recorded (and at least 3 nights with temperatures above 0°C within this period) and when more than 5 females of the complexes Obsoletus, Pulicaris and Nubeculosus are present in the traps. Based on the data obtained from the entomological surveillance program for the *Culicoides* vectors over the period 2009-2018 and on the monthly average temperatures over this period, it was established that in Romania the vector-free period usually starts in December of current year and ends in March of the following year.

Keywords: Biting midges, *Culicoides*, Bluetongue

Insectele culicoide aparțin familiei *Ceratopogonidae*, genul *Culicoides* (Latreille, 1809).

Rolul lor epidemiologic în România este legat în principal de transmiterea virusului Bluetongue, agentul cauzal al bolii limbii albastre, care afectează rumegătoarele domestice și sălbatice.

Urmare a notificării bolii limbii albastre pentru prima dată în România, în anul 2014, au fost luate măsuri pentru aplicarea prevederilor legislației europene privind controlul bolii, care solicită, printre altele, implementarea unui program de supraveghere entomologică pentru insectele culicoide, cu scopul de a determina intervalul sezonier fără activitate vectorială.

Stabilirea debutului și sfârșitului acestei perioade precizează intervalul calendaristic când riscul de transmitere a bolii limbii albastre este redus semnificativ, făcând posibilă reluarea, în anumite condiții, a activităților de comerț cu animale vii.

Strategia pentru determinarea perioadei libere de vectori culicoizi în România, are în vedere evaluarea a doi parametrii relevanți: temperatura și numărul de insecte capturate. În baza acestei strategii, se consideră că perioada liberă de vectori debutează când sunt îndeplinite următoarele condiții: se înregistrează timp de 7 zile consecutiv temperaturi sub 10°C (și un minimum de 3 nopți cu temperaturi sub 0°C în acest interval) și în capcane sunt capturate mai puțin de 5 exemplare de culicoizi din complexele Obsoletus, Pulicaris și Nubeculosus. Sfârșitul perioadei libere de vectori se declară când se înregistrează timp de 7 zile consecutiv temperaturi peste 10°C (și un minimum de 3 nopți cu temperaturi peste 0°C în acest interval) și în capcane sunt capturate cel puțin 5 exemplare de culicoizi din complexele Obsoletus, Pulicaris și Nubeculosus.

În baza datelor obținute în cadrul programului de supraveghere entomologică pentru vectorii culicoizi, din perioada 2009–2018 și a temperaturilor lunare medii din acest interval, s-a stabilit că în România perioada liberă de vectori debutează, în mod obișnuit, în luna decembrie a anului curent și se încheie în luna martie a anului următor.

Cuvinte cheie: insectele culicoide, *Culicoides*, boala limbii albastre

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Culicoides biting midges are insects involved in the transmission of a significant number of diseases that have important impact on domestic and wild livestock. At European level, the main disease transmitted by these insects is bluetongue, which has been constantly evolving since the early 2000s (25). In Romania, the first outbreaks of bluetongue were reported in 2014, in the context of the incursion of the BTV4 in several Balkan countries (27). The disease produces important economic losses, generated by the movement restrictions imposed on susceptible animals, in accordance with the provisions of the European legislation in force (26).

The main purpose of imposing movement restrictions is to limit the contact between the host and the vector, resulting in a reduction of the spread of the virus in free territories. The measure of restricting the movement of animals is useful in those months of the year characterized by sufficiently high positive temperatures. These temperatures favour the biological cycle of the vector leading to an increase of the number of adult specimens (13). The calendar interval characterized by a high presence of adult specimens of biting midges, is considered as the vector activity period. In contrast to the above situation, European legislation foresees a reduction of movement restrictions of receptive ruminants during those months of the year characterized by sufficiently low temperatures, so that the biological cycle of vector development is interrupted. The period with significant reduction in the number of adult specimens of *Culicoides* vectors is designated as the vector - free period (14). The definition of such a period is of great economic importance, as trade in live animals can take place on much more favourable terms, at least between areas with the same epidemiological status (6).

According to the European legislation, three types of criteria are taken into account in defining the beginning of the vector-free period: general, specific and additional criteria. The main general criterion is the existence of a bluetongue disease surveillance program, with an entomological surveillance subcomponent. The most relevant specific criterion establishes the need to provide evidence that the number of *Culicoides* vectors dropped below a maximum threshold (usually the specimens of *Culicoides imicola* should be absent and less than 5 specimens of parous females of other *Culicoides* species should be present in the traps). The additional criterion is related to the specification of thermal conditions (temperatures) that have an impact on the biological cycle of the biting midges.

In Romania, all the previously mentioned criteria were gradually implemented, starting with 2003, when the entomological surveillance network for *Culicoides* vectors was established.

Currently, entomological surveillance is performed in all major administrative units (42 counties).

The beginning and the end of the vector-free period is established taking into account both the number of specimens captured, in relation to the established maximum/minimum thresholds and the temperature values recorded in the areas subject to surveillance.

In this study, we present the strategy for establishing the beginning and end of the vector-free period for bluetongue virus in Romania, given the data on the seasonal abundance of *Culicoides* vectors between 2009-2018 and the average temperature values recorded during this period.

MATERIALS AND METHODS

The study includes catches made between 2009 and 2018, in 1183 localities from all 42 major administrative units (counties) in Romania. OVI type, dark light traps were used, produced in South Africa. Only one trap per locality was used. The capture frequency is shown in Table 1.

Table 1

Operation frequency of the traps used as part of the national surveillance network for *Culicoides*, in Romania, between 2009-2018

Year(s)	Month	Frequency
2009 - 2013	January – December	Weekly
2014	April, November	Weekly
2014	December – March	Monthly
2015 – 2016	March – November	Weekly
2015 – 2016	December – February	Monthly
2017 – 2018	March, November	Weekly
2017 – 2018	April – October	Monthly
2017 – 2018	December – February	Monthly

Insects were identified based on morphological characteristics (particularly the wing pattern), using the stereomiscroscope.

After identification, the insects were kept in 70% alcohol. The taxonomic classification was based on the identification keys provided by Goffredo & Meiswinkel in 2004 (9). Because the morphological taxonomic criteria are often insufficient to achieve an exact separation of species (12,16,22), in the entomological surveillance program the captured specimens were classified in species complexes: Obsoletus, Pulicaris and Nubeculosus.

The monthly temperature values corresponding to the study period were provided by the National Institute of Meteorology and Hydrology.

The arithmetic means of the maximum and minimum monthly temperature values registered in each county, between 2008 - 2018 were taken into account.

RESULTS AND DISCUSSIONS

Between 2009 - 2018, a number of 12,433 catches were made, and 5,196,273 insects were examined. Of these, 2,146,314 specimens (41.3%) were classified as *Culicoides* midges. Within the genus *Culicoides,* the captured specimens were classified into 3 species complexes: Obsoletus (69.84%, n=1,499,145), Pulicaris (11.73%, n=251,824) and Nubeculosus (0.2%, n=4488) (Fig. 1). 18% (n=390,850) of the biting midges was represented by the specimens that could not be included in the three mentioned complexes.

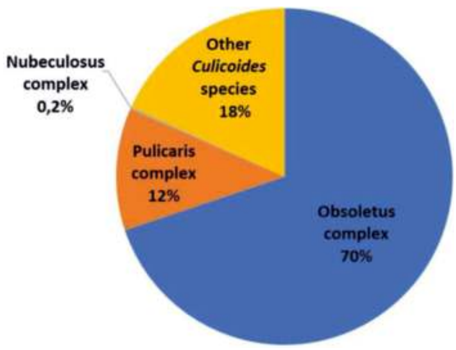

Fig. 1. Proportion of three *Culicoides* species complexes based on the number of specimens captured in Romania between 2009 - 2018

Seasonal abundance, based on the total number of specimens caught between 2009 and 2018, demonstrates that vector activity begins in spring (March), increases to a maximum in summer (June - July) and then gradually decreases in auumn to a minimum (no activity) during the winter season (Fig. 2).

The temperature values from 2008 to 2018 were calculated as arithmetic means of the maximum and minimum monthly temperatures at the level of each county, provided by the National Institute of Meteorology and Hydrology (Fig. 3). A number of 451 values/month were calculated for the period of January - October and 410 values/month for the period of November - December. In December, January and February, the average monthly temperatures are below the threshold of 10°C (except for two values from February 2016 - 10.5°C and 10.7°C respectively). Starting in March, the number of temperature values increases above the 10°C threshold (n = 97 in March and n = 434 in April), reaches a maximum (n = 451) between May and September and decreases during October - November (n = 380 and n = 63). The correlative evaluation of the number of average monthly temperature values above 10°C and of the number of specimens of *Culicoides* midges captured between 2008-2018, demonstrates the direct proportionality between temperature and seasonal abundance/vector activity (Fig 4).

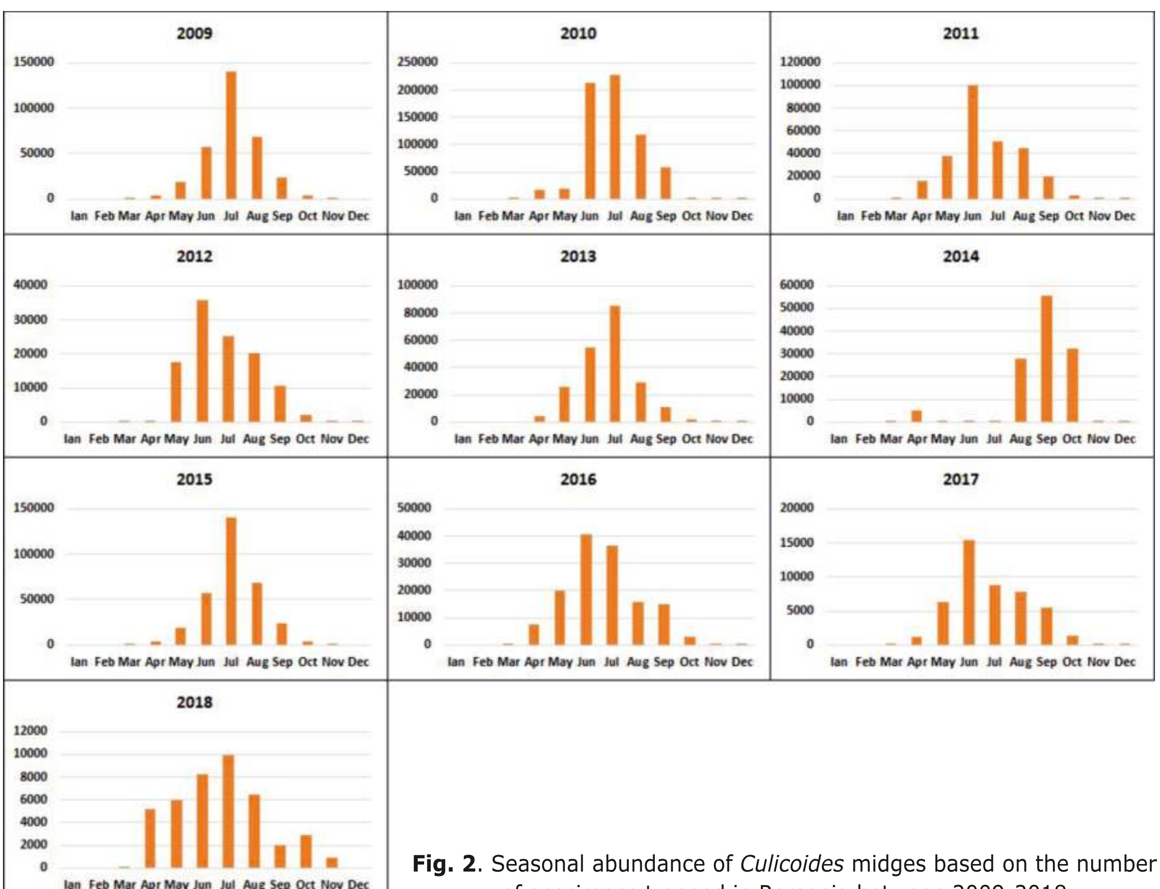

Fig. 2. Seasonal abundance of *Culicoides* midges based on the number of specimens trapped in Romania between 2009-2018

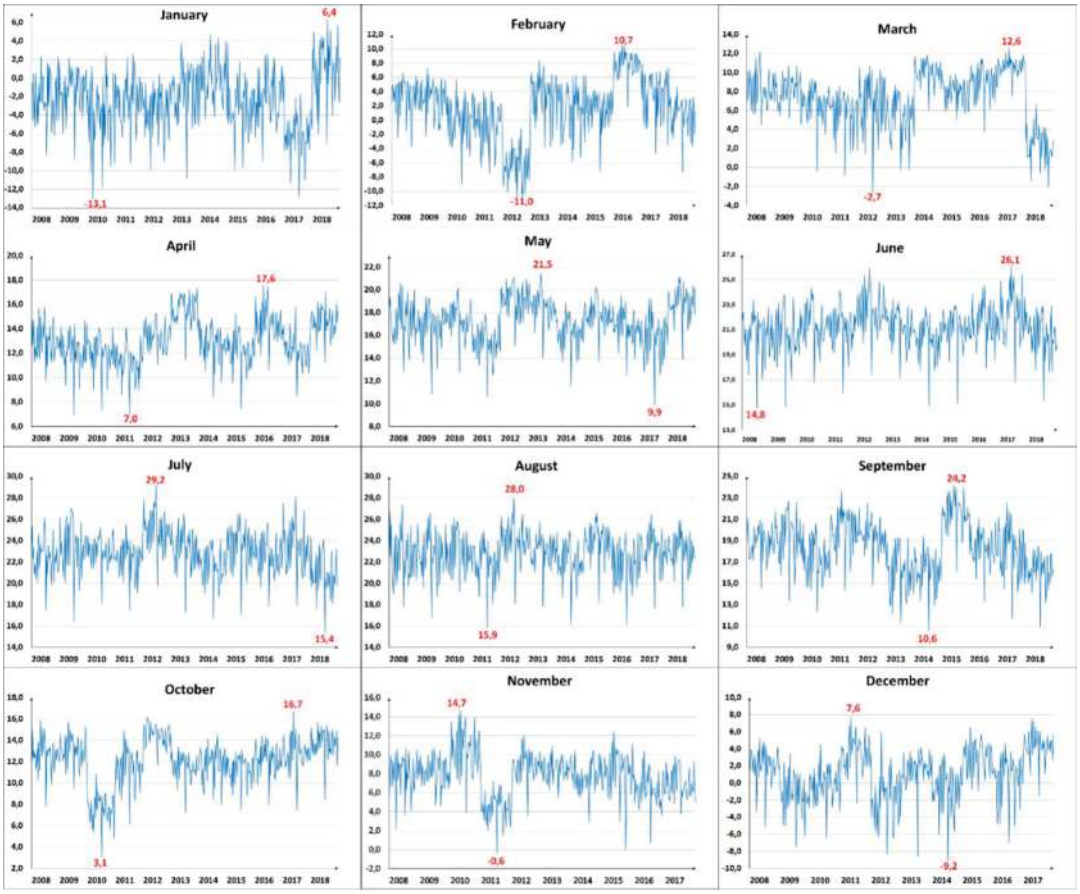

Fig. 3. Monthly average temperatures in 41 counties (2008 – 2018)

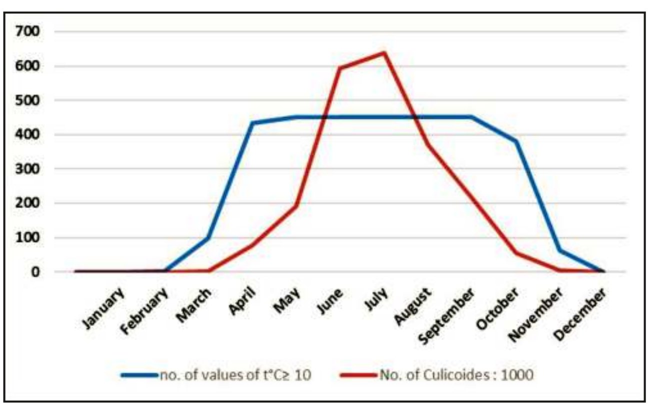

Fig. 4. Corelation between the number of monthly average temperature values above 10°C and the number of *Culicoides* specimens (divided by 1000), between 2008 – 2018, in Romania

Insects of the genus *Culicoides* are involved in the transmission of viral diseases with a significant impact on susceptible domestic and wild animal populations, such as Bluetongue and African horse sickness (4). For bluetongue, direct economic losses are caused by mortality, reduced fertility and decreased milk and meat production (3,10). Indirect economic losses are due to the disruption of trade activities (as a consequence of movement restrictions imposed by current European legislation) as well as the control measures (treatments, disinsection, vaccination) (8,29).

Hanon et al. (2009) estimates for the Walloon region (Belgium) losses of 104.8 million Euro, between 2006 and 2007 (11). In a comparative study conducted by Pinior et al. (2018), it was estimated that between 2007 and 2016, Austria and Switzerland spent 23.6 and 18.3 million Euro respectively on vaccination and surveillance programs for bluetongue disease (21). Velthuis et al. (2010) considers that the cumulative losses caused by bluetongue disease for the period 2006-2007 in the Netherlands are between 192.4-207.4 million Euro (30). Rushton&Lyons (2015) estimate that the financial impact of the evolution of bluetongue disease worldwide would be around 3 billion $ (24). In the context of such economic losses, European legislation on disease control measures (6), provides for the possibility of declaring the so-called "bluetongue seasonally-free zones". These zones are established based on the demonstration of the reduction of the number of *Culicoides* vectors below a certain threshold, so that this time interval is considered as a "vector-free period".

The beginning and end of the vector-free period are established under the following conditions: the existence of an entomological surveillance program, the demonstration of cessation/resumption of *Culicoides* activity using traps and the establishment of temperature thresholds relevant for the biological cycle of *Culicoides* insects. The most frequent interval in which the vector-free period for bluetongue is declared is the coldest part of the year (late autumn - winter - early spring). The importance of establishing such an interval is given by the relaxation of movement restriction of ruminants intended for trade in live animals (2).

Evaluating the conditions mentioned above, we find that temperature is the decisive environmental factor in establishing the vector-free period (1).

Mellor & Leake (2000) found that the duration of the egg-adult cycle decreases with the rise of temperature, increasing the number of generations (17). Experimental research on *C. variipennis sonorensis* has shown that the period between two feeds (hence the gonotrophic cycle) increases from 3 days (at 30°C) to 14 days (at 13°C) (31). In adults, high temperatures are associated with increased mortality (1).

Vector competence, which defines the ability of the vector to multiply and transmit the pathogen, increases in direct proportion to temperature (7). Vectorial capacity, which reflects the ability of a population of vectors to transmit the pathogen, increases with temperature as it increases the number of feeds per time unit and vector competence, while decreasing the extrinsic incubation period (time from ingestion of infected blood and the installation of the ability to retransmit the pathogen to a healthy animal) (15,18). Although the number of vectors may decrease with increasing temperature, the extrinsic incubation period becomes short enough to compensate for the decrease in survival rate. In relation to those mentioned above, research has shown that lower temperatures have negative effects on the life cycle and vector competence/capacity (by mainly affecting feeding behaviour and virogenesis). Purse et al. (2005) states that the optimum temperature range for the development of *Culicoides* insects is 25 - 30°C, and is inhibited at values lower than 8 - 10°C (23). Carpenter et al. (2011) reports that *C. sonorensis*, infected with BTV9, was able to transmit the virus after 3 days at 30°C, 4 days at 25°C, 5 days at 20°C and 20 days at 15°C; at 12°C transmission could not be demonstrated even after 40 days (5). Paweska, Venter & Mellor (2002) demonstrate that the development of the transmission potential for BTV1 of *C. bolitinos* is 2 days at 25°C and 8 days at 15°C; at 10°C the viral multiplication activity in the insect body could no longer be detected (20). Mullens et al. (1995) notes that viral replication of the BTV11 strain in *C. variipennis sonorensis* occurs after 22 days at 15°C and after 4-10 days at 27°C (19). In a study on the vector competence of *C. sonorensis* for viral strains AHSV4, AHSV6, EHDV1, BTV10 and BTV16, Wittmann, Mellor & Baylis (2002) established that the theoretical minimum temperature favourable for virus multiplication is 9.2°C for strain BTV10 and 15.2°C for EHDV1 (32). Research by Dijk & Huismans (1982) has shown that viral particle synthesis is dependent on the activity of the RNA-dependent RNA polymerase, which is optimal at 28-29°C and inhibited at temperatures below 10°C (28). In addition to temperature, the European legislation also provides that the determination of the vector-free period is based on the demonstration of a reduction in the number of biting midges below a maximum threshold. Thus, the total absence of *Culicoides imicola* specimens and the presence of less than 5 females of fertile *Culicoides* per trap are expected. Given the above, in Romania, the strategy for the establishment of the vector-free period states that the beginning of the period is recorded when for 7 consecutive days temperatures are below 10°C (and at least 3 nights with temperatures below 0°C in this interval) and when less than 5 female specimens from the Obsoletus, Pulicaris and Nubeculosus complexes are captured. The end of the vector-free period shall be recorded when temperatures above 10°C are recorded for 7 consecutive days (and at least 3 nights with temperatures above 0°C during this period) and when at least 5 female specimens from the Obsoletus, Pulicaris and Nubeculosus complexes are captured. Due to the difficulties of morphological determination, female specimens are identified at the complex level, without specifying fertility stage.

The assessment of seasonal abundance, based on captures made between 2009-2018, showed that the *Culicoides* vector activity period begins in March, reaches a maximum between June and August and then decreases to no-activity in December. The vector-free period for bluetongue usually begins in December of current year and ends in March of the following year. The vector-free period can be extended or shortened, depending on the temperature dominants in the autumn-winter (early or late winters) and winter-spring (cold or mild winters).

CONCLUSIONS

The appearance and evolution of the bluetongue disease in Romania, since 2014, has generated significant economic losses due to the direct and indirect effects on the domestic and wild ruminants. In this context, the determination of the seasonal abundance of *Culicoides* insects, resulting in the declaration of the vector-free period for bluetongue virus, is relevant for mitigating economic losses, as it allows the relaxation of movement restrictions applicable to trade in live animals. The data collected between 2009 and 2018, demonstrated that, usually, the vector-free period begins in December and ends in March.

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