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SUPPLEMENT ARTICLE

Medical Entomology: A Reemerging Field of Research to Better Understand Vector-Borne Infectious Diseases

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In the last decade, the Chikungunya and Zika virus outbreaks have turned public attention to the possibility of the expansion of vector-borne infectious diseases worldwide. Medical entomology is focused on the study of arthropods involved in human health. We review here some of the research approaches taken by the medical entomology team of the University Hospital Institute (UHI) Méditerranée Infection of Marseille, France, with the support of recent or representative studies. We propose our approaches to technical innovations in arthropod identification and the detection of microorganisms in arthropods, the use of arthropods as epidemiological or diagnostic tools, entomological investigations around clinical cases or within specific populations, and how we have developed experimental models to decipher the interactions between arthropods, microorganisms, and humans.

Keywords. ticks; mosquitoes; fleas; lice; bedbugs.

Arthropods are invertebrate animals encompassing >1 million species and representing >80% of all living animal species [1]. Some of these, which we designate as vectors, can effect the active transmission of a pathogenic microorganism (virus, bacterium, parasite) from one vertebrate to another while taking their blood meal [2]. Mosquitoes are the main vectors of human disease and are known in tropical countries to cause hundreds of individual deaths due to malaria or dengue fever every year. Moreover, in the last decade, the Chikungunya virus (CHIKV) and Zika virus (ZIKV) outbreaks have turned public attention to some examples of the expansion of vector-borne infectious diseases throughout the globe, including parts of the New World [3, 4]. Moreover, the consequences of tick infestation are well known to veterinarians, but their medical importance has reemerged with the description of Lyme disease in the 1980s and the description of several tick-borne rickettsioses over the last 15 years [5]. Ticks are now recognized as the second main vector of human infectious diseases worldwide.

Understanding vector biology helps anticipate the emergence of vector-borne diseases. The last CHIKV and ZIKV outbreaks are representative of this. Indeed, with such widespread anthropophilic mosquito vectors, and in the context of travelers spreading viruses, it is logical to anticipate related outbreaks. Despite our alerts [4, 6], CHIKV and ZIKV became a "public health emergency of international concern" many months or even years after our first reports [7].

The discipline of medical entomology is focused upon insects, and more globally, arthropods that impact human health. It includes many links with veterinary entomology and environmental sciences, in a "one-health" concept. Knowledge of vector biology, arthropod monitoring, and control of vector populations remains essential to preventing and surveying vector-borne infectious diseases. Research and monitoring in medical entomology are therefore essential in fighting arthropod-borne diseases.

Here, we present a number of research projects conducted by the medical entomology research teams of the University Hospital Institute Méditerranée Infection in Marseille, France. We limit this report to recent or representative studies: technological innovations for arthropod identification and the detection of the microorganisms they carry; the use of arthropods as diagnostic or epidemiological tools; entomological investigations of cases or within specific populations; and experimental models used to decipher the interactions between arthropods, pathogens, and humans.

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EMERGENCE OF MALDI-TOF IN ENTOMOLOGY

Vector control remains essential in fighting transmitted diseases. The identification of vectors during entomological investigations, and a knowledge of their distribution, contribute to estimating the risk of infectious diseases in a studied area, and to planning vector control and the protection of exposed populations. In addition, regarding individual cases, the identification of an arthropod collected on a patient is also important. If a vector is recognized, this can influence the care of the patient, but can also suggest an entomological investigation of the case. For example, at our laboratory, we receive ticks collected and sent by both patients and doctors, to evaluate the risks of transmission of human pathogens [8].

Morphological identification based on dichotomic keys is today the most common method used to identify arthropods [2]. However, this approach requires both entomological expertise and comprehensive documentation. Detailed identification keys are available for some arthropods (eg, mosquitoes, ticks) and are generally organized by geographic area [9–11], but access to these documents is limited [12]. Moreover, several events can alter or compromise crucial morphological criteria, such as the deterioration of the specimen during collection or transportation, or the engorgement of the specimen. Morphological criteria can also be absent for the immature stages, but also within a species complex where the species are morphologically indistinguishable [13]. All these events can lead to a misidentification that may have an impact on the interpretation of the infectious risk. Furthermore, in the past 30 years, the number of experts in systematics has decreased, and some arthropod families have become orphans. Indeed, individual entomologists can hardly become experts on a wide range of arthropod families.

During the last 15 years, molecular biology (polymerase chain reaction [PCR], sequencing) approaches have been used for arthropod identification. These methods require molecular biology facilities and, depending on the arthropod, different genes are targeted for the identification [14–17]. Indeed, there are no ideal universal primers to identify any arthropod with certainty, and this approach is completely dependent on the comprehensiveness and reliability of the GenBank database to which the obtained sequences are compared [17].

For the last 4 years, our team has developed the use of matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) for arthropod identification. This technology is based on the thorough comparison of protein profiles of the submitted samples with a database of reference spectra, and has revolutionized the field of clinical microbiology. It is now routinely used for the rapid identification of bacteria and fungi from clinical samples [18]. The use of MALDI-TOF MS for the identification of arthropods involves several steps, including (*i*) determination of the body part of the arthropod which will be used for MS analyses (ideally the smallest, to save the remaining body parts for further analyses); (*ii*) construction of a database of reference protein profiles of definitely identified specimens; (*iii*) completion of blind tests to check if the specimens are correctly identified when compared to the database; (*iv*) continual updating of the database with new species (Figure 1) [19]. We have applied this method to the identification of several hematophagous arthropods: mosquitoes [20, 21], ticks [22], bedbugs, and triatomines (unpublished), using the spectra obtained from their legs, as well as fleas [23] and lice, using their cephalothorax. MALDI-TOF identification was recently validated on sand flies using their thorax, legs, and wings [24]. To date, our database includes reference spectra for >60 arthropod species. This technology has been transferred to our laboratory in Dakar, Senegal, where it enabled the identification of the Ceratopogonidae, which are small insects of veterinary importance whose identification can be challenging [25]. These skills have been taught to laboratory technicians who are now fully trained to identify ticks collected on patients by MALDI-TOF MS, using the legs, while the rest of the body is used for pathogen detection through molecular biology.

More recently, we showed that MALDI-TOF MS was able to distinguish *Rickettsia conorii*–infected *Rhipicephalus sanguineus* ticks and noninfected ticks [26, 27], as well as *Borrelia crocidurae*–infected *Ornithodoros sonrai* ticks [28]. Based on these promising results, MALDI-TOF MS was challenged to detect *Plasmodium* parasites directly from *Anopheles* mosquitoes. Experimentally, *Plasmodium berghei*–infected mosquitoes were successfully distinguished from noninfected mosquitoes based on the spectra from their cephalothorax [29]. Furthermore, the identification of blood meal sources of malaria vectors is key information to obtain to better understand host/vector interactions and malaria epidemiology in endemic areas. Abdomen proteins from *Anopheles gambiae* that were artificially engorged on 7 distinct types of vertebrate blood were submitted for MALDI-TOF MS, resulting in the accurate determination of feeding patterns of freshly engorged mosquitoes up to 24 hours post–blood meal [30].

THE USE OF ARTHROPODS IN CATALOGING VECTOR-BORNE INFECTIOUS DISEASES

Over the last 15 years, the development of molecular tools has enabled the use of arthropods as a tool for epidemiological and geographical monitoring of the microorganisms they carry. This method has been used to obtain specific information regarding the epidemiology of a targeted microorganism, to increase the catalogue of known infectious diseases in a geographical area of interest, and to alert clinicians and microbiologists to the presence of a pathogen in a specific area. As a reference center for rickettsiology, we have used this approach to contribute to

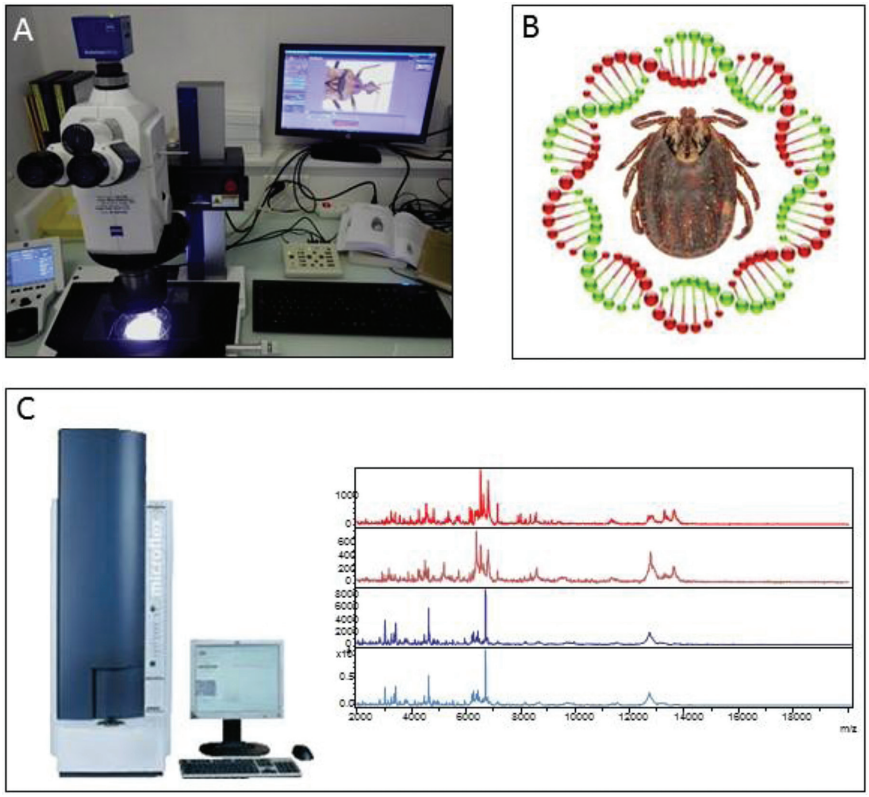

Figure 1. Standard and innovative methods for arthropod identification. *A,* Microscopy platform for morphological identification based on dichotomic keys. *B,* Molecular biology for identification of arthropod-specific genes. *C,* Development of matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry technology for arthropod identification based on protein profiling. Adapted from [19].

the knowledge of rickettsioses [5, 31]. One illustrative example is the description of African tick bite fever in the New World. Having diagnosed a spotted fever group rickettsiosis in a patient who had returned from Guadeloupe, a French Caribbean island, a literature search highlighted the presence of *Amblyomma variegatum*, an African tick introduced to the West Indies in the 18th century along with cattle from Senegal. Known as the vector of *Ehrlichia ruminantium*, the causative agent of bovine cowdriosis, this tick had recently been described as the vector of *Rickettsia africae*, the agent of the human African tick-bite fever in Sub-Saharan Africa. Specific serological analysis targeting *R. africae* confirmed that it was indeed the etiological agent of our index case [32]. An entomological survey enabled the detection and isolation of *R. africae* from *A. variegatum* ticks collected on cattle [33]. Other studies conducted by our team (and others) then confirmed the presence of *R. africae* on neighboring islands [34, 35].

Another example is the major contribution of this approach to the repertoire of rickettsioses in North Africa. In fact, until 1990, only Mediterranean spotted fever, caused by *R. conorii,* was described in this area. With the development and expansion of molecular tools such as PCR and sequencing on arthropods, we identified several new pathogens in ticks collected from North Africa, and therefore complemented the knowledge already collected on arthropod-borne pathogens circulating in Maghreb [5, 36]. We detected *Rickettsia slovaca*, the causative agent of TIBOLA (tick-borne lymphadenopathy), also known as SENLAT syndrome (scalp eschar and neck lymphadenopathy after a tick bite), and several agents of spotted fever group rickettsioses including *Rickettsia aeschlimanni*, *Rickettsia massiliae*, and *Rickettsia monacensis* [36]. When studying fleas from Africa, we also detected *Rickettsia typhi*, the agent of murine typhus and *Rickettsia felis*, an emerging pathogenic *Rickettsia* species [37]. More recently, we detected one of the causative agents of Lyme disease, *Borrelia garinii*, in *Ixodes ricinus* ticks collected in Algeria, where the epidemiology of the disease is not known [5, 38, 39].

In the field of arboviruses, we have used this technique to improve our knowledge on mosquito-borne viruses such as CHIKV and ZIKV, as well as sandfly-borne viruses. Such studies usually complete the description of cases in specific settings, as we did to describe the Toscana virus infection in Southern Europe [40–42]. We demonstrated that this virus was much more widespread than believed, with cases in all countries in North Africa and a presence beyond Europe [43–45]. These techniques also enabled us to detect and/or isolate new viruses from sandflies throughout the world [46, 47]. The latter is a good example of integrated research studies grouping entomologists,

virologists, parasitologists, ecologists, epidemiologists, and medical and veterinary doctors. Classic techniques (cell culture, electron microscopy, seroneutralization) were automated and combined with molecular techniques such as real-time PCR and next-generation sequencing [48]. Collection of phlebotomines in the field (Figure 2) [41] can be oriented from data on (*i*) *Leishmania* parasites, (*ii*) human/canine leishmaniasis cases, (*iii*) seroprevalence results for phleboviruses, and (*iv*) previous data indicative of phlebovirus isolation or detection. During the last decade, our group has discovered and isolated >50% of the newly described viruses transmitted by phlebotomines in the Old World: Punique and Medjerda Valley viruses in Tunisia [48, 49], Massilia virus in France [50], and Zerdali, Toros, and Adana viruses in Turkey [40, 47]. Similar studies have also isolated new viruses in Iran and various countries of the Balkan region (Bosnia-Herzegovina, Albania, Croatia) (unpublished data). Subsequent to the discovery of new viruses, studies aimed at providing evidence for their public health importance are increasingly being conducted; such studies have shown extremely high levels of exposure for populations living in certain regions [51–53].

All this information plays a crucial role in raising the awareness of medical doctors about the presence of pathogens and associated arthropod-borne diseases.

ENTOMOLOGICAL INVESTIGATIONS ASSOCIATED WITH CLINICAL CASES

These investigations have been conducted primarily in the field of rickettsiology, particularly in cases where unusual seasonal or ecological parameters were encountered. Such investigations may enable a better understanding of vector behaviors, as seen when we showed that the aggressiveness toward humans of the brown dog tick, *Rhipicephalus sanguineus*, was significantly greater at warmer temperatures. This may explain why cases of Mediterranean spotted fever caused by *R. conorii* and transmitted by *Rh. sanguineus* are encountered during the warmest months in the Mediterranean area [54]. Also, when investigating 2 cases of infection with *Rickettsia sibirica mongolitimonae* in the same family living in the south of France, we were able to identify *Rhipicephalus pusillus* as the potential vector, which is a tick associated with wild rabbits that can occasionally parasitize dogs and cats. These results may explain the epidemiology of

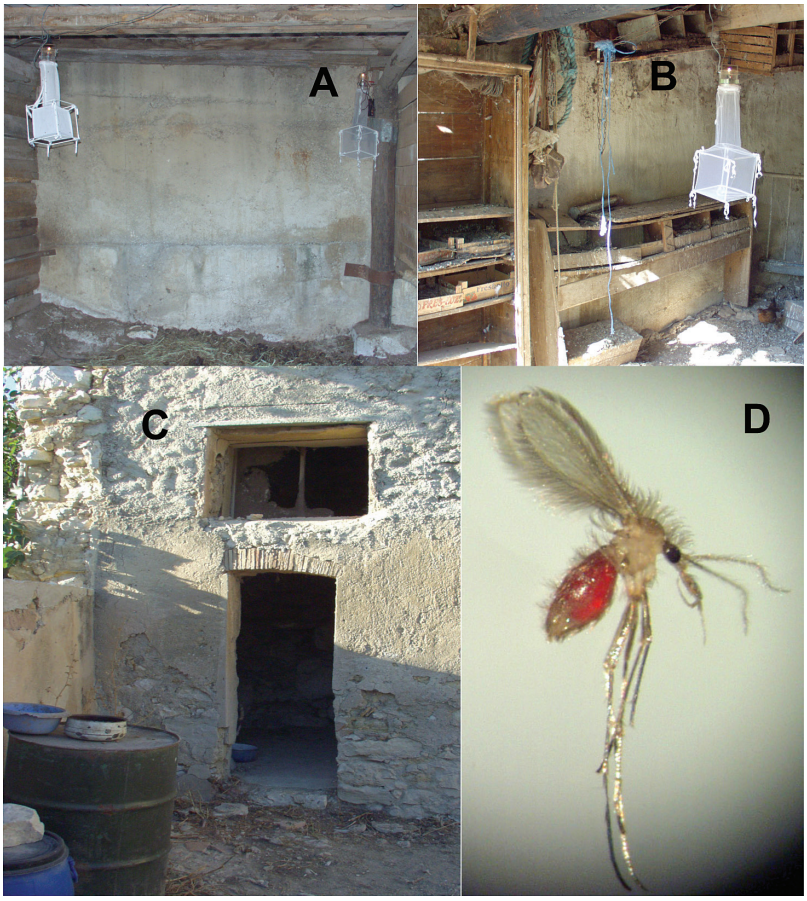

Figure 2. Centers for Disease Control and Prevention light traps, adapted for sandfly trapping. Placed in horse stables (*A*), near rabbit hutches and henhouses (*B*), and in quiet places in the shade of human habitations (*C*), where dogs sleep. *D*, Engorged female *Phlebotomus perniciosus* sandfly trapped in a horse stable. Adapted from [93].

the emerging rickettsiosis in this area, particularly its incidence is in the spring [55].

Our team is also directly solicited by patients following insect and bug bites, thanks to the expertise of one of our team members (J. M. B.). Bedbugs are frequently involved [56]. These hematophagous human parasites have indeed started reappearing since the 1990s. Host reaction to bedbug bites is highly variable, but includes dermatitis [57]. They are closely associated with human dwellings and are easily transported in luggage, allowing the infestation of hotels, boats, trains, etc [58, 59]. Their role in the transmission of vector-borne diseases in the wild is still poorly known. However, recent studies highlighted the vector competence of these bugs in the transmission of *Trypanosoma cruzi* [60] and, following our work, of *Bartonella quintana* [61]. However, investigations in patients' homes sometimes find new bugs that are poorly known both to the public and physicians. Recently, a patient was referred to us by a dermatologist for a suspected bedbug infestation. However, our investigation found straw itch mites, *Pyemotes ventricosus*. These parasites of xylophagous insects colonize wooden furniture, and their exploratory bites are painless but induce a very characteristic linear erythematous macular tract called a "comet sign," which is associated with intense pruritus [62]. We also identified another poorly known pest, the tropical rat mite (*Ornithonyssus bacoti*), as the cause of a patient's multiple bites, which were suffered while working in her office. The building recently underwent rat extermination, resulting in the *O. bacoti* feeding on unusual hosts [63].

ENTOMOLOGICAL INVESTIGATIONS AMONG SPECIFIC POPULATIONS

We have been involved for a long time in surveying and managing infections in the homeless population, particularly louse-borne diseases such as trench fever [64]. In recent years, we have investigated strategies to eradicate lice in the homeless. We demonstrated and characterized the resistance of lice to pyrethrinoids [65]. We carried out a clinical trial on infested clothes, suggesting that permethrin should no longer be used because of its strong ability to select resistance [66]. We also showed that head and body lice on homeless people have the same genotype [67]. Last, we developed molecular tools to distinguish head and body lice [68], which led us later to demonstrate that *B. quintana* is specific to the body louse [69]. Finally, the origins of body and head lice were redefined. Our involvement in tropical areas also enabled the detection of the presence of *B. quintana* DNA in black head lice collected from 3 locations in Senegal [70].

Vector-borne diseases have long been known to severely reduce the fighting capacity of armies, at times causing the suspension or cancellation of military operations. The current situation with many operations overseas increases the risk of vector-borne diseases in deployed troops [71]. We participated in monitoring the entomological status of French military bases in sub-Saharan Africa (Gabon [72] and Ivory Coast [73]) and French Guiana [74]. This monitoring was based on identifying vectors, studying behaviors, and evaluating insecticide resistance. New tools have been developed for vector trapping and the identification of pathogens in vectors, to find the best candidates for vector monitoring for pathogen transmission [75, 76]. Additionally, we participated in the evaluation of antivectorial control programs in several areas, in collaboration with overseas civilian entomology units [77–79].

We showed that the use of remotely sensed environmental and meteorological data enables the prediction of the risk of malaria transmission in Africa [80, 81] as well as dengue fever in French Guiana [82]. Tools have been also developed to evaluate the risk of exposure to vector bites in soldiers by identifying biomarkers of exposure through the analysis of mosquito salivary antigenic proteins, as well as serological responses associated with the level of exposure [83]. These tools enabled the evaluation of the effectiveness of antivectorial strategies, the estimation of the risk of disease transmission, and the monitoring of mosquito populations [84]. Additionally, genetic and environmental contributions to vector competence and viral genotypes or genetic diversity in mosquitoes have been analyzed, to evaluate the risk of emergence of arthropod-borne viruses [85].

Two entomological evaluation missions are being conducted, one in Ivory Coast and one in Gabon, where French troops are based. The main objective will be to evaluate local vector transmission. Catches will be conducted using BG-Sentinel traps and Centers for Disease Control and Prevention light traps; ticks will also be collected. Specimens will be identified using molecular biology and MALDI-TOF technology; pathogens will be sought, as well as resistance markers of the vectors. Finally, the last blood meal will be analyzed to identify the host of the concerned vector (trophic preference). This illustrates the application of all our technologies and research approaches to specific fields and populations.

EXPERIMENTAL MODELS

The molecular detection of a pathogen in a hematophagous vector does not necessarily mean that the arthropod is a competent vector of that pathogen. With the support of our insectary platform, which includes the laboratory rearing of a variety of arthropods (Figure 3), we have the ability to set up experimental models to test the vector competence of arthropods. Often, the first step has been the molecular detection of a microorganism following the cataloging strategy described above, and supported by epidemiological or clinical evidence. For example, *B. quintana*, the causative agent of trench fever, has long been known to be transmitted by body lice [86]. However, we detected this bacterium in bedbugs collected from a prison in Rwanda, raising the question of the potential vector competence of bedbugs for the transmission of *B. quintana* [87].

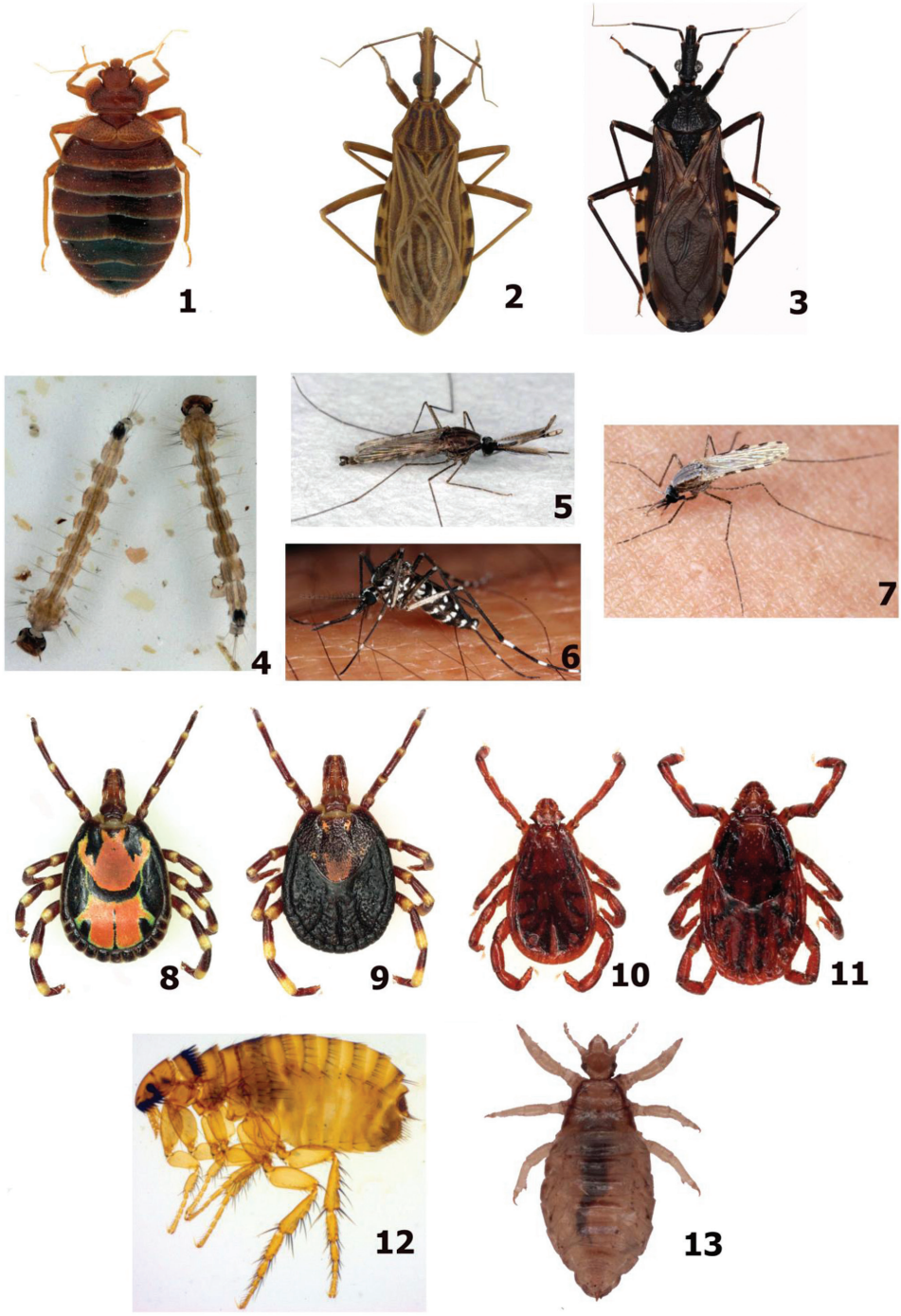

Figure 3. Pictures of arthropods of medical importance reared in the University Hospital Institute Méditerranée Infection insectary: 1. *Cimex lectularius* (bedbug); 2. *Rhodnius prolixus* (*Triatominae*); 3. *Triatoma infestans* (*Triatominae*); 4. *Aedes albopictus* larva; 5. *Anopheles gambiae*; 6. *Aedes albopictus*; 7. *Anopheles stephensi*; 8. Male *Amblyomma variegatum*; 9. Female *Amblyomma variegatum*; 10. Male *Rhipicephalus sanguineus*; 11. Female *Rhipicephalus sanguineus*; 12. *Ctenocephalides felis* (cat flea); 13. *Pediculus humanus corporis* (body louse). Adapted from [19]. See video at https://www.youtube.com/watch?v=BkpWb7CNTQs&sns=tw.

We confirmed this hypothesis with an experimental model using *Cimex lectularius* fed with *B. quintana*–infected blood through an artificial feeding device [61]. Furthermore, these data formed one of the first demonstrations of the potential vector role of bedbugs.

Another illustrative example of an experimental model contributing to knowledge of the emerging pathogen involves *R. felis*, an emerging pathogen described in 2002 [88]. In recent years, a growing number of cases have been reported around the world, and *R. felis* has been detected in several arthropods,

particularly in fleas. However, the only long-established vector has been the cat flea, *Ctenocephalides felis*. In 2012, *R. felis* was detected in mosquitoes in Africa, including *Anopheles* malaria vectors [89]. Moreover, the distribution of fevers of unknown origin associated with *R. felis* (up to 15% of cases) overlapped with malaria-endemic areas [90]. We artificially fed *An. gambiae* mosquitoes with blood or culture medium infected with *R. felis*. The bacterium was later detected in the mosquitoes, particularly in their saliva and salivary glands. Molecular detection of the bacterium's DNA in mice following exposure to *R. felis*–infected *An. gambiae* bites revealed the ability of this mosquito to transmit this *Rickettsia* from one vertebrate to another. This work was the first evidence of transmission of human pathogenic bacteria by mosquitoes and introduced *An. gambiae* as a potential vector of *R. felis* in Africa [91].

Last, an experimental model of infection of *Phlebotomus perniciosus* with bioluminescent *Leishmania infantum* parasites highlighted the variability in infection intensity due to several factors such as the vector and the parasite species, but also the infection method. Artificial feeding was described as the most efficient approach to obtain high parasite loads in the exposed flies [92].

Experimental models also enabled us to assess the powerful antifeeding and insecticidal efficiency of the dinotefuran-permethrin-pyriproxyfen ectoparasiticide on *Triatoma infestans*. Indeed, *T. infestans* bugs are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease, for which dogs are reservoir hosts. The prevention of domestic infection of dogs is a crucial step in the protection of domestic animals and humans (unpublished data).

PERSPECTIVES

The medical entomology studies conducted by our team have enabled us to decipher many aspects of vector-borne human diseases. The vast majority of our master's and doctoral students involved in these studies come from developing countries, predominantly Africa. Most of them are financially supported by the Méditerranée Infection Foundation, and plan to create research units in their countries after being trained in Marseille.

The performance of our insectarium is based on the large diversity of arthropods we breed. It enables quick answers to epidemiological and clinical questions involving known and emerging pathogens or arthropod vectors, by developing experimental models and entomological surveys. The MALDI-TOF tool is continually challenged with identifying new arthropods as well as with the additional detection of their associated microorganisms. Indeed, we recently developed the MALDI-TOF identification of triatomines, the giant bugs responsible for the transmission of *T. cruzi*, the etiological agent of Chagas disease in the Americas. We also aim to assess the performance of MALDI-TOF in the concomitant identification of 2 vector/pathogen couples, mosquitoes/*Dirofilaria* species and *Ct. felis* fleas/*Bartonella* species. Moreover, to improve our knowledge of vector-borne diseases, we are still conducting experimental models of infection. We have shown that *An. gambiae* was able to transmit *R. felis,* but this bacterium was detected in *Aedes albopictus* from Gabon by quantitative PCR, and *Ae. albopictus* cells support *R. felis* growth. These elements raise the question of the ability of *Ae. albopictus* mosquitoes to transmit *R. felis* [7]. This question will be answered by an experimental model of infection where *Ae. albopictus* mosquitoes will be put in a situation to reveal the acquisition and transmission of *R. felis*. Finally, in the national context of the French plan for a better understanding and knowledge of Lyme disease and other tick-borne diseases, we plan to create a so-called tick clinic, where patients bitten by ticks and suspected of having been infected by a tick-borne agent will have access to a doctor, microbiologists, and entomologists to investigate their condition.

Notes

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