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TROPICAL BED BUG, *CIMEX HEMIPTERUS*, ESTABLISHED IN CENTRAL EUROPE

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Abstract *Cimex hemipterus* is spreading to areas where only *C. lectularius* have been historically observed, such as the Middle East, North Australia, but also temperate regions like Russia. In Europe, until now mostly episodic observations of the species were known. We collected material from around 600 bed bug cases from Europe. Six collections from the Czech Republic, Slovakia and Switzerland included *C. hemipterus*. Using cytochrome oxidase subunit I, we linked these infestations to the most common haplotype found globally. The bed bugs possessed two kdr-associated mutations in the sodium channel gene, commonly found across the world, which are responsible for resistance to organochlorines and pyrethroids. The majority of Czech and Slovak cases were made among people whose social background suggest bed bug dispersal within local communities.

Key words *Cimex hemipterus*, *Cimex lectularius*, Central Europe, resistance, insecticides

INTRODUCTION

The occurrence of bed bugs is increasing worldwide, and during the last three decades, they have become one of the major concerns to public health (Zorrilla-Vaca, 2015). Although bedbugs are not vectors for human diseases, they are capable to cause allergic reactions (Goddard and deShazo, 2009), psychological distress, social stigma (Ashcroft et al., 2015) and economic burden (Potter, 2006). The global spread concerns both species that humans had historically associated with, the common bedbug *Cimex lectularius* and the tropical bedbug *Cimex hemipterus*.

C. lectularius was commonly present in temperate regions, while *C. hemipterus* mainly inhabited tropical and subtropical areas (Dang et al., 2017). However, the changes in human mobility have recently led to the expansion of *C. hemipterus* to regions, where only *C. lectularius* was known. In Europe, *C. hemipterus* had been reported only occasionally, and the reports were usually associated with international travels. Such cases are known from Italy (Masseti and Bruschi, 2007; Masini et al., 2020), France (Berenger and Pluot-Sigwalt, 2017; Chebbah et al., 2021), Spain (Pradera and Ruiz, 2020) or Sweden (Vinnersten, 2017), though the last report was suspected to represent a stable local population. *C. hemipterus* have been reported to have replaced the original populations of *C. lectularius* in Moscow and St. Petersburg (Gapon, 2016), although, other study reported only a partial representation of *C. hemipterus* in Moscow (Krivonosov, 2021), along with single records from three other Russian cities. Russia appears to be the first country in Europe colonised by this species, which indicates that the spread of the tropical bed bug is most likely not associated with climate change.

European pest management companies (PMCs) are likely to be dealing with a new pest in the near future. However, the detection and control of a new pest may be challenging. The bed bug control is complicated by multiple insecticide resistance mechanisms developed by the bed bugs. Despite the insecticidal resistance, chemical control is the most common strategy for bedbug control worldwide (Romero et al., 2017), and so, the resistance is considered to be one of the main drivers for the recent spread of both common and tropical bedbugs (Dang et al., 2017). Target site insensitivity increased metabolic detoxication and penetration resistance are the main mechanisms responsible for insecticide resistance in bedbugs. An important resistance mechanism against pyrethroids and some organochlorines is known as the knock-down resistance (kdr-type) (Davies et al., 2007). In this case, mutations in the voltage-gated sodium channel (vgsc) decrease the neural sensitivity of bedbugs (Dong et al., 2014). Various kdr-associated mutations have been identified in *C. hemipterus* including M918I, L1014F (Dang et al., 2015), Y/L995H, V1010L, I1011F, V1016E, L1017F/S, (Punchihewa et al. 2019), I1011T (Soh and Singham, 2021) along several only suspected to be kdr-associated, namely L899V, D953G (Dang et al., 2015) and A1007S (Punchihewa et al., 2019).

In *C. hemipterus,* the first reports of resistance against pyrethroids and organochlorines were recorded in the 1950s around the world from: India (Halgeri and Rao, 1956), Hong Kong, Gambia, Mombasa, Somalia, Tanzania (Busvine, 1958) in other African countries (WHO, 1963), Malaysia (Reid, 1960), and Venezuela (Tonn et al., 1982). Due to human health protection, environmental concern, and the resistance development, the use of organochlorines was restricted in some countries, and organophosphates or carbamates gradually began to be used in pest control. The first case of resistance to organophosphates in *C. hemipterus* was recorded in India in the 1960s (Reid, 1960). Moreover, the resistance to both organophosphates as well as carbamates was reported by some rather recent studies for example in Sri Lanka (Karunaratne et al., 2007) or Thailand (Tawatsin et al., 2011). Neonicotinoids, pyrroles or phenylpyrazoles are among substances recently introduced in bed bug control worldwide While no evidence of resistance of *C. hemipterus* against these substances has been found yet, the resistance of *C. lectularius* against the neonicotinoids and phenylpyrazoles has already been proved (Romero and Anderson, 2016; Gonzales-Morales et al., 2021).

This study reports further expansion of *C. hemipterus* across the European continent, pointing out the presence of kdr-associated mutations in the collected bedbug strains, and summarizing the possibilities for future effective bed bug control practice in Europe.

MATERIALS AND METHODS

The bed bug samples were collected between 2002 and 2020 in cooperation with PMCs, who also provided a brief description of the social background of the affected inhabitants. Specimens from 2002 to 2018 (267 locations) were identified based on morphological characters (Usinger, 1966). The morphological patterns were used for identification of samples collected from 299 locations in 2019-2020, and those believed to be *C. hemipterus* were confirmed by sequencing a 658bp portion of Cytochrome oxidase subunit I (COI, for primers and conditions see Balvín et al., 2012). Specimens identified as *C. hemipterus* were then sequenced for two fragments of the para-type sodium channel gene, to identify mutations previously connected with kdr in *C. hemipterus* (following Dang et al., 2015; Punchihewa et al., 2019).

RESULTS

Overall, 566 samples were obtained from 15 European countries. In 2002 -2018, all the specimens were identified as *C. lectularius*. Since 2019, *C. hemipterus* was recorded in six locations from three countries. Four cases were found in Bratislava, Slovakia (an apartment and a hostel room, both rented to socially disadvantaged people, an apartment inhabited by seniors, and an apartment occupied by a young family). In Prague, the Czech Republic, the infestation of *C. hemipterus* was recorded in the hostel, occupied by workers of mostly Ukrainian origin. In Geneva, Switzerland, *C. hemipterus* was found in a hotel. Two kdr-associated mutations (M918I and L1014F) were detected in the sodium channel gene, consistently in a homozygous state across all populations examined.

DISCUSSION

The expansion of bed bugs connected to increased human mobility and the development of insecticide resistance has become a serious public health concern (Zorrilla-Vaca, 2015). Our research strongly indicated the establishment of

local *C. hemipterus* population in central Europe, based on the information on social background of the inhabitants of the infested properties, suggesting that *C. hemipterus* spreads within the city, not via travelling to origin countries.
The presence of M918I and L1014F mutations suggested that the new European populations of *C. hemipterus* are likely resistant to pyrethroids. Therefore, the European PMCs must be aware of an occurrence of a new synanthropic pest, which is, similarly, to the already known *C. lectularius*, resistant against commonly used insecticides. There is no evidence yet that the effect of chemical treatment differs between the European population of *C. hemipterus* and *C. lectularius*. (Ghavami et al., 2021).

Chemical treatment is still the key element of bed bug control in European states (Romero et al., 2017). The effective active substances available on the European market is limited. Some active substances in the EU are still subjected to a Review programme according to Regulation (EU) No 528/2012. The Review Programme is foreseen to be completed by 2024. The transitional provisions are applied in EU states, and a biocidal product containing an active substance included in the Review Programme could be used according to national rules (ECHA, 2021). A compilation of all active substances allowed for bed bug control in the EU is unavailable. The variety of such substances is assumed not to be very wide (ECHA, 2020).

Considering the risk of development of further resistance, it is important that PMCs should not use chemical treatment exclusively, but also consider the alternative approach of pest control with a variety of other strategies within the integrated pest management (IPM). Bed bug IPM should be based on the combination of methods focused on a thorough inspection of the infested apartment, environmental adjustment such as cleaning, removal of the infested objects and furniture arrangement in the apartments. It should include non-chemical treatments such as vacuum cleaning, trap setting, heat applying and freezing or encasing matrasses (Ghavami et al., 2021; Rukke et al., 2021). The bed bug treatment within the IPM may also include chemical treatment when the appropriate choice of insecticide is crucial. The effectivity of pyrethroid insecticides was reported to be significantly positively affected by synergists such as piperonyl butoxide (PBO) (Dang et al., 2021), essential oils (Gaire et al., 2021), and insect growth regulators (Campbell et al., 2017). According to Rukke et al., (2021), bed bugs can be successfully eliminated using IPM strategy, education, rising awareness and communication with the inhabitants of the infested apartments, and frequent visits and controls.

In conclusion, the results obtained within this study reported a newly established pest in Central Europe, *C. hemipterus*, which currently occurs here along with the original *C. lectularius*. From the evolutionary point of view, further resistance mechanisms may develop in the case of the co-occurrence of two bed bug species. For this reason, it is essential to appeal to the conscientiousness of the PMCs, because the quality of pest control services is crucial for the efficient control of bed bugs (Bennett et al., 2016).

The existence of comprehensive guidelines for bed bug control published by national public health institutes would be very beneficial for European PMCs. Such guidelines are currently only available in some European states. Therefore, we highly recommend publishing a comprehensive review of the recommended strategy of bed bug control, which would be helpful for PMCs in order to set up the proper pest management. This approach should lead to preventing the increase of resistance and finding a solution in case the list of active substances will be restricted.

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