On Viruses, Bats and Men: A Natural History of Food-Borne Viral Infections

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Abstract In this chapter, cross-species infections from bats to humans are reviewed that do or do not use intermediate animal amplification hosts and that lead to human-human transmissions with various efficiencies. Rabies infections, Hendra virus infections in Australia, Nipah virus infections in Malaysia and Bangladesh and SARS coronavirus infection in China are explored from the public health perspective. Factors of bat biology are discussed which make them ideal virus reservoirs for emerging diseases. In line with the book theme, it is asked whether even in these epidemic conditions, viruses can be seen as essential agents of life where host species use their viruses to defend their ecological position against intruders. It is asked whether another essential function of animal viral infections could be the "killing the winning population" phenomenon known from phage biology which would stabilize species diversity in nature.

1 Introduction

> *Ich bin ein Teil von jener Kraft,*
> *die stets das Böse will und stets das Gute schafft.*
> *Ich bin der Geist, der stets verneint!*
> *Und das mit Recht; denn alles, was entsteht,*
> *ist wert, daß es zugrunde geht.*
>
> *(Who then are you?/Part of the power that would/ Alone work evil, but engenders good./ The spirit I, that endlessly denies./And rightly, too; for all that comes to earth/Is fit for over-throw, as nothing worth)*
>
> *Mephisto in Goethe's Faust*

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2   Rabies Virus in Bats

When I learned virology in the early 1980s during my PhD at the Max Planck Institute in Munich, bats were not a big concern. In fact, as a student I knew only about a single virological problem with bats: rabies. In industrial countries, I have never seen a clinical case of rabies, the only victim of rabies whom I knew was the Swiss coordinator of rabies vaccination who had a fatal helicopter accident when dropping the vaccine for foxes. Rabies became even rarer in industrial countries after these vaccination campaigns, but in my consciousness I maintained a deep-seated distrust of bats despite all zoological interest for this fascinating form of mammalian life. I will illustrate this ambiguous attitude towards bats with a trivial personal experience. The author was called to a female neighbor who reported a strange animal in her house. Actually what I found was a rather drowsy bat crawling on the floor. Instead of removing the bat directly, I first went home, searched a big pair of gardening gloves and only then I went back to her to remove the bat. My cautious reaction might have appeared to her as an overreaction, but it probably corresponded to what virologists would have recommended to do. Healthy bats are able flyers and avoid collisions or contact with humans. Bats found on the ground during day time are suspect. Due to my professional education- other would say- deformation, I suspected a rabid bat. Some rabid bats may become aggressive, but others simply become disoriented and loose their flying ability. Insectivore bats have very fine teeth that may lead to so small puncture marks when biting your hands that they are overlooked. Contamination of such a trivial skin wound with bat saliva could lead to infection. Rabies is caused by a rhabdovirus. Worldwide about 55,000 cases of rabies death are reported annually, most are the consequence of bites from rabid dogs in regions where large scale vaccination programs were not conducted. Indigenous rabies was still observed in the USA and Canada with about ten cases per year in the 1950s, most of them were dog-associated. In the 1960s the cases came down to one case per year while a rise to four cases per year – practically all bat-associated– were seen since the 1990s (de Serres et al. 2008). In the USA rabies is still enzootic in foxes, skunks, raccoons and bats. Rabies is also enzootic among bats in Europe, but only 5 human cases of bat-associated rabies were reported from Europe. Rabies is a dreaded disease and the medical literature reports only a single case who has survived an infection without post-exposure prophylaxis. There is thus good reason to be circumspect of bats, but the odds for an infection with a bat-variant rabies virus were not high when I was helping my neighbor.

Today we know that rabies infections represent only one extreme of cross-species infections between bats and humans. It can be described as a single infection "spillover" event, hence the very low number of cases. Were it not for the dreaded consequences of this disease, it would probably not attract medical attention. A major barrier against viral spillover between species is the species barrier. Unfortunately, this concept is more a time confirmed empirical medical concept than a much investigated experimentally phenomenon. What leads to breaches in this barrier? One could imagine that spillovers occur primarily between species with high ecological contact rates. Alternatively, the height of the barrier might be determined by host

genetics factors. Streicker and colleagues (2010) have addressed this question by sequencing the nucleoprotein gene in nearly 400 rabies viruses isolated from 23 bat species. They identified 43 unambiguous cross-species infections. Their observations amount to one trans-species for every 73 within-species transmission events. These authors also observed that the intensity of the trans-species transmission declined continuously with the genetic difference between donor and recipient species. Transmission increased less with the extent of geographical overlap between species habitats. The authors concluded that the vast majority of the trans-species infections of bats with rabies virus are evolutionary dead-ends. From these data it appears that this highly mutable RNA virus does not represent a major concern for introduction of a bat virus into the human population. Can this relatively assuring conclusion be generalized to other viruses of bats? Unfortunately, the answer is No. In subsequent paragraphs, we will recognize cross-infections from bats that led to transient outbreaks (e.g. Nipah virus infections) and even sustained epidemics with the potential of endemic establishment (e.g. SARS corona virus) in the human population. There is another reason not to be complacent with bat rabies. Virologists from the Pasteur Institute in Paris, where Louis Pasteur had developed the first rabies vaccine, had sequenced many lyssaviruses (how rabies virus is called taxonomically) isolated from carnivoran and chiropteran ("finger-wings", the systematical name of bats) hosts. The phylogenetic tree of the surface glycoprotein which is responsible for receptor recognition revealed seven genotypes (Badrane and Tordo 2001). The long branches on the tree were all bat viruses. Genotype 1, the classical rabies group, was found in bats and carnivoran mammals. The carnivoran rabies viruses were all small twigs on the glycoprotein tree suggesting recent introduction. Using the tree, the authors deduced two spillover events, one into raccoons and another, independent event into the other carnivores (dog, fox, wolf, skunk, mongoose) which then spread worldwide without much diversification. Using molecular clock arguments, the authors dated this spillover to the time of the decline of the Roman Empire. They explained the fact that rabies was already described in cuneiform tablets in Mesopotamia 4,000 years ago by the hypothesis that this represented a spillover event with a rabies virus which became in the meanwhile extinct. In an even bolder hypothesis, the Pasteur authors speculated that bats had acquired the lyssaviruses from their insect prey. Indeed, rhabdoviruses are a prominent insect pathogen and another rhabdovirus, Mokola virus (known from two human case reports) was also isolated from an insectivorous mammal (this time a shrew) and this virus could be propagated on insect cells. According to the Pasteur scientists the spillover from insects to bats might have occurred 10,000 years ago.

3   Hendra Virus in Australia

In my PhD thesis at Max Planck, I looked for the potential involvement of a particular paramyxovirus in multiple sclerosis. From my work in Munich I kept a lively interest for neurological diseases caused by this group of viruses. Following the literature, I became witness of the great flexibility displayed by morbilliviruses (measles virus,

rinderpest, canine distemper) with respect to suspected or proven cross-species infections between different mammalian species. Paramyxoviruses that are pathogenic in novel hosts were dolphin, porpoise, and phocine morbilliviruses. However, I had to wait until the mid-1990s to see the first cases where a morbillivirus from bats spilled over into the human population. In 1994 an outbreak of severe respiratory disease was observed in Brisbane (Queensland/ Australia). The animals developed high fever and died. A trainer of the horses became ill with a severe influenza-like disease and died subsequently from interstitial pneumonia. Organ homogenates from two horses yielded a virus that showed typical cytopathic effects in cell culture (syncytia) as well as paramyxovirus-specific nucleocapsids in the cytoplasm. The homogenate could also induce fever with respiratory distress in two healthy horses. The outstanding gross pathology was lung edema. At the histopathological level syncytial giant cells in blood vessel walls were observed (Murray et al. 1995). Also material from the patient yielded a serologically identical virus to the horse virus isolate. Both horses and the trainer developed high-titered neutralizing antibodies in the serum to the virus isolates. Minimal cross-neutralization was seen with known paramyxoviruses and the sequencing of a viral gene confirmed this distant relationship defining a new paramyxovirus group which should get known under the name of Hendra virus from a suburb of Brisbane where the first cases were observed. The researchers investigated 1,600 horses for serological evidence of antibodies to Hendra virus; all were seronegative demonstrating that horses are a new host species that had not previously been exposed to this virus. A second smaller Hendra virus outbreak was observed at the same time point, but in Mackay 1,000 km apart in Queensland: only 2 horses were affected, but again a human contact died. The case report from this fatal encephalitis patient showed again the presence of this novel paramyxovirus in his brain, but the researchers failed to isolate this virus in cell culture. The long symptom-free period that followed the exposure to the equine morbillivirus before the fatal illness set in reminded the authors the behavior of defective measles viruses in SSPE patients (O'Sullivan et al. 1997). Since then about a dozen of further outbreaks of Hendra virus infection was documented in Queensland (Marsh et al. 2010), the largest in 2008 with 5 horses which showed predominantly neurological rather than respiratory symptoms. The attack rate was 10% in contact persons from a veterinary office again with a human fatality. A veterinarian showed influenza-like symptoms followed by a progressive neurological disease (Playford et al. 2010). A veterinary nurse showed also a neurological disease, but recovered. A 2-week incubation period was deduced. The horse-to-human transmission mode was probably from direct contact with respiratory secretions of the infected horses. Since early serosurveys had not provided evidence for Hendra virus infection in 2,100 horses from Queensland, a wildlife source was quickly suspected. A first serosurvey with 5,200 sera from 46 species gave no hit. A true detective story set in: the epidemiologists postulated that the viral source should be a species present both in Brisbane and Mackay, the species should be able to travel between both areas, and the species should have contacts with horses. Two species fulfilled this phantom image: migratory waders (a bird) and flying foxes (a fruit bat).

Queensland has four species of bats belonging to the suborder Megachiroptera all belonging to the genus *Pteropus*. Within 224 serum samples from fruit bats, 20 showed neutralizing antibodies against the equine Hendra virus. Clearly, a virus closely related to Hendra virus was circulating in all four Queensland fruit bat species (Young et al. 1996). These authors extended their searches to virus isolations from fruit bats. They investigated 650 tissue samples from 460 individual fruit bats and obtained one isolate from the uterine fluid of a pregnant female grey-headed fruit bat (*P. poliocephalus*) and one from the lung of a fetal black fruit bat (*P. alecto*). A gene was amplified and revealed an identical nucleotide sequence with the Hendra virus (Halpin et al. 2000). For an RNA virus this group of viruses showed a high degree of sequence conservation: All Hendra virus isolates from Queensland showed less than 1% nucleotide sequence diversity (Marsh et al. 2010).

Epidemiologists tried to understand why it came to the cross-species virus transmission (Plowright et al. 2011). In view of the rare virus isolation rate the likely transmission mechanisms must remain conjectural, but the models are quite plausible. Flying foxes depend on nectar and fruit as food sources. In their native forests, the distribution of food trees is patchy which necessitates wide foraging flights over large habitats to assure a sufficient food supply. On the east coast of Australia nearly three quarters of the initial forest cover has been lost and flying foxes were obliged to seek alternative food sources. Urban gardens became a reliable replacement for the bats. The new food was quite convenient since it made long and energy-expensive foraging flights unnecessary. As a consequence bats became urbanized. Indeed, many major towns from eastern Australia have now daytime roost places for flying foxes. As a consequence of habitat fragmentation and behavioral changes, flying foxes came also in contact with horses held for sport purpose in urban settlements creating new opportunities for cross-species virus transmissions that did not exist in the past.

Hendra virus infections are not a curiosity: Menangle virus (Philbey et al. 1998) and Tioman virus (Yaiw et al. 2007), both also novel paramyxoviruses, caused infections in pigs which acquired the virus from fruit bats and in both cases transmission of mild infection to human contacts were described. The ecological relevance of the link between viruses from fruit bats to pigs to humans was dramatically demonstrated in Malaysia.

4   Nipah Virus in Malaysia

It did not take long until the next spillover of a virus from bats to humans was observed. As in the case of the Hendra virus outbreak it needed an intermediate host for the cross-species transmission. This time it was not horses, but pigs which transmitted the virus. All began in September 1998 with a respiratory illness in pigs from farms in Malaysia. However, except for a loud cough, the disease symptoms were not very distinctive. Only a minority of pigs was noted to be ill and the death rate in pigs was only increased minimally by 5% (Chua et al. 2000). By February 1999,

similar cases in pigs were also seen in other states of Malaysia as a result of transport of infected pigs into the new outbreak areas (Lam 2002). By mid-June 1999 it became clear that Malaysia was struck by an epidemic: more than 265 cases of encephalitis cases were reported in humans and 105 patients died. The first case reports described patients with fever and confusion who developed a characteristic segmental myoclonus leading to a deepening coma and death from hypotension and bradycardia. The histopathology showed vasculitic blood vessels with thrombosis in the brain. Giant syncytia observed in the kidney and the cerebrospinal fluid cells guided the suspicion towards paramyxoviruses. Infected cells showed indeed a strong positive reaction with antibodies to Hendra virus. The first nucleotide sequences from this virus suggested a paramyxovirus related to, but distinct from Hendra virus (Chua et al. 1999). When a larger number of patients from Malaysia were investigated, a clearer clinical pattern emerged. Presenting clinical features were not very distinctive: fever, headache and dizziness. The patients were young (mean of 37 years) and male (4.5:1 female), mostly ethnic Chinese and quite conspicuously 93 % were pig farmers or had occupations which brought them into direct contact with pigs. Furthermore 41 % of the patients reported that they had contact to pigs that died from an unusual respiratory tract infection (Goh et al. 2000). These observations dispelled the initial hypothesis of an infection with the Japanese encephalitis virus. JE virus is endemic in Malaysia, but as a mosquito-borne infection it has no association with particular occupations and is most common among children (Lam 2002). Furthermore most of the new encephalitis patients had been vaccinated against the Japanese encephalitis virus, some of them even quite recently making this hypothesis untenable. Furthermore, JE vaccination and mosquito control programs had no effect on the epidemic. Virus isolation was tried from 18 encephalitis patients of Malaysia, 5 yielded from the cerebrospinal fluid a virus resembling a paramyxovirus. Further viruses were isolated from tracheal and nasal secretions and the urine. The new virus was called Nipah virus from the name of an outbreak site. Seventy per cent of the patients showed serum antibodies against this new virus. Nipah virus infections have a short incubation period. The virus spreads systemically. The patients show some pulmonary involvement, but mainly a predilection for the central nervous system and prominent brain-stem dysfunction in comatose patients. The outbreak in Malaysia ceased when more than 1 million pigs from the outbreak areas were culled inflicting a major economical burden on the small family farms rearing pigs. The Nipah virus was characterized in some detail. It showed the typical pleomorphic membrane-enveloped paramyxoviruses with the "herringbone" nucleocpsid structure. The viral RNA was amplified by reverse transcription polymerase chain reaction and the N protein (the major nucleocapsid protein of the virus) showed that the Nipah virus forms with the Hendra virus a new genus within the paramyxovirus family tree. This genus was called Henipavirus and it was clearly distinct from the known Respirovirus, Morbillivirus and Rubulavirus genera in this virus family. The Nipah virus differed from the Hendra virus by 31 % at the nucleotide sequence level. In comparison, Hendra virus isolates taken 5 years apart differed by only 0.4 % (Chua et al. 2000).

5 Follow-Up in Singapore

In March 1999 an abattoir worker died in Singapore with fever, headache and confusion. The next day a patient showing the same symptoms who also worked in an abattoir was admitted to the same hospital. Family members recalled a third and fourth abattoir worker hospitalized with a neurological disease. The Ministry of Health closed the abattoirs in Singapore and started a screening program. Eleven of thirty-five diseased abattoir workers showed IgM antibodies to Nipah virus. All worked in the same abattoir processing pigs imported from a farm in Malaysia. The index patient showed headache, fever, productive cough, pulmonary involvement and confusion. Necropsy showed widespread systemic vasculitis (Paton et al. 1999). No secondary cases in the family or contacts were observed and the outbreak ceased when the import of pigs from Malaysia was stopped. Exposure to live pigs was the only significant risk factor associated with the disease. However, only few abattoir workers noted coughing pigs or reported lethargic pigs with nasal discharge. Paradoxically, only one of two abattoirs processing Malaysian pigs was affected and just this abattoir had introduced face masks for the workers and blood products from the slaughter pigs were not collected (Chew et al. 2000).

6 Linking Nipah Virus to Bats

Serological studies demonstrated Nipah virus-specific antibodies in dogs, cats and ponies from the outbreak areas in Malaysia (Chua et al. 2000) while wild boar, hunting dogs and rodents were all negative for Nipah virus antibodies (Yob 2001). The researchers then extended the survey to 14 species of bats from Malaysia. Two species of Megachiroptera (fruit bats), namely *Pteropus hypomelanus* and *P. vampyrus* showed relatively high prevalence rates of 31 and 17 % Nipah antibody seropositivity, respectively. No virus reactive with anti-Nipah virus antibodies was isolated. All attempts to amplify Nipah virus RNA were also negative. Subsequently researchers collected urine from *Pteropus hypomelanus* and swabs of their partially eaten fruits. Three viral isolates (two from urine and one from a partially eaten fruit), which caused syncytial cytopathic effect in Vero cells and stained strongly with Nipah- and Hendra-specific antibodies, were isolated. Molecular sequencing confirmed the isolate to be Nipah virus with a sequence deviation of five to six nucleotides from Nipah virus isolated of humans (Chua et al. 2002). More recently, Nipah virus was also isolated from *P. vampyrus* (Rahman et al. 2010). However, 272 throat and 272 urine samples had to be processed to yield a single isolate. This Nipah virus differed from the human, pig and *P. hypomelanus* isolate at 98 nucleotide positions, about twice the difference between the human and *P. hypomelanus* isolates.

The virus isolation data confirm the serological data and point to fruit bats as source of the Malaysian Nipah virus outbreak. However, some points are noteworthy.

The titer of Nipah virus in the urine from the Rahman et al. (2010) study was with 10 TCID$_{50}$ (tissue culture infective doses) very low and probably only induced by stress (confinement in a cage), which might have lowered the immunity of the index animal. Two male bats from the same colony seroconverted during the observation period, but a virus could not be isolated from them. None of the three animals showed any disease symptoms. In its natural host, Nipah virus is not maintained by a boom and bust dynamic typical of acute viral infections, but by repeated, intermittent low virus shedding as a result of a chronic infection characterized by virus recrudescence (Sohayati et al. 2011). Such an infection mode is therefore very difficult to detect for viral ecologists working in the field. This observation is somewhat surprising since a number of non-host species could be infected with Henipa viruses. Natural infections were seen in horses, pigs, dog and cats. Experimental infections were seen in the guinea pig, hamster, ferret and nonhumane primates like the African green monkey (Wong and Ong 2011). In contrast, experimental infections of bats were not very successful. In one series, infected fruit bats developed a subclinical infection characterized by the transient presence of virus within selected viscera, episodic viral excretion and seroconversion (Middleton et al. 2007). The intermittent, low-level excretion of Nipah virus in the urine of bats may be sufficient to sustain the reproduction of the virus in a species where there is regular urine contamination due to mutual grooming and licking and biting during mating. In another series, *Pteropus* bats from Malaysia were inoculated with Nipah virus by natural routes of infection. Despite an intensive sampling strategy, no virus was recovered from the Malaysian bats. Therefore, the probability of a spill-over event to another species is low (Halpin et al. 2011). For spill-over to occur, a range of conditions and events must coincide. These peculiar conditions were apparently met in Malaysia (Pulliam et al. 2012). Two possible precipitating factors were discussed, which are not mutually exclusive, but might have acted synergistically. One factor is a "push" in form of progressive deforestation which put the fruit bats under ecological pressure. Another factor is a "pull" which attracted fruit bats to farms. Malaysia has seen a widespread dual use of agricultural land to produce both pigs and mangoes on the same farm. On the index farm where the Nipah virus outbreak started, 400 mango trees were planted directly adjacent to pig enclosures. Fruit bats were attracted to this "fast food". In fact, bat roost places and the index farm were clearly within the bats' nightly foraging range. Not all Megachiroptera are really fruit eaters, for example some species from the subfamily Nyctimeninae showed in their stomach exclusively remnants of beetles and flies. However the majority of the Megachiroptera are indeed fruit eaters and they show a highly adapted mouth part for their food choice. With their long canines and one foot they grasp the fruit. With their small incisive teeth they open up the fruit and with the flat molars they squash the fruits. The stomach and intestine of *Pteropus* bats was full of a milky and slimy fruit juice while fruit fibres were not found in the gut. In fact, the squeezed fruit is normally discarded and falls on the ground. On the index farm, these discarded fruits contaminated by the saliva and urine of the bats fell into the pigsties and became a welcome supplementary food to the pigs. This unfortunate chain of events probably allowed the cross-species infection to occur. The dynamics of pig movements

through the farm from the breeding to the growing to the finishing section mixed up the pig population and permitted to maintain infection chains. The movement of pigs from farm to farm led to a spread of the infection between geographically separated areas of Malaysia. Pig farmers had too close contact with the pigs resulting in a lethal bat-borne zoonosis of humans with pigs as an amplifying intermediate host. The export of Malaysian pigs to slaughterhouses in Singapore finally led to the spread of the disease to abattoir workers in Singapore. Consistent with this model identifying pigs as infection source was the observation that 92% of the infected patients reported close contact to pigs and that the outbreak stopped after pigs in the affected areas were slaughtered and buried. Human-to-human virus transmission was not observed. To assess the possibility of nosocomial transmission, 288 unexposed and 338 health care workers exposed to outbreak-related patients were surveyed, and their serum samples were tested for anti-Nipah virus antibody. Needle stick injuries were reported by 12, mucosal surface exposure to body fluids by 39 and skin exposure to body fluids by 89 workers. All serum samples were negative for Nipah virus-neutralizing antibodies (Mounts et al. 2001).

Thus far, one could conclude that the threat from bat viruses is rather low and that it needs very special conditions for an intermediate host to get in close contact with bats to serve as infection source for humans. The dimension of an outbreak with the tragedy of more than 100 human deaths and the enormous economic outfall from the culling of more than a million pigs should not be minimized. However, as long as no infection chains can be maintained in the human population, the outbreak cannot get out of control. One should, however, not take too much comfort from these reflections for two reasons. First, satellite telemetry studies have shown that bats are highly mobile and can move between Indonesia, Malaysia, Singapore and Thailand. Second, *Pteropus* has a wide geographical range covering the north-eastern coasts of Australia, Indonesia, South-East Asia, South Asia and Madagascar (but notably not Africa, which is an unexplained enigma of *Pteropus* biology). One might fear Nipah outbreaks within this geographical range and wherever peculiar ecological conditions are met putting humans in close contact with Nipah virus from bats. Unfortunately, one had not to wait too long to get this concern confirmed.

7 Nipah Virus in Bangladesh

The next outbreak was observed in February 2001 in India close to the northern border of Bangladesh. Overall 66 cases were observed resulting in 45 deaths (Harit et al. 2006). Retrospective investigations by the Centers for Disease Control and Prevention (CDC) demonstrated Nipah virus infection by the detection of Nipah virus-specific antibodies in the serum and the isolation of Nipah virus from the urine of patients. No concomitant veterinary outbreak was detected, nor had the patients contacts to diseased animals. Shortly after this outbreak, 7 outbreaks with Nipah virus infection were documented in Bangladesh during the time period between 2001 and 2007. The infection was confirmed by all patients developing IgM antibodies to Nipah virus.

The clinical presentation of the Bangladeshi patients differed substantially from that of the Nipah virus patients in Malaysia. When the first four outbreaks were analyzed, fever, an altered mental status, headache, cough and breathing difficulties determined the clinical picture (Hossain et al. 2008). Some patients showed symptoms more compatible with acute respiratory distress syndrome than encephalitis. Case fatality rates were with 73% very high; death occurred within a week after the onset of the disease. The most striking and distinctive feature was that the predominantly male patients were with a median age of 12 years very young. Another important observation was the lack of exposure to pigs which served as intermediate host in Malaysia. In fact, Bangladesh is a traditional Muslim society where pork is not eaten and even the contact with pigs is avoided for religious reasons. These peculiar characteristics pointed to a different mode of Nipah virus introduction into the Bangladesh population than in Malaysia. Therefore, epidemiologists from the CDC together with collaborators from the International Centre for Diarrhoeal Diseases Research Bangladesh (ICDDR,B) in Dhaka and the World Health Organization (WHO) conducted a risk factor analysis with a case-control study (Montgomery et al. 2008). Contact with domesticated animals was excluded. The occurrence in young boys suggested an association with some childhood activity; one outdoor activity, namely climbing trees, was significantly associated with infection risk. Most notably, the only other significant risk factor was having contact with an infected person and visiting a hospital. Since under-nutrition is widespread in Bangladesh, the epidemiologists suspected that the boys gathered fruits from trees and also consumed partially eaten fruits contaminated with Nipah virus from saliva of infected fruit bats. Fruits are indeed a major food source in rural Bangladesh. Further epidemiological investigations shed more light on the Nipah virus outbreaks in Bangladesh (Luby et al. 2009a). Overall, ten infection clusters were identified with a median of 10 persons who were affected. Infections occurred with a clear-cut seasonality: nearly all cases were observed during the first 4 months of the year. Geneticists provided further hints about the outbreaks. When they sequenced Nipah virus genomes even from patients living in a limited geographical area and sampled over a few months time period, higher levels of sequence heterogeneity was observed than from Nipah viruses in pigs and humans of Malaysia (Lo et al. 2012). This observation was interpreted as repeated and independent introduction of Nipah virus into the human population in Bangladesh from different sources. However, there are also sequence data from an outbreak in Bengal /India in 2007 that shared 99% nt sequence identity with viral isolates from Bangladesh obtained in 2004 pointing to a common source (Arankalle et al. 2011). The investigation of a 2004 Nipah virus outbreak in Bangladesh by a joint CDC-ICDDR,B team of epidemiologists led to the likely source of the infection. Twelve case patients with a serologically confirmed Nipah virus infection leading to 11 deaths were compared with 33 neighbourhood controls in a case-control study. The only exposure significantly associated with disease was drinking raw date palm sap (Luby et al. 2006). This link can explain a lot of the observed epidemiology of Nipah virus infections in Bangladesh. Date palm sap collection is a seasonal occupation: it begins in mid-December with the cold season and ceases in mid-February overlapping the seasonality of Nipah virus infections in Bangladesh. Collectors climb the tall trees, the bark is shaved off near the top, a

hollow bamboo tap is inserted and directs the palm sap that rises during the night through the tree into a clay pot. Up to 3 L of sap is harvested per night and sold as fresh sap in the next morning by street vendors. Fresh date palm sap is a national delicacy for millions of Bangladeshis in the winter. However, fruit bats also appreciate this palm sap and drink from the clay pots fixed to the trees. In fact, fruit bats of the species *Pteropus giganteus* living in close association with the human population in northern India and Bangladesh are a nuisance to date palm sap collectors. They not only drink the collected sap, but bat excrements are occasionally found floating in the sap. About half of captured *P. giganteus* bats from India indeed showed antibodies to Nipah virus making them likely sources for these infections (Epstein et al. 2008). Veterinarians from the ICDDR,B then caught the fruit bats in action. They installed motion sensor-tripped infrared cameras on tapped palm trees and observed bats licking the sap running into the jug. Thus, the sap can be contaminated with the bat virus contained in saliva and urine of infected animals (Stone 2011). The ICDDR,B is a remarkable research hospital in Bangladesh. It not only conducts internationally recognized research in clinical sciences, microbiology, epidemiology and nutrition, but its scientists are striving to find practical low cost solutions with means accessible to the poor local population which are as easy as effective. A recent proposal was to use the sari cloth of women in Bangladesh to filter the drinking water. In a controlled test, the researchers could demonstrate a nearly 50% reduction in cholera incidence with this practice. In 2007 the ICDDR,B scientists deployed bamboo skirts on palm trees and could demonstrate by their infrared cameras that this fences off the fruit bats.

A survey was conducted in 100 health care workers who provided care to Nipah patients at a Dhaka hospital during the 2004 outbreak with minimal use of protective personal equipment. This study did not provide evidence for nosocomial transmission of Nipah virus even when using sensitive serum antibody tests (Gurley et al. 2007b). However, a case-control study from this 2004 outbreak in Bangladesh painted a different picture. Contact with an index patient carried the highest risk for infection in this survey followed by having contact to a family member harvesting palm sap. A diseased religious leader having many social contacts and sick visits became a "super-spreader" infecting more than 20 contacts. Two contacts infected four and two further contacts, respectively, but then the infection died out (Gurley et al. 2007a). Another case-control study conducted during the 2007 outbreak in Bangladesh also identified as risk factors the visit of a Nipah virus patient in a hospital, touching the index case or being in the same room with a diseased person (Homaira et al. 2010). The person-to-person transmission was likewise demonstrated by virologists who isolated Nipah viruses with practically identical genome sequence from an index case from West Bengal, India, who was an addict to liquor from palm juice, and three diseased family members (Arankalle et al. 2011). There might be cultural and social reasons why person-to-person transmission was seen in Bangladesh and not in Malaysia. Social norms in Bangladesh require family members to maintain close physical contact to the diseased person (Luby et al. 2009b). Poverty induces also the sharing of eating utensils and drinking glasses with the diseased person. Leftovers of food from the patient are commonly distributed to family members. Sleeping in the same bed as the patient even at local hospitals is not unusual in Bangladesh.

However, the Bangladesh Nipah viruses differ also genetically from the Malaysian virus isolates, which might be responsible for the pronounced respiratory symptoms seen in Bangladeshi patients. Since Nipah virus is present in respiratory secretions of diseased patients, transmission of the Nipah virus in aerosol droplets might have induced a marked person-to-person transmission of Nipah infections in Bangladesh. In fact, when eight Nipah patients in an early infection stage were investigated, virus was isolated from the throat in six of them, but only from the urine of three patients (Chua et al. 2001).

With Nipah infection in Bangladesh we saw the possibility for a bat virus to be transmitted directly to humans without the need of an intermediate host, but the potential of the bat virus to circulate in the human population was very limited since the infection chains broke after a few human-to-human transmissions. However, another bat virus demonstrated that this is not an intrinsic property of bat viruses. SARS showed the potential for extended human transmission and wide geographical spread of what was initially a food borne viral infection.

8 SARS in China

SARS (Severe acute respiratory syndrome) emerged 2002 as a new human disease in the Guangdong Province of China. After an incubation period of less than a week, patients showed fever, malaise, headache and myalgias followed by cough and dyspnea. The respiratory problems could progress to frank adult respiratory distress syndrome with multiorgan dysfunction. The virus infects the respiratory tract using the angiotensin-converting enzyme 2 receptor leading to a systemic illness with virus being present in the blood, urine and the feces. The patients are infectious for 2–3 weeks with peak titer excretion 10 days after symptom onset. The patients were treated with ribavirin antiviral and glucocorticoids, but beneficial effects could not be documented. Supportive care to maintain pulmonary functions was the only therapeutic option.

The early phase of the epidemic passed largely unrecognized. The disease attracted attention in 2003 when a major outbreak occurred in a hospital of Guangzhou and a hotel in Hong Kong. Epidemiologists identified a super spreader, who infected 300 other individuals (Dye and Gay 2003). Under such conditions, outbreaks would show an explosive growth. Fortunately, during the middle phase of the epidemic (Chinese SARS Molecular Epidemiology Consortium 2004), the transmission dynamics remained with 2.7 secondary infections per case less dramatic such that public health interventions could finally cope with the epidemic (Riley et al. 2003) leading to the decline of the case numbers in the third late phase. However, at that time the disease had already spread to 25 countries around the world with epicenters as far away as Canada, the virus had infected over 8,000 individuals and killed nearly 800 patients. The epidemic ended in July 2003- the nightmare of a pandemic running out of control did not become a reality. Despite all disruption of international travel and economical exchange, the international research community, assisted by the WHO,

could thus prevent the worst. A contributing factor was certainly the early warning by avian influenza infections in Hong Kong, which led to fatalities in humans and heightened the alert of virologists for the possible emergence of devastating viral epidemics in China.

Is SARS a food borne infection like Nipah infections (Brüssow 2007)? The connection became clear when laboratories in the United States, Canada, Germany, and Hong Kong isolated and then sequenced a coronavirus as the causative agent of this epidemic (Rota et al. 2003; Marra et al. 2003). The agent turned out to be a known virus. It belonged to the coronavirus group, which comprises large, enveloped, positive-strand RNA viruses, where the viral genome encodes the information for the viral proteins. Coronaviruses cause respiratory and enteric diseases in humans and animals. Human coronaviruses were up to that epidemic only associated with mild upper respiratory tract infections, but some animal coronavirus like Transmissible Gastroenteritis virus (TGEV) cause deadly enteric infections in swine. Coronaviruses contain the largest genomes of any RNA virus: the SARS isolates showed genome lengths around 29,750 nucleotides. The genome organization resembled closely that of the known coronaviruses, but its sequences constitute a distinct group on the coronavirus tree.

A review of the early patient data by the WHO revealed that nine of the 23 early patients worked in the food industry. Also, people working in the vicinity of food markets and workers in specialty food restaurants were over-represented in the cases (Normile and Enserink 2003). These data were later substantiated by serological surveys. During the outbreak in May 2003, 13 % of 500 animal traders tested positive for serum IgG antibodies in the quickly developed SARS virus immunoassay. Control groups showed only 1 to 3% prevalence rates. Notably, traders that handled the masked palm civet were the most likely to show SARS-specific antibodies (Enserink and Normile 2003). This is not an entirely unplausible finding since civets are traded as a food delicacy in China. Wealthy consumers praise their tasty meat. In China, civets are also believed to strengthen the body against winter chills. The demand for wildlife cuisine in China is thus high and farming of wildlife is widespread. Many families in the rural area make a living by providing this wildlife to cities (Liu 2003).

Guided by the epidemiological data, a Chinese virologist went into live animal markets where he borrowed animals from vendors (Guan et al. 2003). None of them was found to be ill, but PCR diagnosis tools showed that from the many sampled species four of the six palm civets scored positive, the two negative animals yielded a live virus from nasal secretions. They were sequenced and turned out to be 99.8% identical to the human isolates and differed from them mainly by a 29-nt insertion upstream of the structural N gene. Interestingly, the earliest human SARS virus isolates still contained this 29-nt segment, but later isolates lost this segment possibly as an adaptation to human-to-human virus transmission (Chinese SARS Molecular Epidemiology Consortium 2004). The researchers cautioned that their isolation of the SARS virus from civets might not have identified the true animal reservoir of the virus. Civets might have contracted the infection in the markets and much larger investigations in feral animals were needed to settle the question of the virus reservoir. In fact, also a racoon dog from the investigated market yielded a closely related virus.

Paradoxically, the very close similarity of the civet isolate with the human isolates was a major argument against civets as the SARS virus reservoir. In that case, virologists would have expected a much larger diversity of civet coronavirus sequences and only one out of the many would have made it into the human patients. Other arguments concurred with this reasoning. For example, experimental infection of civets with human SARS virus resulted in overt clinical disease, which is not expected for a viral reservoir where asymptomatic infection should be the rule. Finally, when the researchers looked more closely into civet coronavirus isolates recovered only one year apart, they found again very similar sequences, but within the few single-nucleotide variations a very high rate of non-synonymous over synonymous nucleotide substitutions was detected. These major genetic changes occurred in the spike gene which is essential for the transition between hosts suggesting an adaptation to a new host. This phenomenon was also seen in coronaviruses from the human host in the early 2002–2003 epidemic (Song et al. 2005). Such a process would not be expected in the natural host.

Therefore the Chinese virus hunters went for other virus sources and targeted bats. This is not an odd choice. Also bat meat is eaten in delicacy restaurants of southern China and bat feces are used in traditional Chinese medicine to cure asthma and kidney ailments. Two groups found what they were searching for. One group sampled 408 bats representing nine species which they trapped in their natural environment. They investigated blood, fecal and throat swabs. Three species of communal, cave-dwelling horseshoe bats (genus *Rhinolophus*) showed the high seroprevalence levels of SARS-neutralizing antibodies expected for a virus reservoir ranging from 28% in *R. pearsoni* to 71% in *R. macrotis* (Li et al. 2005). Five stool samples from three species (*R. pearsoni, macrotis and ferrumequinum*) yielded coronavirus RNA and the complete genome sequences could be obtained for SL-CoV Rp3 (SARS-like Coronavirus isolate Rp3), while a live virus could not be recovered. The overall nucleotide sequence identity with human SARS isolates was 92%. However, the domain of the S protein involved in the receptor binding showed only 64% sequence identity explaining why bat sera failed to neutralize SARS virus. Another group of Chinese virologists screened nasopharyngeal and anal swabs of 120 bats, 60 rodents and 20 monkeys from rural areas. The conserved polymerase gene from coronaviruses gave a positive signal in the feces of 29 bats. They detected a coronavirus sequence related to the SARS virus in 23 anal swabs from the insectivorous Chinese horseshoe bats (*Rhinolophus sinicus*) using PCR technology (Lau et al. 2005). The sequences showed 88 % nucleotide sequence identity with the SARS virus again with a sharp drop in similarity over the S gene. The phylogenetic distance from the SARS virus and the presence of the 29-bp insertion sequence missing in the human isolates made a transmission of the SARS virus from humans to bats unlikely. Instead, bat SL-CoV and civet SARS-like CoV are likely to have a common ancestor. None of the positive bats showed clinical symptoms, but many showed an antibody response and high serum titers correlated with low anal virus excretion. Both studies showed closely related sequences for this coronavirus, much closer related to SARS than to another recently isolated bat coronavirus. *Rhinolophus* roosts in caves and feeds on moths and beetles. However, also the cave-dwelling fruit

bat *Rousettus leschenaulti* showed serological evidence for coronavirus infection. These fruit bats were found by the virus detectives on markets in southern China. One hypothesis imagines that they were the asymptomatic source for virus spill-over to susceptible animals exposed on the markets like the civet. The spread of the virus to susceptible animals might have provided the necessary amplification to achieve intrusion into the human population.

The search for the direct ancestor phage for the SARS virus is still ongoing. Additional bat coronavirus isolates point to *R. sinicus* as likely bat source species, which yielded an isolate closely related to Rp3 (Yuan et al. 2010). These researchers proposed that the bat ancestor to the SARS virus might have resulted from a recombination event near the S gene which occurred in a bat viral lineage that experienced a transfer to civets 4 years before the SARS outbreak (Hon et al. 2008). The link to *R. sinicus* was confirmed by recent ecological surveys. Of 1,400 horseshoe bats trapped near Hong Kong, 9 % showed a SARS-related virus in the feces. Peak activity was in spring. All positive animals appeared healthy, but they showed lower weight and they cleared the virus within a few weeks. Tagging experiments showed that these animals had foraging ranges of up to 17 km. The mobility of the host allows for recombination events between coronaviruses from bats of different geographical locations provided that their foraging ranges overlap (Lau et al. 2010). The divergence time between human/civet and bat SARS-like strains was estimated to date 8 years ago.

According to current hypotheses, palm civets were simply conduits rather than the fundamental reservoirs of SARS virus in the wild. In fact, mutational analysis identified at least two separate transmission events that occurred between palm civets and humans: one in the main SARS epidemic in 2002–2003 and another during sporadic infections occurring during the next winter season. In view of the large coronavirus reservoir in bats, the ecological framework, the high mutation rate of RNA viruses and the recombination potential of coronaviruses, the emergence of another pathogenic human coronavirus from bats might be more a question of "when" rather than "if" (Graham and Baric 2010). One needs to remain aware of this risk. The rapid deployment of classic tools of public health that brought the SARS epidemic to an end like air passenger control and strict quarantine measures will be as instrumental in containing future outbreaks as an increased research into the virology of bats as an early warning system. That this consideration is not a moot point can be illustrated with two recent virus isolates.

9 Bats as Reservoir Hosts of Further Emerging Viruses

Equatorial Africa in 2001 and 2005 experienced human Ebola virus outbreaks that decimated gorilla and chimpanzee populations. Researchers captured more than 1000 small animals near the primate carcasses (Leroy et al. 2005). Serum antibodies specific for Ebola virus were found in three different bat species with the highest prevalence of 25 % in *Hypsignathus monstrosus*. Viral nucleotide sequences were

found in liver and spleen samples from all three species, with *H. monstrosus* again leading with a 20% prevalence rate. Animals were either seropositive or virus positive, the viral titers were generally low and no bat showed disease symptoms. The sequencing of the isolated genomes revealed a clustering with the Zaire clade of human Ebola virus isolates. Since the identified bat species are eaten by people in central Africa and the three bat species have a broad geographical range over equatorial Africa, opportunities for cross-species transmission are manifold. Another incident linked a further filovirus with bats. The CDC investigated an outbreak of Marburg hemorrhagic fever which occurred in a gold-mining village in the Republic of the Congo in 1998. Sporadic cases that continued to occur until September 2000 and short chains of human-to-human transmission were observed in 154 patients of whom more than 80 % died. Only a quarter reported a contact with another patient. Nine distinct lineages of viruses were observed excluding a clonal outbreak. The researchers suspected a heterogeneous virus reservoir host that inhabited the mines (Bausch et al. 2006). The scientists examined the fauna of the mine and found Marburg virus nucleic acid in 12 bats, comprising two species of insectivorous bat and one species of fruit bat. The link was further substantiated by finding antibody to the Marburg virus in the serum of 10 % of one insectivorous and in 20 % of the fruit bat species (Swanepoel et al. 2007).

To document the intensity of this viral hunt, just by opening the current issue of a scientific journal, I saw a report describing the isolation of a distinct lineage of an influenza A virus from a Phyllostomidae bat in Guatemala (Tong et al. 2012). The bat virus displayed a novel hemagglutinin H17 antigen and a highly divergent neuraminidase extending the genetic range of known influenza A viruses. However, its genome replication complex was able to function in human cells suggesting that this bat virus could achieve genetic exchanges with human influenza viruses.

The story is not ending here. Thus far, virologists have demonstrated that bats harbour more than 60 viruses. Virus hunting is a time-consuming and dangerous business. Frequently it does not yield a live virus isolate by lack of suitable cell culture systems. Therefore, virologists are now increasingly using nucleic acid-based analytic methods for virus detection. RT-PCR methods can only reveal viruses for which the researchers have matching primer sets and will thus only reveal known viruses. Metagenome analyses of the virome has the potential to reveal the entire diversity of viral sequences present in a given host species. One study investigated the bat guano from caves in California and Texas. About half of the sequences were related to eukaryotic viruses. The largest sequence fraction corresponded to insect viruses, reflecting the diet of the investigated insectivorous bats. The second fraction represented sequences from viruses that infect plants and fungi, which probably reflects the diet of the herbivorous insect prey of the bats. The last fraction corresponded to viruses infecting mammals. This group comprised Parvo-, Circo-, Picorna-, Adeno-, Pox-, Astro- and Corona-Viridae (Li et al. 2010). However, no close relatives of human viral pathogens were identified. Another group investigated fecal, oral, urine and tissue samples from individual captured bats. They confirmed these observations and identified in addition three novel group 1 bat coronaviruses and bacterial viruses (Donaldson et al. 2010).

10 Why Are Bats Special?

In fact, one might question why bats are special with respect to zoonosis. Aren't pigs, ducks or chicken as dangerous reservoirs for viral cross-species transmission from animals to humans? With our current attention focus on the next influenza pandemic, one could probably argue that bats should not represent our primary concern with respect to zoonosis, particularly in view of the limited resources that can be allocated to this type of research. However, bats are special in several respects (Halpin et al. 2007) and it is worth to repeat the arguments of US virologists on this issue (Calisher et al. 2006).

   With 925 recognized species bats represent about 20% of the species diversity of mammals. In addition, bats are an old branch of mammalian evolution, which can be traced back into the Tertiary Period 50 million years ago and the overall design of bats have essentially not changed over this time period testifying a successful evolutionary solution. This evolutionary success is also documented by other facts. Bats have colonized all continents with the exception of the Antarctic. Except for humans, no other group of mammals has such a broad geographical range. Bats are also extremely numerous. Literally millions of individuals can be found in single caves and roost trees teem with bats. Like humans, bats are very social and this combination of sheer numbers with physical proximity creates enormous possibilities for viruses. Airborne rabies transmission is observed under these conditions. There are still further characteristics of bats that favour viral transmissions. Bats are the only mammal that learned to fly. Bats fly in their daily quest for food, but some bats also fly up to nearly 1,000 km between their summer caves and winter hibernation sites. These regular long distance migration paths open possibilities for wide range dispersal of viruses. In their caves, different species of bats frequently intermingle such that bat viruses have ample possibilities to "learn" how to cross species barriers. To conserve energy, two bat families including the Rhinolophidae developed hibernation reducing their body temperature down to 8 °C. Under these cold conditions, viral viremia can be maintained for 100 days. Persistent viral infections are also furthered by the long life span of bats. For the little brown bat weighing a minuscule 7 g, a life span of 35 years was documented. Once persistently infected, an individual has many years to pass its viral passengers. Bats are also the only land mammals that developed echolocation for their pursuit of food. At first glance, this physiological trait might not impact on virus transmission. However, when considering that the echolocation signals are produced by the larynx of these animals and emitted with high acoustical energy from mouth and nostril, this trait creates again substantial possibilities for aerosol virus transmission. It should therefore not come as a surprise that bats have repeatedly been linked to cross-species viral infections.

11 Viruses: Essential Agents of Life?

Bats have important roles in folklore, both positive and negative. Both angels and demons are winged reflecting this dual role. Bats reflect the angel functions as plant pollinizer and seed disperser and the demon function when spreading disease.

However, what can be said about the role of viruses in nature- the subject of the present book? The entrance verses of this chapter are a quotation from a demon and the ambiguity of his verses are perhaps also a valuable image for the role of viruses in general. Since viruses live, by definition, on the metabolism of other cellular organisms, they are frequently considered as the force of annihilation and destruction in biology. Yet in Goethe's Faust, God the creator gave humans the devil as companion since

Des Menschen Tätigkeit kann allzu leicht erschlaffen,
er liebt sich bald die unbedingte Ruh;
Drum geb ich gern ihm den Gesellen zu,
Der reizt und wirkt und muß als Teufel schaffen.

(Man's efforts sink below his proper level,/ and since he seeks for unconditioned ease, /
I send this fellow, who must goad and tease/ and toil to serve creation, though a devil)

The evil force is thus perceived by the poet as a dynamic principle. Only from the dialectics of creation and annihilation, thesis and anti-thesis, anabolism and catabolism is a synthesis possible. In the end, evolution as understood by biologists is not too far from these old philosophical ideas. The destructive force gets thus a positive dimension. To avoid speculative thinking, let's finish by asking what we know about the role of viruses in bats as biologists that possibly fits into this framework. Certain viruses are clear-cut evils (pathogens) for bats. Rabies virus is an example. Rabies virus is found in about 70 % of drowned, dead or dying bats. Despite that fact, rabies has not threatened bats with extinction. This does not mean that bats are immune against extinction. Currently, part of the bat population in the eastern USA collapses under the pressure of a fungal infection ("white nose syndrome") (Frick et al. 2010). Ecologists state that such devastating diseases are not the equilibrium situation; normally "old, adapted" viruses coexist with the host causing only minimal symptoms- just enough to be maintained in nature. As we have seen, asymptomatic infections with low level virus production seem to be the rule in virus-bat relationship. Large epidemics are evolutionary accidents where a virus enters in a susceptible host that has not yet learned to live with the virus. A host coexisting with an "adapted, domesticated" virus might also use the latter as a weapon to defend its turf. If an intruder enters the same ecological niche, it might get into the way of the "domesticated" virus coexisting with host 1, which might become a dangerous pathogen for host 2. Viruses can thus be used for defense, but also use for attack is imaginable. Host 1 might "use" its viral flora to compete with host 2 when intruding into the niche of the latter. Viruses might have an important role in reestablishing equilibria. Phage biologists have shown that viruses interfere with the transfer of organic matter in the food chain, assuring enough nutrients in the microbial loop. Bacteria profit thus from their bacterial viruses. Phage biologists have also introduced the concept of a virus killing the winning population (Wommack and Colwell 2000). This concept means that phages cannot infect bacteria below a threshold density. However, bacteria that start to dominate a niche become excellent targets for phage infection. This way, phages are believed to maintain diversity of bacteria in any environment. Animal viruses might play a similar role in animal populations. Humans are a winning population in the ecosphere and occupy more and more niches. However, by doing so

and changing the ecological framework, we are getting into a viral cross-fire. The evolutionary "sense" of this viral cross-fire could be to maintain biological diversity. In that ecological "logic," humans are getting "too" numerous and we do not come alone- together with our domesticated animals and plants we are striving for agricultural surfaces and thereby geographical dominance on the globe. Viruses might be an in-built safety valve against this monopolization of the ecosphere by a dominant species. There are some speculations that climate change are behind all these emerging infectious diseases, which we have seen in recent decades. However, we might only "feel" the pressure of viruses that nature has "designed" to maintain organismal diversity. The sad prediction of such a hypothesis would be that we will see more and more viral accidents as described in this chapter, simply because we are getting in the way of too many species that compete with us for a place under the sun. If correct, we will need both a lot of science to defend our dominance against the viruses of our competitors and wisdom to refrain from our desire to subjugate the entire earth and to deny other organisms their ecological niche. In this sense, viruses might indeed be essential and constructive elements of life, even if we perceive them from our perspective as destructive demons. Viruses could have spoken the words of Mephistopheles quoted at the beginning of the chapter.

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