Virology

Invaders and infections

by Martin Thurau

Source: Lee Jin-man/Picture Alliance/AP Photo

Our globalized transport networks make it possible for hitherto unknown viruses to travel the world. LMU virologist Gerd Sutter has developed a platform for the production of vaccines against these emerging threats.

The first novel virus to go global got its big chance on February 21st 2003. On that day, the man experts would later call "the super spreader" checked into a Hong Kong hotel on that day. He wasn't feeling well, was feverish and had a cough. So the next morning the occupant of Room 911, himself a doctor, went to the nearest hospital. He didn't get around much in the city, but he managed to infect 16 people with a viral pathogen that had been circulating for months in Guangdong Province on the Chinese mainland, less than 200 km away. Based on the diffuse symptoms associated with it, the illness was later dubbed 'SARS' an acronym for "Severe Acute Respiratory Syndrome". At that point, its cause was still unknown.

Over the following 12 hours, the 16 people infected by the index case would bring the infectious agent from Hong Kong halfway round the world, to Vietnam, Singapore and Canada. In the succeeding weeks, it would reach some 30 countries on six continents. Nearly 8500 people were infected, of whom more than 900 died. Epidemiologists would later succeed in tracing nearly half of these cases back to the hotel guest in Hong Kong, who himself succumbed to the disease.

"It suddenly became clear to everyone just how fast an infectious agent can be propagated in the modern world," says Gerd Sutter, Professor of Virology at LMU. The globe is now one enormous network of commercial interactions: Business people are in Hong Kong today, London tomorrow and New York the next day – and vast numbers of tourists are on the go. With some 35 million flights annually, it is no wonder that airports have become a point of focus for epidemiologists.

"The process has accelerated"

Fear of the SARS virus spread even faster than the pathogen. It was rapidly identified as a member of the so-called coronaviruses, but this did little to allay fears of emerging pathogens and further epidemics, as coronaviruses had been regarded as essentially harmless. People hurriedly cancelled planned trips, airlines lost business, and social life in those locations the virus had reached came to a standstill, with pronounced economic consequences. In the German media, use of the term 'SARS' became pervasive, even though not a single fatal case of SARS occurred in the country. Ten years later, the media were recalling the time when "death flew around the world".

But SARS was only the first global epidemic of the new millennium. Less than a year later, bird flu was making headlines, to be followed by swine flu, Middle East Respiratory Syndrome (MERS) and then the unprecedentedly large outbreak of Ebola fever in West Africa. The latest in the sequence is the Zika virus. Discovered nearly 70 years ago in Africa, it recently reached the Americas, and is now endemic in parts of South America. The rate of emergence of new viral threats has increased in recent years – and this is not just an impression from improved surveillance or media hype. "The process has indeed accelerated," Sutter confirms.

With globalization, the world has effectively shrunk, which promotes not only the dissemination of goods and services, but also that of viruses and infectious illness. But where do the emerging viruses actually come from, and what factors turn a previously unremarkable virus into a killer? As the SARS example shows, viral breakout is both serendipitous and inevitable. Can these inherently unpredictable interactions be effectively prevented? Do we have reliable strategies to guide the development of vaccines against such threats?

These are some of the questions that concern Gerd Sutter. He works at the Institute for Infectious Diseases and

Zoonoses, which is part of LMU's Faculty of Veterinary Medicine. Around two-thirds of human pathogens originated in animals, and are often harmless to their normal hosts. Over millions of years of evolution, virus and animal host have developed a modus vivendi that enables both to survive and reproduce. In a sense, the new challenges have re-directed Sutter's research interests from veterinary to clinical medicine. He is now primarily involved in the design of vaccines to protect people from zoonotic viruses like SARS, MERS, bird flu or Ebola. And in this capacity, he has made a name for himself worldwide.

MERS turns up in South Korea: An outbreak of MERS in Seoul in June 2015 severely disrupted public life. *Source: Lee Jin-man/Picture Alliance/AP Photo*

Severe birth defects

Only a few months ago, a panel of experts compiled a list of the most urgent threats for the World Health Organization (WHO). They came up with what one could call "The Hateful 8", a set of lethal viruses that includes the coronaviruses SARS and MERS, and the Ebola virus and its close relative Marburg virus, both of which induce a hemorrhagic fever leading to severe internal bleeding. Similar symptoms characterize the syndromes caused by Crimea-Congo, Lassa and Rift Valley Fever viruses, while the Nipah virus also causes encephalitis.

The current scare concerns the Zika virus, which is strongly suspected to be responsible for severe birth defects in human neonates. In adults, infections usually take a mild course, sometimes accompanied by a skin rash, headaches and joint or muscular pain. But pregnant women, who may not even realize they are infected, can transmit the virus to the fetus. The health authorities in Brazil have linked the virus to a recent increase in the incidence of microcephaly, a congenital condition characterized by severe perturbation of brain development and malformation of the skull, in newborns. This link is supported by experiments in laboratory models which have shown that the virus infects neuronal precursor cells, which give rise to the nerve cells that populate the cerebral cortex.

The rapid spread of the Zika virus in Central and South America prompted the WHO to declare it a "public health emergency of international concern", in February 2016. Meanwhile, Brazil has initiated a campaign to inform the public of the risk, and mobilized thousands of soldiers who are using insecticides to reduce the numbers of *Aedes aegypti*, the mosquito that transmits the virus. According to a bulletin from the Robert Koch Institute (RKI) in Berlin, the agency tasked with monitoring the incidence of infectious diseases in Germany, the virus may have reached South America during "an international sporting event" (i.e. the World Cup) "in 2014," a suggestion that Sutter refers to as "an interesting speculation."

A cozy ecological niche

A. aegypti has long been known to transmit the yellow fever virus, which is endemic in large areas of the tropics and subtropics worldwide. But, as Sutter points out, the mosquito is "a competent vector" for a number of infections. Apart from acting as the intermediate host for the virus of Yellow Fever – an outbreak of which is currently raging in Angola – it also transmits the virus responsible for Dengue Fever. The viruses replicate in the insect and are transmitted when the mosquito bites its next victim. The Zika virus may also be transmissible by other mosquitos, such as the Asian tiger mosquito (*A. albopictus*), which is now found in Southern Europe, and "in certain localities in Southern Germany", such as the Upper

Rhine Rift near Freiburg, as the RKI reports. "However, no one believes that they currently represent a serious threat," says Sutter.

Only a few years ago, entomology was regarded as a rather old-fashioned subject. "But it is now booming," says Sutter. The renewed prominence of tropical diseases raises many questions. Which insect species might be candidate hosts, and what range of viruses might they carry and transmit? What habitats and dispersal mechanisms do they use? What impact will a milder climate have on the overall risk of infection in Central Europe? How will it affect the distribution of mosquitos hitherto restricted to the tropics and subtropics? "So far, there is little cause for alarm." Even if average temperatures were to rise by 1°C or more, we would still have periods of cold severe enough to prevent the establishment of permanent populations of these insects. "In many cases, a week of frost is sufficient to wipe out the interlopers."

Mosquitos act as intermediate hosts for many viruses. But the ultimate source, or reservoir, of most potentially transmissible viruses is the warm-blooded host to which it is specifically adapted. In these animals, the virus occupies a cozy ecological niche in which it can replicate and undergo genetic variation – which in turn enables it, over thousands of years, to evolve and become ever more attuned to the host's physiology. In the end, a stable equilibrium is established, a kind of live-and-let-live situation in which the virus does not harm the host. That such a virus should infect a human is usually an evolutionary accident, and often an error. If the virus kills its new host, its own chances of propagation are drastically reduced. This remains true until it acquires the ability to pass directly from one human to another, but that again involves a process of adaptation.

Birds serve as reservoirs for myriad viruses, and many of these hosts are migratory species that spend time in Europe each year. And indeed, as Sutter explains, stopover sites for water birds – such as the Ebro Delta in Northern Spain – have become hotspots for the dissemination of West Nile virus in Europe. In rare cases, exotic pets bought from online suppliers have turned out to harbor infectious viruses from the tropics. "Viral taxonomy is being rewritten at this very moment," says Sutter. "We are discovering a previously unknown world."

Be prepared! Gerd Sutter, shown here with his coworker Asisa Volz, hopes to begin a Phase I clinical trial of a new MERS vaccine this year. *Source: LMU*

Inadvertent intimacy

In addition to birds, rodents, apes and monkeys, bats are now being recognized as important intermediaries in viral life cycles, such as those of the SARS and MERS viruses – and the Ebola virus. Many bat species live in huge colonies, often in inaccessible caves, and are widely distributed in many regions around the world. Under such circumstances, viruses can circulate freely, filling a kind of metareservoir that provides an ideal laboratory for viral evolution. The same holds for many other animal hosts, says Sutter. "They harbor a wide spectrum of pathogens, and serve as breeding grounds for many emerging infections."

Epidemiological studies have traced the catastrophic outbreak of Ebola in West Africa in 2014 to a fruit-eating bat, a so-called hammerhead, which infected a toddler. The youngster may have come into contact with the dead animal in the bush, or he may have played with a mango that the bat had fed on. A few days later, in December 2013, the child died in his village, Meliandou, in the heart of Guinea. The neighboring villages have grown rapidly in recent years, as thousands of refugees have settled in the region, having fled civil wars in neighboring Sierra Leone and Liberia. They have degraded the forest cover

around existing villages, thus breaching the natural barrier between its denizens and human settlements. Such inadvertent intimacy can be catastrophic – as in the case of other infections, Sutter remarks. Indeed, the disturbance of natural habitats is a recurring pattern: "It extends opportunities for contact with endemic pathogens – and note that it is a matter of humans invading the territory of the viruses, not the other way round."

Based on epidemiological studies, researchers believe that the Ebola virus has infected humans on around 30 separate occasions since the 1970s. Most of the resulting outbreaks have occurred in isolated areas, and rapidly subsided because chains of infection are short. But this time, the virus – although not easily transmissible from person to person – managed to provoke a wholesale epidemic. Initially circulating within a small localized population, it took months to break out into more densely settled areas. Then it established itself in small towns located at the intersections between trade routes. Inexperienced personnel and understaffed hospitals may have unwittingly promoted the further spread of the virus. Months after the primary infection, the virus arrived in the urban centers on the coast, such as Freetown, Monrovia and Conakry – gateways to the wider world.

Blueprint for vaccines

It is not surprising that the WHO has undertaken a survey of the greatest viral threats , nor is it just a statistical exercise. The agency realized that it must be better prepared to respond when the next public health emergency appears. Project Blueprint, in which Sutter is directly involved, is designed to help speed up the development of vaccines and antiviral agents in the event of an outbreak of a lethal viral infection. It typically takes 10 to 15 years before a vaccine is ready for widespread use – far too long to enable health authorities to respond to an acute emergency. The idea now is to prepare specific blueprints for vaccines in advance, which can be rapidly manufactured when needed. In principle, this model could make it possible to develop a practical vaccine within 18 months to 2 years. "But at the moment, it's pie in the sky."

Draining the reservoirs

In the case of MERS, Sutter and his partners at the German Center for Infection Research (DZIF) will make the transition from candidate vaccine to initial trials in humans in the space of 4 years – provided the planned clinical tests can get underway in the autumn. "And even that is still far too long to be of much use for a situation like the Ebola outbreak," Sutter readily admits. MERS remains an urgent problem, as localized outbreaks continue to occur. The MERS virus first appeared in humans in Saudi Arabia in 2012. Since then it has broken out in the Gulf States and in neighboring regions. Some 1400 cases have been recorded, about a third of which ended fatally. Last year, the virus turned up in South Korea; life in the capital Seoul was severely disrupted.

The dromedary camel is thought to be the intermediate host for the MERS coronavirus, though direct person-to-person transmission is also possible. The fact that the MERS and SARS viruses are closely related has turned out to be "a lucky break" for researchers, Sutter points out. The family resemblance meant that he and his colleagues had a good idea which of the viral proteins was most likely to induce formation of protective antibodies, and could therefore serve as the basis for a vaccine. The viral products present in a vaccine provoke the production of antibodies that react with and induce the destruction of virus particles.

Having worked for many years with MVA (Modified Vaccinia virus Ankara), an attenuated poxvirus (which the Bavarian State Laboratory for Vaccines had used in the 1970s to raise a vaccine against smallpox), Sutter also had a suitable carrier for the MERS-specific target molecules in his toolbox. Using genetic engineering techniques, his team inserted the gene for the MERS spike protein into the MVA genome, such that the MERS protein was expressed by the MVA particles. The MERS genome had already been sequenced, so no coronavirus particles were needed for the experiments. The relevant nucleotide sequence was chemically synthesized by a commercial supplier.

In December 2015, Sutter and his collaborators reported in the journal Science that their candidate vaccine was effective in dromedaries. "We showed for the first time that, upon subsequent infection with MERS, virus replication was significantly inhibited in vaccinated camels, he explains. The infected animals also showed none of the typical symptoms of MERS – inflammation of the nasal epithelia and sinuses – because they had developed neutralizing antibodies against the virus. According to the researchers, the results suggest that one could, in principle, use the vaccine to eliminate the virus reservoir by inoculating all the camel herds.

However, the Munich virologists are also pursuing the standard approach: In collaboration with experts at the Hamburg University Hospital in Eppendorf and at Marburg University, the DZIF is gearing up for a Phase I clinical trial, which is scheduled to begin this year. This test is designed to show that the MERS vaccine is safe for use in humans,

and that it generates antibodies against the spike protein.

Sutter hopes that the MERS vaccine could serve as a template for the Blueprint initiative. Preliminary work with Ebola virus proteins indicates that the MVA also provides a promising framework for the development of vaccines against other viruses on the WHO list, he says.

The dying swans

In Sutter's view, the fact that the SARS epidemic did not claim even more victims was a fortunate accident. In the end, the virus was successfully contained using conventional measures – isolation of those infected and their immediate contacts. "But it was a close call," he adds. Luckily, transmission of SARS requires direct contact with secretions; it is not spread by means of aerosols. "However, when people talk of a pandemic their classical standard of reference is influenza. The influenza virus reached all parts of the world without the help of the latest wave of globalization, which is why we think of it as being in a different class." But the Ebola epidemic has once again demonstrated that a subtropical virus that is difficult to transmit can cause infections in Europe and the US.

Perhaps the distressing images of patients are responsible for the fact that a hemorrhagic fever, like that caused by Ebola, gets so much more attention than the recurring waves of the common flu which claim the lives of thousands of people in Germany alone every year. Subtypes of the flu virus that can be transmitted from birds to humans on poultry farms or in markets are, however, of greater concern. That avian influenza viruses pose a real threat to humans became clear only around the turn of the century, says Sutter, with the appearance of the subtype H5N1, which first appeared in 1997 in Hong Kong, and has extended its range since 2003.

We are very fortunate in two important respects, says Sutter, who is now working on MVA-based vaccines directed against new forms of influenza virus: In order to contract a severe and potentially fatal pneumonia, one must be exposed to a very high dose of H5N1. "The nose really has to be stuffed with the virus." Even more importantly, H5N1 cannot be passed directly from person to person – at least not yet. But flu viruses are highly mutable and can adapt rapidly to a new host. In laboratory experiments, the bird virus has indeed developed the capacity for direct transfer within one mammalian species, the ferret.

In 2013, a new influenza virus appeared in China, the subtype H7N9. In the case of H5N1, "swans fell out of the sky here in Germany." For subtype H7N9, we have no such early warning system. Infected poultry show no obvious symptoms. But H7N9, says Sutter, has not yet set out to conquer the world …

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