Ebola virus (*Filoviridae: Ebolavirus: Zaire ebolavirus*): fatal adaptation mutations

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Ebola virus disease (EVD) (former Ebola hemorrhagic fever) is one of the most dangerous infectious diseases affecting humans and primates. Since the identification of the first outbreak in 1976, there have been more than 25 outbreaks worldwide, the largest of which escalated into an epidemic in 2014–2016 and caused the death of more than 11,000 people. There are currently 2 independent outbreaks of this disease in the eastern and western parts of the Democratic Republic of the Congo (DRC) at the same time. Bats (*Microchiroptera*) are supposed to be the natural reservoir of EVD, but the infectious agent has not yet been isolated from them. Most animal viruses are unable to replicate in humans. They have to develop adaptive mutations to become infectious for humans. In this review based on the results of a number of studies, we hypothesize that the formation of adaptive mutations occurs directly in the human and primate population and subsequently leads to the development of EVD outbreaks.

Key words: *Ebola virus; adaptation mutations*

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Вирус Эбола (*Filoviridae: Ebolavirus: Zaire ebolavirus*): фатальные адаптационные мутации

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Болезнь, вызванная вирусом Эбола (БВВЭ) (прежнее название – геморрагическая лихорадка Эбола), – одно из самых опасных инфекционных заболеваний, поражающих человека и приматов. С момента идентификации первой вспышки в 1976 г. в мире было зарегистрировано более 25 аналогичных эпизодов, самый крупный из которых в 2014–2016 гг. перерос в эпидемию и унёс жизни свыше 11 тыс. человек. В настоящее время одновременно в восточной и западной частях Демократической Республики Конго (ДРК) протекают 2 независимые вспышки БВВЭ. Считается, что естественным резервуаром её возбудителей являются летучие мыши (*Microchiroptera*), однако инфекционный агент из них до сих пор не выделен. Известно, что большинство вирусов животных не способно реплицироваться в человеческом организме. Для того чтобы произошло заражение человека, необходимо наличие адаптационных мутаций (АМ). В данном обзоре на основании результатов ряда исследований сформулирована гипотеза о том, что формирование мутационных изменений подобного рода происходит непосредственно в популяциях людей и приматов, приводя в дальнейшем к развитию вспышек БВВЭ.

Ключевые слова: *вирус Эбола; адаптационные мутации*

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Adaptive mutations in the structure of Ebola virus glycoprotein tend to increase viral infectivity in human and primate cells

Ebola virus disease (EVD), one of the deadliest viral diseases, affects both humans and primates. It is characterized by a severe condition, development of overall intoxication and a high fatality rate reaching 90% [1–3]. Ebola viruses belong to the genus *Ebolavirus*, family *Filoviridae* [4]. Currently, the 6 known species of this genus are *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SUDV), *Bundibugyo ebolavirus* (BDBV), *Reston ebolavirus* (RESTV), *Tai Forest ebolavirus* (TAFV), *Bombali ebolavirus* (BOMV), out of which the first 3 are most pathogenic for humans.

The Ebola virus (EBOV) has a complex structure. Its virion consists of a lipid envelope with transmembrane proteins, a nucleocapsid containing genomic RNA and polymerase complex, and a matrix layer consisting of VP24 and VP40 proteins [5]. The viral genome is represented by a negative-polarity single-stranded RNA molecule, which encodes structural and nonstructural proteins. It is located in the central part of the virion, being bound to a nucleoprotein (NP) and nucleocapsid proteins (VP30). The same location hosts VP35 proteins and a viral polymerase catalytic subunit L [6–8]. Through matrix proteins VP24 and VP40, the nucleocapsid is attached to the inner side of the lipid bilayer of the virus envelope, which is formed from the plasma membrane of the host cell during the budding of a virion [6–8]. Envelope glycoprotein (GP) molecules anchored in the bilayer form spikes and play a critical role in the virus life cycle by mediating the internalization process.

The study of different mutations in EBOV proteins has shown that the most effective mutations associated with virus replication are those that involve its full-length glycoprotein. Wong G. *et al*. demonstrated that acquired mutations in the GP structure increased the pathogen's ability to perform internalization, thus affecting the growth rate and, consequently, an increased viral progeny output per cell [9]. It results in increased infectivity of the agent both *in vitro* and *in vivo*. Similar results were obtained by different researchers [10, 11] studying adaptive mutations (AMs) in the EBOV glycoprotein by using cell cultures of various mammals. For example, Kurosaki Y. *et al*. showed that during culturing of the above pathogen or the vesicular stomatitis virus (VSV) whose glycoprotein was replaced with the EBOV glycoprotein, AMs of the glycoprotein structure were developed in the Vero E6 cell culture, causing the increased viral internalization. In its turn, it causes an increase in the growth rate and in viral progeny output per cell. Therefore, the occurrence and fixation of such mutations lead to increased EBOV infectivity for human and primate cells [12–15].

The study of AMs of the above infective agent during the 2014–2016 sweeping outbreak identified several key mutations resulting in the widespread disease. Topping the list, there were mutations in the full-length viral glycoprotein, which considerably enhanced viral internalization [16, 17].

From the first EVD outbreak to fatal adaptive mutations

Since the virus was first identified, there have been more than 25 EVD outbreaks (**Table 1**); the 2014–2016 outbreak was the largest one that rapidly escalated into an epidemic and claimed more than 11 thousand human lives [18, 19].

The first outbreak was reported in Nzara (Sudan) at the end of June 1976, in 3 cotton factory workers; however, the route of infection was not described [20]. Later, in September of the same year, another outbreak occurred in Zaire (now the Democratic Republic of Congo (DRC)) in the vicinity of Yambuku village [21]. The first patient diagnosed with malaria was treated with injec-

tions at the Yambuku Mission Hospital (YMH). Then, the infection was transmitted through used needles and syringes in the hospital and clinics located in the area as well as through direct human-to-human contact.

In 2014, the outbreak that rapidly spiraled into an epidemic started with an 18-month-old boy from the village of Meliandou in southern Guinea [22, 23]. The boy is believed to have been infected by bats or their body fluids (urine, feces, saliva).

The detailed analysis of EVD cases has shown that frequently the primary case is a person or a small group of people who are first to spread the disease to others. The documents available at sites of the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDCs) state that the primary cases may have come from contact with infected/dead monkeys (*Haplorhini*) or bats (*Microchiroptera*). Those who had contact with monkeys displayed the disease symptoms at the same time, while symptoms appeared only in a few people who had contact with bats [24]. A human can contract the virus (causing an infection) only when there are AMs, as most of the animal viruses are not able to replicate in humans [25].

To date, no isolation of EBOV that would have high infectivity has been successful from bats, though the tested animals were PCR-positive [26]. The viral agent isolated from their tissues tends to reproduce very poorly in human and primate cell cultures; therefore, it needs AMs to replicate in humans [17]. The discovery of the fact that mutations cause infectivity loss in bats was an important step in the study of AMs in the structure of EBOV glycoprotein. Urbanowicz R.A. et al. showed that such mutations caused increased viral infectivity for humans, while resulting in decreased infectivity for bat cells [17].

Table 1. Chronology of the EVD outbreaks since 1976 [19]

Country/region	Cases of disease, *n*	Lethal outcomes, *n*	Type of pathogen	Years
Democracy Republic of Congo (DRC), Uganda	3228	2157	ZEBOV	2018–2019
DRC	54	33	ZEBOV	2018
DRC	8	4	ZEBOV	2017
DRC	66	49	ZEBOV	2014
Guinea, Sierra Leone, Liberia	28 652	11 325	ZEBOV	2014–2016
Uganda	6	3	SUDV	2012
DRC	36	13	BDBV	2012
Uganda	11	4	SUDV	2012
Uganda	1	1	SUDV	2011
DRC	32	15	ZEBOV	2008
Uganda	149	37	BDBV	2007
DRC	264	187	ZEBOV	2007
South Sudan	17	7	SUDV	2004
Republic of the Congo	35	29	ZEBOV	2003
Republic of the Congo	143	128	ZEBOV	2002
Republic of the Congo	57	43	ZEBOV	2001
Gabon	65	53	ZEBOV	2001
Uganda	425	224	SUDV	2000
South Africa	2	1	ZEBOV	1996
Gabon	60	45	ZEBOV	1996
Gabon	37	21	ZEBOV	1996
DRC	315	250	ZEBOV	1995
Côte d'Ivoire	1	0	TAFV	1994
Gabon	52	31	ZEBOV	1994
South Sudan	34	22	SUDV	1979
DRC	1	1	ZEBOV	1977
South Sudan	284	151	SUDV	1976
DRC	318	280	ZEBOV	1976

AMs can occur when EBOV persists in the body of monkeys having contact with body fluids of infected bats (saliva, feces) and eating bats. In these situations, the viral agent can mutate, and this ability is demonstrated by the cultivation of kidney epithelial cells extracted from an African green monkey (*Chlorocebus sabaeus*) – Vero E6 [10, 11] as well as when studying its mutational variability in infected monkeys [27]: mutations have been detected in different genomic regions of the pathogen (including regions responsible for the glycoprotein structure) and contributed to the increased efficiency of the virus internalization. The adapted EBOV is able to actively reproduce in monkey and human cells; when this pathogen enters the body, it causes EVD in the individual. It should be noted that all contacts of a human with infected monkeys resulted in contracting the disease. It is well illustrated by the case in Central Africa, when a group of hunters brought back a dead (the cause of death had been unclear) animal to the village for consumption. An EVD outbreak started a few days later.

Most likely, AMs can also occur in a human body. Interestingly, the population of endemic areas can have EBOV-specific antibodies, while displaying no symptoms. In Sudan, specific antibodies were detected in blood serum in 19% of people having contact with EVD patients and having not been exposed to the virus previously. In DRC, 1% of people living in villages outside the epidemic zone, having no contact with EVD patients and displaying no EVD symptoms have these antibodies as well [21]. In endemic areas of the country (villages in the vicinity of Tandala), EBOV-specific antibodies were detected in 7% of the population, and their presence has a direct correlation with the age: from 1% in children under 4 years to 21% in adults over 60 [28]. A number of other studies have also shown people's seropositivity to ebolaviruses: 20.8% in the Central African Republic (CAR) [29], 22% in Sudan [38], 13% in Liberia [40], 11% in Gabon [34–37], 10% in DRC [28, 30–33], 7% in Cameroon [41, 42], 4% in Madagascar [39], 2% in Nigeria [43], 1% in the Federal Republic of Germany (FRG) [44] and 1% in Kenya [45]. The recent studies published in July 2020, state that in Uganda, those who live and work in high-risk areas were 5.4 times more likely to be filovirus seropositive compared to residents of central Uganda [46]. Thus, representatives of the population of EVD endemic regions have contacts with EBOV carriers (bats) when hunting and catching them, cooking, eating them, etc. As a result, those who were exposed to the virus can develop a specific immune response, which apparently prevents the disease from progression in the index case, though the pathogen can persist in such individuals and acquire AMs. They do not develop a disease, as their immune system bridles the development of an infection process. The assumption can be proved by a comparative analysis of EBOV genomes isolated from bats and genomes of the virus isolated from sick/dead people and monkeys; however, at the moment, it does not seem to be realistic. Public databases (GeneBank) have information about EBOV glycoprotein nucleotide sequences; the information is based on studies of the RNA isolated from tissues of sick people and monkeys, but there are no comparable data on biological material from bats being a natural reservoir of the virus. There are only 7 sequences encoding the L polymerase of the virus isolated from the animals.

Crossing the interspecies barrier

Thus, we can offer several possible models, following which the virus can cross the interspecies barrier.

Model 1. Infection is transmitted to humans from bats or other animals carrying an adapted pathogen.

The 2014–2016 epidemic started from the primary case – a little boy who played with other children in a hollow tree housing a colony of free-tailed bats (*Mops condylurus*). Such bats are a potential EBOV reservoir (the recently discovered new species of ebolaviruses, *Bombali ebolavirus*, was isolated from animals of this species). Bats usually live in colonies; each of them can have a bat-carrying virus with mutations. The affected child may have had contact with such bats [25].

This model implies that adaptation occurs in bats, then the adapted virus is transmitted to a human or a monkey; mutations can differ, as humans differ from primates (**Fig. 1**). Monkeys, in their turn, are genetically closer to humans as compared to bats; therefore, the monkey-human interspecies barrier is easier to cross than the barrier involving a bat and a human. Currently, it is an established (classical) model. However, it is still not clear if the pathogen can adapt to the structures absent in the source body, taking into account that tissues of bats do not contain cell receptors of humans, monkeys or representatives of other species. If the answer is negative, the occurrence of an adapted virus should be seen as impossible.

Model 2. Infection is transmitted to humans (or other animals) from bats carrying an unadapted virus.

This model is contrary to the first one; it is postulated that AMs occur in a human body (**Fig. 2**). The virus adapts to the surrounding cell structures or, specifically, to human cells. Yet, the model has a number of pitfalls, namely:

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1) The infective agent adapted to bat cells is not able to get attached to human cells, to enter them, to replicate, etc., as previously discussed. There must be AMs, which occur during the viral genome replication, i.e. after all the above stages have been passed.

2) This model implies that the non-virulent virus develops into a highly virulent pathogen, i.e. its lethality in a human body tends to increase gradually rather than go down. In the meantime, nothing of the kind is observed in nature or even in experimental laboratories. After the interspecies barrier is crossed, the virus lethality is always high and decreases gradually. In the meantime, no EBOV-caused epidemics have demonstrated any decrease in the virulence (which may be as a benefit, as high virulence results in the eradication of infection); however, the same phenomenon is frequently observed in other viral diseases.

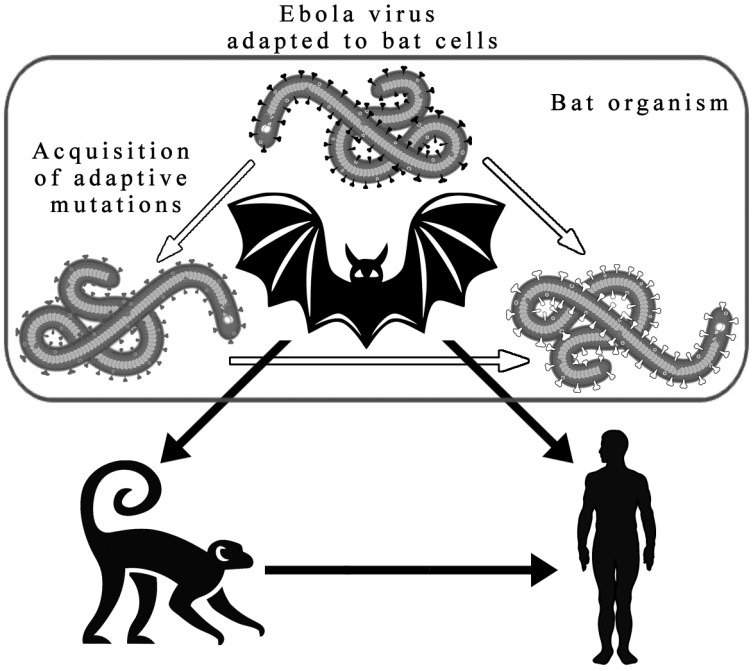

Fig. 1. The presumable route of infection in humans or monkeys from bats carrying the mutant virus.

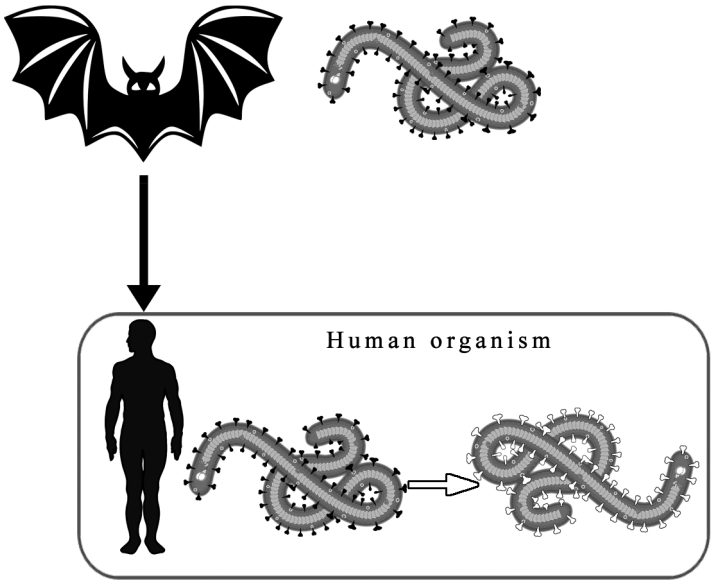

Fig. 2. The presumable route of infection in humans or other animals from bats carrying the unadapted virus.

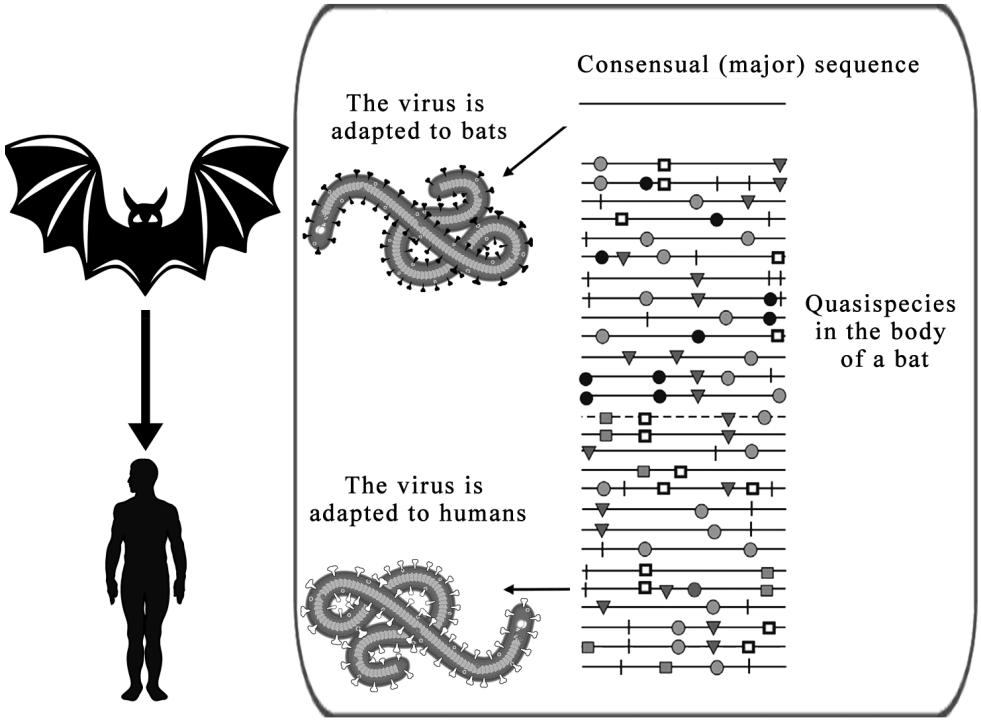

Fig. 3. The presumable route of infection in humans from animals with part of the virus micropopulation having AM.

Model 3. Infection is transmitted to humans from animals whose viral micro-population, or its part, has AMs.

This model of crossing the interspecies barrier combines all the features of the above models; thus, it is appealing to many present-day researchers and is based on the quasispecies model offered by Nobel laureate Manfred Eigen in 1982 [47]. He came up with a theory suggesting the existence of quasispecies – viral micro-populations. The core idea is that RNA viruses, unlike other organisms (including DNA-containing viruses), mutate very rapidly and, therefore, do not exist as exact clones of the same virion, but as a micro-population of particles, which are highly similar, though slightly different in nucleotide and protein sequences.

Thus, a human can be infected in the event of a high concentration of virions and/or in the event of frequent contacts with the source of infection, if there is the EBOV variant moderately adapted to human cells due to random mutations (**Fig. 3**).

The virus gains access to reproduction and, consequently, acquires a number of other AMs, which are highly specific for replication in human cells. This model provides an explanation to the above problems and to the findings obtained after some experimental observations:

• In contrast to the first model of crossing the interspecies barrier, in this scenario the virus in bats does not go through adaptation to human cell structures; at the same time, the EBOV micro-population has virions with low adaptation (low fitness) to them.

• It would be reasonable to assume that in all the infected, the infection may be acute (when the virus is adapted) or inapparent – when the virus is unable to replicate or the disease cannot develop into an acute form before the specific immunity is established. If the acute infection develops and an epidemic begins, the virulence will gradually decrease.

Many people who do not display any symptoms of the disease caused by EBOV have antibodies against it. In other words, there was an inapparent infection process, when the infective agent entered the body and resulted in antibody production; however, the disease did not develop, as the virus was not adapted to human cells.

Model 4. Infection is transmitted to humans from various animals, and the index case does not display any disease symptoms.

We are looking into another hypothetical way of crossing the interspecies barrier, when infection is caused by an unadapted virus carried by various animals. Since the pathogen is not adapted to human cells, the infection can be temporary (transient) or inapparent, possibly developing – due to AMs in the viral genome – into true persistence characterized

by virus shedding and absence of disease symptoms. Such an individual (the index case) develops AMs to the cell structures. The persisting infection accompanied by virus shedding means that the infection can be transmitted to the primary case – the first individual with disease symptoms (**Fig. 4**).

The above model is supported by some observations and facts, namely:

• The infection process can take a form of true persistence (existing virus shedding and absent symptoms). There are cases when the virus remained in the body after treatment of the acute form of infection. This may be explained by the occurrence of genetic EBOV variants that for some unknown reasons can persist, while the infective agent is always shed by immune privileged organs. It can be assumed that changed viral variants can penetrate into them; however, as there are no favorable conditions for replication, the pathogen disappears completely over time. The phenomenon does not seem to have any epidemiological significance, though it implies the possibility of infection of another individual who can start an epidemic outbreak. One of the studies shows that EVD human and monkey survivors may develop persistent infection [48]. Other researchers have demonstrated that EBOV can persist for a long time in inter-articular fluid and semen, in the anterior chamber of the eye, bone marrow, breast milk, sweat and other biological media of a human [49]. The examination of 93 EVD male survivors showed that 100% of males had EBOV RNA in the semen 2–3 months after the recovery; the proportion went down to 65% in 4–6 months and to 26% in 7–9 months [49]. In another examination, 11 (8%) out of 137 male survivors had a viral RNA detected in the semen 2 years after EVD [50].

Notably, EVD can take a chronic form, when the disease is reactivated in survivors [51, 52]. There is the evidence of relapse that occurred a few months after the recovery and that most likely was caused by the virus persisting in the body. The nurse who contracted and recovered from the disease was readmitted to hospital 9 months later with symptoms of acute meningitis; EBOV was detected in her blood and cerebrospinal fluid; 9 weeks after the recovery, a doctor developed uveitis, and the infective agent was detected in the ocular fluid [53]. There is no information about reinfection cases in published studies.

• Existence of immune responses to ebolaviruses among the population of endemic areas. As discussed previously, EBOV-specific antibodies are detected in residents of EVD endemic areas. Therefore, the in-

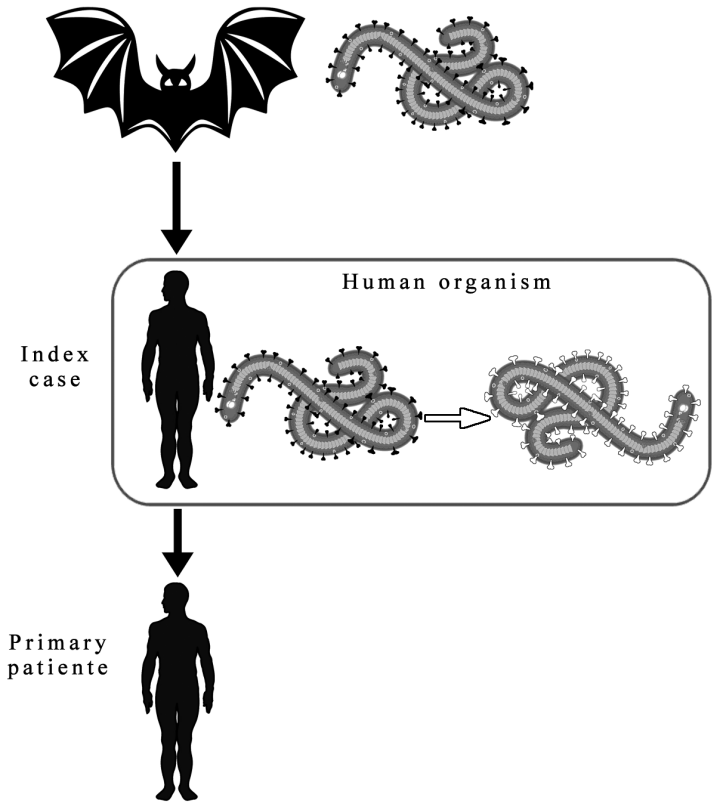

Fig. 4. The presumable route of infection in humans from various animals without showing signs of disease in the index case.

dex case can have virus-specific antibodies and, when infected, the individual may not display any disease symptoms, while retaining the capability of infection transmission. We assume that residents living in these areas may have contacts with animals carrying ebolaviruses: during hunting and catching, cooking and eating them, etc. As a result, those who had contacts, first of all, develop specific antibodies, which prevent the disease development in the index case; secondly, ebolaviruses can persist in those who had contact with them and acquire the required AMs. Then, index cases act as a potential source of infection. Such carriers can migrate actively from one area to another (for example, during hostilities, which are not infrequent in Central and East Africa), thus causing disease outbreaks in areas previously known for the favorable epidemiological situation for EVD.

Conclusion

Viruses of the *Ebolavirus* genus are highly pathogenic infective agents having a substantial epidemic potential; therefore, their study is among top-priority objectives focusing on epidemiological safety of mankind. However, the lack of information about ebolavirus variants (mainly, about those that exist in natural environments) makes it difficult to delve into the evolution of this taxonomic group and mechanisms involved in crossing the interspecies barrier by its representatives. Consequently, there can be problems associated with the assessment of the epidemic potential of a specific pathogen variant as well as with the selection of the type of glycoprotein as the main protective antigen for new vaccines. Therefore, the study of filovirus isolates obtained from natural reservoirs should be among the main research areas. The human-involving crossing of the interspecies barrier may most likely be achieved by using a single mechanism, which should be thoroughly studied and described as a combination of the above scenarios. A clear understanding of this route will not only help predict the occurrence and spread of EVD outbreaks but also will play a crucial role in minimizing the number of ebolavirus epidemics.

REFERENCES

1. Sanchez A., Geisbert T.W., Feldmann H. Filoviridae: Marburg and Ebola viruses. In: Knipe D.M., Howley P.M., eds. *Fields Virology.* Philadelphia: Lippincott Williams & Wilkins; 2006: 1409–48.
2. Taylor D.J., Leach R.W., Bruenn J. Filoviruses are ancient and integrated into mammalian genomes. *BMC Evol. Biol.* 2010; 10: 193. https://doi.org/10.1186/1471-2148-10-193.
3. Geisbert T.W. Marburg and Ebola hemorrhagic fever (Filoviruses). In: *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases.* Philadelphia: Elsevier; 2015: 1995–9.
4. International Committee on Taxonomy of Viruses. Available at: https://talk.ictvonline.org/taxonomy/p/taxonomy-history?taxnode_id=201901569 (accessed January 14, 2021).
5. Bharat T.A., Noda T., Riches J.D., Kraehling V., Kolesnikova L., Becker S., et al. Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. *Proc. Natl. Acad. Sci. USA*. 2012; 109(11): 4275–80. https://doi.org/10.1073/pnas.1120453109.
6. Ascenzi P., Bocedi A., Heptonstall J., Capobianchi M.R., Di Caro A., Mastrangelo E., et al. Ebolavirus and Marburgvirus: insight the Filoviridae family. *Mol. Aspects Med.* 2008; 29(3): 151–85. https://doi.org/10.1016/j.mam.2007.09.005.
7. Gutsche I., Desfosses A., Effantin G., Ling W.L., Haupt M., Ruigrok R.W., et al. Structural virology. Near-atomic cryo-EM structure of the helical measles virus nucleocapsid. *Science*. 2015; 348(6235): 704–7. https://doi.org/10.1126/science.aaa5137.
8. Beniac D.R., Booth T.F. Structure of the Ebola virus glycoprotein spike within the virion envelope at 11 Å resolution. *Sci. Rep.* 2017; 7: 46374. https://doi.org/10.1038/srep46374
9. Wong G., He S., Leung A., Cao W., Bi Y., Zhang Z., et al. Naturally occurring single mutations in Ebola virus observably impact infectivity. *J. Virol.* 2018; 93(1): e01098-18. https://doi.org/10.1128/JVI.01098-18.
10. Ruedas J.B., Arnold C.E., Palacios G., Connor J.H. Growth-adaptive mutations in the Ebola virus Makona glycoprotein alter different steps in the virus entry pathway. *J. Virol.* 2018; 92(19): e00820-18. https://doi.org/10.1128/JVI.00820-18.
11. Ruedas J.B., Ladner J.T., Ettinger C.R., Gummuluru S., Palacios G., Connor J.H. Spontaneous mutation at amino acid 544 of the Ebola virus glycoprotein potentiates virus entry and selection in tissue culture. *J. Virol.* 2017; 91(15): e00392-17. https://doi.org/10.1128/JVI.00392-17.
12. Kurosaki Y., Ueda M.T., Nakano Y., Yasuda J., Koyanagi Y., Sato K., et al. Different effects of two mutations on the infectivity of Ebola virus glycoprotein in nine mammalian species. *J. Gen. Virol.* 2018; 99(2): 181–6. https://doi.org/10.1099/jgv.0.000999.
13. Diehl W.E., Lin A.E., Grubaugh N.D., Carvalho L.M., Kim K., Kyawe P.P., et al. Ebola virus glycoprotein with increased infectivity dominated the 2013-2016 epidemic. *Cell*. 2016; 167(4): 1088–98.e6. https://doi.org/10.1016/j.cell.2016.10.014
14. Ueda M.T., Kurosaki Y., Izumi T., Nakano Y., Oloniniyi O.K., Yasuda J., et al. Functional mutations in spike glycoprotein of Zaire ebolavirus associated with an increase in infection efficiency. *Genes Cells*. 2017; 22(2): 148–59. https://doi.org/10.1111/gtc.12463.
15. Wang M.K., Lim S.Y., Lee S.M., Cunningham J.M. Biochemical basis for increased activity of Ebola glycoprotein in the 2013–16 epidemic. *Cell Host Microbe.* 2017; 21(3): 367–75. https://doi.org/10.1016/j.chom.2017.02.002.
16. Dietzel E., Schudt G., Krähling V., Matrosovich M., Becker S. Functional characterization of adaptive mu-

tations during the West African Ebola virus outbreak. *J. Virol.* 2017; 91(2): e01913-16. https://doi.org/10.1128/JVI.01913-16.
17. Urbanowicz R.A., McClure C.P., Sakuntabhai A., Sall A.A., Kobinger G., Müller M.A., et al. Human adaptation of Ebola virus during the West African outbreak. *Cell.* 2016; 167(4): 1079–87.e5. https://doi.org/10.1016/j.cell.2016.10.013.
18. WHO. Ebola Situation Report-21; 2015. Available at: https://apps.who.int/ebola/current-situation/ebola-situation-report-21-october-2015 (accessed January 14, 2021).
19. CDC. Ebola virus disease distribution map: Cases of Ebola virus disease in Africa since 1976. Available at: https://www.cdc.gov/vhf/ebola/history/distribution-map.html (accessed January 14, 2021).
20. Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. *Bull. World Health Organ.* 1978; 56(2): 247–70.
21. Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. *Bull. World Health Organ.* 1978; 56(2): 271–93.
22. Baize S., Pannetier D., Oestereich L., Rieger T., Koivogui L., Magassouba N., et al. Emergence of Zaire Ebola virus disease in Guinea. *N. Engl. J. Med.* 2014; 371(15): 1418–25. https://doi.org/10.1056/NEJMoa1404505.
23. CDC. 2014-2016 Ebola Outbreak in West Africa. Available at: https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak/index.html (accessed January 14, 2021).
24. Marí Saéz A., Weiss S., Nowak K., Lapeyre V., Zimmermann F., Düx A., et al. Investigating the zoonotic origin of the West African Ebola epidemic. *EMBO Mol. Med.* 2015; 7(1): 17–23. https://doi.org/10.15252/emmm.201404792.
25. Warren C.J., Sawyer S.L. How host genetics dictates successful viral zoonosis. *PLoS Biol.* 2019; 17(4): e3000217. https://doi.org/10.1371/journal.pbio.3000217.
26. Edenborough K.M., Bokelmann M., Lander A., Couacy-Hymann E., Lechner J., Drechsel O., et al. Dendritic cells generated from Mops condylurus, a likely filovirus reservoir host, are susceptible to and activated by Zaire ebolavirus infection. *Front. Immunol.* 2019; 10: 2414. https://doi.org/10.3389/fimmu.2019.02414.
27. Guedj J., Piorkowski G., Jacquot F., Madelain V., Nguyen T.H.T., Rodallec A., et al. Antiviral efficacy of favipiravir against Ebola virus: A translational study in cynomolgus macaques. *PLoS Med.* 2018; 15(3): e1002535. https://doi.org/10.1371/journal.pmed.1002535.
28. Heymann D.L., Weisfeld J.S., Webb P.A., Johnson K.M., Cairns T., Berquist H. Ebola hemorrhagic fever: Tandala, Zaire, 1977–1978 external icon. *J. Infect. Dis.* 1980; 142(3): 372–6. https://doi.org/10.1093/infdis/142.3.372.
29. Meunier D.M., Johnson E.D., Gonzalez J.P., Georges-Courbot M.C., Madelon M.C., Georges A.J. Current serologic data on viral hemorrhagic fevers in the Central African Republic. *Bull. Soc. Pathol. Exot. Filiales.* 1987; 80(1): 51–61 (in French).
30. Nkoghe D., Padilla C., Becquart P., Wauquier N., Moussavou G., Akué J.P., et al. Risk factors for Zaire ebolavirus-specific IgG in rural gabonese populations. *J. Infect. Dis.* 2011; 204(Suppl. 3): S768–75. https://doi.org/10.1093/infdis/jir344.
31. Busico K.M., Marshall K.L., Ksiazek T.G., Roels T.H., Fleerackers Y., Feldmann H., et al. Prevalence of IgG antibodies to Ebola virus in individuals during an Ebola outbreak, Democratic Republic of the Congo, 1995. *J. Infect. Dis.* 1999; 179(Suppl. 1): S102–7. https://doi.org/10.1086/514309.
32. Becquart P., Wauquier N., Mahlakõiv T., Nkoghe D., Padilla C., Souris M., et al. High prevalence of both humoral and cellular immunity to Zaire ebolavirus among rural populations in Gabon. *PLoS One.* 2010; 5(2): e9126. https://doi.org/10.1371/journal.pone.0009126.
33. Van der Groen G., Pattyn S.R. Measurement of antibodies to Ebola virus in human sera from N.W.-Zaire. *Ann. Soc. Belg. Med. Trop.* 1979; 59(1): 87–92.
34. Allela L., Bourry O., Pouillot R., Délicat A., Yaba P., Kumulungui B., et al. Ebola virus antibody prevalence in dogs and human risk. *Emerg. Infect. Dis.* 2005; 11(3): 385–90. https://doi.org/10.3201/eid1103.040981.
35. Heffernan R.T., Pambo B., Hatchett R.J., Leman P.A., Swanepoel R., Ryder R.W. Low seroprevalence of IgG antibodies to Ebola virus in an epidemic zone: Ogooué-Ivindo region, Northeastern Gabon, 1997. *J. Infect. Dis.* 2005; 191(6): 964–8. https://doi.org/10.1086/427994.
36. Lahm S.A., Kombila M., Swanepoel R., Barnes R.F.W. Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994-2003. *Trans. R. Soc. Trop. Med. Hyg.* 2007; 101(1): 64–78. https://doi.org/10.1016/j.trstmh.2006.07.002.
37. Georges A.J., Leroy E.M., Renaut A.A., Benissan C.T., Nabias R.J., Ngoc M.T., et al. Ebola hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and health control issues. *J. Infect. Dis.* 1999; 179(Suppl. 1): S65–75. https://doi.org/10.1086/514290.
38. Baron R.C., McCormick J.B., Zubeir O.A. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull. World Health Organ.* 1983; 61(6): 997–1003.
39. Mathiot C.C., Georges A.J., Fontenille D., Coulanges P. Antibodies to haemorrhagic fever viruses in Madagascar populations. *Trans. R. Soc. Trop. Med. Hyg.* 1989; 83(3): 407–9. https://doi.org/10.1016/0035-9203(89)90519-1.
40. Van der Waals F.W., Pomeroy K.L., Goudsmit J., Asher D.M., Gajdusek D.C. Hemorrhagic fever virus infections in an isolated rainforest area of Central Liberia. Limitations of the indirect immunofluorescence slide test for antibody screening in Africa. *Trop. Geogr. Med.* 1986; 38(3): 209–14.
41. Bouree P., Bergmann J.F. Ebola virus infection in man: a serological and epidemiological survey in the Cameroons. *Am. J. Trop. Med. Hyg.* 1983; 32(6): 1465–6. https://doi.org/10.4269/ajtmh.1983.32.1465.
42. Paix M.A., Poveda J.D., Malvy D., Bailly C., Merlin M., Fleury H.J. Serological study of the virus responsible for hemorrhagic fever in an urban population of Cameroon. *Bull. Soc. Pathol. Exot. Filiales.* 1988; 81(4): 679–82 (in French).
43. Tomori O., Fabiyi A., Sorungbe A., Smith A., McCormick J.B. Viral hemorrhagic fever antibodies in Nigerian populations. *Am. J. Trop. Med. Hyg.* 1988; 38(2): 407–10. https://doi.org/10.4269/ajtmh.1988.38.407.
44. Becker S., Feldmann H., Will C., Slenczka W. Evidence for occurrence of filovirus antibodies in humans and imported

monkeys: do subclinical filovirus infections occur worldwide? *Med. Microbiol. Immunol*. 1992; 181(1): 43–55. https://doi.org/10.1007/BF00193395.

45. Johnson B.K., Ocheng D., Gichogo A., Okiro M., Libondo D, Tukei P.M., et al. Antibodies against haemorrhagic fever viruses in Kenya populations. *Trans. R. Soc. Trop. Med. Hyg.* 1983; 77(5): 731–3. https://doi.org/10.1016/0035-9203(83)90216-x.
46. Nyakarahuka L., Schafer I.J., Balinandi S., Mulei S., Tumusiime A., Kyondo J., et al. A retrospective cohort investigation of seroprevalence of Marburg virus and ebolaviruses in two different ecological zones in Uganda. *BMC Infect. Dis.* 2020; 20(1): 461. https://doi.org/10.1186/s12879-020-05187-0.
47. Eigen M., Schuster P. The Hypercycle. A Principle of Natural Self-Organization. Springer-Verlag. 1979.
48. Zeng X., Blancett C.D., Koistinen K.A., Schellhase C.W., Bearss J.J., Radoshitzky S.R., et al. Identification and pathological characterization of persistent asymptomatic Ebola virus infection in rhesus monkeys. *Nat. Microbiol.* 2017; 2: 17113. https://doi.org/10.1038/nmicrobiol.2017.113.
49. Deen G.F., Broutet N., Xu W., Knust B., Sesay F.R., McDonald S.L.R., et al. Ebola RNA persistence in semen of Ebola virus disease survivors – final report. *N. Engl. J. Med.* 2017; 377(15): 1428–37. https://doi.org/10.1056/NEJMoa1511410.
50. Fischer W.A., Brown J., Wohl D.A., Loftis A.J., Tozay S., Reeves E., et al. Ebola virus ribonucleic acid detection in Semen more than two years after resolution of acute Ebola virus infection. *Open Forum Infect. Dis.* 2017; 4(3): ofx155. https://doi.org/10.1093/ofid/ofx155.
51. Forrester J.V. Ebola virus and persistent chronic infection: when does replication cease? *Ann. Transl. Med.* 2018; 6(Suppl. 1): S39. https://doi.org/10.21037/atm.2018.09.60.
52. Varkey J.B., Shantha J.G., Crozier I., Kraft C.S., Lyon G.M., Mehta A.K., et al. Persistence of Ebola virus in ocular fluid during convalescence. *N. Engl. J. Med.* 2015; 372(25): 2423–7. https://doi.org/10.1038/nri2940.
53. MacIntyre C.R., Chughtai A.A. Recurrence and reinfection – a new paradigm for the management of Ebola virus disease. *Int. J. Infect. Dis.* 2016; 43: 58–61. https://doi.org/10.1016/j.ijid.2015.12.011.