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Searching for the source of Ebola: the elusive factors driving its spillover into humans during the West African outbreak of 2013–2016

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Summary

The natural ecology of Ebola virus infection remains enigmatic. No clear reservoir species has been confirmed but there is evidence of infection in a wide spectrum of mammals; including humans, non-human primates, domestic and wild ungulates and a variety of bat species, both frugivorous and insectivorous. Humans and most other species examined appear to be spillover hosts and suffer disease. Bats are the exception and are tolerant to infection in some laboratory studies. Some surveys show a low prevalence of antibodies against Zaire Ebola virus (ZEBOV) strains in bats during human outbreaks and inter-epidemic periods, and this order of mammals is considered to be the likely reservoir for the virus. Other putative sources include insects but this hypothesis is unproven in the field or laboratory. Moreover, some potential sources, such as aquatic species, have yet to be investigated. There are a number of environmental, human behavioural and ecological risk factors proposed with respect to spillover and spread. In the West African outbreak, which was unprecedented in scale and geographic spread, the source of the spillover remains unproven, although an association exists between the proposed index case and a colony of insectivorous bats. In all but a few Ebola virus disease events, spillover has only been superficially investigated and this was also the case in the West African epidemic. The authors suggest that, to address risks at the human–animal–environmental interface, using a One Health approach, more effort is needed to investigate spillover factors at the time of a ZEBOV epidemic, in addition to conducting inter-epidemic surveys in peri-domestic environments. The true prevalence of ZEBOV infection in any species of bats remains unknown. Large-scale, expensive, non-randomised surveys, with low sampling numbers per species, are unlikely to provide evidence for Ebola virus reservoirs or to improve our epidemiological understanding.

Keywords

Ebola virus – Reservoirs – Spillover – West Africa – Wildlife disease.

Introduction

Putative wildlife reservoirs of Zaire Ebola virus

To date, the only wildlife species in which evidence for Zaire Ebola virus (ZEBOV) infection has been detected, either by direct (antigen) or indirect (antibody) diagnostic methods, are non-human primates (NHPs), duikers and bats. The full epidemiological role which each of these might play in maintaining and/or circulating the virus is still unclear. The high mortality rates reported in western lowland gorillas (*Gorilla gorilla gorilla*) and common chimpanzees (*Pan troglodytes*) in western and Central Africa, and in duikers (*Cephalophus* spp.) in Central Africa (1, 2, 3), suggest that these species act only as dead-end hosts (4), although they also act as a bridge to human infection through the consumption of bushmeat (2). Additionally, Leroy and others found that infectivity is brief in apes and physical contact with other groups of apes is rare, resulting in difficulties in virus transmission between different groups of animals. This also seems to indicate that NHPs are not reservoir species (2).

On the other hand, experimentally infected frugivorous (*Epomophorus wahlbergi*) and insectivorous (*Chaerephon pumilus* and *Mops condylurus*) bats have been shown to tolerate ZEBOV infection without clinical signs under experimental conditions (5). Several studies subsequently detected antibodies at a very low prevalence in many different frugivorous and insectivorous species living in Central and West Africa (*Eidolon helvum, Epomops franqueti, Epomophorus gambianus, Hypsignathus monstrosus, Micropteropus pusillus, Mops condylurus [=Tadarida condylura], Myonycteris torquata, Rousettus aegyptiacus, Rousettus leschenaultia*) (4).

To the best of the authors' knowledge, true prevalence has not been established in any species of animal thought to be susceptible to ZEBOV and in which the virus might be cycling. Viral RNA from ZEBOV has been found in only one published study, detected by reverse transcription polymerase chain reaction (RT–PCR) from homogenised liver and spleen samples (6). Recent unpublished reports of partial sequences from bats in Liberia remain to be confirmed. These findings suggest that bats may play a central role that warrants further investigation. However, considerable efforts to find populations of bats harbouring or excreting ZEBOV have been surprisingly unproductive to date, if these species do indeed act as a reservoir.

Haematophagous and non-haematophagous arthropods have also been proposed as possible host or reservoir species (7): this hypothesis has been only partially investigated. To date, no field (8) or laboratory (5, 9) studies have proven the involvement of insects in the viral transmission chain.

Similarly, the role of aquatic and semi-aquatic animals, including aquatic insects, has never been assessed and cannot be excluded (10).

In the case of the West African outbreak, which originated in Guinea in 2013 (11), a wildlife survey did not reveal any obvious decline in populations of large mammals living in two protected areas close to the site where the epidemic originated (12). Large autochthonous fauna are rarely hunted in the region and most bushmeat is smoked and imported from distant locations. These findings, coupled with the lack of involvement of hunters in the first cases of the disease, suggest that large mammals are an unlikely source of the spillover (12).

However, in the area affected by the epidemic, frugivorous bats are a common source of meat and insectivorous bats, although not commonly hunted because of their bad smell and the belief that they feed on human excreta, have been reported by an anthropological investigation as occasionally being captured by children and grilled over fires (12). A sampling of 13 different species of bats close to the index case did not detect ZEBOV RNA or antibodies (12). The only clue about a possible spillover event is the presence of insectivorous bats (*M. condylurus*) in the area frequented by what is assumed to be the index case: a two-year-old boy who was reported to play in a hollow tree inhabited by a colony of these bats. This species was previously reported to be seropositive for antibodies against ZEBOV in Gabon (13) but, in the case of the West African outbreak, the link remains speculative.

Spillover and host spectrum in relation to zoonotic events

The peculiar tolerance of bats to viral infection is presumably mediated by a unique behaviour (flight) (14); seasonality (periods of torpor); and an unusual immune system (15). As regards their immune system, long-standing co-evolution with these viruses has generated multiple adaptive mechanisms. On the one hand, certain species seem to minimise and accurately modulate the antiviral and inflammatory response to prevent cell and tissue damage. On the other hand, other species seem to manifest a strong innate antiviral response, when compared to primates, which is able to limit viral replication earlier in the infection (16).

Even if the exact mechanism underlying this tolerance is not yet understood, these features suggest that bats may be a key element in the epidemiology of several different zoonotic viruses, including ZEBOV (17, 18, 19, 20). Bat species that have a greater body mass, a long lifespan, gregarious habits and larger but fragmented distribution areas seem more likely to host zoonotic viruses (21, 22). However, analyses of the influence of sympatry with other bat species generated conflicting results (21, 22), and the variance in the number of zoonotic viruses per bat species remains largely unexplained (22). Bats are implicated in the ecology of the virus but their association with human Ebola virus disease (EVD) events (13) remains speculative and unclear. No bat hunter has ever been reported to be an index case and, even when human outbreaks occur, people involved in massive bat hunting and slaughtering in the same region have not been affected by the disease (23).

Certain surveys suggest that bats are able to clear ZEBOV infection (6, 23) but the mechanisms for virus persistence at a population level are not clearly understood. It is uncertain if a particular physiological state, e.g. pregnancy, or certain environmental conditions could suppress the immune system, triggering epidemic viral shedding (6). Large surveys of bats (4,022 blood samples and 665 tissue samples from 39 species) associated with ZEBOV outbreak areas in Central and West Africa (24) have shown very little evidence of the virus, either by antibody or antigen detection methods, with levels of antibody at less than 1% and no samples testing positive by PCR (24). Essentially, there is an absence of evidence for virus circulation in these populations.

Over all, these sample sizes are still relatively small, since bat populations number in the millions, and the data are still deficient. As a result, it remains impossible to determine the role of bats in ZEBOV outbreaks, at least for now (17, 23). For the same reason, genetic sequencing of the isolated viral strains can only provide partial knowledge of the phenomenon, which is insufficient to prove or disprove the various transmission theories (25). Bats have also been proposed as a source of ZEBOV transmission to NHPs and duikers, which subsequently act as bridge species, transmitting the virus to humans when these animals are hunted and slaughtered or handled when found dead (2). As an alternative, NHPs and duikers could simply be dead-end hosts, without transmitting ZEBOV to humans.

One theory is that primates and duikers are infected by the ingestion of fruits contaminated by bat saliva, urine or faeces that fall on the ground around the trees where bats feed. This is a proven mode of transmission for other bat viruses, such as Nipah virus. It has even been shown that humans can become directly infected with Nipah virus from bats via harvested fruits (26). In five different cases, Ebola outbreaks among the Great Apes of Gabon have been reported to occur at the beginning of the dry season (2), and it is suggested that this might be associated with the increased contact between the different species that compete for food during this period (6). This mechanism has been proposed as a possible functional link between plant phenology and spillover events, to explain the detected association between local flowering and fruiting patterns and reported EVD outbreaks in humans and non-human animals (27).

Given the possibility of fruit-mediated transmission, it may be that agricultural production in forest zones has the potential to act as a driver for outbreaks, and this has been postulated in the case of the West African outbreak (28). In this particular case, it is suggested that global economics and development responses have led to rapid environmental change, new agriculture, and expanding and shifting food sources. It is speculated that this, in turn, might influence bat and other wildlife distributions and behaviours, and thus the risk of spillover. This was proven as a major factor in the Nipah virus spillover to pigs and humans in the Philippines (29) and, since this risk factor was addressed, there has been no repetition of this devastating event.

Another food-related transmission chain might involve the scavenging for meat of dead apes by other species, potentially including humans. Duikers could also be infected in this way (2). Direct predation

of monkeys was also reported to be a risk factor for ZEBOV infection in wild chimpanzees in Côte d'Ivoire (3).

The chain of factors needed to produce a spillover event is likely to be complex. In the case of ZEBOV, it may require the presence of intermediate host species that not only transmit the virus but also amplify it (18). Generally speaking, in such a complex system, a spillover event results from the alignment of multiple, hierarchical enabling conditions that also involve environmental and ecological factors (18). The role of human behaviour may also be central to the spillover event, bringing humans into contact with the reservoir, whether as a hunter or gatherer, or through agricultural, recreational or other activities. Interest in the influence of climate and phenology on viral dynamics is also growing rapidly; an association between seasonal and environmental factors and spillover has already been revealed for Ebola virus (20, 27). Understanding the exact mechanism by which this effect is produced could help to explain why certain outbreaks, such as the 2013–2014 West African outbreak, were traced back to a single spillover event (11, 12), while others were associated with multiple and independent transmission events (2, 30).

Viral adaptations to human hosts were also described in the 2013–2016 West African outbreak. These adaptations increased viral transmissibility between humans; at the same time reducing tropism for bats (31, 32). Despite the impossibility of assessing the role played by these mutations in producing the most extensive human outbreak of ZEBOV thus far, this finding highlights the necessity of considering a range of factors that affect the dynamics of viral transmission. It also underlines the relative importance of human-to-human transmission in the epidemic, as opposed to zoonotic transmission.

In any case, the significant number of reported spillover events suggests that host adaptation is not a prerequisite for animal-to-human infection (31), and that, once such transmission has occurred, an urban setting and socio-economic factors have a strong influence on the development of the outbreak (33).

Bushmeat as a possible source of the West African Ebola virus disease outbreak

Bushmeat, including bats, is widely eaten in West Africa (34). There is evidence that, despite attempts to eliminate this custom (for example, a government ban on bushmeat in Guinea in March 2014), it continues as a traditional practice. There is also evidence that individuals will deny consumption when questioned by officials, perhaps because of widespread criticism or criminalisation of this activity (35). Unpublished work (R. Suluku, personal communication, 2018) conducted in 31 villages among 6,000 people in the north, south and east of Sierra Leone revealed that a majority of the people in these areas ate bats during the Ebola outbreak because they had never seen or heard of anyone dying after eating bats since the time of their ancestors. However, it was common for people to deny eating bats when asked by representatives of non-governmental organisations (NGOs) and government officials.

There is no evidence from the West African outbreak that bushmeat was the source of infection to humans. This statement is based on epidemiological investigations of the emergence and spread of the virus. Samples were taken from bats soon after, and in the locality of, the presumed index case: 169 bats were captured from 13 species and all tested negative for ZEBOV antibody and antigen (12).

Wildlife as a source of the West African Ebola virus disease outbreak

The evidence gathered from the first known infected household in Meliandou suggests a possible zoonotic source (12). A two-year-old child, the purported index case, is described as playing in a tree hole before the infection, where a colony of insectivorous bats (*M. condylurus*) were living. It is ironic that this colony was burned out before any investigation could take place and the only confirming evidence, found using forensic techniques, was that the remnant DNA found in the charred areas of the tree was consistent with the species described by the householder. In this *post hoc* study, attempts to measure perturbations in the local wildlife population were crude. A short transect survey was undertaken in local protected areas, including broad categories of mammal (described as carnivores, chimpanzees, primates, duikers and other unspecified mammals). This survey was then compared to earlier biodiversity samples that used a similar method. This was interpreted as indicating that there had been no major change in any of these populations that might suggest a large decline associated with disease mortalities. However, the lack of specificity and sensitivity of this method and the absence of a bat census should be noted. No attempt was apparently made to identify the virus in other peri-domestic animal species (e.g. small ruminants, dogs, cats, poultry or peri-domestic rodents) that were present in the village. In general, during this extended and extensive outbreak, no effort was made to establish any secondary epidemiological cycles other than the one in humans. This was perhaps understandable, given the severity of the epidemic in humans and the small number of veterinarians involved in the management of the crisis. Nevertheless, it was a missed opportunity and somewhat ironic, given that this was a disease of animal origin.

Other possible socio-ecological factors driving spillover, such as ecological niche, forest fragmentation, agriculture, and settlement

Ebola has long been associated with the Central African forest. Attempts at defining the ecological niche and environmental co-variates of outbreaks (19) bring various parameters to the fore, including: vegetation, elevation, evapotranspiration, temperature and suspected reservoir bat distribution. However, none of these factors seemed to be consistent with the West African outbreak, which defied prediction along these co-variates**.** Others have suggested that forest fragmentation, slash-and-burn subsistence agriculture and settlement, and/or commercial agriculture are characteristic of all EVD outbreaks since the 1970s (28, 36, 37).

Discussion

The various observations and studies that emerged from the unpredicted and catastrophic EVD epidemic in West Africa have all focused on the post-spillover event. Most publications examined the role of socio-cultural factors in the virus's spread, and the failure of Health Services to prevent the virus from moving along a socio-ecological gradient from the rural villages to the cities. Interventions were basically too late to prevent the wide dissemination of cases in the first place but, as the outbreak grew larger, efforts rapidly improved. Epidemiological control efforts concentrated on tracing cases in the infected human population and isolating infected individuals as a means of stamping out Ebola's spread, essentially through preventing contact. This, along with the recovery or death of infected hosts, eventually led to the elimination of the virus (38).

Attempts by medical authorities to limit its spread through communications by text and other forms of messaging were initially confused. They emphasised bushmeat as a possible source of infection, despite the fact that this was clearly not a significant risk in this event.

A series of by-laws instigated by national tribal chiefs helped to reduce the spread of the virus in the provinces but, because people in the cities did not observe these by-laws, the epidemic persisted in urban areas. Moreover, the disease spread rapidly because people were denied burial of their relatives in accordance with their traditions and beliefs in transcendence. Once this policy was reversed, and people were allowed to witness the burial of their family members, without physical contact, the situation improved (39).

Local medical personnel had no knowledge about the disease and thus it killed many healthcare workers. In Nigeria, the medical staff left the hospital; it was only after involving a virologist and receiving specific training that they were able to control the virus and prevent any further spread. Vaccine development was fast-tracked at huge expense, and the resulting vaccine came into use late in the outbreak, when it was close to ending. However, this did provide an opportunity to evaluate the vaccine's efficacy and safety (38, 40).

Clearly, all of these aspects were and are extremely important for the future management of this disease. Nonetheless, the facts demonstrate a failure to stop the disease at its source. Moreover, due to the unprecedented nature of the virus's spread, public messaging was often confused and sometimes inappropriate, and it took some time for both the national and international health systems to begin to be able to cope. The recent outbreaks in the Democratic Republic of the Congo in 2018 are also worrying, in their multiplicity of events and the extended period of the epidemic, suggesting that perhaps a similar trend in scale and impact is emerging there (41, 42).

The absence of detailed studies on the presumed spillover of the virus from animals, and the relatively crude examination of the animal component of the West African outbreak, is concerning and,

unfortunately, consistent with efforts in most earlier outbreaks. After such outbreaks, studies and inter-epidemic surveillance have included a number of broad, relatively unfocused attempts to identify infected and/or reservoir species for the virus, with – to date – little reward. It is clear from serological results that bats and other species are infected at times, but there is still only one convincing study on the presence of virus antigen, published by Leroy in 2005 (6).

In this study, positive results for ZEBOV by nested PCR provided some confidence that a low level of viral RNA was present in three sampled populations of fruit bats (*H. monstrosus, E. franqueti* and *M. tourquata*) around an affected village in Mbomo, Gabon (6). None of the animals that tested positive by PCR returned positive results for immunoglobulin G (IgG), and animals giving positive IgG results tested negative by PCR. This result was reversed in a sampling from the same population five months later.

These results were evidence that the first sample was taken early in an infection cycle in this population and the second was taken during the post-epidemic phase. This hypothesis cannot be rigorously tested as this was a convenience sample: it was not taken randomly and there is no certainty that the same population was sampled on the second occasion. *H. monstrosus* is known to make seasonal movements (43). The evidence for bats being the reservoir for Ebola virus remains weak and unsubstantiated, in Leroy's or any other study, at least for the present.

Nevertheless, the Leroy study remains the most convincing evidence yet for viral circulation in the environment at around the time that infection spilled over into humans. The fact that Leroy and his colleagues apparently sampled the bats during the early phase of the infection is key. A timely purposive sample around a known human (and Great Ape) outbreak site provided results, and this supports the proposition that, in the future, animals should immediately be sampled around such events, and in a structured manner. Despite this obvious interpretation, the Leroy study seems not to have been replicated.

Although the number of screened species, both in the West African outbreak and other studies, was remarkable, the number of sampled individuals from each species was limited. Moreover, since sampling takes place after the event and is *ad hoc* in all cases, this could affect the value of the results from all studies. The single investigation of a longitudinal sample of infection in animals around a human Ebola epidemic, again by Leroy (6), showed that prevalence was apparently very low: ~2% in the bats sampled within a few months. This was conducted in the precise period after human infection had first occurred in Guinea and before sampling of animals in the West African outbreak began, so perhaps it is not unexpected that negative results ensued. If a delayed response to the outbreak failed to provide solid evidence of infection at the animal source, it is not surprising that inter-epidemic studies are also fruitless. Discovering the reservoir of ZEBOV is proving to be rather like finding a needle in a haystack and, despite considerable expenditure of time and money, there is still little concrete evidence to explain this enigmatic virus's natural ecology.

The evidence of apparently unaffected bats in the environment of the index case in Guinea in 2014, and of similarly unaffected chimpanzees in adjacent protected areas, provides the only significant wildlife survey data of interest in the West African outbreak of Ebola. The chimpanzee data, at least, suggest that this outbreak was not similar in epidemiology to that reported in outbreaks of the virus in the Great Apes in Central Africa, by Leroy (6). This does not rule out the possibility of spillover from wildlife, bushmeat or other animals, but it remains unproven. The efforts made to sample wildlife and take more general environmental samples in the West African outbreak were not extensive and may not have been adequate to prove the absence of a wildlife source or cycles of virus infection. An additional, more detailed survey of flora and fauna from the peri-domestic area, cleared secondary forest, agricultural areas and protected forest zones, during and after the outbreak, might have shown something but there was apparently little capacity for this with only one limited study.

In 2016, for the first time in West Africa, a serological survey in Sierra Leone reported that three out of 400 samples from pigs (*Sus scrofa domesticus*) showed the presence of Ebola-virus-specific antibodies. Even if epidemiological analysis shows that pigs did not play a role in the West African outbreak, this finding highlights once again the need for new investigations in the peri-domestic area, especially before and during an active outbreak (44). There is little mention of environmental factors (for example, fruiting, flowering or other seasonal conditions) that might have been relevant in this event or that have been reported as being associated with EVD elsewhere. It is possible to infer that the West African outbreak occurred after the beginning of the plant fruiting season (around November, in this region) and during the dry season (December to May). This is a time when bats are very active, feeding on ripe fruit, and it is also consistent with the timing of all the Great Ape-associated outbreaks in the Congo Basin.

One observation made in the West African outbreak that is consistent with other outbreaks is that there was evidence of considerable development and agricultural activity in the index-case zone, including various new settlements, clearances, roads, and planting of agricultural crops, such as palm oil, which is a preferred food of fruit bats. This habitat fragmentation and reduction of buffer areas might influence bat population distribution and concentration, generally bringing these animals into closer proximity to humans and domesticated species (45, 46).

It appears that, understandably, the main focus in the West African Ebola outbreak was on human-to-human transmission and controlling the outbreak. However, this meant that an opportunity was missed to take our understanding of the epidemiology of Ebola spillover events forward. This remains a contemporary challenge to the new One Health framework, now adopted by the World Health Organization (WHO), the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), and covered extensively in this publication. In future outbreaks, a more source-oriented study is warranted; one that includes the range of known risk factors for spillover in the immediate investigation, as well as in communications with communities at risk.

In the final analysis, finding the triggers for spillover using a One Health approach might be the key to preventing EVD in humans in the future, and for all time. The cost of *not* addressing the underlying drivers for EVD over the longer term might well outweigh any immediate costs involved in obtaining a fuller understanding of the disease, through more rigorous investigations and research into the outbreak's source. This approach should be seriously considered, even while current efforts remain firmly focused on a vaccine, risk communication, and other response measures. The ecological complexity of EVD is daunting but, if sufficient One Health resources are not invested into clarifying its epidemiology, humanity might end up with a truly global disease challenge to rival human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV AIDs).

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Résumé français: titre

Résumé

Mots-clés

Resumen español: título

Resumen

Palabras clave

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