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Review

Bats as reservoirs of severe emerging infectious diseases



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ABSTRACT

In recent years severe infectious diseases have been constantly emerging, causing panic in the world. Now we know that many of these terrible diseases are caused by viruses originated from bats (Table 1), such as Ebola virus, Marburg, SARS coronavirus (SARS-CoV), MERS coronavirus (MERS-CoV), Nipah virus (NiV) and Hendra virus (HeV). These viruses have co-evolved with bats due to bats' special social, biological and immunological features. Although bats are not in close contact with humans, spillover of viruses from bats to intermediate animal hosts, such as horses, pigs, civets, or non-human primates, is thought to be the most likely mode to cause human infection. Humans may also become infected with viruses through aerosol by intruding into bat roosting caves or via direct contact with bats, such as catching bats or been bitten by bats.

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Contents

1. Introduction	2
2. Special features of bats in viral transmission and maintenance	2
3. Evidence for bat origins of emerging infectious diseases	2
3.1. Hendra virus (HeV)	2
3.2. Nipah virus (NiV)	3
3.3. SARS coronavirus (SARS-CoV)	3
3.4. MERS coronavirus (MERS-CoV)	3
3.5. Ebola virus	3
4. Routes for bat-borne viruses to transmit to humans	3
4.1. Intermediate host	3
4.2. Direct contact with bats	4
4.3. Aerosol transmission	4
5. Ecological drivers of bat-borne emerging infectious diseases	4
5.1. Factors that increase interaction between bats and humans	4
5.2. Factors that accelerate transmission in human society	4
6. Future directions	5
7. Conclusion	5
Acknowledgements	5
References	5

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1. Introduction

Bats have been known to be reservoirs of Rabies virus for a long time (Pawan, 1959a,b), and bats have also been considered as natural hosts of many common human and animal viruses, such as measles, mumps, parainfluenza, canine distemper and hepatitis C viruses (Drexler et al., 2012; Quan et al., 2013). However, bats have caught our attention very recently, due to their association with several severe emerging infectious diseases. Currently, bats have been considered to be natural reservoirs of SARS-CoV, MERS-CoV, NiV, HeV, Ebola virus, and Marburg viruses. These viruses have attracted global attention in recent years for their severity and/or easy transmission. This article reviews special features of bats in viral transmission and maintenance, bat's role as the reservoirs of some important viruses, the methods for bat-borne viruses' transmission to humans, and ecological drivers of bat-borne emerging infectious diseases.

2. Special features of bats in viral transmission and maintenance

Bats belong to the order *Chiroptera* (Greek means Hand-Wing) and can be further classified into two suborders: the *Yinpterochiroptera* (*Megachiroptera*, commonly known as megabats) and the *Yangchiroptera* (*microchiroptera*, commonly known as microbats) (Simmons, 2005). Megabats eat fruit and microbats live on insects, small mammals, fish or blood. Megabats have no echolocation and microbats possess echolocation (except for *Rousettus* and its relatives) (Table 1).

Bats have many features that enable them to carry a diversity of viruses. With approximately 1240 recognized species worldwide, bats account for about 25% of all mammalian species, making them the second largest order of mammals (Jones et al., 2005; Turmelle and Olival, 2009). The huge diversity of bat species can provide a large breeding ground for viruses. In addition, bats are ancient species and can be traced back to 52.5 million years ago (Clyde et al., 2001; Jepsen, 1966). The long evolutionary history provides long period for a variety of viruses to co-evolve with bats to make bats their natural reservoirs. In order to facilitate flying, bats developed hollow bones to reduce body mass; as a result, they do not have bone marrow as other mammals to produce B cells. This special immunological characteristics of bats may enable bats to carry a large number of viruses without obvious illness (Dobson, 2005). During winter time, some bats hibernate to conserve energy. Reduced body temperature and metabolic rate may

suppress immune responses and delay viral clearance from bats (George et al., 2011; Sulkin and Allen, 1974).

Some features of bats may keep viruses circulating among the bat population. Bats are social animals, millions of individuals can be found in a single cave. The large number of bats in a colony with physical proximity facilitates viral transmission among bats, maintaining viruses circulating stably among bats. A study showed that several emerging viruses could be amplified in a bat colony during the breeding season (Drexler et al., 2011). Microbats developed echolocation for navigation. Apart from ultrasonic waves, the vibration of the larynx can also generate aerosols, which may also play an important role in viral transmission among bats (Calisher et al., 2006).

Some features of bats can contribute to viral dispersal. With a large number of species, bats can be found in all continents except the Antarctica and inhabit various ecological niches, including trees, caves, and man-made structures, such as tunnels, deserted houses, even occupied houses in rural areas. The worldwide distribution and various habitats of bats pose the public to a general risk of infection with bat-borne viruses. Bats are special as the only mammals that can actually fly. Bats fly in their daily quest for food and seasonal migration, some of which can fly up to nearly 2000 km (Fleming and Eby, 2006). The ability to fly enables bats to carry viruses to distant areas. The eating behavior of bats can also be linked to viral transmission. Fruit bats cannot take a large amount of food, and to meet their demand for energy, instead of swallowing, they just chew to absorb sugars and higher energy components, and spit out the partially digested fruits. Discarded fruits contaminated by viruses in bat saliva may be eaten by other animals and infect them (Dobson, 2005). In addition, despite their small size, bats have a relatively long life span, most of the species live for 10–20 years and some can live up to 30 years (Brunet-Rossinni and Austad, 2004). The longevity of bats also increases the possibility of viral dispersal.

3. Evidence for bat origins of emerging infectious diseases

3.1. Hendra virus (HeV)

HeV (formerly known as equine morbillivirus) was first recognized in Australia in 1994, when it caused severe respiratory or neurological diseases in horses and humans (Murray et al., 1995). Many investigations were done in search for the natural reservoir of this new highly pathogenic virus. Initial screening of 2411 horses for HeV-specific antibodies showed negative results (Ward et al., 1996), and a later extensive serosurvey of 5264 sera from

Table 1
Summary for selective bat-borne viruses.

Virus	Putative host	Intermediate host	Modes of transmission	Drivers
Nipah virus	Flying foxes	Pigs	Close contact with the sick ones, drinking date palm juice contact with pigs	Climate changes, changes of farming practices (dual land use), transportation of pigs as merchandise, social/cultural practices, habitat destruction
Hendra virus	Flying foxes	Horses	Contact with horses	Climate changes, Urbanization, Social/cultural practices
SARS-CoV	Horseshoe bats	Palm civets	Slaughtering, farming of wildlife	Economic growth, desire for game meat, wildlife trading in wet markets, international travel
MERS-CoV	Bats ^a	Dromedary camels	Direct contact with camels, consumption of camel milk/meat	Not known
Ebola virus	Egyptian fruit bats	Non-human primates	Slaughtering, hunt for bush meat,	Preference for bush meat, burial practices, poor healthcare practice

^a Specific bat species not identified.

46 animal species, including both domesticated and wild animals, showed no evidence of infection. An epidemiological investigation pointed to fruit bats as a candidate natural reservoir, and serosurvey of 224 serum samples of fruit bats showed that 20 contained HeV-specific antibodies (Young et al., 1996). Three virus isolates were later obtained from urine and lung tissue from two of 460 individual fruit bats, and serological testing and sequencing of 200 nucleotides in the matrix gene indicated that the three isolates were identical to HeV (Halpin et al., 2000). Isolation of HeV, together with serological and epidemiological evidence indicates that fruit bats are natural reservoirs of HeV in Australia.

3.2. Nipah virus (NiV)

NiV first emerged in Malaysia in 1998, when it caused an outbreak of respiratory illness in pigs and encephalitis in humans. NiV and HeV were classified into a new genus, *Henipavirus*, within the family *Paramyxoviridae*. Studies also tend to link NiV to bats. Serological evaluation in outbreak areas of Malaysia showed that domesticated animals, such as dogs, cats and ponies had NiV-specific antibodies (Chua et al., 2000), while wild boar, hunting dogs and rodents were all negative (Yob et al., 2001). An extended survey of 14 species of bats highlighted that two fruit bat species had relatively high seropositivity, 31% and 17% for small flying fox (*Pteropus hypomelanus*) and large flying fox (*P. vampyrus*), respectively. NiV was isolated from *P. hypomelanus* urine and their partially eaten fruits, which was nearly identical to isolates from pigs and humans (56 nt changes) (AbuBakar et al., 2004; Chua et al., 2002b). Recently, NiV was isolated from *P. vampyrus*, which was about twice the difference between the human and *P. hypomelanus* isolates (98 nt changes) (Rahman et al., 2010). Serological findings and viral isolation indicate that fruit bats are natural reservoirs of NiV.

3.3. SARS coronavirus (SARS-CoV)

SARS-CoV is the causative agent of SARS (severe acute respiratory syndrome), which emerged as a new clinical severe human disease in Guangdong Province of China in 2002. Initially, palm civets sold in live animal markets were demonstrated to carry SARS-CoV and were considered as the reservoirs of SARS-CoV (Guan et al., 2003), but later studies pointed to bats as the source of SARS-CoV. One group of researchers took bat samples from the natural environment, with 408 bats representing nine species. Three species of horseshoe bats (genus *Rhinolophus*) showed relatively high seroprevalence of SARS-neutralizing antibodies, ranging from 28% to 71%. In addition, bat-CoV RNA was demonstrated 92% identity with human SARS-CoV isolates (Li et al., 2005). Another group of researchers screened nasopharyngeal and anal swabs of 120 bats and found that bat-CoV sequences detected in 23 Chinese horseshoe bats (*Rhinolophus sinicus*) manifested 88% identity with the SARS-CoV, with great differences in spike genes (Lau et al., 2005). These bat SARS-CoVs may be the progenitors of SARS-CoV, but cannot be closely related, since they were phylogenetically disparate from SARS-CoV and were unable to use the SARS-CoV cellular receptor molecule, human angiotensin converting enzyme II (ACE2), to enter cells (Ren et al., 2008). Later, a study reported the whole-genome sequences of two novel bat-CoVs detected in Chinese horseshoe bats, which were far more closely related to SARS-CoV than any previously identified ones, especially in the receptor binding domain of the spike protein, and they also isolated live SARS-like CoV from bat fecal samples. *In vitro* testing showed that this virus had a broad species tropism and used ACE2 from humans, civets and Chinese horseshoe bats for cell entry (Ge et al., 2013). Thus Chinese horseshoe bats are considered to be the natural reservoirs of SARS-CoV.

3.4. MERS coronavirus (MERS-CoV)

MERS-CoV is a novel coronavirus, which was isolated from a man with acute pneumonia and subsequent renal failure in Saudi Arabia in 2012 (Zaki et al., 2012). Full genome sequence analysis of MERS-CoV and serological data provided evidence for transmission of MERS-CoV from camels to humans (Azhar et al., 2014), and MERS-CoV specific antibodies and RNA have also been detected in camels during MERS-CoV outbreak investigations (Reusken et al., 2013). However, phylogenetic analysis showed that MERS-CoV belonged to lineage C of the genus *Betacoronavirus*, along with the bat-CoV HKU4 and HKU5 (van Boheemen et al., 2012). Hence, bats are supposed to be the natural reservoirs of MERS-CoV, while camels may just act as intermediate animal hosts that facilitate the spillover from bats to humans. Recent studies have revealed a diversity of MERS-related CoVs in bats from Saudi Arabia (Memish et al., 2013), Africa (Ithete et al., 2013), Europe (Annan et al., 2013), and Asia (Yang et al., 2014). Studies on receptor usage by MERS-CoV can also give a clue of bat origins. Initial study found that the receptor use by MERS-CoV was different from the one used by SARS-CoV. The receptor may be a conserved one, since MERS-CoV can replicate in both bat and human cells (Muller et al., 2012). Subsequently, dipeptidyl peptidase 4 (DPP4 or CD26) was identified as the cellular receptor for MERS-CoV (Raj et al., 2013). Bat coronavirus HKU4, which is closely related to MERS-CoV, can also use DPP4 as receptor to initiate cellular entry, and this discovery indicated that bats might be the natural reservoirs of MERS-CoV (Wang et al., 2014).

3.5. Ebola virus

Ebola virus first came into our knowledge in the Democratic Republic of Congo in 1976 (Johnson et al., 1977; Report of an International, 1978). Since then, this notorious virus has been decimating gorilla, chimpanzee and human populations in Africa with high mortality. During the 2013–2014 epidemic, Ebola virus has caused 21,121 human infections and 8304 deaths (as of January 9th, 2015, <http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/index.html>). During Ebola outbreaks in humans and great apes between 2001 and 2003 in Gabon and the Republic of Congo, to investigate the natural reservoirs, more than 1000 small vertebrates were captured. Serum antibodies specific for Ebola virus were found in three different bat species with prevalence as high as 25% (Leroy et al., 2005). Given the lack of overt disease after being infected, bats are considered as likely natural reservoirs of Ebola virus.

4. Routes for bat-borne viruses to transmit to humans

The viruses discussed above tend to be restricted to certain geographic regions with a particular bat reservoir, such as HeV and NiV associated with flying foxes in Australia and Southeast Asia and Ebola virus associated with Egyptian fruit bats in Africa. Most bat population roost in tropical forests or caves and they do not make frequent contact with people. How bats transmit viruses to humans is a mystery until now. Below are the hypotheses of how bat-borne viruses are transmitted to humans.

4.1. Intermediate host

A popular theory for bats to transmit viruses to humans is through intermediate hosts, which are in close contact with humans and can amplify viruses. When fruit bats eat fruit, they contaminate the fruit with viruses in their saliva and urine; discarded fruit fall to the ground and is consumed by intermediate hosts, such as pigs, horses, and nonhuman primates; humans become

infected by contact with or consuming products of the intermediate animal hosts. Flying foxes are thought to transmit NiV/HeV in Southeast Asia and tropical Australia. During the 1998 NiV outbreak in Malaysia, it is thought that pigs were infected by NiV by consuming mangoes contaminated by flying foxes. Mangoes were a food source for flying foxes, and discarded mangoes contaminated by the saliva and urine of the bats fell into the pigsties and were consumed by pigs, resulting in the cross-species infection of pigs and subsequently humans (Chua et al., 2002a). HeV is endemic in flying-foxes in Australia. HeV spills over from bats to domestic animals, primarily horses, which amplify the virus and subsequently infect humans (Murray et al., 1995). Bats contaminate a drip zone around trees where they feed or roost by excreting urine, feces and saliva (with partially eaten fruit). Horses may be exposed to HeV when consuming contaminated grass, fruit, feed or water; or when browsing or sniffing contaminated surfaces within this drip zone (Plowright et al., 2015). Fruit contaminated by bats is also thought to transmit Ebola virus to apes in Central Africa (Leroy et al., 2005). In the case of the 2003 SARS outbreak in China, the transmission of SARS-CoV from bats to humans was made possible by the special taste for wildlife cuisine, including civets, which acted as intermediate hosts for SARS-CoV. In South China, civets are thought to strengthen health in winter, and the demand was so high that farming of this wildlife species was widespread. Trade of civets in live wet markets exposed other susceptible animals. Therefore, bat SARS-CoV was likely introduced into humans through civets and other animals (Liu, 2003). As for MERS in Middle East, dromedary camels are hypothesized as intermediate animals to transmit MERS-CoVs from bats to humans (Memish et al., 2014). Camels are popular in Middle East countries both for entertainment and transportation. People may be infected through direct contact with infected camels, which can shed virus in respiratory secretions (Azhar et al., 2014). MERS-CoVs were also detected in camel milk samples from mares infected with the virus (Reusken et al., 2014). Whether the viruses is excreted in the milk or is introduced as contaminant during the milking process, it poses a risk for people to become infected by consumption of unpasteurized camel milk.

4.2. Direct contact with bats

Although bats are rarely in contact with people, people may become infected with bat-borne viruses by consuming bat meat. Wild animal meat or 'bush meat', including bats, are taken as delicacies in some regions of Africa. The capture and selling of wild animals increases the risk of being infected by zoonotic viruses. Consumption of infected bats may transmit such bat-borne viruses as Ebola virus to humans. In 2007 Ebola hemorrhagic fever re-emerged in the Democratic Republic of Congo (DRC) causing 186 deaths. Epidemiological investigation showed that the outbreak was due to consumption of fruit bats, which were migrating towards the southeast and were massively hunted as a major source of protein by local villagers (Leroy et al., 2009). Consumption of well-cooked bat meat may not be a problem, but the handling of bats may increase the risk of infection.

In addition, accidental bite by a bat may result in Rabies. One example is the case of an elderly man in South Africa who died of Duvenhage virus (DUVV) infection after being scratched by a bat (Adjemian et al., 2011).

4.3. Aerosol transmission

Some bats with a large population roost in caves. Bat-borne viruses may contaminate air in caves where bats live and people may become infected by inhaling viruses in the air when they enter caves. Research showed that cynomolgus macaques could suffer a lethal viral hemorrhagic fever after exposure to small-particle

aerosols containing MARV-Angola (Alves et al., 2010). There was a report that humans were infected by Marburg virus after visiting or working in caves in Africa, and transmission by aerosol could not be excluded as a possible mode of infection (Timen and Koopmans, 2008).

5. Ecological drivers of bat-borne emerging infectious diseases

For a spillover to occur, a range of conditions and events must be met. Bats must be present and be infected, in most cases shedding viruses. Viruses excreted by bats must survive in the environment (if transmitted indirectly), with access to intermediate animals. Human or intermediate animals must be exposed to a sufficient dose of virus for an infection to be established, and humans or intermediate animals must be susceptible to the virus (Plowright et al., 2015). However, of all these conditions required for a spillover, it is of utmost importance for bats and humans or animals in close contact with humans to be present in the same habitat, which can be a result of intrusion into each other's living environment. Once infected, some factors can accelerate transmission in human society. Below we describe ecological factors that increase interaction between bats and humans and factors that accelerate transmission in humans.

5.1. Factors that increase interaction between bats and humans

In human history, the emergence of new pathogens has been accompanied by increased density of humans and other animals. With human population explosion, more space and resources are needed to meet ever-increasing demand for food and housing. Factors that contribute to the intrusion of bats into human living environment can be summarized into a 'push' and a 'pull' (Brüssow, 2012). A 'push' refers to the enormous demand for more space and resources brought by the human population explosion, which leads to the destruction of bat habitats and shortage of food. Natural environmental changes, such as typhoons and droughts, can also place stresses on bats. A 'pull' involves the living environments built by humans, characterized by urbanization, intensive agriculture and food animal breeding, which attracts bats into human living environments for an abundant of food supply. The 1998 NiV outbreak in Malaysia was thought to be a combined result of anthropogenic deforestation and drought caused by *El Nino*, which pushed flying fox bats from their natural habitats into farms (Chua et al., 2002a).

5.2. Factors that accelerate transmission in human society

The rapid development of modern transportation, and the mobility of people, animals and goods lead to the rapid spread of emerging infectious diseases. Local transportation is important in sustaining endemics, while international air travel may facilitate cross-continent spread (Bobashev et al., 2008). During the 1998 NiV outbreak in Malaysia, the transport of infected pigs led to the expansion of outbreak areas in Malaysia (Lam and Chua, 2002), and the follow-up NiV outbreak in Singapore among abattoir workers (Paton et al., 1999). Due to air transit, the SARS outbreak that initially emerged in Guangdong, China in November of 2002, quickly spread to 25 countries as far away as Canada by the end of March of 2003 (CDC, 2003). Another example is the 2014 Ebola hemorrhagic fever outbreak, the largest Ebola virus outbreak to date, which quickly spread in West Africa and resulted in massively affected areas including Guinea, Liberia, Sierra Leone and Nigeria. Ebola virus also spread to the USA by infected patients who traveled from West Africa (Von Drehle, 2014).

Cultural practices can also play an important role in the transmission of emerging infectious diseases. During the 2001

NiV outbreak in Bangladesh, person-to-person transmission was attributed to the social norms that require family members to maintain close physical contact with the sick one (Luby et al., 2009). In Africa, traditional burial practices require the living ones to make contact with the deceased, which also facilitates transmission during Ebola virus outbreaks (Dowell et al., 1999).

Socioeconomic factors may also contribute to transmission, associated with poor health care. One example is the 2014 West Africa Ebola virus outbreak, first-line health care workers were infected, because of lack of knowledge as well as the unavailability of proper personal protective equipment (Kilmarx et al., 2014). Person-to-person transmission during the 2001 Bangladesh NiV outbreak was also thought to be a result of poverty-induced sharing of eating utensils and food with the infected person (Brüssow, 2012).

6. Future directions

With ever-increasing interest in bat-borne viruses, and the availability of molecular biotechnology, novel viruses are continually detected or isolated from bats all around the world. In recent years, viral metagenomics may provide a glimpse of bat viral diversity (Dacheux et al., 2014; Donaldson et al., 2010; Ge et al., 2012; He et al., 2013). So far, viruses detected in bats can be classified into as many as 22 families and many are novel viruses (<http://www.mgc.ac.cn/DBatVir/>). The pathogenicity of many of these novel viruses for humans remains unknown, and further efforts are needed to determine their potential threats to humans. In addition, previously known bat-borne viruses are also being detected in more bat species, and the geographic distribution of these viruses is also expanding. With human activity increasingly overlapping the habitats of bats, emerging infectious diseases from bat-borne viruses will undoubtedly increase. Transmission modes discussed above are just current hypothesis that could theoretically explain some of the spillover events. However, the spillover process is still something of a black box that is scarcely understood and much more research is needed to expand our understanding of the spillover events. There are many questions that remain to be answered about the ecology of bat-borne infections: (1) Are bats the natural reservoirs or just transient carriers of these novel viruses? (2) How long can bats harbor these viruses? (3) What are the routes of virus shedding? (4) How long can viruses excreted by bats survive in the environment? (5) and What are the risk factors leading to infection of humans or intermediate animals exposed to bat-borne viruses?

7. Conclusion

With special social, biological and immunological features, bats provide unique niches for many viruses to co-evolve with them. Spillover of bat-borne viruses to humans may cause severe diseases and panic. Bats have been proposed as the natural reservoirs of viruses causing severe diseases in humans, such as NiV and HeV in Southeast Asia and Australia, Ebola and Marburg viruses in Africa, SARS-CoV in Asia and MERS-CoV in Middle East. However, the role of bats in transmission of these infectious diseases needs to be further investigated because of lack of direct experimental data on transmission of the viruses from bats to intermediate animal hosts. In addition, bat-borne viruses may have other reservoirs. In a recent study a novel Henipa-like virus was detected in rats and was responsible for 3 deaths of mine workers in Yunnan Province, China, suggesting that bats may not be the only reservoir of these viruses (Wu et al., 2014).

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