Chinese Science Bulletin 2003 Vol. 48 No. 12 1179—1182

Fruit bats as a natural reservoir of zoonotic viruses

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Abstract **As a natural reservoir of manifold zoonotic viruses, fruit bats have been involved in at least three emerging zoonoses in recent years. This paper aims to introduce the epidemiological characteristics of these diseases emerged in the Australasian region between 1994 and 1999, transmission pathways of the newly discovered viruses and the relationship between the changed entironment of fruit bats and occurrences of these emerging diseases and provide a clue for the epidemiological investigations of SARS.**

Keywords: fruit bat, natural reservoir, zoonotic virus.

DOI: 10.1360/03wc0177

The outbreak of a cluster of three zoonotic diseases in Australasian region has caused devastating loss of human life and economy between 1994 and 1999. The pathogens of these diseases are newly discovered viruses and have caused a worldwide regard. The result of epidemiological research indicates that the outbreak is related to the natural history of the viruses that caused these diseases and fruit bats are the natural reservoir of these agents. The appearance of atypical pneumonia referred to as severe acute respiratory syndrome (SARS) that first emerged in Guangdong Province, China in the winter of last year is a severe epidemic in recent years and it is spreading worldwide. The identification of the pathogen is still in question, but the agent also probably comes from wildlife. This paper seeks to summarize six points thereinafter: i) Epidemiological characteristics of the three zoonotic diseases; ii) origins of the viruses and possible transmission pathways; iii) viruses hosted by bats and their latent endangerment; iv) fruit bats ecology and distribution; v) interference of human into nature and outbreak of diseases; and vi) fruit bats distribution in China and surveillance of the pathogens.

1 Epidemiological characteristics of the three zoonotic diseases

In September 1994, there was a sudden outbreak of an acute respiratory syndrome in thoroughbred horses in a training complex at Brisbane in Queensland. The syndrome was characterized by severe respiratory signs and high mortality. Fourteen horses and a trainer died. A virus, the putative causal agent, had been identified. The virus is a previously undescribed member of the family *Paramyxoviridae*, which was initially named equine morbillivirus, but was subsequently re-named Hendra virus (HeV) [1,2].

From April to September 1997, a new virus of the *Paramyxoviridae* family, called Menangle virus, was recognized in a commercial piggery in New South Wales of Australia. It led to abortion of pregnant sows and stillborn piglets[3]. In addition, the agent also caused influenza-type illness in two workers at the piggery[4].

Nipah virus (HiV) led to an outbreak of disease in pigs in Peninsular Malaysia between September 1998 and April 1999 and had caused thousands of deaths. It spread to human in several weeks since the emergence of this virus and resulted in the death of 105 humans among 265 reported cases[5,6]. Indirect immunofluorescent test of the cerebrospinal fluid samples of the died patients indicated that anti-NiV antibody was positive. IgG and IgM capture ELISA showed that patients had IgG or IgM antibodies to NiV. The agent was proved a HeV-like virus, but the epidemiological characteristics and clinic symptom were distinct from that caused by HeV. So the new virus was named Nipah virus[7,8]. According to the molecular phylogenetic study, NiV is most closely raleted to HeV within *Paramyxoviridae* and both are considered belonging to the same new genus[9].

2 Origins of the viruses and possible transmission pathways

After the emergence of HeV, extensive investigations (including targeted serological surveys of livestock, and traces of livestock movements) were undertaken, but no anti-HeV antibodies were detected in over 5000 domestic animals (including 4000 horses, plus cattle, dogs, cats and poultry)[10—12]. Later, the targeted species were aimed to wildlife that can move between outbreak locations, and anti-HeV antibodies were identified in a black flying fox (*Pteropus alecto*), and within weeks, in grey-headed flying foxes (*P. poliocephalus*), little red flying foxes (*P. scapulatus*), and spectacled flying foxes (*P. conspicillatus*)[10—12]. And then, HeV was isolated from the reproductive tract of a pregnant grey-headed flying fox (*P. poliocephalus*)[14]. A serologic survey of 1043 non-randomly sampled flying foxes from multiple Queensland locations revealed a crude HeV seroprevalence of 47%. The described occurrence and frequency of anti-HeV antibodies in flying foxes is consistent with an endemic pattern of infection Australia-wide, indicating that infection in flying foxes may be largely sub-clinical. Transmission from flying foxes to horses has not been demonstrated; however, experimental infections in a range of species, and investigations of natural infections in flying foxes and in horses have suggested possible modes of transmission[15]. It has been hypothesised that spillover from flying foxes to horses is effected by contact with infected foetal tissues or fluids, most probably via the ingestion of recently contaminated pasture[14,16,17]. This hypothesis was initially based largely on the temporal overlay of disease events in horses with the birthing period of species of flying fox in Queensland[15], and it is also supported by the fact of isolation of virus from foetal tissues of naturally infected[14] and experimentally infected flying foxes[18]. Another possible spillover mechanism is the ingestion by horses of the masticated pellets of residual fruit pulp spat out by flying foxes feeding on fibrous fruit[15]. The possibility of contact with nasal discharge or infected urine is a means of horse-to-horse transmission and the infection of humans is contact with infected horses[15]. The transmissibility of HeV in laboratory infections has been low[19].

When Menangle virus emerged, a large breeding colony of grey-headed flying foxes, as well as little red flying foxes roost within 200 m of the affected piggery from October to April, therefore flying foxes were investigated as a potential source of infection. In a preliminary study, 42 of 125 serum samples collected from fruit bats in New South Wale and Queensland were positive in the virus neutralization test with titres of 16 to 256[3]. Flying foxes are the most probable natural reservoir of this virus though it has not been isolated from these fruit bats as yet.

NiV spread very quickly in the infected piggeries, and transmission among pigs in the same stable was via contact with the secretion or egesta of infected pigs, such as saliva, urine and secretion from throat and trachea, especially in the closed piggery. The disease in pigs was highly contagious, and characterized by acute fever with respiratory involvement and sometimes nervous signs in all age classes. The predominant clinical syndrome in humans was encephalitic, and a small amount of patients appeared atypical pneumonia. The majority of human cases had a history of direct contact with live pigs. Most were pig farmers or slaughterhouse workers. Transmission was via wound, secretion or egesta of infected pigs, such as saliva, urine, dejection, nasal mucus, and also blood or breathed out gas. The main presenting features were fever, headache, dizziness, and vomiting. Some patients had a reduced level of consciousness and prominent brain-stem dysfunction. Distinctive clinical signs included segmental myoclonus, areflexia and hypotonia, hypertension, and tachycardia. Typical cases died in 6 d after infection. No spreading of Nipah virus among humans was reported[6]. Besides the outbreak of NiV disease, there was also Japanese encephalitis in Malaysia. The workers probably had been infected with Japanese encephalitis virus before NiV infection, so there was also antibodies to Japanese encephalitis virus in NiV infected patients. But Japanese encephalitis virus is not a causitive agent in the outbreak of NiV disease[20]. Based on the fact that NiV has a close relationship with HeV whose natural hosts are flying foxes, so Malaysia bat species became a logical surveillance priority. Malaysia has a great diversity of bat species. There are at least 13 species of fruit bats, and at least 60 species of insectivorous bats described in Peninsular Malaysia alone. Blood and tissue samples were collected from 324 bats from 14 species and submitted for serological test. Neutralising antibodies to NiV were found in 21 bats from five species (four species of fruit bats, and one insectivorous species) [21]. Later, NiV was isolated from urine of Malaysia flying foxes (*P. hypomelanus*)[22]. All these proved that fruit bats are the natural reservoir of NiV.

3 Viruses hosted by bats and their latent endangerment

In recent years, a novel paramyxovirus named Tioman virus was isolated from the urine of fruit bats (*P. hypomelanus*) after the cluster emergences of HeV, Menangle virus and NiV, and this virus has a close relationship with Menangle virus[23]. In addition, a novel virus of the genus *Lyssavirus* was isolated from an Australian black fly fox (*P. alecto*). This virus had a unique serotype and was named Australian bat lyssavirus (ABL)[24]. In fact, bats likely act as a natural reservoir of viruses. Some 40 viruses have been isolated from bats during the past four decades. The list of isolated viruses includes members of the *Rhabdoviridae*, including rabies[25] and rabies-related virus[26], smaller numbers of representatives from among the *Alphaviridae*[27,28], *Reoviridae*[29], *Arenaviridae*[30] and *Herpesviridae*[31]. Quite a mount of these are zoonotic viruses and their dissemination and transmission are far out of being imagined, forming a latent endangerment to health of human and animals.

4 Fruit bats ecology and distribution

Bats belong to the Order Chiroprera, which is the second large Order in mammalia. At present there are about 1000 recognized species worldwide, making up almost a quarter of all known mammal species. Bats are divided into two suborders, the Megachiroptera and the Microchiroptera. All of the Megachiroptera consume fruits, flowers and/or flower products, so they are called fruit bats, while the Microchiroptera feed on insects. There are 41 genera containing a total of 173 species in Megachiroptera and the largest and best known genus is *Pteropus* commonly referred to as flying foxes, which has 59 species[34,35].

The single megachiropteran family, the Pteropodidae, ranges from Africa, the eastern Mediterranean, Madagascar and the Indian Ocean islands in the west, across mainland southern Asia, throughout the islands of the west Pacific from the Ryukyu Archipelago and Ogasawara-shato in the north, to coastal eastern Australia, new Caledonia and the Loyalty Islands in the south, and east to Fiji, Tonga, Samoa and the Cook Islands[33]. Many species are restricted to islands, while a number of them are widespread. Fruit bats range in body weight from 300 g to over 1 kg, and in wingspan from 600 mm to 1.7 m. They are the largest bats in the world, most of them do not echolocate and navigate at night by eyesight and their keen sense of smell. Females of *Pteropus* usually have only one young a year after a 6-month pregnancy. The young grow rapidly but are dependent on their mother for up to 3 months. All species eat plant products (most commonly fruits, flowers and pollen) and roost communally in trees or den[33—36]. Fruit bats are known to travel over considerable distances. There is a record of a little red flying fox in New Zealand, 2000 km from its southernmost Australian occurrence, and a sighting of India flying foxes (*Pteropus giganteus*) 320 km from land[35,36]. Some kinds of fruit bats move from one camp to another continuously on feeding habits. These movements cause fluctuating numbers in their camps and indicate that over their whole range the population is in a state of flux[32—35].

5 Interference of human into nature and outbreaks of emerging diseases

Though HeV and NiV are newly discovered viruses, the molecular characterization and phylogenetic analysis suggest that both are actually "old" viruses[1,37]. In fact, most emerging infections appear to be caused by pathogens already present in the environment, brought out of obscurity or given a selective advantage by changing conditions and given an opportunity to infect new host populations (on rare occasions, a new variant may also evolve and cause a completely new disease)[38].

Fruit bats are very important seed dispersers and pollinators in the virgin forest of tropical and subtropical regions. Most young plants cannot develop normally in the shadow of parent trees or when restrained by toxins secreted by parent trees. Therefore, seed dispersal away from parent trees is an absolute necessity. Fruit bats consume a large amount of fruits and eject the seeds through the mouth or anus, thus transporting them to remote places, where these seeds can germinate and grow up to full size. Some seeds, such as those contained in many figs, pullulate only when seeds have passed through the intestinal tract of birds or fruit bats. Finally, flower-visiting fruit bats which are attracted by the flower's pollen and nectar for food, also often pollinate these flowers in the ecosystem. Unfortunately, in wide areas of South East Asia, the beneficial effects from many species of fruits have dramatically declined because the numbers in fruit bat populations have plummeted[33].

These days, the continuous extension of human civilization seriously disturbs the natural environment for fruit bats, and this has caused significant habitat shifts in fruit bat populations, placing fruit bats in contact with humans and their livestock. This favors the spread of certain viruses which originally were hosted by fruit bats and can result in the infection of domestic animals or even humans. The outbreak of NiV in Malaysia, for instance, was closely related to the destruction of natural habitat of fruit bats caused by deforestation and consequently food shortage. This forced fruit bats to move from traditional forest environment to orchards nearby for food. In rural Malaysia, piggeries in human habitations are often placed right next to orchards. The half-eaten or digested fruits contaminated by fruit bats could then easily be ingested by pigs, thus eventually spreading the NiV, which is fatal, among humans as well[38]. In some areas, people could have been infected directly by fruit bats while hunting them for food[39].

6 Fruit bats distribution in China and surveillance of the pathogens

According to Wang[40], there are 11 species of fruit bats in China, which are *Rousettus leschenaulti*, *R. amplexicaudatus*, *Pteropus dasymallus*, *P. lylei*, *P. vampyrus*, *P. giganteus*, *Cynopterus sphinx*, *C. brachotis*, *Sphaerias blanfordi*, *Eonycteris spelaea*, *E. sobrinus*. However, the ecology and behaviour of these bats remain, despite some very patchy preliminary work, largely unknown. Fruit bats generally home in the tropics or subtropics[41]. Hence, the records of *Pteropus dasymallus* from Shannxi and *P. giganteus* from Qinghai do not represent their natural distribution. Instead, these specimens must have reached these locations either by having been drifted there by strong winds or by having been displaced by unnatural means by humans. In recent years, the only species we usually encounter in our field surveys are *Rousettus leschenaulti* and *Cynopterus sphinx*. Adult *Rousettus leschenaulti* have a body weight of about 100 g and wingspan of 450 mm, with the female being slightly larger than the male. The fur on the back is brown and the flanks are dust-colored. During the day, they communally roost in caves or in forests of bamboo, bananas or other trees providing cover and fly out to forage at night. *Cynopterus sphinx*, which is smaller in size, seeks cover amidst branches of coco or palm trees, forming small round clusters. *Rousettus leschenaulti* can still be found often forming large colonies, thousands of individuals strong in a single cave. They consume large amounts of longan and lichee, sometimes causing significant losses for the farmers.

It is obvious then that the lives of humans and fruit bats become more and more intertwined, which as a consequence exposes us to manifold zoonotic viruses. Until now, there have been few studies in China which address the issue of pathogens hosted by fruit bats. Huang[42] has isolated Japanese encephalitis virus in two of 136 samples obtained from a fruit bat (*Rousettus leschenaulti*), indicating that fruit bats can play an important role in reservoir and spread of this virus.

Nowadays, the global spread of SARS cost hundreds of lives and infected thousands. Although there are still different results in identification of the SARS pathogen, many researchers attribute its source and potential reservoir to wildlife. As the reservoir of many zoonotic viruses, flying foxes have potentially played an eminent role in several previous epidemics. The time has come to begin including fruit bats in the surveillance of pathogen-carrying reservoirs.

Acknowledgements We are very grateful to Dr. Walter Metzner for the English revision. This work was jointly supported by a special grant for the "Study of the Origin of SARS" by the Institute of Zoology, Chinese Academy of Sciences; the National Natural Science Foundation of China (Grant Nos. 30025007 and 30270169) and the Innovation Program of the Chinese Academy of Sciences (Grant Nos. KSCX3-IOZ-03 and KSCX2-1-03).

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(Received April 23, 2003; accepted June 3, 2003)