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Bats and Viruses: a Brief Review

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Abstract: Bats, probably the most abundant, diverse and geographically dispersed vertebrates on earth, have recently been shown to be the reservoir hosts of a number of emerging viruses responsible for severe human and livestock disease outbreaks. Flying foxes have been demonstrated to be the natural reservoir for Hendra and Nipah viruses. Evidence supporting the possibility of bats as potential reservoirs for SARS coronavirus (SARS-CoV) and Ebola virus has also been reported. The recent discovery of these viruses and other viruses occurring naturally in the bat population provides a unique insight into a diverse pool of potentially emergent and pathogenic viruses. The factors which influence the ability of zoonotic viruses to effectively cross the species barrier from bats to other animal populations are poorly understood. A brief review is provided here on the recently emerged bat viruses and on current and future strategies for research in this area.

Key words: Bats; Animal reservoir; Spillover; Emerging zoonoses; Virus

Bats always have had a mysterious presence in almost every ancient culture for no clear reason other than that they are the only flying mammals. Bats represent approximately 20% of all known mammals on earth and are divided into two suborders, Megachiroptera and Microchiroptera (27). Bats evolved and diverged from other mammals very early. Although there is no conclusive scientific data to pinpoint their first appearance, it is estimated that they originated about 50 million years ago (28).

The first recognition of bats as carriers of viruses came during the 1920s when rabies virus was identified in bats in South and Central America. Although the study of rabies and bats remains active even to this present day, the importance of bats as reservoirs of zoonotic viruses have been largely unappreciated until the middle 1990s (3).

EMERGING BAT VIRUSES

Hendra virus

The first contemporary bat zoonotic virus emerged in 1994 in Australia, resulting in the death of more than ten horses and one human (21). The virus was named Hendra virus, and identified as a novel paramyxovirus. It was classified as a Biosafety Level 4 (BSL4) agent due to its high mortality and lack of treatment. Fruit bats in the genus *Pteropus* were later identified as the reservoir of Hendra virus (14; 33). Since the first recognised outbreak, there have been at

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least ten additional outbreaks of Hendra virus in Australia and several of them involved human infections. The latest outbreaks in 2008 resulted in the death of a veterinarian working with infected horses.

Nipah virus

Approximately five years after the first Hendra virus outbreak, a very closely related virus, the Nipah virus, emerged in Malaysia, believed to be the result of a bat-to-pig spillover event(s). This was followed by massive pig-to-pig transmission and eventually led to pig-to-human transmission, and claimed more than 100 human lives in Malaysia and Singapore (4). A different strain of the Nipah virus has since emerged in India and Bangladesh, causing outbreaks almost on a yearly basis, with a mortality of up to 70 to 90%. Epidemiological investigations suggest that this Nipah virus strain is capable of direct bat-to-human and human-to-human transmission, raising the possibility of a much larger outbreak in humans (13; 15).

Hendra and Nipah viruses are now classified into a separate genus, *Henipavirus*, in the family *Paramyxoviridae* (8).

Virus isolation, PCR and serological studies indicated that related henipaviruses are widely spread among different bat populations, from Australia, Indonesia, Papua New Guinea, Thailand, Malaysia, Cambodia to Madagascar and western Africa. A related virus(es) may also be circulating among bats in China (19).

Menangle virus

This virus was isolated in 1997 from stillborn piglets with deformities at a large commercial piggery in New South Wales, Australia and was found to be responsible for a single outbreak of reproductive disease, causing reduced farrowing rates and stillbirths with deformities. Serological investigation of persons in contact with pigs revealed that two humans, who were in close contact with infected pigs and suffered an influenza-like illness, had high levels of convalescent neutralizing antibodies to Menangle virus (24). A recent study found that neutralizing antibodies against Menangle virus can be detected 46% of black flying foxes (*Pteropus alecto*), 41% of greyheaded flying foxes (*P. poliocephalus*), 25% of spectacled flying foxes (*P. conspicillatus*) and 1% of little red flying foxes (*P. scapulatus*) in Australia. In addition, virus-like particles were observed by electron microscopy in faeces from *Pteropus* spp. and reactivity was detected in pooled faeces and urine by immunogold EM using sera from sows that had been exposed to Menangle virus, although no live virus was isolated from these samples (25).

Tioman virus

During a search for the reservoir host of Nipah virus, urine samples were collected from bats on Tioman Island off the eastern coast of peninsular Malaysia. A novel paramyxovirus (named Tioman virus) was isolated in addition to Nipah virus and Pulau virus (a novel orthoreovirus, see below). Molecular and antigenic studies indicated that Tioman virus is most closely related to Menangle virus and both are placed within the genus *Rubulavirus* in the family *Paramyxoviridae* (7). The potential of Tioman virus to infect and cause disease in human or other animals is unknown, however our recent studies have demonstrated that pigs are susceptible for infection by Tioman virus (31) and that there is serological evidence for infection of humans by this virus on Tioman Island (32). It is highly possible that Tioman virus can cause mild flu-like symptoms which are Mis-

diagnosed or undiagnosed.

SARS-like coronaviruses

The severe acute respiratory syndrome (SARS) virus was responsible for the first serious and widespread zoonotic disease outbreak of the 21st century which exhibited efficient human-to-human transmission (23). Although zoonotic SARS virus strains were isolated from civets and raccoon dogs (12), and viral genomic materials were detected in cats, pigs and other animals (30), none of these animals were the natural reservoir of the virus. In 2005 two groups independently found that horseshoe bats in the genus *Rhinolophus* carry a group of coronaviruses which are closely related to the outbreak strains (16, 18). These viruses, named SARS-like coronaviruses, have an almost identical genome organisation and a high sequence similarity with the SARS virus strain responsible for the outbreaks of disease in humans. The spike protein (S), responsible for virus-receptor recognition, contains the only major difference between the human SARS virus strain that of the bat SARS-like coronaviruses. In a recent study, it has been shown that a recombinant bat SARS-like coronavirus was able to infect susceptible cells and mice when its receptor binding site in the S protein was replaced with that of the human SARS virus (1). Taking together, these studies suggest that bats carry a variety of coronaviruses and some of them are capable of spill over into human populations, probably via an intermediate adapting and amplifying host, and cause severe disease like SARS.

Ebola virus

As one of the most deadly viruses known to infect humans, the origin of Ebola virus remains a mystery. However, a recent study has shown that bats of three different species (*Hypsignathus monstrosus, Epomops franqueti* and M*yonycteris torquate*) captured in Gabon and the Republic of Congo seemed to be susceptible for Ebola virus infection, with evidence provided by the detection of virus-specific RNA or antibodies in several individual bats (2, 17). Since similar findings have been reported previously in rodents (20), further research is needed to define the exact natural reservoir host(s) of Ebola viruses.

Marburg virus

Soon after the discovery of Ebola virus RNA and antibodies in fruit bats, the same group conducted similar studies for Marburg virus and reported the detection of virus-specific RNA and antibodies in individual bats of the common species (*Rousettus aegyptiacus*) in Gabon (29). These results provide further evidence that bats are natural reservoir of filoviruses.

Melaka virus and related orthoreoviruses

First discovered in the early 1950s, reoviruses (respiratory enteric orphan viruses) were not known to be associated with any disease, and hence named orphan viruses. During an investigation of a flu-like disease outbreak in 2006 in a family in Melaka, Malaysia, a novel orthoreovirus was isolated and named Melaka virus (5). Serological and epidemiological studies confirmed that Melaka virus is the causative agent of the acute respiratory diseases suffered by multiple members of the family and that the virus is capable of human-to-human transmission. Epidemiological tracing indicated that the virus was most likely introduced from a bat "visiting" the residential house of the family about a week prior to the onset of disease in the first patient of the outbreak cluster. Molecular sequencing further revealed that

Melaka virus is closely related to two previously isolated bat orthoreoviruses, the Nelson Bay virus isolated in the 1970s in Australia (11) and the Pulau virus isolated in 1999 on Tioman Island, Malaysia (26). A retrospective serological study revealed that 13% of human volunteers (n=109) from Tioman Island were seropositive to this group of viruses, indicating that bat orthoreoviruses may infect humans more frequently than reported. This notion is supported by recent studies from two independent groups as described below.

A Melaka-like virus, named Kampar virus, was isolated from a throat swab of a male patient in Kampar, Perak, Malaysia who was suffering from high fever, acute respiratory disease and vomiting at the time of virus isolation (6). Serological studies indicated that Kampar virus was transmitted from the index case to at least one other individual and caused respiratory disease in the contact case. Sequence analysis indicated that Kampar and Melaka viruses are closely related. This was confirmed by virus neutralization assay, showing an effective cross neutralization between the two viruses.

Recently, another related virus was isolated in Hong Kong from a human subject who had potential exposure to bats in Bali (viral genomic sequences deposited in GenBank with Accession number EU165526).

These studies highlight the increasing trend of emergence of bat zoonotic viruses and the need to expand our understanding of bats as a source of many unknown viruses.

WHY NOW AND WHY BATS?

These are obviously very important questions which are difficult to obtain a definitive answer. In general, it is believed that the following factors contribute to the emergence of new zoonotic pathogens including bat viruses: 1) Environmental changes; 2) Anthropogenic activities; 3) Virus evolution; 4) Improved detection technology.

Using the emergence of henipavirus as example, we can see these factors all play a role to some degree. In Australia, it has been shown that the distribution of *Pteropus alecto*, the main reservoir host for Hendra virus, has undergone a rapid southward shift in the last eighty years, extending its presence from approximately 200 km north of Brisbane in the 1920s to the Sydney area in the 2000s (A. Breed, personal communication). In another word, there would have been little or no chance for Hendra virus spillover in the Brisbane area in the early 20th century since there was no *P. alecto* in the region. For Nipah virus outbreaks, the intensive pig farming practice and the business practice of combining pig farming with fruit growing in the same geographical area were mainly responsible for the initial spillover of Nipah virus from bats to pigs. Since the banning of fruit growing in the vicinity of pig farms, there has not been any spillover events detected in the last decade (9). The genetic evolution /diversity of henipaviruses provides the clue to why Nipah viruses from the Bangladesh/India region exhibited significantly different properties to those in Malaysia (15). Finally, modern virus detection/isolation, electron microscopy and molecular techniques played a vital role in the rapid discovery of Hendra virus as the causative agent of both horse and human diseases (21, 22), which in turn facilitated the rapid identification of Nipah virus as the aetiological agent of the major disease outbreaks in pigs and humans (4).

Is it unusual that so many emerging zoonotic viruses

originated from bats? The answer to this question will be "yes" or "no" depending on how the question is examined. From an absolute "number game" point of view, bats represent approximately 20% of all known mammals, yet there is only about 2% of human pathogens that are of bat origin. It seems that bat pathogens are underrepresented. However, if one looks at the major emerging zoonotic viral diseases of the last few decades, bat viruses are over represented. For example, there are many more "new" bat paramyxoviruses than "new" rodent viruses identified in the last 20-30 years, although there are more rodent species than bat species (10). Also, rodents are expected to have more chance of contacts with other land mammals than bats.

Another approach is to examine bat biology to see if there is anything unique in bats which may make them more suitable as virus reservoir host. Unfortunately, due to the very limited knowledge we have in bat biology, it is premature to make any conclusion in this respect. Nevertheless, the following combination of features has been identified as unique to bats. Bat species are the second most diverse among all mammals on earth (next only to rodents); they are most abundant in terms of individual numbers; they can have a very long life span in relative to their physical sizes; they live in large colonies (up to a few millions at one time and in one location); they can fly long distances and are distributed worldwide with the only exception, Antarctica; they feed on insects, mammals, fish, blood, fruit and pollen; most of them use echolocation to navigate; they are capable of hibernation and torpor; and last but not least, they have hollow bones as in birds. Whether any or some of the above -listed features make bats a more suitable viral host remains to be elucidated.

FUTURE RESEACH DIRECTIONS

As discussed above, with the increasing trend of novel zoonotic viruses emerging from bats of different species, there is an urgent need to conduct basic studies into bat biology, bat immunology and bat ecology in order to better understand bat-virus interaction. The future direction of research in this area should therefore be directed to the following:

1) Bat genomics and transcriptomics: many of the basic biological questions can be addressed in much greater detail if the genome sequence of selected bat species is known.

2) Development of reagents for bat immunological studies: for example, we are not able to determine IgM levels for bats due to the lack of IgM-specific reagents. This is also true for T-cell immunology studies. So there is an urgent need to develop these basic reagents before any immunological studies can be carried out.

3) Establishment of bat cell lines: currently there are only two bat cell lines available in the international community, which are not suitable for in-depth immunological studies. We are in the process of establishing different cell lines of pteropid bats (reservoir species of many known zoonotic viruses) to address this shortage.

4) Combination of reverse genetics and animal infection studies: to better understand the pathogenesis and virus-host interaction, we believe that it is essential to establish reverse genetics systems for key bat viruses so that well-defined molecular markers can be tested in conjunction with infection of live animals (bats and other mammals).

5) International network: many of the epidemiology studies need very close collaboration of different groups in different countries, each with a complimentary skills base. Although we have enjoyed such networks in the past for our hunt for new bat viruses, there is a need to expand and formalize such networks so that a more rapid and effective response can be mounted should there be new bat virus outbreaks.

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