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Ebola Virus and Arthropods: a Literature Review and Entomological Consideration on the Vector Role

Virus Ebola et arthropodes : revue de la littérature et considérations entomologiques sur le rôle de vecteurs

M. Dutto · M. Bertero · N. Petrosillo · M. Pombi · D. Otranto

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Abstract Ebola virus is a pathogen responsible for a severe disease that affects humans and several animal species. To date, the natural reservoir of this virus is not known with certainty, although it is believed that fruit bats (Chiroptera: Pteropodidae) play an important role in maintaining the virus in nature. Although information on viral transmission from animals to humans is not clear, the role of arthropods has come under suspicion. In this article, we review the potential role of arthropods in spreading Ebola virus, acting as mechanical or biological vectors.

Keywords Ebola virus (EBOV) · Arthropods · Mechanical vectors · Biological vectors · Chiroptera

Résumé Le virus Ebola est un pathogène responsable d'une maladie grave qui touche les humains et plusieurs espèces animales. Nous n'avons à ce jour aucune certitude sur le réservoir naturel de cette maladie, mais les roussettes (Chiroptera : Pteropodidae) pourraient jouer un rôle important dans le maintien du virus dans la nature. Bien que la transmission virale des animaux à l'être humain reste encore précisément à élucider, les arthropodes peuvent jouer un rôle dans cette transmission. Dans cet article, nous étudions le rôle potentiel des arthropodes dans la propagation du virus Ebola en tant que vecteurs mécaniques ou biologiques.

Mots clés Virus Ebola · Arthropodes · Vecteurs mécaniques · Vecteurs biologiques · Chiroptères

M. Dutto (✉)
Former Entomology Consultant Department of Prevention ASL CN1, Cuneo, Via Papò 4, 12039 Verzuolo (CN), Italy
e-mail : moreno.dutto@gmail.com

M. Bertero
Department of Dermatology, General Hospital "S. Croce e Carle", Cuneo, Italy

N. Petrosillo
National Institute for Infectious Diseases "L. Spallanzani", Rome, Italy

M. Pombi
Department Public Health and Infectious Disease, Parasitology unit, "Sapienza" University of Rome, Italy

D. Otranto
Department of Veterinary Medicine, University of Bari, Bari, Italy

Introduction

Ebolavirus (EBOV) is a genus composed of five genospecies, four of which cause a severe disease in humans characterized by high transmission and mortality (70–90%) rates [3] and the other for which an effective specific treatment is still unavailable [4,10]. Nonhuman primates are also susceptible to infection [22].

The virus replicates in insectivorous and fruit-eating bats (particularly Pteropodidae) [30,39], suggesting that these groups of animals may act as reservoirs for enzootic infections [20,28,29,32]. However, given the high susceptibility of mammals to the pathogen, an epizootic cycle is possible/suspected from bats to many species of animals [27]. Transmission to humans occurs when a suitable host becomes part of the enzootic cycle, or in the case of epizootic cycles that could include farmed and huntable species in the spreading of viruses. Human infection occurs through direct contact with infected secretions and/or excretions, or, indirectly, through contaminated objects [26]. In addition, direct exposure of hunted fruit bats has been considered as a possible cause of an epidemic in the Congo [19].

Arthropods represent a large and heterogeneous group of animals which may also display significant vector capacities, being responsible for the biological or mechanical transmission of numerous human and animal pathogens such as viruses, bacteria, protozoa and helminths that can be

acquired through ingestion or by contact [14,16,33,37,42]. In the case of Ebola virus, arthropods may potentially come into contact with the virus by ingesting viremic host's blood (hematophagous arthropods) or through infected secretions and excretions (blood, feces, saliva, and vomit) in the host environment (i.e., saprophagous, necrophagous, and omnivorous arthropods).

Hypotheses and evidence of the role of hematophagous arthropods

Hematophagous arthropods have been suspected in the past of being either the reservoir and/or biological vector of EBOV due to the possibility that they may acquire the pathogen from an infected host. Since 1968 the role of Culicidae, particularly *Aedes aegypti* (Diptera, Culicidae), has been investigated as potential biological vector of Marburg virus (MARV) [17], a Filovirus taxonomically similar to EBOV. A study done in 1968 showed that MARV could persist for more 3 weeks in *Ae. aegypti* mosquitoes after experimental inoculation [17,35].

For this reason a considerable number of ticks (Acari, Ixodida) and insects (e.g., Culicidae, Psychodidae, Cimicidae) have been screened for EBOV during epidemics in Africa, returning negative results for the viruses in all cases [5,11,36].

Further studies on the competence of hematophagous arthropods (i.e., mosquitoes and Argasidae soft ticks) as spreaders of EBOV have also been conducted under laboratory conditions [40]. However, in an experimental study based on the intrathoracic inoculation of EBOV in mosquitoes and ticks species (i.e., *Culex pipiens*, *Aedes albopictus*, *Aedes taeniorhynchus*, and *Ornithodoros sonrai*) EBOV failed to replicate [40].

Finally, species belonging to the Hippoboscoidea superfamily (Diptera) have been suspected to be vectors of EBOV and MBGV (Marburg Virus) among bats, but no studies were able to identify the presence of EBOV in these insects [8,20,23,41].

Hypotheses and evidence of the role of nonhematophagous arthropods

In 1975 lycosid (Aracnida, Araneae) and other spider or scorpion species, together with horseflies (Diptera, Hippoboscidae), were suspected as a chain in the route of transmission of MARV infection through biting behaviour [6,35]. However, no further investigation was conducted linking the infection to arthropod bites.

In 1996, a study was conducted to investigate the natural source of EBOV infection experimentally by inoculating the virus *in vivo* into several animal and plant species. To identify possible natural EBOV reservoirs in arthropods, *Periplaneta americana* (Blattaria, Blattidae), *Stegodyphus dumicola* (Araneae, Eresidae), *Messor capensis* (Hymenoptera, Formicidae), and *Alloporus* sp. (Diplopoda, Spirostreptidae) were experimentally by inoculated with doses of EBOV without evidence of viral replication [39].

Lastly, Darriet [7] hypothesized that Homoptera (Rhynchota, Hexapoda) may play a role as reservoirs and vectors of EBOV. These insects feed on plant sap and could therefore transmit viruses to humans and primates through contaminated honeydew that can spread on vegetation manipulated and used for industrial purposes, such as cotton.

Accordingly, aquatic insects may also be involved in the ecology of EBOV infection of animals [18]. This is the case for mayflies (Ephemeroptera), which live as eggs or larvae in sediment and other aquatic material, whilst adults live as swarms on river banks. It is hypothesized that these insects can carry the virus in a "latent" form ; when these insects are preyed upon by bats, EBOV can be spread through the bat's stool. Fishes, reptiles, and amphibians are refractory to this infection because they are unable to activate EBOV during digestion (buffer species).

Concluding remarks

Undoubtedly, the transmission of viruses by nonhematophagous arthropod vectors is limited when compared to other pathogens such as bacteria, protozoa, and helminths, which are able to develop forms of resistance to the external environment (e.g., spores, eggs, larvae) during their life cycle [1,15]. However, the likelihood of an arthropod to act as a vector is related to the specific resistance of the viral agent to environmental conditions, the viral load, and, in the case of transmission of viruses via arthropod feces or the feeding on host, by the specific enzymatic digestive capability [33]. For example, poliovirus was isolated after 13–15 days in the common green bottle fly *Phaenicia sericata* (Diptera, Calliphoridae) and after 50 days in the American cockroach *Periplaneta americana* (Blattaria, Blattidae) [33].

Biological transmission from hematophagous arthropods implies viral replication in the vector's body. The interaction between specific viral surface components and receptors on target cells plays a fundamental role in viral tropism. Cell membrane components serve normal cellular functions and are used by viruses for attachment and entry into the host cell [25]. For example replication of human immunodeficiency virus in insects is impaired by the absence of T4 antigen on the insect cell surface [13].

Although available data suggest that bloodsucking insects do not act as biological vectors of EBOV in humans, the role of these arthropods as potential biological or mechanical

vectors needs further investigation. In particular, the spread of the virus by insects should be investigated soon after they interrupt their trophic activity on an infected host and when they conclude it on a secondary host. In the latter case, pool-feeder hematophagous insects could potentially be capable of transmitting the virus via their feeding apparatus. By this route, due to the limited amount of blood that can contaminate the mouthparts of hematophagous insects, the main determinants for the efficacy of transmission of the infection should include a very high and persistent viral load in the host and good environmental survival of the virus itself. EBOV survives in the environment up to several (~3–6) days depending on the substrate (i.e., blood, water) and environmental conditions [9,38]. In bats viremia occurs for a short period (e.g., in MARV) [31]. In humans EBOV viremia is prolonged in the acute phase of infection, with a variable persistence. As a consequence, the mechanical transmission from a vector, even if extremely unlikely, cannot be ruled out in cases of high and persistent viremia in the host. In the past years several transmission scenarios have been hypothesized for EBOV [23]. Nonetheless, based on present knowledge, no evidence is available that could attribute a role to hematophagous arthropods in the replication and diffusion of this virus. However, it would be worth investigating the role of Hippoboscoidea (Diptera) parasites of bats in the occasional transfer of the virus from infected bats (natural reservoirs?) to humans. As an example, the bed-bug *Cimex lectularius* has been described as a potential mechanical vector of human hepatitis B virus [14].

It could also be interesting to evaluate the natural presence of EBOV in necrophagous insects, considering that this virus has been found in feces of experimentally infected fruit bats over 21 days postinfection [39] and that some insects (e.g., Coleoptera and Diptera) could feed on the feces and infected remains. Theoretically the virus, once ingested by necrophagous insects, could be transmitted to other invertebrate or vertebrate predators, thus potentially circulating in various habitats and animal species.

Surely, the role of insects and arachnids of dermatological/toxicological interest in EBOV transmission can be ruled out, considering the constant incidence of spiders and scorpions bites in Ebola endemic countries as well as in the rest of Africa [12,24] and, more importantly, there being no indication of unequivocal relationships between arthropod sting intoxications/lesions and EBOV infections.

The hypotheses that nonparasitic species of vertebrates, i.e., phytophagous [7], or aquatic insects in fluvial environments [18] could be potentially involved in the maintenance and spread of EBOV are supported by the already known diffusion of bacterial organisms from the environment to susceptible species. This is the case of *Neorickettsia risticii* that is transmitted to horses following the ingestion of adult insects that have been infected at the aquatic larval stage [2] or is the case of *Mycobacterium ulcerans* that is transmitted to humans through the bite of aquatic insects such as Naucoridae (Hemiptera) [21,34].

Conclusion

- Hematophagous arthropods (Anoplura, Siphonaptera, Cimicidae, Culicidae, etc.) have not been demonstrated to be natural reservoirs of EBOV.
- However, a mechanical transmission of EBOV from bats to humans through Hippoboscoidea (Diptera) in particular environments (i.e., bat roost) cannot be ruled out. These insects could hypothetically act as potential "bridge" mechanical vectors.
- Further studies are needed on what happens to EBOV when it is ingested by necrophagous species of insect that feed on excrement or corpses of infected bats.
- The hypothesis that EBOV could be present in environments and organisms ecologically different from those up to now investigated cannot be excluded, considering also other parameters such as seasonality of epidemics, microhabitats, etc.
- Even though the role of anthropophilic hematophagous (e.g., mosquitoes) and necrophagous (e.g., Calliphoridae and Sarcophagidae) insects in epidemic conditions is unclear, a marginal epidemiological role on EBOV transmission cannot be completely ruled out.

Conflict of interest : The authors do not have any conflict of interest.

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