BATS: CARRIERS OF ZOONOTIC VIRAL AND EMERGING INFECTIOUS DISEASES

Koushlesh Ranjan¹*, Minakshi Prasad² and Gaya Prasad³

¹Department of Veterinary Physiology and Biochemistry, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India, 250110
²Department of Animal Biotechnology, LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India, 125004
³Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India, 250110

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ABSTRACT

Bats are reported as reservoir host for several viruses, which cause significant illness in human and animals. Some of the bat transmitted zoonotic viral diseases such as Ebola, Hendra, Nipah and rabies may cause severe human casualties. They also harbor several other viruses such as MERS and SARS corona viruses, which may cause disease in human through direct spillover to human or through an intermediate host or vectors. Being reservoir hosts bats do not get affected by these viruses. This probably may happen due to the specificity of bat immune system, which reacts differently with viral pathogens in comparison to their other mammalian counterparts. Although bats are important reservoir hosts for several zoonotic viruses, very little information is available regarding host/virus relationships as only few experimental studies have been done on bat colonies, lack of expertise for study of bat immunology and antiviral responses and difficulty in conducting field work. However, with the advancement in epidemiology and molecular biology, these problems can be addressed, which will provide the insight into interactions of bats and zoonotic viruses. It may also clarify regarding virus persistence in nature and various associated risk factors which might facilitate viral transmission to animals and humans.
1 Introduction

Bats are the most diverse, abundant and geographically dispersed member of vertebrate family. Despite enough information since ancient age, still reliable information is required to explain the diversity in their lifestyle, anatomy, role in ecosystems ecology and as reservoir hosts for viruses of medical and veterinary importance. Bats can survive in diverse climate. They are found in all continents except Antarctica. Different bat species may feed on several food materials such as mammals, blood, insects, fish, pollen and fruit. Bats are also recognized as reservoir hosts for several zoonotic viruses which can infect humans and animals (Hayman et al., 2013). Although they can carry several zoonotic viruses, they are also valuable elements of terrestrial biotic communities. They play a significant role in insect control, pollination of plants and reseed the cut forests which are essential for survival of human and animal life (Hill & Smith, 1984; Kunz & Fenton, 2003).

Bats harbor a range of emerging infectious viral pathogens. Many of such emerging infectious diseases (EIDs) are zoonotic in nature (Woolhouse & Gowtage-Sequeria, 2005; Jones et al., 2008; Dharma et al., 2013). In developing countries, the zoonotic viral infections especially caused by RNA viruses such as rabies, Ebola etc. have been recognized as significant threats for human health (Maudlin et al., 2009; Dharma et al., 2015). In addition to rabies and other lyssaviruses (Streicker et al., 2010), bats have also been reported as reservoir hosts for several other viral pathogens such as Hendra virus (HeV) (Halpin et al., 2000; Edson et al., 2015), severe acute respiratory syndrome-coronavirus (SARS-CoV) (Li et al., 2005; Vijaykrishna et al., 2007), Ebola virus (EBOV) (Leroy et al., 2005), Nipah virus (NiV) (Chua J et al., 2002a; Chua et al., 2002b; Reyes et al., 2005) and Marburg virus (MARV) (Peterson et al., 2004; Towner et al., 2007). In USA, a new lineage of influenza A virus has also been reported from little yellow shouldered bats (Sturnura lilium) (Tong et al., 2012). Several other Paramyxoviruses have also been reported from bats from various regions of the globe (Drexler et al., 2012). Since, bats are reported as reservoirs for several viral EIDs (Table 1), it is crucial to understand that how bat ecology may influence zoonotic disease outbreaks and their role as reservoirs for emerging viral pathogens (Messenger et al., 2003; Calisher et al., 2006; Wong et al., 2007; Hayman et al., 2013). Several new viral pathogens are identified in bats every year which need to be characterized for their zoonotic potential to human population. Most of such studies are mainly focused on zoonotic infectious diseases of medical and veterinary importance.

This review paper is focused on bat associated zoonotic viruses causing diseases to animals and human. Several bat species play important role in maintenance and transmission of zoonotic viruses which explains the requirement of special consideration for characterization of bats from other mammals.

2 Bat Immunology

It is observed that several viruses which are highly pathogenic for human and animals can infect and persist in healthy bats without causing significant harm to them. Possibly it may be due to the fact that bats were evolved earlier among mammalian species and their acquired and innate immune responses have significant differences from other animal species such as rodents and primates. It is also assumed that bat’s immune system react differently with pathogens which lead to control virus replication with persistence of infectious virus in bat tissues (Schountz, 2014). This results in prevention of immunopathological responses in infected bat tissues. However, within several bat species significant differences in immune responses against viral infection may be found.

Although very little is known about bat immunology, several studies have shown that bat immune responses also have some similarities with mammals that evolved after bats. Several immunoglobulin classes such as IgG, IgA, and IgM found in mammals have also been purified from great fruit-eating bat (Artibeus lituratus) sera (McMurray et al., 1982). The lymphoid development of bats and other mammals are also very similar which is evidenced by identification of B and T lymphocytes, Macrophages and cells expressing surface Ig in bone marrow of Indian bats (Pteropus giganteus) (Schountz et al., 2004). The serological assays against several viruses such as severe acute respiratory syndrome-coronavirus (SARS-CoV) like viruses, Hendra virus and Ebola virus in bats (Lau et al., 2005; Leroy et al., 2005) indicate that virus specific adaptive B and T cell responses might occur despite persistent virus infection. However, further study is required to understand the mechanism of antibody synthesis, cytokine synthesis, lymphocyte proliferation etc. in bats.

3 Zoonotic viruses in bats

Bats harbor several viruses as reservoir host. Many of these viruses have not been reported to transmit from bats to human or other mammals. However, several viruses of bats such as Nipah and Hendra virus, rabies virus and related lyssaviruses, SARS-CoV-like virus etc may be transmitted to human and animals and lead to highly pathogenic disease (Table 1). Some other viruses such as certain flaviviruses, alphaviruses and bunyaviruses may also infect bats via vectors. However, it is not established that whether bats act as important reservoir hosts for such viruses.

3.1 Rabies Virus

A lot of scientific information is available regarding rabies virus, its transmission and pathogenesis in human and animals. Rabies was described in ancient literature in around 4000 years ago. However, its scientific study started in late 19th century. Louis Pasteur amplified the rabies virus in spinal cord of rabbit and prepared vaccine for post exposure prophylaxis.
Table 1 Zoonotic viruses causing disease in human and their bat reservoir hosts.

| S. No. | Disease                     | Virus                                         | Reservoir Host                                      | References                  |
|--------|-----------------------------|-----------------------------------------------|---------------------------------------------------|-----------------------------|
| 1      | Rabies                      | Rabies virus and other lyssaviruses           | Several bat species distributed worldwide         | Rupprecht et al., 1995; Calisher et al., 2006; López-Roig et al., 2014 |
| 2      | Ebola virus disease         | Ebolaviruses                                  | Franquet’s epauletted fruit bat (Epomops franquetti), Hammer headed bat (Hypsognathus monstrosus), little collared fruit bat (Myonycteris torquata) | Leroy et al., 2005          |
| 3      | Marburg virus disease       | Marburg virus                                 | Egyptian fruit bat (Rousettus aegyptiacus)         | Towner et al., 2007         |
| 4      | Middle east respiratory syndrome | MERS-CoV                                      | Egyptian tomb bat (Taphozous perforatus)           | Memish et al., 2013         |
| 5      | Severe acute respiratory syndrome | SARS-CoV                                      | Chinese horseshoe bat (Rhinolophus spp.)          | Lau et al., 2005            |
| 6      | Severe acute febrile disease | Sosuga virus                                  | Rousettus spp.                                    | Albarino et al., 2014; Amman et al., 2015b   |
| 7      | Encephalitis                | Nipah and Hendra viruses                      | Some flying foxes (Pteropus spp.)                 | Chua et al., 2002; Halpin et al., 2000 |
| 8      | Encephalitis                | Tioman virus                                  | Pteropus hypomelanus                               | Chua et al., 2001; Yaiw et al., 2008 |
| 9      | Menangle virus disease      | Menangle virus                                 | Little red flying foxes and gray headed flying foxes | Philby et al., 1998; Barr et al., 2012 |

Rabies virus belongs to the family Rhabdoviridae, genus Lyssavirus and transmitted between several mammals, including bats. Rabies transmission is primarily mediated by bite inoculation of virus available in saliva of rabies infected individuals. Three species of bats viz. Diaemus youngi (white-winged vampire bat), Diphylia caudata (hairy-legged vampire bat) and Desmodus rotundus (vampire bat) have been reported to be involved in rabies transmission. However, further studies have shown that mainly Desmodus rotundus (vampire bat) is important in rabies transmission (Turner, 1975; Anderson et al., 2014). In USA, bats have been reported as reservoir vector in over 90% of human rabies cases. Among bats tricolored bat (Perimyotis subflavus) are reported as major reservoir host (Gilbert et al., 2015). The bat rabies virus variants isolated from Latin America in free tailed bats (genus Tadarida) and vampire bats (Desmodus rotundus) have been found to be close to earliest rabies virus. The study also suggest that adaptation of rabies virus in bats occurred earlier in colonial genera (Myotis and Eptesicus) than in bats of solitary genera (Pipistrellus, Lasionycteris, and Lasiusius) (Hughes et al., 2005).

Globally, approximately 55,000 annual human deaths are caused by rabies virus which can be associated with bats (Knobel et al., 2005). Rabies viruses of bat origin may sporadically spill over to infect human and other mammals. It has been reported in USA that most rabies victims do not recall the incidence of bitten by bat. This may be due to unusual circumstances during bat bite or being small size of the biting animal (Rupprecht et al., 2004). Recent studies also suggest that all rabies virus variants affecting terrestrial carnivores might be originated from cross-species transmission and genetic exchange from bat associated rabies virus.

3.2 Other lyssaviruses

Bats are also reservoir for several other lyssaviruses including Duvenhage virus (DUVV), Shimon bat virus (SHIBV), Irkut virus (IRKV), West Caucasian bat virus (WCBV), Australian bat lyssavirus (ABLV), European bat lyssavirus 1 (EBLV-1) and European bat lyssavirus 2 (EBLV-2). EBLV-1 and EBLV-2 are reported in Europe from Eptesicus fuscus and Myotis spp of bat respectively. Some of the sporadic cases of human rabies have been reported from EBLV-1 and EBLV-2 (Kuzmin & Rupprecht, 2007; Kuzmin et al., 2011). However, in terrestrial mammals some of the sporadic cases of EBLV-1 infection were also reported which might be a potential source for human exposure (Dacheux et al., 2009). In France, neutralizing antibodies against EBLV-1 were detected in six species (Pipistrellus pipistrellus, P. kuhlii, Hypsugo savii, Plecotus auritus, Eptesicus serotinus and Tadarida teniotis) of bats (López-Roig et al., 2014). Recently, in Germany, EBLV-1 and EBLV-2 were detected from Eptesicus serotinus and Myotis daubentoni species (Schatz et al., 2014). The complete genome sequences of EBLV-1 have been extracted from Eptesicus isabellinus bat in Spain (Marston et al., 2015).

Some of the insectivorous bat species such as Murina leucogaster harbor IRKV (Botvinkin et al., 2003). IRKV may also cause human rabies. IRKV was reported from a human rabies case in Russia. The human patient was a victim of an
insectivorous bat bite (Leonova et al., 2010). Some of the suspected human rabies cases caused by IRKV were also detected in Ukraine and China (Botvinkin et al., 2006).

IRKV was also first time isolated in China from brain of northeastern bat (Murina leucogaster) which showed maximum nucleotide and amino acid identity with IRKV isolated from Russia. Virus produced rabies like symptoms in adult mice (Liu et al., 2013a). On experimental pre-exposure prophylaxis (PrEP) and postexposure prophylaxis (PEP) analysis with rabies vaccine against IRKV in hamster model showed that routine PEP with three doses of vaccine may generate complete protection. However, for complete protection from IRKV higher doses of PEP agent such as anti-rabies immunoglobulins are required (Liu et al., 2013b).

The WCBV was isolated in south-eastern Europe from insectivorous bat Miniopterus schreibersii. Since, WCBV are most divergent Lyssavirus, all the anti-rabies biological are inefficient in providing protection against this virus (Hanlon et al., 2005). Although, the public health significance and ecology of WCBV is still unknown, the experimental infection in bats and laboratory animals, developed typical rabies symptoms which led to death (Kuzmin et al., 2008).

Other member of Lyssavirus, SHIBV was also isolated from a bat (Hipposideros commersoni) (Kuzmin et al., 2011). The biological significance of SHIBV for public health is unknown. However, they may cause pathogenesis in experimentally infected laboratory animals, which develop rabies and finally die (Markotter et al., 2009; Kuzmin et al., 2010). Due to their antigenic differences, current rabies biologicals cannot protect from SHIBV (Hanlon et al., 2005).

DUVV also causes dreadful human rabies in Africa. Despite availability of anti-rabies biological, it still causes human casualties because of inadequate knowledge of disease. Some of insectivorous bat species such, Miniopterus sp may transmit DUVV to human (Markotter et al., 2008). In 2007, a Dutch tourist was bitten by a bat in Kenya. The patient was allowed for medical help. However, due to lack of adequate anti-rabies PEP administration, rabies symptom was developed and patient died from DUVV infection (van Thiel et al., 2009; Koraka et al., 2012).

The ABLV was discovered in ‘rabies-free’ Australia in 1996. The ABLV was identified first in black flying fox (Pteropus alecto) (Fraser et al., 1996). Now, it is assumed that all bats in Australia may potentially carry ABLV (http://www.health.nsw.gov.au/). Later on, it was reported that some of the insectivorous bat species such as Saccolaimus flaviventris may also harbour ABLV (Gould et al., 2002). Two fatal human cases of ABLV infection with clinical symptoms compatible with rabies have been detected (Gould et al., 2002; Warrilow et al., 2002).

3.3 Henipavirus (Hendra and Nipah virus)

An outbreak of acute respiratory illness was reported in human and horses during 1994 to 2004 in Hendra, Australia (Field et al., 2011). The etiological agent reported was from genus Henipavirus and family Paramyxoviridae. Later on it was named as Hendra and Nipah virus (Murray et al., 1995). Several bat species such as fruit bats (flying foxes) of the genus Pteropus, including gray headed flying fox (Pteropus poliocephalus), black flying fox (P. alecto), spectacled flying fox (P. conspicillatus) and little red flying fox (P. scapulatus) were reported as probable reservoir hosts of Hendra virus (Field et al., 2011; Wang et al., 2013). The qRT-PCR assay showed that P. alecto is potent reservoir host than P. poliocephalus and P. scapulatus for Hendra virus in Australia (Edson et al., 2015). However, a little knowledge is available about the dynamics of Hendra virus infection and maintenance in bat. The horses probably get Hendra virus infection from flying foxes by spillover (Field et al., 2011). The periodic outbreaks of Hendra virus in local flying fox population lead to an increased incidences of spillover infection to horses. The Hendra virus infection in flying foxes increases when threshold number of susceptible flying foxes is reached and virus enters the flying fox population from a nomadic individual or group. This concept was well studied for related morbilliviruses (Bolker & Grenfell et al., 1996; Swinton et al., 1998).

Nipah virus was isolated form adult male human and pigs showing symptoms of respiratory illness, fever and encephalitis in Malaysia in 1999 (Chua et al., 1999). The disease was found highly fatal for human patients. Further, investigation showed that most of the human patients were having history of direct pig contact (Chua et al., 2000). Later on, variable flying fox (Pteropus hypomelanus) and large flying fox (P. vampyrus) were found as natural reservoir hosts for Nipah virus (Chua et al., 2002a; Chua et al., 2002b). Nipah virus associated disease was also reported from human in Bangladesh (Sazzad et al., 2013; Chakraborty et al., 2016). Nipah virus outbreak in Bangladesh was very similar to Malaysian outbreak in several aspects such as fever, central nervous system signs, delayed recognition and a high case fatality rate. However, in Bangladesh human cases were not directly associated with disease in pigs, and some evidence of human to human transmission was also reported (Hsu et al., 2004). The serological surveys in Bangladesh suggested that Nipah virus is transmitted by only the Indian flying foxes (Hsu et al., 2004). Nipah virus infections were also reported from human in India (Chadha et al., 2006). Later on, neutralizing antibodies against Nipah virus was also reported from large flying foxes in Cambodia (Olson et al., 2002) and Indonesia (Sendow et al., 2006). Thus, henipaviruses are reported from human and bats in several countries across the globe (Halpin et al., 2000).

The detail molecular genetics study also evidenced that Nipah and Hendra viruses are circulating in their natural hosts, flying foxes since ancient days (Gould, 1996). However, the recent outbreak of Nipah and Hendra virus in human population suggests some major changes in behavior and habitat change in bats. The emergence of flying fox populations under stress
conditions due to habitat loss altered the foraging and behavioral patterns which results in virus niche expansion and closer proximity to livestock and human population. This may be the pathway of Nipah virus outbreak in human (Chua et al., 2002a).

3.4 Menangle and Tioman Viruses

Menangle virus of genus Rubulavirus and family Paramyxoviridae was originally isolated from stillborn piglets near Menangle in Australia in 1997 (Philbey et al., 1998). The affected litters were characterized by mummification, autolysis, stillborn and live piglets. Several teratogenic defects such as brachygnathia, arthrogryposis and kyphosis were also reported (Barr et al., 2012). It has been proved that Menangle virus has significant tissue tropism for secondary lymphoid organs in pigs and humans and for intestinal epithelium in weaned piglets (Bowden et al., 2012). Serological analysis of persons in contact with the infected pigs also showed the high titers of antibodies against Menangle virus along with clinical signs of febrile illness with measles like rash. However, none of the persons were in direct exposure to flying foxes (Chant et al., 1998). Further study showed that bats living in mixed colonies of little red flying foxes and gray headed flying foxes near the pig farm had neutralizing antibodies against the virus (Philbey et al., 1998). Although the virus isolation from flying foxes was unsuccessful, the paramyxoviruses like virion particles labeled with antibody against Menangle virus was reported from flying fox feces collected near the pig farm and a convalescent sow by electron microscopy.

The Tioman virus is a rubulavirus and is distinct from Menangle virus. It is antigenically related to Menangle virus and harboured by Pteropid fruit bats (Yaiw et al., 2008). It was isolated from variable flying foxes in Malaysia. It was discovered accidentally during identification of natural host of Nipah virus which caused large scale outbreaks of encephalitis in pigs and humans in Singapore and Malaysia in 1998-1999. It is a newly recognized paramyxovirus and little is known about its pathogenesis and host range (Chua et al., 2001).

3.5 SARS-CoV like viruses

An unrecognized corona virus from family Coronaviridae was reported as causative agent of severe acute respiratory syndrome in humans in 2002 (Rota et al., 2003). The virus was later named as severe acute respiratory syndrome-corona virus (SARS-CoV), which is a distant relative of group 2 coronaviruses of rodents, dogs, cattle, pigs, and humans (Gorbalenya et al., 2004). The epidemiologic studies suggested that SARS outbreaks were directly associated with wildlife meat industry. The SARS-CoV like viruses were also isolated from some of the wildlife species such as raccoon dogs (Nyctereutes procyonoides) and masked palm civets (Paguma larvata). The SARS-CoV specific antibodies were also detected in hog badger (Arctonyx collaris) in China (Guan et al., 2003). The viral RNA was detected by reverse transcription-PCR (RT-PCR) from some of the seronegative animals suggesting acute infection. However, the continuous virus shedding from seropositive animals also suggested the presence of persistent infections in some animals (Guan et al., 2003). Further study also proved the palm civets act as an incidental host for SARS-CoV rather than principal host.

Later on, it was reported that some of the bats (Chinese horseshoe bats; family Rhinolophidae and genus Rhinolophus) possessed either antibody against SARS-CoV or infected with SARS-CoV like viruses (Li et al., 2005). The genome sequences of SARS-CoV from humans and civets were also found phylogenetically close to bat SARS-CoV like viruses (Li et al., 2005). It suggests the origin of humans and civets SARS-CoV is associated with bat viruses in China. Further study also suggest origin of human SARS-CoV might be from unrecognized SARS-CoV like virus of bat origin which was transmitted to amplifying hosts viz. raccoon dogs, masked palm civets and hog badger and spilling over to human population through close contact with these animals or their tissues. Later on adaptive mutations in virus genome lead to human to human transmission of virus (Song et al., 2005).

The disease potential of a SARS like virus, SHC014-CoV from Chinese horse shoe bat (Rhinolophidae) was studied using reverse genetics system where a chimeric virus was prepared which expressed spikes of bat coronavirus SHC014 in a mouse adapted wild type SARS-CoV backbone (Menachery et al., 2015). In mouse, chimeric virus developed severe pathogenesis which was found untreatable with anti-SARS immunotherapeutics. Moreover, chimeric virus replicated in primary human airway cell line and produced an equivalent titer of SARS-CoV outbreak from human (Ge et al., 2013; Menachery et al., 2015) which indicates a vital threat of re-emergence of human SARS-CoV from wild bat population.

3.6 Middle East respiratory syndrome (MERS)

MERS causes severe respiratory illness in human. MERS was first time reported from Saudi Arabia in 2012 (Bermingham et al., 2012). Later on, it has been spread to several other countries. Most people suffered with this disease develop symptoms of severe acute respiratory illness such as cough, fever and shortness of breath. MERS is caused by a corona virus called MERS-CoV. For MERS the case-fatality rate is reported as about 45%. It may cause infection to pregnant woman and develop severe respiratory signs (Alsereh et al., 2016). MERS-CoV and SARS-CoV are very similar, which suggests that bats may also play a role in transmission of MERS CoV to human population. The partial RNA sequence of betacoronavirus from faecal pellet of an Egyptian tomb bat Taphozous perforates showed 100% nucleotide identity with virus isolated from human index case patient (Memish et al., 2013). One of the camel species (Camelus dromedarius) may harbor this virus in nature, because MERS-CoV can be experimentally established in camel (Adney et al., 2014; Raj et al., 2014; Omrani et al., 2015).
3.7 Ebola Virus

The Filoviridae family of virus consists of genus Ebolavirus (Ebola Sudan virus, Ebola Zaire virus, Ebola Reston virus and Ebola Ivory Coast virus) and Marburgvirus (Marburg virus). The natural reservoirs of these viruses are not yet confirmed. However, the RNA genome of Ebola virus has been identified in terrestrial mammals in Central African Republic (Morvan et al., 1999). Ebola virus may cause highly fatal haemorrhagic disease in human, which may also infect other mammals (Dhama et al., 2015). The high viral loads in body fluids allow virus transmission from human to human (To et al., 2015). A serious Ebola virus outbreak was started in December 2013 in West Africa which also reached to other continents (Gumusova et al., 2015). Experimentally, Ebola Zaire virus was also replicated in little free-tailed bat (Chaerephon pumilus), Angola free tailed bat (Mops condylurus) and Wahlberg’s epauletted fruit bat species (Epomophorus wahlbergi) (Swanepoel et al., 1996). The serological surveillance also showed presence of IgG immunoglobulin in 4% of bat population of six species viz. Hypsipithecus monstrosus, Epomops franqueti, Myonycteris torquata, Mops condylurus, Micropteropus pusillus and Rousettus aegyptiacus (Pourrut et al., 2009). Later on Ebola virus RNA was also detected in spleen and liver tissues of some fruit bats species viz. Hypsipithecus monstrosus, Epomops franqueti and Myonycteris torquata (Leroy et al., 2005). The qPCR assay have successfully detected the Reston ebolavirus (RESTV) specific RNA segments from oropharyngeal swabs of several bat species (Miniopterus schreibersii, M. australis, C. brachyotis and Ch. plicata) from Philippines (Jayme et al., 2015). The detection of Ebola virus RNA from bats is a fascinating finding, but only based on nucleic acid detection it is difficult to establish the bat as reservoir host. It is also suggested that there might be a nonpathogenic undetected Ebola virus spreading in bat population which may give rise to pathogenic strain by mutations in other mammals (Monath, 1999). However, until and unless virus is isolated from bat species, the experimental infections unambiguously demonstrate that virus is persisting as well as transmitting from bat species to other mammals.

3.8 Marburg virus

Marburg virus was first reported from an epidemic in Frankfurt and Marburg in Germany and Belgrade in the former Yugoslavia. Marburg virus belongs to Filoviridae family. It causes highly fatal disease in human called Marburg virus disease (MVD). Although it is a rare disease, it may cause high fatality in human during outbreak. The case fatality rate of MDV was reported from 25% in the initial laboratory based study in 1967, to more than 80% during outbreaks in Democratic Republic of Congo in 1998-2000 and in Angola in 2005 (http://www.who.int/csr/disease/marburg/en/). This virus is transmitted either by direct contact with the tissues, blood and other body fluids of infected persons or handling dead or ill infected animals such as fruit bats and monkeys. Some of the study in Uganda showed that fruit bat of Rousettus aegyptiacus species might be a natural reservoir for Marburg virus (Amman et al., 2012). The Marburg virus specific IgG and nucleic acid (RNA) was detected in naturally infected individual fruit bat (Rousettus aegyptiacus) in Gabon indicating the Rousettus aegyptiacus as natural reservoir for Marburg virus (Towner et al., 2007). Later on, serological surveillance also revealed the presence of antibody against Marburg virus in 1% of bat population of Hypsipithecus monstrosus and Rousettus aegyptiacus species (Pourrut et al., 2009). The experimental infection of Marburg virus to Rousettus aegyptiacus species of bats also showed the wide distribution of virus in bat tissues followed by recovery of large quantity of viral RNA which suggested the natural reservoir potential of Rousettus aegyptiacus species of bat (Jones et al., 2015; Amman et al., 2015a).

3.9 Sosuga virus

Sosuga virus is a novel paramyxovirus which may cause severe acute febrile condition in human. In 2012, a female wildlife biologist reported the malaise, fever, generalized myalgia, headache, arthralgia, neck stiffness and sore throat after a short field expedition for collection of bats and rodents in South Sudan and Uganda (Albarino et al., 2014). However, the patient recovered successfully with adequate medical support. The metagenomics studies of pathogen nucleic acid suggest that the etiological agent might be a novel paramyxovirus related to rubula like viruses of fruit bats origin (Albarino et al., 2014). The new virus was named as Sosuga virus (on name of South Sudan and Uganda). It was also established that virus is most likely originated in bats. However, the efforts to virus detection in African bats are still under way.

To establish the fact regarding bat as potential reservoir, the bat tissues collected during the last three week period prior to onset of clinical symptoms were tested for presence of Sosuga virus (Amman et al., 2015b). It was reported that several Egyptian rousette bats (Rousettus aegyptiacus) were found positive for Sosuga virus. Further analysis of Egyptian rousette bat tissues collected from other locations in Uganda were also found positive for Sosuga virus (Amman et al., 2015b). This suggests that Egyptian rousette bats could be a potential natural reservoir for Sosuga virus.

4 Routes for transmission of bat-borne viruses to human

Many of the bat associated viruses are restricted to specific geographical regions with availability of bat reservoir host, such as Egyptian fruit bats associated Ebola virus in Africa and flying foxes associated Hendra and Nipah virus in Australia and Southeast Asia. However, how bat transmit diseases to human is a mystery because most of the bat species remain away from human dwellings in tropical rain forests and in caves. The studies of bat transmitted zoonotic diseases revealed that most probably these diseases are transmitted to humans either via intermediate host or direct contact with bats (Figure 1). Therefore some of the hypotheses for transmission of bat borne disease to human have been proposed.
4.1 Transmission through direct contact

Bats usually reside in dark caves and deep forests. Therefore the direct contact of bat with human is a rare incidence. However, people may get infection of bat associated viruses by bat bite and handling of live bats during capture and consumption of bat meat (Mari Saéz et al., 2015). The capture and selling of wild animals including bats increases the risk of zoonotic virus outbreak in human population (Figure 1). In 2007, Ebola hemorrhagic fever virus outbreak costs life of 186 human in Democratic Republic of Congo (DRC). The epidemiological investigation reported that infection reach to human population by consumption of infected fruit bats meat (Leroy et al., 2009). The transmission by direct contact or ingestion of food infected with bat droppings, is an important source because several viral nucleic acid have been extracted from bat droppings (Halpin et al., 2000; Mari Saéz et al., 2015). Sometimes, accidental bat bite may also result in human rabies. In South Africa human death was reported by Duvenhage virus (DUVV) infection by bat scratch (Adjemian et al., 2011).

4.2 Transmission through intermediate host

It is proposed that bats may transmit disease to human through an intermediate host which is close to human and may amplify the virus. The remaining contaminated fruits eaten by fruit bats may be consumed by intermediate hosts such as horses, pigs and non-human primates. Human may get infection from these intermediate hosts by direct contact or consuming their products. In tropical Australia and Southeast Asia, Hendra and Nipah viruses are transmitted by flying foxes. During Nipah virus outbreak in 1998 in Malaysia, it was hypothesized that pigs get infection of Nipah virus by consuming the half consumed mangoes by flying foxes. Mangoes were a major food for flying foxes, and half consumed mangoes contaminated by urine and saliva of bats was accidently consumed by pigs (Figure 1). This results in cross-species infection of pigs followed by subsequent infection to human (Chua et al., 2002a).

Horses may get Hendra virus infection by consuming contaminated fruit, grass, feed or water by bat’s saliva, urine and feces and subsequently infection may reach to human (Plowright et al., 2015). Camels play major role in human life in Middle East countries for transportation as well as entertainment. It was hypothesized that dromedary camels act as intermediate host for MERS-CoV infection from bats to humans (Memish et al., 2014). MERS-CoV was also detected in camel milk (Reusken et al., 2014). Thus, virus may be excreted in milk and poses a high risk of infection for people either during milking process or consumption of unpasteurized milk. In 2003, severe SARS outbreak was reported in China. The SARS-CoV was transmitted from bat to palm civet and
subsequently to human (Liu, 2003). In Central Africa, Ebola virus was transmitted to apes by consumption of fruit contaminated by bats (Leroy et al., 2005).

4.3 Transmission through aerosol

Bat may spread large number of viruses in air. Thus, air may get contaminated by bat borne viruses especially in caves. People may get infection by bat borne viruses by inhalation of contaminated air (Figure 1). The lethal viral hemorrhagic fever outbreak in Cynomolgus macaques was reported by inhalation of aerosols containing Marburg virus (MARV-Angola) (Alves et al., 2010). Some reports suggest that human may get infection of Marburg virus by visiting in caves in Africa. The most probable route of transmission in this condition might be by aerosol transmission (Timen et al., 2009).

5 Isolation and characterization of virus

Viruses from several tissues samples can be grown in a variety of cell culture system in laboratory. For molecular diagnostic study nucleic acid isolation from cell culture material is a good choice. Nucleic acid isolation followed by PCR assays is extremely rapid and sensitive technique. Several other sensitive diagnostic assays such as multiplex PCR, RT-PCR, Real-time PCR etc. are also used for viral emerging infectious diseases (EIDs) diagnosis (Rihtaric et al., 2010; Huang et al., 2012; Freuling et al., 2013; Suin et al., 2014). For identification of a newly recognized virus, PCR amplification of viral nucleic acid followed by nucleic acid sequence data analysis is used. The nucleic acid sequence data of viral pathogens are compared with available sequences in GenBank database (http://www.ncbi.nih.gov/GenBank/) to search for sequence similarities with nucleic acid sequences of known viruses. Moreover, recombinant viral proteins expressed in other expression system can also be used for serodiagnostic tests. During diagnosis of EIDs extra precaution should be taken to avoid misdiagnosis. For example, the first report of Nipah virus infection in Malaysia in 1999 was misdiagnosed as Japanese encephalitis virus (JEV) infection (Calisher et al., 2006). Although, all the human patients were of adult age male already vaccinated against JEV and pigs also suffered a fatal disease, the disease was misdiagnosed as JEV. Later on the failure of intensive vaccination in clinical disease control forced the medical and scientific community to think about new emerging disease. But, by the time there is a huge economic and human life loss was reported. Such incidences force us for certain degree of intellectual preparedness in terms of reagents, equipments and scientific knowledge that could be used for development of rapid diagnostic assays during outbreak of newly emerged viruses.

6 Diagnostic limitations

Several diagnostic assays based on serological techniques such as ELISA, immunofluorescence assay etc and molecular techniques viz. PCR, Real-Time PCR, multiplex PCR, nucleic acid sequencing etc are available for sensitive detection of viruses of bat origin. However, for diagnosis of previously unrecognized viruses, new assays and reagents are required. For identification of new viruses PCR will be useful which needs knowledge of nucleic acid sequences of recognized bat associated viruses such as viruses of Mononegavirales order (family Bornaviridae, Filoviridae, Rhabdoviridae, and Paramyxoviridae) for suitable primer designing (Pringle, 1991). In addition, specific antibody conjugates may also be required for enzyme-linked immunosorbent assays or immunofluorescence assays to identify either virus specific antibodies in sera samples or antigens in tissue samples.

Some of the classical methods such as hemagglutination or hemagglutination inhibition tests were also used for viral diagnosis. However, these assays are broadly cross-reactive. Several cell cultures and animal inoculations can also be used for virus isolation. However, for bat associated zoonotic viruses this technique is potentially hazardous, and it should not be used without appropriate biocontainment. With the advancement in molecular biology techniques for viral nucleic identification, virus isolation technique is not much appreciated. However, virus isolation technique will provide us virus in bulk quantity which may be used in many areas of research and development such as development of vaccines, suitable diagnostics and animal disease model to study the pathogenesis of virus.

7 Bats and emerging viruses

More than 200 different viruses under 27 families are detected in some species of bats (Moratelli & Calisher, 2015). However, only few viral diseases such as SARS, MERS, Ebola virus disease etc are transmitted from bats to human (Moratelli & Calisher, 2015). Because a large proportions of bats under mammalian species (about 20%), their diverse habitats, biology and natural history, it assumed that bats may harbor several other viruses of human and animal importance (O'Shea et al., 2014; Brook & Dobson, 2015).

However, the transmission of zoonotic viruses through bat is mostly based on assumptions. Proper investigation is still required for establishment of role of bat in zoonotic virus transmission (Fenton et al., 2006). In most of the study same viruses are detected both in bats and humans, but this does not prove the bats as reservoir host. Many of the viral nucleic acid sequences have been isolated from bat tissues or excreta. The virus might be entered to bat body through food chain. It only indicates that bats may act as temporary host for those viruses (Calisher et al. 2006; Melaun et al., 2014).

Bats share several immunophysiological parameters to human. This probably occurred due to the fact that bats are in close contact with human population since several years in many parts of the world for habitat and food requirements. Such interaction of bat with human and other animals favors the chance of potential spillover of diseases. Some of the phylogenetically related species of bats may act as intermediate host for bat transmitted viruses. It explains the
transmission of Hendra and Nipah diseases to human. However, in some of the cases spillover infection is also caused by other animal species such as palm civets, pigs, raccoon dogs and horses. In Malaysia, it was established that Nipah virus was spliced over to human population through pigs from fruit eating bats (Chua et al., 2002b; Dobson, 2006). Some of the insects such as Haematophagous sp. may also transmit virus from bat to human (Melaun et al., 2014). It is also reported that mechanical transmission of bat associated zoonotic viruses to human population is also possible.

8 Control and prevention of bat-associated emerging infectious diseases (EIDs)

Several factors progressing from primary to more proximate drive disease emergence from bats. For bat originated viral disease control such factors should be taken in consideration. Several steps should be taken for control of bat transmitted zoonotic viruses. Such steps should be initiated at individual level, population level and at societal level.

8.1 Individual level control

In most of the cases no specific medical therapy has been found beneficial in bat associated viral EID. In human rabies therapeutic measures are very challenging and in most of the cases they fail to save the patient life. The early diagnosis i.e. before onset of fulminant stage in animal may allow effective prophylaxis in human. The prognoses of fulminant rabies carry a very poor and unfavorable result. In medical history the first case of successful experimental rabies treatment (Milwaukee Protocol) was reported in a 15 year old girl bitten by a bat in 2004 (Willoughby et al., 2005). Later on, extension of Milwaukee Protocol (consisting of antiviral drugs therapy, therapeutic coma and intensive medical care) did not show much successful in many other patients (Rupprecht, 2009; Rubin et al., 2009). The suitable prophylaxis measures before the onset of illness, has proved a much higher success rate in several other bat associated EIDs. For treatment of viral diseases modern molecular biology approach may also be used. The currently untreatable infection of henipaviruses may be treated with small interfering RNA (siRNA) molecules homologous to viral RNA (Mungall et al., 2008). Although, siRNA has capability to treat several viral infections, it is still under developmental phase. Several issues related to siRNA such as its delivery, efficacy in humans and cost effectiveness has yet to address.

Moreover, the possibility of potential use of Ebola virus as bio-weapons has forced scientific community for development of an effective vaccine product for any emergency outbreak. In mouse model of hemorrhagic Ebola virus infection, the vesicular stomatitis virus based recombinant vaccine has proved its safety and efficacy in preventing clinical signs of disease (Jones et al., 2007). Ebola virus vaccines can also be delivered through mucosal surface route. This vaccine delivery approach is very rapid and may prove advantageous during sudden disease outbreak.

8.2 Population level control

The bat associated viral EIDs should be addressed intensively at population level. The population level study of several bats associated EIDs have been carried out. Rabies is studied in depth and public health guidelines including vaccination of pets and other animals on public display, vaccination of humans in high risk groups, separation of domestic and pet animals from the wildlife reservoirs of rabies, public awareness regarding rabies etc was issued for rabies control. The current recommendation also advocates about pre and post exposure prophylaxis for high risk group individuals such as animal handlers, veterinarians, rabies researchers and laboratory workers and long term travelers to rabies endemic areas (NASPHV, 2009). Despite advances in epidemiology, molecular biology and vaccination science the proper control of bat associated viral EIDs remains challenging in many parts of the world. To reduce the bat associated viral EIDs outbreaks in human population, measure should be taken to control either the bat population or viral infection to bat population. In one of such measure anticoagulant on vampire bats can be applied and subsequently bats should release in wild condition (Kuzmin & Rupprecht, 2007). This will lead to consumption of anticoagulant by other vampire bats during grooming. It is well established that vampire bats can digest only coagulated blood. Thus, they may die by blood feeding which will remain uncoagulated in their digestive system. The anticoagulant can also be applied on animal skin to control bat population (Kuzmin & Rupprecht, 2007).

8.3 Societal level control

The recent global emergence of Henipavirus and SARS coronavirus of Bat origin has started a new discussion on how to control disease emergence. The possible reasons of emergence of bat associated viral EIDs are environmental changes, increased human mobility and overpopulation. Therefore, to control viral EIDs monitoring of increased global mobility with other practical measures such as surveillance of transportation can be initiated. The intensive monitoring of borders and ports can be initiated for ill passengers and animals. Proper care and management facility should be provided which will benefit the ill animal and human as well as population moving from there. Moreover, for international travelers specific health measures such as pre-travel vaccination as well as post-travel health checkup should be initiated.

Environmental conservation is also essential for sustenance of biodiversity and natural habitat. It is reported that many of the wild animals including bats are now reaching to human dwellings for food and shelter which also carry the EIDs to human population. The evidence show that environmental degradation play a major role in increased rates of disease emergence especially EIDs. However, the exact role of loss of environmental conservation in EIDs is still not understood, therefore further study is needed to establish the facts.
Conclusion and future perspective

Bats cover the diverse group of mammalian species. They harbor several zoonotic viruses in their body than any other animal species (Hayman et al., 2013; Luis et al., 2013). It explains the necessity of knowledge of immune resistance mechanisms of bat that allow bats to harbor viral pathogens, mechanisms underlying disease emergence and pathogenic basis of viral diseases in bats (Dobson, 2006; Daszak et al., 2013; Mandl et al. 2015). To address such issues field epidemiological studies along with intensive laboratory experiments on bat associated virus using live bats and bat cell cultures are required. The bat cell lines from bats of different species need to establish to facilitate the in vivo and in vitro experiments.

There is always a threat of experimental introduction and release of virus through laboratory animals, especially laboratory animals of foreign origin in new geographical area. Therefore, to reduce such risks only native laboratory animals should be used for animal experimentation for viruses. These animals should be kept for captive breeding. After few generations of captive breeding physiologically uniform animal strains could be obtained, that can be used in study of zoonotic diseases (Eger & Gardner, 2008).

It is also necessary to design field studies for continuous search regarding new viral pathogen circulation in bats, their zoonotic potential, role of various abiotic and biotic factors affecting bat populations and their role in disease spillovers to humans (Parrish et al., 2008, Daszak et al., 2013, Marí Saéz et al., 2015). For adequate control of bat associated EIDs epidemiologists as well as wildlife experts should work together to minimize the risk of viral outbreak in human population. The joint expertise of bat biologists, veterinary and medical professionals and molecular biologists can be utilized for control and prevention of bat associated zoonotic viruses. After all, it is difficult to say that bats are responsible for emergence of zoonotic viruses. Only the intensive laboratory research using epidemiological and ecological approaches conducted by molecular biologists, bat biologists, veterinary and medical researchers may provide useful and satisfactory evidence regarding zoonotic virus transmission from bat. The accurate statement will be based on concrete laboratory evidence only.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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