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The new invasive mosquito species *Aedes koreicus* as vector-borne diseases in the European area, a focus on Italian region: What we know from the scientific literature

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The increased mobility of goods, people, and animals worldwide has caused the spread of several arthropod vectors, leading to an increased risk of animal and human infections. Aedes koreicus is a common species in South Korea, China, Japan, and Russia. Due to its cold-resistant dormant eggs, the adults last from the late summer until the autumn seasons. For these reasons, it seems to be better adapted to colder temperatures, favoring its colonization of hilly and pre-alpine areas. Its first appearance in Europe was in 2008 in Belgium, where it is currently established. The species was subsequently detected in Italy in 2011, European Russia, Germany, the Swiss-Italian border region, Hungary, Slovenia, Crimea, Austria, the Republic of Kazakhstan, and the Netherlands. The role of A. koreicus in the transmission of vector-borne pathogens remains unclear. The available scientific evidence is very old, often not available in English or not indexed in international databases, and therefore difficult to find. According to the literature reviewed, A. koreicus can be considered a new invasive mosquito species in Europe, establishing populations on the European continent. In addition, experimental evidence demonstrated its vector competence for both Dirofilaria immitis and Chikungunya and is relatively low for ZIKA but not for Western Nile Virus. On the other hand, even if the field evidence does not confirm the experimental findings, it is currently not possible to exclude with absolute certainty the potential involvement of this species in the spread, emergence, or re-emergence of these vector-borne disease agents.

KEYWORDS

Korean bush mosquito, nematodes, Flavivirus, Alphavirus, hematophagous arthropod

Introduction

Aedes koreicus (Edwards, 1917), also known as the Korean bush mosquito, is naturally distributed in South Korea, China, Japan, and Russia (Knight, 1968; Gutsevich et al., 1974; Tanaka et al., 1979). The first occurrence in Europe was recorded in Belgium in 2008, where it is currently established (Versteirt et al., 2012, 2014). The species was subsequently detected in Italy in 2011 (Capelli et al., 2011; Marcantonio et al., 2016; Ballardini et al., 2019; Negri et al., 2021), European Russia (Bezzhonova et al., 2014), Germany (Werner et al., 2016; Pfitzner et al., 2018; Zotzmann et al., 2019; Hohmeister et al., 2021), Swiss-Italian border region (Suter et al., 2015), Hungary (Kurucz et al., 2016), Slovenia (Kalan et al., 2017), Sochi, Russia (Ganushkina et al., 2016), Crimea (Kovalenko and Tikhonov, 2019; Ganushkina et al., 2020), Austria (Fuehrer et al., 2020), and Republic of Kazakhstan (Andreeva et al., 2021) (see Figure 1) for further details). A. koreicus is nowadays established (Jansen et al., 2021) in all the countries mentioned above except Slovenia and Switzerland (ECDC, 2020). In 2021, the first finding of A. koreicus larvae in the Netherlands was made (Teekema et al., 2022). Until now, nothing is known about the initial pathways of the introduction of A. koreicus into the different European areas, although international trade has been suggested as a possible route (Versteirt et al., 2012). For what concerned A. koreicus arrival time in Italy, the second European A. koreicus population was detected in 2011 in Belluno province. However, it has been supposed that this species has been present for several years (Pfitzner et al., 2018) (Figure 1).

The females lay their drought-resistant eggs in artificial breeding sites such as containers, like Aedes (Stegomyia) albopictus (Skuse) and Aedes (Hulecoeteomyia) japonicus japonicus (Theobald), so its introduction could be due to the trade of small containers, ornamental plants or used tire (Versteirt et al., 2012; Medlock et al., 2015; Kampen et al., 2017; Ibáñez-Justicia et al., 2020). A. koreicus and the Japanese bush mosquito A. japonicus lived in sympathy and, for a long time, were confused. Indeed, according to phylogenetic studies, both mosquito species belong to the same monophyletic group (Miyagi, 1971; Cameron et al., 2010; Hohmeister et al., 2021). However, A. koreicus adults differ from the latter in having a pale basal band at the hind tarsomeres IV, the typical scutal pattern, the presence of a subspiracular patch of pale scales, and the base of the posterior femur is completely pale (Cameron et al., 2010; Werner et al., 2016; Pfitzner et al., 2018).

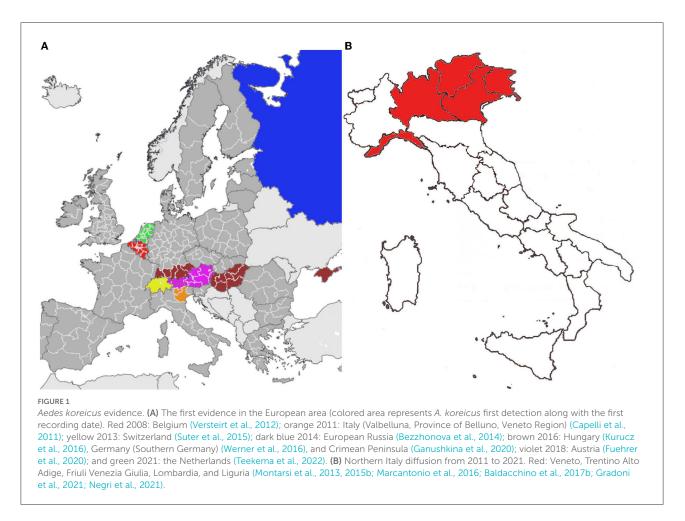
Aedes koreicus has settled in areas partially occupied by other mosquito species, including its most likely competitor, *A. albopictus*, which uses similar larval habitats for its development (Montarsi et al., 2013). Although larval coexistence between these two species is possible, it was not expected. Indeed, field observations carried out in the Province of Belluno, and the neighboring provinces demonstrated that *A. albopictus* larvae number was higher than the *A. koreicus* ones during the fall and toward the south. Moreover, they were often found alone and developed earlier than *A. albopictus* ones. According to the authors, northern Italy is highly likely to be invaded by *A. koreicus* in the next decade and other parts of Europe (Montarsi et al., 2013, 2015b). Laboratory experiments have also revealed a weak larval competition between *A. albopictus* and *A. koreicus*, with a slight advantage in favor of the first one (Baldacchino et al., 2017a).

At the end of the annual season, when daylight becomes shorter, A. koreicus females, like other females of the Aedes species, lay desiccated and cold-resistant eggs that can survive during the winter and hatch in the spring (Medlock et al., 2015). Compared to A. albopictus, A. koreicus dormant eggs are more resistant to cold temperatures. Furthermore, adults have a more remarkable persistence during the late summer and autumn seasons, and the species is active earlier. For these reasons, A. koreicus seems to be better adapted to colder temperatures, favoring its colonization of hilly and prealpine areas (Knight, 1947; Miyagi, 1971; Capelli et al., 2011; Montarsi et al., 2013; Baldacchino et al., 2017a). In particular, laboratory trials demonstrated that A. koreicus's optimal thermal range is placed between 23°C and 28°C, and the authors found that warmer seasonal temperatures provoke an increase in values of the adult abundance curve (Marini et al., 2019). This last evidence is especially relevant concerning climate change causing increased temperatures in the Alpine area (Coppola et al., 2018).

Moreover, *A. koreicus's* ability to colonize areas with harsh winter temperatures allows the species to avoid competition with other species, such as *A. albopictus*, showing a considerable advantage in terms of number and speed of replication (Baldacchino et al., 2017a).

Several studies indicate that *A. koreicus* is well adapted to urban settlements and colonize gardens and urban areas, where various artificial containers can be found as breeding sites (Gutsevich et al., 1974; Tanaka et al., 1979; Montarsi et al., 2013). However, this species was also frequently found in natural habitats, like forests, far from human settling (Baldacchino et al., 2017b; Pfitzner et al., 2018), suggesting that *A. koreicus* can complete its life cycle by feeding on animals other than humans (Montarsi et al., 2013). Until now, only three species of mammals (*Homo sapiens, Canis lupus*, and *Bos taurus*) have been detected as hosts, and adult females seem to bite humans mainly during the daytime (Montarsi et al., 2014, 2015b; Tripepi, 2014; Cebrián-Camisón et al., 2020).

According to the European Center for Disease Prevention and Control (ECDC, 2020), *A. koreicus* in transmitting vectorborne pathogens remains unclear. Thus, this narrative review aims to provide a broad view in light of the latest scientific evidence about *A. koreicus* vector competence, focusing on the re-emergence of vector-borne diseases in the European area.



The bibliographic research for scientific papers specialized in the field of interest was conducted from 1st January 2000 to 31st January 2022 on PubMed (the MEDLINE database), using the following keyword: *Aedes koreicus*. As a preliminary result, more than 57 documents were found. Of these, 8 papers, 5 on filed evidence and 3 on laboratory experiments, were selected for review due to their relevance. The search and selection criteria for reviewed scientific articles can be found in Supplementary Figure S1.

Literature review

The selected manuscripts were subdivided according to the study type (namely, field or experimental) and reviewed according to the publication date (from oldest to most recent).

Field evidence

Concerning field research data, the results are not entirely consistent. Of the five publications reviewed, only one by

Kurucz et al. (2018) detected the presence of filaria DNA from captured mosquitoes. A possible explanation of these results can be found in how these studies were carried out and their procedural limitations.

According to Lee et al. (2007), the low number of mosquitoes collected from Gyeonggi Province and Gangwon Province in the Republic of Korea can justify the low number of *A. koreicus* that was retrieved in the study. Among the limiting factors found by the authors were the trapping methods and the sampling periodicity (only one season, from 25th May to 25th September, was considered). For these reasons, it was impossible to determine the species' vector competence and the prevalence of infection in the mosquito population. Despite the negative results (Supplementary Table 1), the involvement of *A. koreicus* as a vector of *Dirofilaria immitis* and *Dirofilaria repens* could not be excluded (Lee et al., 2007).

Another single season (May–October 2009) surveillance study, conducted by Cho et al. (2012) in the Republic of Korea over an extensive area previously considered endemic for filariasis, did not detect *Brugia malayi* DNA in a large sample of captured mosquitoes (Supplementary Table 1). Even if these data demonstrated the disease eradication from previously endemic areas, given the many vector species isolated, the authors pointed out the potential risk of re-emerging filariasis. In addition, the small number of *A. koreicus* captured (Supplementary Table 1) did not allow valid conclusions about the vector status of this species (Cho et al., 2012).

On the other hand, between 2016 and 2017, Kurucz et al. collected 68 specimens of *A. koreicus* adult female mosquitoes from 46 different pools out of 1,123 near the city of Pécs (Baranya County, Hungary). Molecular investigation for filarial DNA revealed 25 specimens positive for nematodes. As this is the first recent field study to detect the *D. immitis* DNA, further research confirming the possible vector role of this mosquito is needed. However, the presence of this species near urban areas could facilitate the spread of these vector-borne pathogens (Kurucz et al., 2018).

The first Italian record of *A. koreicus* was reported in Genoa in September 2015, when a male specimen was caught, and genetic analysis confirmed the identity of the species. Mosquito sampling, conducted in different periods until 2018, led to trapping some mosquito species, and morphological and molecular studies allowed us to identify some samples as *A. koreicus* females.

A subsequent biomolecular assay to detect *Flavivirus* infection showed the DNA absence in females. The authors explained this result by the low density of the species in the sampling area and the low meeting rate with infected hosts (Ballardini et al., 2019).

Similar evidence was reported from a 3-year surveillance study conducted by Jegal et al. (2020) in several provinces of the Korean republic (Jegal et al., 2020). Even though there was geographical proximity to the previous studies reviewed (Lee et al., 2007; Cho et al., 2012), the research procedures were implemented to obtain more comprehensive epidemiological results (i.e., mosquito collection procedures and Flavivirus detection analysis). Thus, sampling was carried out for three consecutive seasons (24 h every fortnight from March to November 2016-2018), and the trap type was adjusted according to the locations analyzed (downtown or cowshed). Furthermore, the authors established the distribution of total population densities of each captured mosquito species according to the collection time. This could explain the significant increase in A. koreicus specimens caught in the field (Supplementary Table 1). Molecular analyses on mosquitoes showed the absence of DNA belonging to the three Flavivirus species investigated (WNV, JEV, and dengue fever virus). Nevertheless, even in this study, the possible role of Ae koreicus as a vector was not wholly rejected (Jegal et al., 2020).

Experimental evidence

In a detailed laboratory study conducted by Montarsi et al. (2015), *A. koreicus* adults were experimentally administered with

D. immitis microfilariae. Parasites at the third larval stage (L3) have been detected in the mosquito's malpighian tubules, thorax, salivary glands, palp, and proboscis, demonstrating that infective larvae could develop within the species. The authors also observed L3 emerging from the mosquito proboscis. However, the L3 host transmission would confirm the role of *A. koreicus* as a *D. immitis* vector and requires further experimental evidence (Montarsi et al., 2015a).

In laboratory experiments, Ciocchetta et al. (2018) explored the potential of A. koreicus to transmit CHIKV "La Reunion" under different temperature regimes: at a constant temperature of 23°C and fluctuating temperatures (12-27°C). These fluctuations simulated a typical summer in Belluno, Italy, where thriving populations of A. koreicus are established. The experiments highlighted that, at fluctuating temperatures, despite the very favorable infection conditions, CHIKV "La Reunion" was detected in a tiny percentage of mosquito bodies; the virus dissemination to the legs and wings was also recorded on a low number of mosquitoes, and for the salivary dissemination. However, CHIKV "La Reunion" disseminates to the A. koreicus at a constant temperature from wings and legs reaching the saliva. Based on the results, A. koreicus may transmit CHIKV. However, only a tiny proportion of mosquitoes may vector the virus under optimal rearing temperatures, and more natural temperature fluctuations might further mitigate transmission risks (Ciocchetta et al., 2018).

Jansen et al. (2021), demonstrated that experimentally infected A. koreicus specimens were able to transmit CHIKV, and, according to Ciocchetta et al., the transmission was temperature-dependent. The mosquitoes were capable of transmitting the virus at a higher temperature ($27^{\circ}C \pm 5^{\circ}C$), with no transmission at $24^{\circ}C \pm 5^{\circ}C$. Moreover, the infection rate at the higher temperature (68.2%) was four times higher than at the lower one (17.6%). Regarding the ZIKA virus, the vector competence was relatively low (4.7%) and temperaturedependent; in particular, ZIKV transmission occurred only at a higher temperature. No transmission of WNV could be detected at both temperatures. Although able to cross the midgut barrier and infect the whole mosquito body, the authors hypothesized that the virus could not pass the salivary gland barrier. They also hypothesized that both the salivary glands' infection and the virus's escape from the tissue into the saliva failed (Jansen et al., 2021).

Discussion

The increased mobility of goods, people, and animals worldwide has caused the spread of several arthropod vectors, leading to an increased risk of animal and human infection (Genchi et al., 2009).

In recent years, several vector-borne diseases have reemerged and spread in Europe due to global and/or local changes that have led to the invasion of new arthropod vectors (Hendrickx and Nicolaij, 2004). The direct consequences of climatic variations include habitat alterations and a lengthened mosquito season with an increased incidence of mosquitoborne diseases (Harrus and Baneth, 2005). Mosquito survival is strongly influenced by the environmental temperature and the pathogens carried by them (Purse et al., 2005). In this scenario, *A. koreicus* adaptation to colder temperatures ensures longer persistence during late summer and autumn, leading to the colonization of hilly and prealpine areas (Knight, 1947; Miyagi, 1971; Capelli et al., 2011; Montarsi et al., 2013; Baldacchino et al., 2017a). In addition, anthropogenic factors such as water shortages due to human consumption or irrigation, pollution, insecticides, and drug-resistance development can impact the vector-borne parasites (Harrus and Baneth, 2005).

The scientific papers reviewed suggest a possible involvement of *A. koreicus* as a vector of both lymphatic filariae and *Dirofilaria* and several viruses belonging to the genus *Flavivirus* and *Alphavirus*.

In 1927, Yamada found that this species can collect microfilariae but does not allow the development of *Wuchereria bancrofti* (Yamada, 1928).

Lymphatic filariasis is an underestimated tropical disease caused by parasitic nematodes known as filarial worms. Currently, approximately 856 million people in 52 countries live in endemic areas. *B. malayi*, one of the nematode species for lymphatic filariasis, is an endemic nematode in Southeast Asia and Indonesia. It develops through four larval stages into an adult male or female, entirely within one of two host species: a mosquito vector and humans (Ghedin et al., 2004). For this reason, the possible involvement of a new mosquito species as a vector is crucial to set up the necessary preventive measures to contain infections.

Dirofilariasis is a zoonotic disease transmitted by mosquitoes (family *Culicidae* belonging to the genus *Aedes*, with domestic dogs and some wild canids as definitive hosts. In 1938, Feng et al. experimentally proved the transmission of *D. immitis* to dogs (Feng, 1938). The *Dirofilaria* species reviewed in this article (*D. immitis* and *D. repens* are occasionally transmitted to humans through the bites of infected mosquitoes. Although *D. repens* is the principal agent of human dirofilariasis, causing a subcutaneous infection, *D. immitis* larvae can occasionally encapsulate in lung tissue or, more rarely, in the eyes, brain, and/or testes, producing nodules (Simón et al., 2012).

Italy is one of the European countries endemic to canine dirofilariasis, with the highest number of human cases described so far. In recent years, an underestimated increase in the number of dirofilariasis cases (*D. immitis* and *D. repens*) has been recorded (Mendoza-Roldan et al., 2021).

According to a study by S. Pampiglione et al., between 1990 and 1999, out of 60 cases of human dirofilariasis caused by *D. repens*, forty-six were from Piemonte (one of the most affected geographical areas in the world), while the remaining were from Emilia-Romagna, Sardinia, Sicily, Tuscany, Apulia, and Lombardy (Pampiglione et al., 2001). In 2018–2019, eight cases were reported from Central Italy, suggesting the current increase in the spread of the parasite in the peninsula (Mendoza-Roldan et al., 2021).

Concerning *A. koreicus* virus transmission, field evidence collected in 1964 and 1966 by Russian researchers has raised the suspicion that *A. koreicus* could be a vector for the Japanese encephalitis virus (Miles, 1964; Shestakov and Mikheeva, 1966). Furthermore, experimental virus transmission was reported in Russia in 1970 (Gutsevich et al., 1974).

Although *A. koreicus* vector competence for ZIKV and WNV has been investigated by experimental studies (Ciocchetta et al., 2018; Jansen et al., 2021), only ZIKV transmission was demonstrated in laboratory conditions without any field evidence (Jansen et al., 2021). These experimental findings on vector competence agree with those obtained for the related species *A. japonicus* which can transmit ZIKV temperature-dependent (Jansen et al., 2018; Abbo et al., 2020; Glavinic et al., 2020).

According to a 2020 study by Durand et al., the first European ZIKV cases were reported in August 2019 in France (city of Hyeres). Although the infection origin was not clarified, the authors established the source of the viral strain, namely, South-East Asia (Durand et al., 2020). Since 1st February 2016, the WHO classified Italy as at moderate risk of ZIKV transmission. ZIKV is not endemic to date as no locally contracted infection has been recorded but only imported cases have been documented (WHO, 2022).

Regarding the *Alphavirus* genus, the first autochthonous outbreak of CHIKV in Europe was reported in Emilia-Romagna (Italy) in 2007 (Marano et al., 2017). In addition, a large number of confirmed autochthonous cases were reported in France from 2010 to 2014 (Zeller et al., 2016).

In Italy, in September 2017, a new outbreak of CHIKV was detected in the Lazio region (central Italy) and subsequently in Calabria (southern Italy). The activity of vector mosquitoes is mainly linked to the summer season (usually between June and October), and outbreaks could be triggered by the arrival of imported cases from endemic areas causing, consequently, secondary cases (McCormack et al., 1995; Hassoun et al., 1999; Silva and Dermody, 2017).

Along with its role as an enzootic vector, another critical aspect of mosquito-related diseases is its allergic potential. There are limited epidemiological data on the prevalence of mosquito allergy, although reactions to mosquito bites are common. Recently, studies have proven an association between unusual, largely local, or exaggerated reactions after mosquito bites and allergic diseases in children. The severity of reactions increases with age, particularly in children with an atopic background (Yavuz et al., 2021). Moreover, a family history of allergy, and atopic dermatitis (Magnifico et al., 2020), together with a self-reported allergy to mosquito bites and residence in an area of high mosquito exposure, were associated with positive IgE levels. In contrast, positive IgG levels were significantly associated with male sex, blood donation after the "mosquito season," and residence in the area of high exposure but inversely related to self-reported asthma (Peng et al., 2002).

In conclusion, according to the reviewed literature data, *A. koreicus* can be granted as a new invasive mosquito species in Europe. Furthermore, the experimental evidence has demonstrated its vector competence for both *D. immitis*, CHIKV, and ZIKA but not for WNV (Montarsi et al., 2015a; Jansen et al., 2021). On the other hand, although field evidence did not confirm the experimental evidence, it is currently not possible to exclude with absolute certainty the potential involvement of this species in the spread and re-emergence of these vector-borne pathogens. In this scenario, it is imperative to increase research activity to conclusively understand the infectious potential of this mosquito, which is a public health threat worldwide.

Author contributions

AD, RD, SG, and GPP contributed conception and design of the study. CL, AG, LP, and NV organized the database. SG and GPP wrote the first draft of the manuscript. DD,

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SP, and CL wrote sections of the manuscript. All authors contributed to manuscript revision, and read and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb. 2022.931994/full#supplementary-material

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