Insects and the Transmission of Bacterial Agents

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ABSTRACT Arthropods are small invertebrate animals, among which some species are hematophagous. It is during their blood meal that they can transmit pathogenic microorganisms that they may be harboring to the vertebrate host that they parasitize, which in turn will potentially develop a vector-borne disease. The transmission may occur directly through their bite, but also through contaminated feces. Zoonotic diseases, diseases that can naturally be transmitted between humans and animals, are a considerable part of emerging diseases worldwide, and a major part of them are vector-borne. Research and public attention has long been focused on malaria and mosquito-borne arboviruses, and bacterial vector-borne diseases remains today a neglected field of medical entomology. Despite the emphasis on Lyme disease in recent decades, and despite the major outbreaks caused by bacteria in the last few centuries, this field has in fact been poorly explored and is therefore relatively poorly known, other than the most famous examples such as the plague and epidemic typhus outbreaks. Here we propose to review the state of knowledge of bacterial agents transmitted by arthropod vectors.

INTRODUCTION

Arthropods are a phylum of invertebrate animals with an exoskeleton, including >1 million species and accounting for >80% of all known living animal species ([1]).

Some hematophagous arthropods known as vectors possess the capacity to transmit infectious agents to human and other vertebrate animals ([2]). To date, the majority of arthropod vector species belong to the classes of insects and arachnids, which include mosquitoes and ticks, respectively ([3]).

Mosquitoes are the primary vectors of human infectious diseases, including malaria, dengue, and filariasis ([4]). Moreover, the recent epidemics of Chikungunya and Zika throughout the world are some examples of the expansion of mosquito-borne diseases ([5]). Dissemination is now seen as a global problem. Most of the vector-borne diseases that have been brought to the attention of the public in recent years are arboviral diseases. Arthropod-borne bacterial diseases are considered neglected, with the exception of tick-borne diseases, thanks to the identification and emergence of Lyme disease and many rickettsial diseases in recent years ([6], [7]). However, knowledge of the transmission of bacterial disease agents by insects, and how some agents might disseminate, is poor.

As a matter of fact, the potential for dissemination is linked to the so-called vectorial capacity of the insect. This term includes several factors linked to the role of an arthropod in microorganism transmission, including arthropod abundance and longevity, host specificity, the time for the microorganism to develop and/or multiply within the vector, the route of transmission, but also environmental, ecological, behavioral, cellular, biochemical, and molecular factors. The vector competence of an arthropod is a subcomponent of vectorial capacity. It corresponds to the intrinsic ability of the arthropod to acquire a microorganism, allow its replication, and subsequently transmit it to a susceptible host.

Although microorganisms enter their arthropod vector during the blood meal, their transmission to an

animal is more varied. Furthermore, a pathogen often needs to overcome several physical barriers in the arthropod before finding an exit and a pathway to be transmitted. Furthermore, the immune response of the arthropod can limit the development of microorganisms and thus the success of the transmission.

Salivation is the most common way for vectors to transmit infectious agents. As an illustration, all arthropod-borne viruses are transmitted this way, and the virus is directly injected into the host during probing or blood feeding. A key step in the transmission of most vector-borne diseases is therefore the entry of the microorganism into the salivary glands of the arthropod (8). For the success of their blood feeding, the salivary glands of arthropods produce a variety of substances, including enzymes and vasodilators, as well as anti-inflammatory, antihemostatic, and immunosuppressive substances (9). However, salivation cannot be employed for the transmission of microorganisms that remain in the gut or the hemocoel of an arthropod, as this would require their entry into the salivary glands. The two major alternate mechanisms of horizontal transmission that can be used include exit with arthropod feces (stercoration) and regurgitation.

Here we focus on bacterial disease agents that are or at least can be transmitted by insects (mosquitoes, sand flies, lice, fleas, and bugs), with illustrative examples of pathways of transmission and the potential for dissemination.

MOSQUITOES

The ability of mosquitoes to transmit bacterial diseases to humans is poorly understood and has not been a topic of research or even of interest until recently. Particularly *Wolbachia* spp., bacteria known as endosymbionts in many arthropods, have been associated with mosquitoes (10). Mosquitoes have also been presented as the mechanical vectors of *Francisella tularensis*, the agent of tularemia in humans, and possible transmission through adult mosquitoes that have acquired the pathogen from their aquatic larval habitats has been suggested (11).

In opposition to mechanical transmission, biological transmission is the way in which mosquitoes transmit arboviruses such as dengue, Chikungunya, and yellow fever viruses through their bite. The competence of mosquitoes in transmitting arboviruses has long been well known; however, nothing was known about their potential competence in transmitting bacterial agents until attempts to decipher *Rickettsia felis*' epidemiology. *Rickettsia felis* is an obligate intracellular bacte-

rium that differs from other officially recognized rickettsial species. Many aspects of the ecology and epidemiology of *R. felis* are not completely understood and remain to be uncovered. Our team definitively described this organism in 2002 (12). A growing number of recent reports have implicated *R. felis* as a human pathogen, paralleling the increasing detection of *R. felis* in arthropod hosts across the globe (13). In Sub-Saharan Africa, epidemiological studies revealed that *R. felis* was detected in up to 15% of patients with fever of unknown origin (14). Various arthropods, but primarily fleas, have been associated with *R. felis*, and more specifically, the cat flea *Ctenocephalides felis* is the arthropod in which *R. felis* has been most frequently detected (15). For a long time, it was the sole confirmed biological vector of *R. felis*. Interestingly, *Aedes albopictus* and *Anopheles gambiae* mosquito cells support *R. felis* growth. In 2012, we found that *A. albopictus* (a major vector of Chikungunya, dengue, and Zika virus) from Gabon and *An. gambiae* (the primary African malarial vector) from Ivory Coast both tested positive for *R. felis* by species-specific real-time quantitative PCR (15). Also, we found several mosquito species from Senegal that harbor *R. felis*. These data raised new issues with respect to the epidemiology of *R. felis* in Africa, including the degree of vector competence of mosquitoes. Recently, we demonstrated that *An. gambiae* mosquitoes, the primary malarial vectors in Sub-Saharan Africa, have the potential to be vectors of *R. felis* infection (16). *An. gambiae* mosquitoes were fed with either *R. felis*-infected blood meal or infected cellular media administered through an artificial membrane feeding system. In an *in vivo* model, mosquitoes were fed on *R. felis*-infected mice. The acquisition and persistence of *R. felis* in *An. gambiae* were demonstrated by quantitative PCR detection of the bacteria up to day 15 postinfection. Furthermore, *R. felis* was visualized by immunofluorescence in the salivary glands and in the ovaries, although no vertical transmission was observed. *Rickettsia felis* was also found in the cotton used for mosquito sucrose feeding, which implies potential transmission through the mosquito's bite. Bites from *R. felis*-infected *An. gambiae* were able to cause transient rickettsemia in mice, indicating that this mosquito species has the potential to be a vector of *R. felis* infection (16).

LICE

Three louse ecotypes are known to parasitize humans: pubic lice, which are a sexually transmitted disease but not known vectors of infectious diseases; as well as head

lice and body lice. The latter are two very close ecotypes on both the genetic and morphological levels ([17](17)). Infectious diseases transmission mainly involve body lice. Until the 19th century, the presence of body lice in the general population was extremely common. Today, these arthropods are only found in poor populations such as homeless populations or in critical situations such as war zones. Lice were involved in the most explosive outbreaks known, such as louse-borne relapsing fever, epidemic typhus, and trench fever, which were caused by *Borrelia recurrentis*, *Rickettsia prowazekii*, and *Bartonella quintana*, respectively ([18](18)). Although one might presume that these diseases belong to the past, very peculiar situations have allowed them now to resurge. Rwanda and Burundi had civil wars that allowed the proliferation of body louse populations, along with an epidemic typhus resurgence, causing 10,000 deaths toward the end of the 20th century ([19](19)). At the same time, it was identified that *B. quintana* was cocirculating with *R. prowazekii* in the same population ([19](19)). *Rickettsia prowazekii* is thought to have been introduced in Europe from Mexico, where it has been detected in ticks. The first human cases of epidemic typhus were detected by paleomicrobiology and reported from the War of the Spanish Succession of the early 18th century. Moreover, paleomicrobiology allowed the detection of *B. quintana* DNA in a 4,000-year-old human tooth ([20](20)), and also in Napoleon's soldiers buried in Vilnius, causing an outbreak of trench fever that might have impacted the French retreat from Russia ([21](21)).

More recently, two major questions emerged regarding the role of lice in the transmission of infectious diseases. The first is the ability of the louse to transmit *Yersinia pestis*, the causative agent of plague. Indeed, paleomicrobiology demonstrated that both plagues (of Justinian and the Middle Ages) were caused by *Y. pestis* ([22](22), [23](23)). However, the admitted biological cycle of transmission of the pathogen is not able to explain the dynamics of the outbreak in Europe, which involved 90% of the population at phenomenal speed. For these reasons, the validity of *Y. pestis* as the etiological agent of the Middle Ages plague was challenged. Rats and fleas alone can't explain the violence of the outbreak. However, this type of explosive outbreak is common for louse-borne infectious diseases. Ancient studies reported that lice could be infected by *Y. pestis*, and we demonstrated in an experimental model that the louse is competent for the transmission of *Y. pestis* in laboratory conditions. Finally, more recently, we reported *Y. pestis* in body lice collected in areas where plague is endemic in Democratic Republic of the Congo (DRC) ([24](24)). Here it appears highly plausible that lice were the origin of the infection. The data suggest that rural plague is probably followed by urban plague cases that are further amplified by body lice.

The second question regarding lice and infectious diseases concerns the inability of head lice to transmit pathogenic microorganisms. *Bartonella quintana*'s DNA was detected in head lice, and its presence in human cases in Dielmo, Senegal, has been reported. It appears that head lice may be vectors of *B. quintana*, which was considered impossible until now. Finally, we recently identified *Y. pestis* in head lice collected in DRC, which raises the question of the potential role of head lice in the transmission of this pathogenic bacterium.

Lice probably played a major role in the outbreaks of the last centuries. The potential role of head lice in the transmission of infectious disease is of far greater concern in the 21st century because of its wide distribution, and how outbreaks could spread. Lice are not very specific, since the microorganisms are transmitted through their feces, not directly by the bite. The itching induced by the louse's saliva allow the pathogen to penetrate the skin through scratching lesions. Therefore, it appears that the transmission of infectious disease by the louse is more likely guided by the opportunism of the ingested microorganism than true host-bacterium specificity.

FLEAS

There are currently ~2,500 species and subspecies of fleas ([25](25)). These insects are small, wingless, bilaterally flat parasites, principally of mammals and sometimes of birds ([26](26)). Fleas are holometabolous arthropods, which means that their development from the egg to the hematophagous adult includes a larval, then a pupal stage. Like many other blood-sucking insects, fleas are involved in the transmission of vector-borne pathogens. However, current data suggest that fleas transmit pathogens through their feces and not through their bite. The microorganisms later enter the vertebrate skin through scratch lesions.

Despite the large number of known flea species, only a few tend to bite humans ([27](27)). Even *Pulex irritans*, which is known as the human flea, is more frequently associated with swine and dogs in some parts of the world ([26](26)). Yet because of their high affinity for domestic animals, *C. felis* fleas are very frequently found associated with human dwellings.

We still have much to decipher about flea-borne diseases. However, much is already known regarding the competence of these arthropods in transmitting human pathogens, and many microorganisms have been

molecularly detected in fleas (28). Indeed, fleas have been and still are involved in the transmission of *Y. pestis*, the causative agent of plague (29). *Yersinia pestis* multiplies in the flea's proventriculus, a structure between the esophagus and the midgut, and therefore obstructs the passage of ingested blood. Infected fleas then regurgitate infected blood during their attempts to feed (30). Around 30 flea species, particularly of the *Xenopsylla* genus, are demonstrated vectors of *Y. pestis* (31). Fleas are also recognized as vectors of *Rickettsia typhi*, an agent of murine typhus and of endemic typhus. Transmitted through flea feces, the bacteria then infect the mammalian host's endothelial cells (32). The disease then manifests with fever, headache, myalgia, and nausea (33).

If the role of mosquitoes in the transmission of *R. felis* is now in the spotlight, this bacterium was initially detected and isolated from the cat flea (15). *Rickettsia felis* transmission was shown both vertically and horizontally in fleas, making them a probable reservoir of the bacterium (34, 35). Since *R. felis* was detected in the salivary glands of fleas, transmission through the bite is highly suspected (36).

Fleas have recently been implicated in the transmission of bartonelloses. Cat scratch disease, caused by *Bartonella henselae*, is transmitted through cat scratch, but likely also by cat bite and by *C. felis* (37). Although most cat cases resolve spontaneously (37), immunocompromised patients may develop a potentially fatal bacillary angiomatosis (38). Moreover, infections with *B. henselae* in patients with valvulopathies may lead to fatal endocarditis, since the mortality can reach 25% (39). *Bartonella quintana* infections were quite frequently reported during previous centuries and are now resurging among disadvantaged populations, among which the louse, its primary vector, is thriving (18). This bacterium has been detected in cat fleas (40), and an experimental model was designed to assess the ability of *C. felis* to transmit *B. quintana* in laboratory conditions. The pathogen was excreted alive in the flea's feces, allowing further contamination of the vertebrate host through scratching (41).

Because of their high affinity for their animal hosts and low attraction to humans, it appears unlikely that fleas could be the origin of explosive outbreaks.

TRUE BUGS

The true bugs order is the largest insect order, composed of ~90,000 species distributed worldwide. These insects are generally characterized by two pairs of wings and a wing morphology dividing the order in two suborders: homopterans, which possess two highly similar pairs of wings; and heteropterans, for which the wing is composed of two thickened and two membranous parts. They are also characterized by piercing mouthparts that enable them to feed on different biological fluids such as plant juice, hemolymph, or vertebrate blood. Although homopterans are mostly phytophagous, heteropterans include hematophagous insects of medical and veterinary importance (26).

Kissing bugs (*Triatominae*) and bed bugs (*Cimicidae*) are the *Hemiptera* subfamily and family, respectively, involved in human health issues. All developmental stages of these flattened insects are hematophagous, and while bed bugs are wingless and relatively small, around 6 mm, kissing bugs are winged insects reaching up to 5 cm long. The ability of these insects to naturally transmit bacteria to humans is still unknown.

Triatomines (*Reduviidae*; *Triatominae*) are among the largest blood-sucking arthropods and live in diverse ecotopes. They can be totally sylvatic and feed on small mammals or they can be invasive, even domiciliated, and then feed principally on humans (42). When they are infected, they can transmit the flagellate protozoan *Trypanosoma cruzi* through their feces, which is the causative agent of Chagas disease. A major parasitic disease in the Americas, it is responsible for heart failure in up to 30% of individuals 10 to 30 years postinfection (43). Around 10,000 people died in 2014 from the manifestations of Chagas disease in 2014, and more than 25 million people are at risk of acquiring the disease. For a long time, the vectors and parasites were restricted to the New World. However, due to the intensification of human exchanges, arthropods are now disseminated all over the world, and triatomines are no exception. *Triatoma rubrofaciata* is now fully implanted in Vietnam (44), and the parasite itself is also present outside America in emigrant communities such as the Latin American migrant community of Italy (45). Because of the public health impact and the rising threat of Chagas disease worldwide, almost no investigations have been conducted to assess the presence of other arthropod-borne pathogens in triatomines. Recent studies reported the molecular detection of a new *Bartonella* species, closely related to *B. bacilliformis*, the etiological agent of Carrion's disease, in invasive triatomines (42). The pathogenicity of this bacterium, however, remains to be demonstrated.

Bed bugs (*Cimex* species) are strict parasites of humans. They feed on human blood and are therefore a major pest when they infest human dwellings, hotels,

trains, etc. (46). The host reaction following the bite is variable, but bed bugs are responsible for severe allergic reactions and dermatitis (47). Their implication in vector-borne pathogen transmission in the wild is still poorly known. Recent studies pointed out their vector competence in transmitting bacteria. The molecular detection of *B. quintana* in bed bugs collected from a prison in Rwanda raised the question of their role in the transmission of this louse-borne pathogen (48). Trench fever, caused by *B. quintana*, was broadly reported during World War I and II and is now resurging among homeless populations. In experimental models of infection, bed bugs were able to eliminate living bacteria through their feces, which allowed further cultivation of the microorganisms (49). To date, *B. quintana* is the only bacterial pathogen detected in bed bugs with demonstration of transmission in laboratory conditions.

CONCLUSION

Arthropod-borne bacteria will constitute an important reservoir of emerging infectious diseases in the future. Regarding the behavior and pathway of transmission, mosquito-borne diseases have the best potential for global transmission and causing large outbreaks. For example, the vectorial competence of *A. albopictus*, the Asian tiger mosquito, remains unknown but requires investigation. *Rickettsia felis* has already been detected in these highly anthropophilic mosquitoes, which played a major role in the spread of the Chikungunya and Zika outbreaks, and *R. felis* might be the next *A. albopictus*-borne outbreak agent (50).

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