AIMSurv: First pan-European harmonized surveillance of *Aedes* invasive mosquito species of relevance for human vector-borne diseases.

List of Authors

Miguel Ángel Miranda[1*], Carlos Barceló[1], Daniele Arnoldi[2], Xenia Augsten[3], Karin Bakran-Lebl[4], George Balatsos[5], Mikel Bengoa[6], Philippe Bindler[7], Kristina Boršová[8], Maria Bourquia[9], Daniel Bravo-Barriga[10], Viktória Čabanová[8], Beniamino Caputo[11], Maria Christou[12], Sarah Delacour[13], Roger Eritja[14], Ouafaa Fassi-Fihri[9], Martina Ferraguti[15], Eleonora Flacio[16], Eva Frontera[10], Hans Peter Fuehrer[17], Ana L. García-Pérez[18], Pantelis Georgiades[12], Sandra Gewehr[19], Fátima Goiri[18], Mikel Alexander González[20], Martin Gschwind[21], Rafael Gutiérrez-López[1], Cintia Horváth[22], Adolfo Ibáñez-Justicia[23], Viola Jani[24], Përparim Kadriaj[24], Katja Kalan[25], Mihaela Kavran[26], Ana Klobucar[27], Kornélia Kurucz[28], Javier Lucientes[13], Renke Lühken[29], Sergio Magallanes[15], Giovanni Marini[2], Angeliki F. Martinou[30], Alice Michelutti[31], Andrei Daniel Mihalca[22], Tomás Montalvo[32], Fabrizio Montarsi[31], Spiros Mourelatos[19], Nesade Muja-Bajraktari[33], Pie Müller[21], Gregoris Notarides[34], Hugo Costa Osório[35], José A. Oteo[36], Kerem Oter[37], Igor Pajović[38], John R.B. Palmer[39], Suncica Petrinic[27], Cristian Răileanu[40], Christian Ries[41], Elton Rogozi[24], Ignacio Ruiz-Arrondo[36], Isis Sanpera-Calbet[39], Nebojša Sekulić[42], Kivanc Sevim[43], Kurtesh Sherifi[44], Cornelia Silaghi[40], Manuel Silva[35], Nikolina Sokolovska[45] , Zoltán Soltész[46], Tatiana Sulesco[47], Jana Šušnjar[25], Steffanie Teekema[23], Andrea Valsecchi[32], Marlen Ines Vasquez[34], Enkelejda Velo[24], Antonios Michaelakis[5], William Wint[48], Dušan Petrić[26], Francis Schaffner[7], Alessandra della Torre[11]

Affiliations

1. Applied Zoology and Animal Conservation group, University of the Balearic Islands (UIB), Ctra Valldemossa km 7.5, 07122 Palma, Spain

2. Research and Innovation Centre, Fondazione Edmund Mach, Via Edmund Mach 1, 38098 San Michele all'Adige (TN), Italy

3. Kommunale Aktionsgemeinschaft zur Bekämpfung der Schnakenplage (KABS) e.V. Georg-Peter-Süß-Str. 3, 67346 Speyer, Germany

4. Austrian Agency for Health and Food Safety (AGES), Division for Public Health, Währinger Strasse 25a, 1090 Vienna, Austria

5. Laboratory of Insects & Parasites of Medical Importance, Benaki Phytopathological Institute, St. Delta 8, Kifisia 14561, Athens, Greece

6. Anticimex Spain, Carrer Jesús Serra Santamans 5 Planta 3, 08174 Sant Cugat del Vallès, Barcelona, Spain

7. Brigade Verte du Haut-Rhin, Service démoustication, 92 rue Mal. de Lattre de Tassigny, 68360 Soultz, France

7. Francis Schaffner Consultancy, Lörracherstrasse 50, 4125 Riehen, Switzerland

8. Institute of Virology, Biomedical Research Center of Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.
8. Institute of Virology, Biomedical Research Center of Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.
9. Agronomic and Veterinary Institute Hassan II, BP 6202, Rabat-Instituts 10100, Rabat, Morocco

10. Department of Animal Health, Veterinary Faculty, University of Extremadura, Av. de la Universidad, s/n, 10003 Cáceres, Spain.

11. Dep. Public Health and Infectious diseases, University Sapienza, Piazzale Aldo Moro 5, 00185 Roma, Italy

12. Environmental Predictions Department, Climate and Atmosphere Research Centre, The Cyprus Institute, 20 Konstantinou Kavafi Street, 2121, Nicosia, Cyprus

13. Animal Health Department. Faculty of Veterinary Medicine of Zaragoza, C/Miguel Servet 177, 50013 Zaragoza. Spain

14. Consell Comarcal del Baix Llobregat, 08980 Sant Feliu de Llobregat, Barcelona, Spain

15. Department of Biology, Faculty of Sciences, University of Extremadura Av. de Elvas, s/n, 06006 Badajoz, Spain.

16. University of Applied Sciences and Arts of Southern Switzerland, Institute of microbiology, Via Flora Ruchat-Roncati 15, 6850 Mendrisio, Switzerland

17. University of Veterinary Medicine, Institute of Parasitology, Vienna.

18. NEIKER-Basque Institute for Agricultural Research and Development, Berreaga 1, 48160 Derio, Bizkaia, Spain.
18. NEIKER-Basque Institute for Agricultural Research and Development, Berreaga 1, 48160 Derio, Bizkaia, Spain.
19. Ecodevelopment S.A., PO Box 2420, Thesi Mezaria, 57010 Filyro, Greece

20. Universidad Iberoamericana (UNIBE), Avenida Francia 129, Santo Domingo 10203, Rep. Dominicana

21. Swiss Tropical and Public Health Institute (Swiss TPH), Kreuzstrasse 2, 4123 Allschwil, Switzerland.

22. Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

23. Centre for Monitoring of Vectors, National Reference Centre, Netherlands Food and Consumer Product Safety Authority, Geertjesweg 15, 6706 EA Wageningen, The Netherlands

24. Vectors' Control Unit, Epidemiology and Control of Infectious Diseases Department, Institute of Public Health, Rruga Aleksander Moisiu 80, Tirana, Albania

24. Vectors' Control Unit, Epidemiology and Control of Infectious Diseases Department, Institute of Public Health, Rruga Aleksander Moisiu 80, Tirana, Albania

25. Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Glagoljaška ulica 8, 6000 Koper, Slovenia.

26. University of Novi Sad, Faculty of Agriculture, Laboratory for Medical and Veterinary Entomology, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Serbia.

27. Andrija Stampar Teaching Institute of Public Health, Mirogojska c. 16, 10000 Zagreb, Croatia.

28. University of Pécs, Ifjúság útja 6, 7624 Pécs, Hungary

29. Bernhard Nocht Institute of Tropical Medicine, Department of Arbovirolog, Hamburg, Bernhard-Nocht-Straße 74, 20359 Hamburg, Germany

30. Joint Services Health Unit, British Forces, RAF Akrotiri BFPO 57, Cyprus.

31. Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (Padua),Italy

32. Agencia de Salud Pública de Barcelona, Plaça Lesseps 8 entresol, 08023 Barcelona, Spain

33. Departament of Biology, Faculty of Mathematic and Natural Sciences, University of Prishtina, Str. Eqrem Qabej 9, Pristina, Republic of Kosovo

34. Cyprus University of Technology, Limassol, Archiepiskopou Kyprianou 30, Limassol 3036, Cyprus

35. National Institute of Health/ Centre for Vectors and Infectious Diseases Research, Avenida Padre Cruz, 1649-016 Lisboa, Portugal

36. Center for Rickettsiosis and Arthropod-Borne Diseases, Hospital Universitario San Pedro-CIBIR, C/ Piqueras 98, 26006 Logroño, La Rioja, Spain.

37. Istanbul University - Cerrahpasa, Faculty of Veterinary Medicine, Department of Parasitology, Buyukcekmece Yerleskesi, Alkent 2000 Mah., Yigitturk Cad. 5/9/1, 34500 Buyukcekmece, Istanbul Turkey.

38. University of Montenegro. Biotechnical Faculty, Mihaila Lalića 15, 81000 Podgorica, Montenegro

39. Universitat Pompeu Fabra - Mosquito Alert, C/ Ramon Trias Fargas, 25-27. 08005 Barcelona, Spain

40. Friedrich-Loeffler-Institut, Suedufer 10, 17493 Greifswald Isle of Riems, Germany.

40. Friedrich-Loeffler-Institut, Suedufer 10, 17493 Greifswald Isle of Riems, Germany.

41. Luxembourg National Museum of Natural History, Rue Münster 25, L-2160, Luxembourg.

42. Institute for Public Health of Montenegro, bb John Jackson Street, Podgorica, Montenegro.

43. Hacettepe University, Faculty of Science, Department of Biology, Ecology Section, Ankara.

44. Department of Veterinary Medicine, Faculty of Agriculture and Veterinary, University Hasan Prishtina, M546+72H, Prishtinë, Republic of Kosovo.

45. PHI Center for Public Health-Skopje, blv.3rd Macedonian brigade 18, Skopje, North Macedonia.

46. Centre for Ecological Research, Eötvös Lóránd Research Network, Alkotmány út 2-4, 2163 Vácrátót, Hungary

47. Institute of Zoology, Ministry of Education and Research st. Academiei 1, Chisinau MD-2028, Republic of Moldova.

48. Environmental Research Group Oxford. c/o Department of Zoology, Mansfiled Road, Oxford, UK

Abstract

Vector-borne diseases (VBDs) of human and animal importance are emerging or re-emerging worldwide, particularly mosquito-borne diseases such as chikungunya, dengue, West Nile and Zika. Six *Aedes* invasive mosquito (AIM) species have been introduced to Europe in the last 50 years: *Aedes aegypti, Ae. albopictus*, *Ae. japonicus, Ae. koreicus*, *Ae. atropalpus* and *Ae. triseriatus*. There is still a need for harmonizing field surveillance methodologies and strategies for sampling different life stages of AIM (eggs, larvae/pupae and adults). Since 2020, the *Aedes* Invasive Mosquito COST Action (https://www.aedescost.eu) implemented the first pan-European surveillance effort for AIMs harmonizing the sampling methods, frequency, minimum length of the sampling period and reporting (AIMSurv protocol; https://www.aedescost.eu/aimsurv). There were minimum requirements about the type of samples (i.e., eggs in oviposition traps) and recommended requirements for those teams having more resources (i.e., sampling of adults). Forty-two teams from 24 countries (23 from Europe and one from North Africa) participated on a voluntary basis. Results are reported herein and data are published as a Darwin Core Archive. The core data file contains 19130 records (EventID) and the occurrences file 19743 records (OccurrenceID). The AIM species recorded in the AIMSurv2020 were *Ae. albopictus, Ae. japonicus* and *Ae. koreicus*. Native mosquito species collected during AIMSurv2020 are also reported.

Data Description

Background

Vector-borne diseases (VBDs) are induced by a pathogen that needs a vector (often an arthropod) to be transmitted from one vertebrate host to another. Emerging or re-emerging VBDs in humans and animals are of increasing concern for public health worldwide [1], particularly mosquito-borne viral diseases such as chikungunya, dengue, West Nile fever and Zika [2,3].

Some mosquito species capable of transmitting pathogens of public health importance are relevant invasive species at global scale[4,5]. They are generally introduced into new areas by global trade (i.e., used tires, plants) (Lounibos 2002; Schaffner 2003; Tatem et al. 2012) and have spread within Europe through human-assisted pathways favoured by environmental and climate change [9–11].

In Europe, six *Aedes* invasive mosquito (AIM) species [12,13] have been introduced in the last 50 years: the yellow fever mosquito *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762); the Asian tiger mosquito, *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894); the Japanese bush mosquito *Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald, 1901); the Korean bush mosquito *Aedes* (*Hulecoeteomyia*) *koreicus* (Edwards, 1917); the American rock pool mosquito *Aedes* (*Georgecraigius*) *atropalpus* (Coquillett, 1902) and the American tree-hole mosquito, *Aedes* (*Protomacleaya*) *triseriatus* (Say, 1823).

Aedes aegypti is a major vector of yellow fever, dengue and chikungunya viruses and it is commonly found in tropical and subtropical areas. [14]. Recent re-establishment of this species in Europe was recorded in Madeira Island (Portugal) [15], in parts of southern Russia, Georgia and Turkey [16–18], as well as in Fuerteventura (Canary Islands, Spain) [19], from where it was successfully eradicated in 2019. This species has also been detected at several locations of Western Europe such as the Netherlands [20], where it has not been able to establish.

The Asian tiger mosquito originates from Southeast Asia and is currently widespread in large areas of Africa, Europe, Australia, the Americas, and the Middle East [14,21]. It is one of the most invasive species in the world, according to the Invasive Species Specialist Group of the IUCN [4]. In Europe, the invasion of *Ae. albopictus* was first detected in Albania in 1979 [22] and nowadays this species is found in more than 27 countries. The oviposition by females in small containers and the transport of the dry-resistant eggs via commodity trading have favoured its rapid expansion in Europe [23]. Transport of adults inside vehicles has contributed to the local expansion of *Ae. albopictus* in Europe [24]. Since 2007, the Asian tiger mosquito has been linked to several outbreaks of arboviral diseases, such as dengue and chikungunya that were introduced by travellers in different areas of Europe (i.e., Italy, France, Croatia, and Spain) and then locally transmitted by *Ae. albopictus* [5,25]. Further, in laboratory trials, *Ae. albopictus* has been shown to be a competent vector of more than 26 arboviruses (Paupy et al. 2009) and it is also well-known to cause a nuisance to humans, in particular because of its outdoor daylight feeding behaviour [27].

Aedes japonicus originates from eastern Asia and has become established in North America, Central Europe and areas in southern Europe (i.e., Spain and Italy) [28–30]. It is also an artificial container breeder and means of introduction and dispersal are similar to those of *Ae. albopictus* [29]. It is not considered a major vector of VBDs in nature, even if laboratory trials showed that it is a competent species of West Nile virus, among others [31].

Aedes koreicus originates from Korea, Japan, and northeast China and is present in some regions of Austria, Belgium, Germany, Hungary, Italy, Slovenia and the Swiss- Italian border [32–39]. It is not considered a major vector of VBDs although there is field evidence that it is a potential vector of the Japanese encephalitis virus [40], and laboratory trials have showed low-level transmission of chikungunya and Zika viruses [41,42]. Moreover, it is likely a competent vector of *Dirofilaria immitis* [43]

Aedes atropalpus is an invasive mosquito species originating from North America detected in European countries such as Italy, France and the Netherlands without any evidence of prolonged establishment [44–47]. It is not considered a major vector, but laboratory trials showed it is a competent vector of viruses such as West Nile, among others [48].

Finally, *Aedes triseriatus* is a species originating from North America that was detected in a single incursion in France in 2004 and was successfully eradicated [46]. La Crosse [49] and West Nile [48] viruses have been detected in field-collected mosquito adults in the USA.

Context

There are different initiatives across the EU to map the distribution of invasive mosquitoes. The European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) have established VectorNet, a community network for medical entomologists and public health experts. VectorNet produces and periodically updates distribution maps of invasive mosquitoes in Europe [50]. These maps result from the analysis of literature records on the distribution of AIM species in Europe and the contribution of public, academia and research institutions that freely share their data with the VectorNet community. Another initiative is Mosquito Alert [51], a Spanish originated citizen science campaign aiming at monitoring and mapping *Aedes* invasive species.

Early detection and surveillance of invasive mosquito species are challenging in terms of coordination and resources. Detection of AIM species may include different means and roles, from national surveillance programmes aiming to detect invasive species at points of entry and establish early warning rapid response systems to monitor AIM populations. Surveillance is commonly organized at the local and regional levels by public agencies, universities and research institutions (ECDC 2021) , leading to different methodologies and strategies for sampling life stages of AIM (eggs, larvae/pupae and adults). Valuable guidelines for conventional surveillance have been produced by the ECDC and the World Health Organisation Europe regional Office (EU-WHO) (ECDC 2012). However, to date, they have never been harmonized and used simultaneously by different entomologist teams across Europe.

To increase harmonization among European entomologists, the *Aedes* Invasive Mosquito species (AIM) COST Action (https://www.aedescost.eu) was initiated in 2018 including three major objectives: i) developing Pan-European networking and collaboration in monitoring and surveillance of AIM species; ii) increasing preparedness and capacity to fight against AIMs by triggering optimisation and innovation in AIM control strategies; iii) disseminating, customising and communicating the AIM-COST Action outcomes.

AIM-COST Action aims to promote data sharing and harmonisation. A particularly important objective is to ensure that vector sampling is consistent and compatible throughout Europe so an accurate continental picture of vector distributions can be obtained. For this, AIM-COST organised a training course in Cyprus in January 2020 on harmonising AIM surveillance across Europe. As a result of the course, trainers and trainees developed a protocol for surveillance of AIM species that can be applied across Europe. Forty-two teams from 24 countries (23 from Europe and one from North Africa) agreed on participating in the first-ever pan-European surveillance of AIMs using a harmonized protocol (AIMSurv protocol; https://www.aedescost.eu/aimsurv). The AIMSurv protocol was first implemented in 2020 and extended to 2021 and 2022. The main aim was to provide longitudinal data enabling comparison of seasonality and abundance across Europe and, in a subsequent phase, to compare field data with reports obtained by citizen science (i.e., Mosquito Alert App https://www.mosquitoalert.com/). Accordingly, both the presence and absence results of AIMs species were considered equally important to improve the information at the continental level.

Methods

The sampling protocol for pan-European surveillance of AIM species (AIMSurv) harmonized the sampling methods, frequency, minimum length of the sampling period and the form of reporting. There were minimum requirements (Minimum Requirements Protocol; MRP) about the type of samples (i.e., eggs in ovitraps), number of sampled sites, number of traps and frequency of collecting samples. Teams with more resources were suggested to follow a Recommended protocol (RP) to either increase number of samplings and/ or, additionally to eggs, sample other life stages such as adults.

The use of a common platform for data collection was also suggested, the VECMAP® App system was made freely available by Avia-GIS to all participants during AIMSurv activities.

For the **MRP**, all teams performed the survey in three sampling sites separated by 10 Km or more. Five oviposition traps (ovitraps) per site were placed and separated by 15 to 100 m. The type of ovitrap was selected by each team according to their availability in the region but usually consisted of 250 to 1000 ml capacity black containers filled with tap water. One scratched wooden tongue depressor (1.7x15 cm) per ovitrap, was used as a substrate for oviposition. Some teams used similar size pieces of Masonite board (when part of a pre-existing surveillance network was in place).

The selected sampling sites shared a similar ecology, when possible, in urban and/or suburban areas (e.g., a garden of single-family houses in residential urban/suburban areas, public parks near residential areas, recreational areas). The frequency of sample collection was biweekly over a minimum of three months that must include the population peak of the targeted AIM species (e.g., in Spain: from September to November for *Ae. albopictus*).

The following parameters were recorded: latitude and longitude of the position of each trap; the name of municipality/county/district (according to the country) and locality; start and end date of each trapping event (e.g., a period of 14 days for ovitraps); land use categories (urban, suburban and others); count of each life stage collected (egg and adult), including absences (0 values).

The more ambitious **RP** sampling included additional sampling sites sampled by five ovitraps per site, weekly sampling frequency and sampling length during the whole seasonality of the AIM species including start, peak and end of the mosquito season (e.g., May to November in Central Europe for *Ae. albopictus*). In addition, sampling adults using one BG-Sentinel™ (Biogents, Germany) trap baited with BG-Lure™ (Biogents, Germany) and/or CO_2 per site under a sampling frequency of one trap/night per week was also included. The use of VECMAP® (AVIA-GIS, Belgium) to report the data was also suggested in the RP. Parameters to record were the same as for the MRP plus the daily or weekly record of meteorological parameters (maximum, minimum, average temperature) per site, collected using data loggers or local weather stations.

The trap status per trapping event was recorded as follows: “Valid” when the trap (either oviposition or BG-Sentinel) was fully functional during the sampling event; “Trap altered” when oviposition trap was found dry or turned over or objects or animals, such as snails and lizards, were found inside, but still sample was possible to be collected. “Trap altered” also referred to

BG-Sentinel traps when they were found battery off or unplugged or funnel blocked, but still sample was possible to be collected.

For the processing of samples, collected eggs of AIM species were counted. When needed, for every location a sub-sample (2 out of 5 ovitrap substrates per locality) of eggs was reared to confirm the species by larva/adult morphology, particularly in those areas where several AIM species are present (i.e., *Ae. albopictus* and *Ae. japonicus*). Alternatively, when possible and depending on the team's resources, species were identified using MALDI-TOF MS or other molecular methods (e.g., DNA sequencing).

Adults of AIM species collected in BG-Sentinel™ were identified by morphology, sexed and counted. Suggested identification keys were ECDC (2012) and MosKeyTool V2.1 [54]. Samples of adults were preserved in 96% ethanol and/or cold preserved at -20/-80 °C to confirm identification if needed (e.g., via molecular tools).

Data Quality Control

All participants in AIMSurv reported data using a harmonized template. All data reported has been curated and the terminology has been homogenized. Data has been validated using the validator available in GBIF.

Reuse potential:

Records presented herein represent the first Pan-European data on field surveillance of AIM species conducted harmonically across 24 countries in terms of methodology and time scale. The records allow the accurate comparison of AIM surveillance, abundance and seasonality among countries and/or regions. Data can also be compared to other sampling strategies of AIM species, such as citizen science.

Data availability

https://doi.org/10.15470/vs3677

Declarations

List of abbreviations

AIM: Aedes Mosquito Invasive

COST: European Cooperation in Science and Technology

DwCA: Darwin Core Archive

ECDC: European Centre for Disease Control

EFSA: European Food Safety Authority

EU: European Union

EU-WHO: World Health Organisation Europe regional Office

IUCN: International Union for the Conservation of Nature

MALDI-TOF MS: Matrix-Assisted Laser Desorption - Ionisation-Time of Flight Mass Spectrometry

MRP: Minimum Requirements Protocol

RP: Recommended protocol

VBD: Vector Borne Disease

Competing Interests

The authors declare that they have no competing interests.

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Author’s Contributions

MAM and AdT conceived this work; MAM, AM, WW, DP and FS designed this work; CB, DA, XA, KB-L, GB, MB, PB, KB, MB, DB-B, VČ, BC, MC, SD, RE, OF-F, MF, EF, EMF-C, HPF, ALG-P, PG, SG, FG, MAG, MG, RG-L, CH, AI-J, VJ, PK, KK, MK, AK, KK, JL, RL, SM, GM, AFM, AM, AM, TM, FM, SM, NM-B, PM, GN, HCO, JAO, KO, IP, JRBP, SP, CR, CR, ER, IR-A, IS-C, NS, KS, KS, CS, MS, NiS, ZS, TS, JŠ, ST, AV, MIV, EV, AM, DP, FS collected the samples and reported results; MAM wrote the original draft and coordinated AIMSurv data compilation and curation; AdT coordinated AIMCost Action. All authors read, revised, and approved the final manuscript.

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