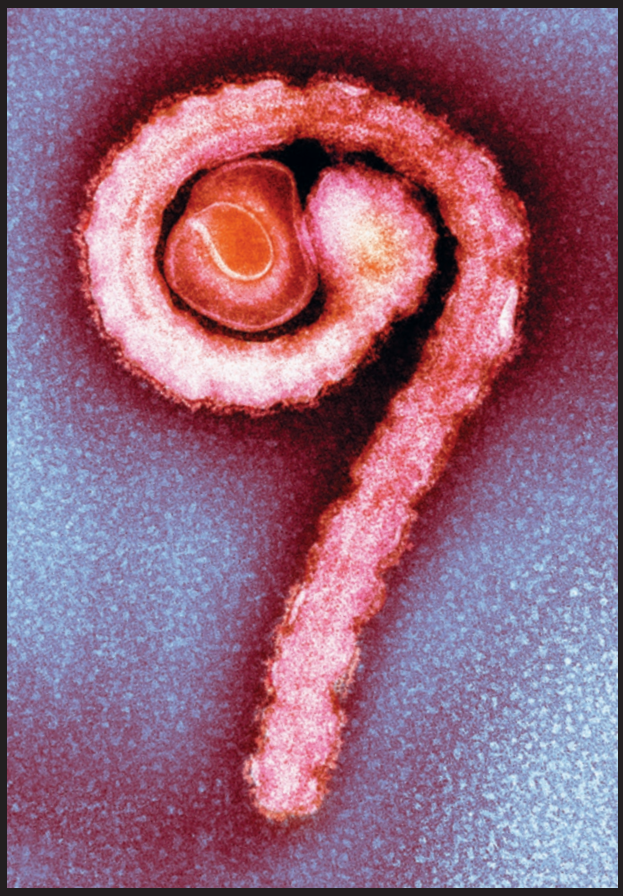


CENTRE FOR INFECTIONS/PUBLIC HEALTH ENGLAND/SPL

THE EBOLA QUESTIONS

Scientists know a lot about the virus that causes Ebola — but there are many puzzles that they have yet to solve.

BY ERIKA CHECK HAYDEN

An Ebola virus particle from the 2014 outbreak.

Too much of the world, the virus behind the devastating Ebola outbreak in Africa seems to have stormed out of nowhere. But Leslie Lobel thinks we should have seen it coming.

In 2012, Lobel and a team of researchers spent six months in Uganda studying the Ebola virus and related viruses. Over the course of their stay, these pathogens caused at least four separate outbreaks of disease in central Africa, affecting more than 100 people. To Lobel, a virologist at Ben-Gurion University of the Negev in Beer-Sheva, Israel, the outbreaks felt like the small tremors that can precede a major earthquake. "We all said, something is going on here; something big is going to happen," he says.

Like Lobel, other scientists have predicted that these viruses would one day cause a major epidemic — and the current outbreak, which has so far killed nearly 5,000 people, has proved them right. There are five species of closely related viruses that scientists refer to as 'ebolaviruses'; the species behind the current outbreak, *Zaire ebolavirus*, is more generally known as 'the Ebola virus'. Along with Marburg virus and Lloviu virus, the ebolaviruses make up the filoviruses, a family that was unknown before the 1960s. All of the filoviruses share a common structure, and most of them cause life-threatening haemorrhagic fevers in humans.

Research on these once-ignored viruses took off after the 2001 anthrax attacks in the United States, which prompted officials to sink money into investigating lethal pathogens that might be used in bioterror attacks and to build dedicated laboratories where they can be

safely studied. Scientists have learned how these viruses work and have created the first experimental vaccines and therapies that might stop them. "The biodefence funding has been huge," says microbiologist Thomas Geisbert of the University of Texas Medical Branch at Galveston, who has studied ebolaviruses for 26 years.

But the advances in knowledge about filoviruses have also exposed the gaps. Scientists suspect that more members of the filovirus family remain to be discovered and in other parts of the world. They are also working to understand which animals naturally harbour filoviruses and why human filovirus outbreaks seem to be rising in frequency: they have occurred in 19 of the past 21 years, and three times this year alone. Finding answers is difficult because outbreaks are unpredictable and laboratory work with filoviruses requires the highest security measures.

In the past few months, research has necessarily taken a back seat to efforts to control the Ebola outbreak — but as the epidemic escalates, science is coming to the fore. Researchers are recognizing that they might be able to stop this Ebola virus only if they understand its biology and how to control it. "We need a lot more information about the virology, the clinical presentation and the epidemiology of this virus," says Michael Osterholm, a public-health scientist at the University of Minnesota's Center for Infectious Disease Research and Policy in Minneapolis. "Nobody underestimates the difficulty of doing that research in these settings, but it is really important to get this information." With this in mind, *Nature* asked leading researchers to discuss the most urgent scientific questions about the Ebola virus and other filoviruses — the questions that, if answered, might prevent another disastrous outbreak, or help to contain the current one. This is what they said.

WHERE DO FILOVIRUSES COME FROM?

In July 2007, a miner who had been prospecting for lead and gold in a Ugandan cave became infected with Marburg virus. Officials closed the cave, and a team of researchers led by the US Centers for Disease Control and Prevention (CDC) swept in to investigate. They hoped to answer a decades-old question: what animal is the natural host for filoviruses? The mystery had lingered since 1967, when Marburg virus — the first filovirus to be discovered — sickened European lab workers who had handled imported monkeys. The high lethality of filovirus infection in monkeys, humans and other apes suggested that primates were not the natural hosts: if a virus kills too many of its hosts, then it cannot propagate and dies out. There were clues that bats might be the 'reservoir' species. But to prove it, scientists needed to find an infected bat.

The researchers captured some 1,300 bats roosting in the cave and tested their blood for Marburg virus[1]. They finally found what they were looking for: infectious Marburg viruses isolated from five Egyptian fruit bats, none of which showed symptoms of disease. The team also found more infected bats in a nearby cave that had been linked to a previous Marburg virus outbreak.

It is not entirely clear how the virus is transmitted from bats to people, although the most likely route is through contact with bodily fluids. Bats infected with Marburg virus in the lab shed the virus in their mouths, so wild bats might spread it by leaving traces on fruit that is later eaten by other animals[2]. Knowing the host species for the other filoviruses is crucial. "Until we understand what that reservoir is, it is difficult to limit your encroachment on that species," says virologist John Dye of the US Army Medical Research Institute of Infectious Diseases in Fort Detrick, Maryland.

Researchers now strongly suspect that bats are the natural host for ebolaviruses, too. In 1976, during one of the first known Ebola outbreaks, the six people who were initially infected worked in a factory room in Sudan that was home to roosting bats[3]. Researchers have since isolated antibodies to ebolaviruses from bats, as well as snippets of genetic material from the viruses. But proving that bats are the reservoir has been maddeningly difficult — no one has isolated an infectious ebolavirus from a wild bat, and it has been difficult to trace rare and sporadic outbreaks back to a source. The ebolavirus outbreaks have originated in many locations, only sometimes among people or animals who have had contact with bats. "You're pretty much looking at the entire tropical forest," says Jonathan Towner, a molecular virologist at the CDC in Atlanta, Georgia, who trapped bats in Uganda for the Marburg investigations.

The current outbreak is thought to have begun in southeastern Guinea in December 2013, when a two-year-old boy died of a mysterious illness that quickly spread to family members and health-care workers. So far, the response to the outbreak has been much more focused on containing it than tracing its source. "The public-health crisis is unprecedented," Towner says. "There's no room for an ecology investigation at this point."

HOW WIDESPREAD ARE FILOVIRUSES?

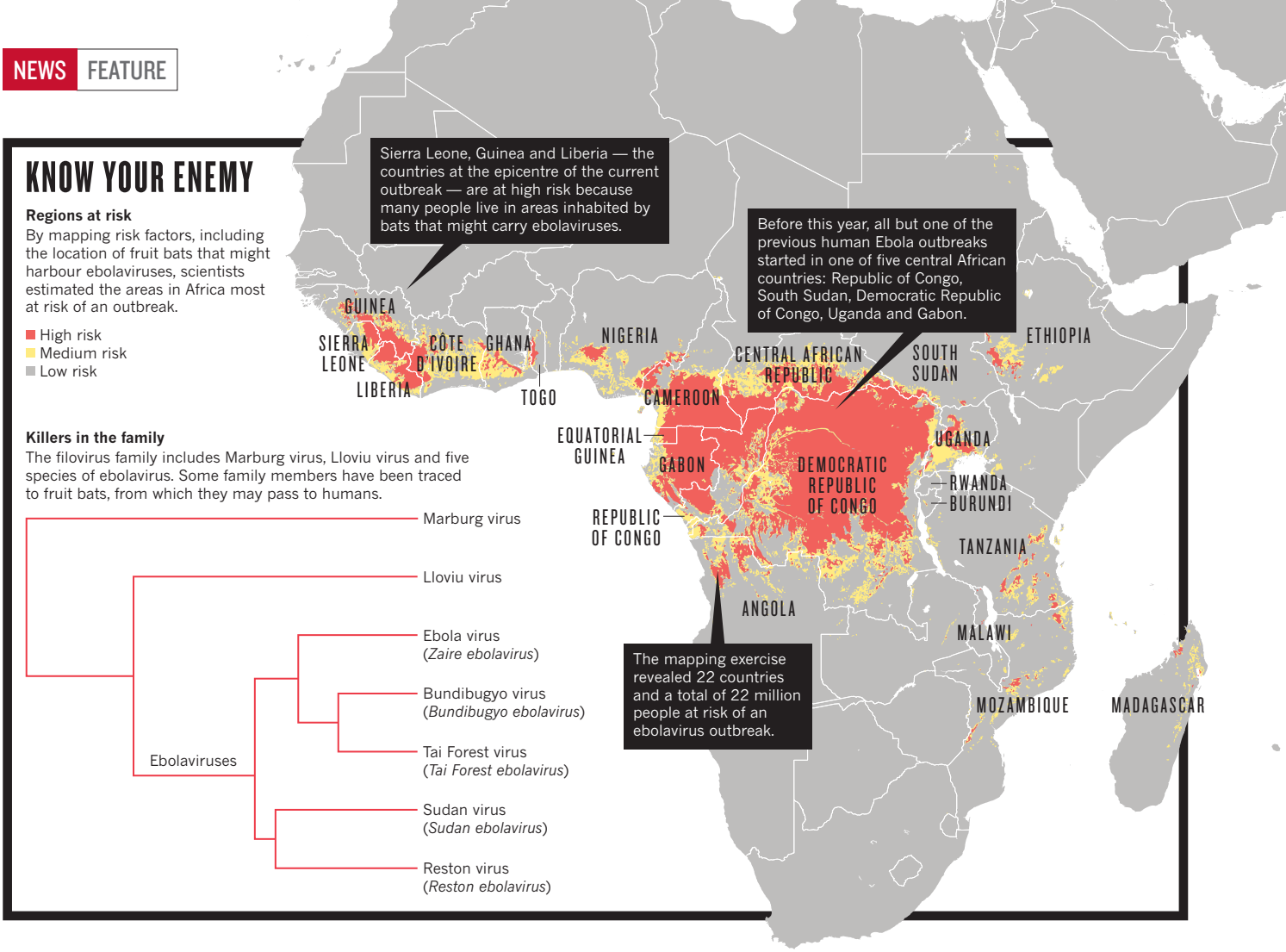
Filoviruses are not just found in bats and primates. That became clear in 2008, when Philippine officials called for help to investigate an outbreak of disease in pigs. When researchers arrived, they found that the pigs were infected with *Reston ebolavirus*[4] — a species that was first discovered in monkeys imported to the United States from the Philippines in 1989. The pig discovery was a shock because until then, no ebolavirus had been known to naturally infect a farm animal. And it was not a freak event: *Reston ebolavirus* was also reported[5] in pigs in China in 2012. However, the Reston virus seems to be relatively harmless to humans. People working on the Philippine pig farms harboured antibodies to it — a sign that the pigs had transmitted the virus to them — but no humans fell ill.

In 2011, scientists confirmed that pigs can also become infected with *Zaire ebolavirus*[6]. The concern now is that pigs could serve as a mixing vessel for filoviruses. Pigs could be simultaneously infected with several filoviruses, which might exchange genetic material to eventually produce new versions that are capable of sickening humans. "The relevant practical question is, do we need to worry about Reston? If it is truly not pathogenic in humans, is there a potential that it could change?" says Erica Ollmann Saphire, a structural biologist who studies filoviruses at the Scripps Research Institute in La Jolla, California.

> "We all said, something is going on here; something big is going to happen."

Scientists are probably just beginning to understand the different types of filoviruses and their geographic reach. The list of known filoviruses only recently expanded: the fifth ebolavirus (*Bundibugyo ebolavirus*) was discovered in Uganda in 2007, and the Lloviu virus was identified from dead bats in Spain in 2011. "We may find more members of the filovirus family elsewhere in the world," says virologist Ayato Takada of Hokkaido University in Sapporo, Japan. The fact that the viruses are more common than was once thought suggests that they have been around for a very long time — perhaps for most of human history. And it could be that scientists are detecting only a small fraction of events in which they cross over from animals to humans. Researchers are now trying to work out how frequently they jump into people and, when they do, how often they cause disease. In 2010, one team reported that as many as 20% of people in some areas of Gabon carry antibodies to *Zaire ebolavirus* in their blood, indicating that they were exposed to the virus in the past without becoming ill[7].

Lobel says that these data must be taken with a pinch of salt, and that the assays could actually be detecting immune responses to viruses similar to ebolaviruses. In his studies, people known to have survived definite Ebola virus infections had a different immune response from



SOURCE: REF. 9

those who have never been infected. He and other researchers are now surveying immune responses among more filovirus survivors.

A pressing question in the present outbreak is how the currently circulating *Zaire ebolavirus* might be changing. Its rapid spread hints that there could be something different about this strain — possibly that it has become easier to pass from one person to another. "We have to start investigating whether the 2014 strain behaves like previous outbreak strains, or whether it could potentially be more transmissible," says Kristian Andersen, a virologist at the Broad Institute in Cambridge, Massachusetts.

Andersen is careful to note that there are no data to suggest this yet. Although a handful of researchers have raised fears that the virus might mutate into an airborne form, most think it is unlikely that the virus could change so dramatically in such a short time. Genetic analyses have shown that this strain of *Zaire ebolavirus* has mutated hundreds of times since it diverged from an ancestral virus about ten years ago[8] (see *Nature* http://doi.org/vsd; 2014), but no one yet knows whether any of these mutations have altered important properties of the virus. Instead, its unprecedented spread is thought to be due to the fact that it emerged in an area of Africa where people were unfamiliar with the virus and how to control it, allowing it to escape to urban centres. Researchers are now carrying out more-detailed studies of the virus to better understand its origins and characteristics.

ARE WE MAKING EBOLA OUR OWN WORST ENEMY?

In September, epidemiologists published an analysis in which they mapped the locations of all ebolavirus outbreaks in Africa along with the known ranges of three bat species that are candidate reservoirs for the viruses[9]. They also plotted changes in African populations and mobility — for instance, the proportion of each country that lives in rural and urban areas. The team wanted to pinpoint the areas that might be at highest risk of future outbreaks.

Before this year, all but one of the human ebolavirus outbreaks could be traced back to central Africa, and the *Zaire ebolavirus* species had never been seen in West Africa. But "we shouldn't have been massively surprised that it was there", says epidemiologist Simon Hay of the University of Oxford, UK, who led the analysis. Even when he and his team did not include data from the current outbreak, they predicted that the three hardest-hit countries — Sierra Leone, Guinea and Liberia — would be at high risk for an ebolavirus outbreak because they have large numbers of people living in areas inhabited by the bats. In all, the analysis highlights 22 African countries where ebolavirus outbreaks are likely to start, putting 22 million people at risk (see 'Know your enemy').

The study also begins to explain why filovirus outbreaks seem to be growing more frequent, widespread and larger in size. Human populations in countries that are likely to harbour filoviruses have nearly tripled since the viruses were first discovered, and flight traffic has increased by one-third since 2005. The viruses are not coming to us; instead, we are encroaching on the viruses, as population growth and increasing travel put humans in contact with viral hosts, and then people unwittingly transport the viruses around the world. "There's this perception that these outbreaks occur in completely isolated, remote parts of Africa where they burn out before they hit major population centres," Hay says. "Obviously we've seen that this is not the case with the latest outbreak." His team has released its data[10] and hopes that others will now use the information to look for more specific environmental factors — combinations of climate and geography that might pinpoint the precise places where future outbreaks are most likely to occur.

NATURE.COM
For more on the spread of the 2014 Ebola outbreak, see:
go.nature.com/m55ual

WHY IS EBOLA SO LETHAL?

Ebola virus is one of the most lethal viruses known. In the current outbreak, an estimated 60–70% of those infected have died, and in previous outbreaks the figure has reached almost 90%. (Only rabies, smallpox and a handful of other viruses are as fatal if left untreated.) The reason that Ebola virus and other filoviruses are so lethal is because they turn the body's own defences against it.

Normally when a virus invades the body, it triggers cells in the 'innate' arm of the immune system, which cause inflammation and other reactions to fight off the infection. The Ebola virus, however, infects and cripples innate immune cells, taking out this first line of defence. These dying cells also trigger a destructive flood of chemicals, called a cytokine storm, and cause the downstream death of cells that normally make protective antibodies.

Other highly pathogenic viruses also trigger cytokine storms, but filoviruses are thought to be particularly lethal because they affect a wide array of tissues. As well as the immune system, Ebola virus attacks the spleen and kidneys, where it destroys cells that help the body to regulate its fluid and chemical balance and that make proteins that help the blood to clot. At its worst, Ebola virus causes the liver, lungs and kidneys to shut down, other organs to fail and the blood vessels to leak fluid into surrounding tissues. Too often, this ends in death.

If scientists can understand how the immune systems of survivors were able to fight off the virus, they might be able to encourage this form of defence through a vaccine. Researchers have found that survivors of previous outbreaks managed to make antibodies to Ebola virus, avoid the cytokine storm and preserve their immune cells during the course of infection. But why they can do this, and others cannot, is a mystery. "The question in our mind is, how did they survive?" Lobel says.

The right treatment can raise the chance of survival. In the current outbreak, people infected with Ebola virus who are treated in developed countries have been more likely to survive than patients treated in Africa, because they receive much more intensive care. There is no targeted treatment for filoviruses, but doctors can closely monitor and correct blood chemistry and protein imbalances caused by organ failure and fluid loss — with intravenous drips or kidney dialysis, for example. "Right now, the Ebola virus has such a high lethality because of the level of care provided in the places where it is occurring," says Dye. "If health care gets better, the lethality rates will decrease."

Sadly, Osterholm says, even the most basic interventions that could help people infected with Ebola virus are not being provided right now in the hardest-hit areas. For instance, oral rehydration therapy is being widely used in place of intravenous fluid replacement because of the fear that health-care workers could be infected in the process of inserting an intravenous line. Osterholm says that it is urgent to ask how these choices are affecting patients: "In this setting, what treatments are working? Is what we're doing having any clinical impact?"

CAN THE VIRUS BE STOPPED?

Dozens of previous filovirus outbreaks have been halted using the same basic tools: isolating and treating patients, and tracing and monitoring their contacts. Public-health officials have also used this method to contain the spread of Ebola virus in Nigeria and Senegal. But across West Africa as a whole, the public-health response was completely inadequate at the start of the outbreak, which has allowed the virus to spread rapidly.

If, as some epidemiologists predict[11], the disease infects tens or even hundreds of thousands of people by January 2015, then it could become nearly impossible for tried-and-tested public containment measures to bring the epidemic under control. For one thing, it could be unfeasible to recruit and train the numbers of medical staff required. At this stage, some experts say that a new plan is needed.

Aid agencies and non-profit organizations are already trying new types of control. In Sierra Leone, officials are building isolation centres that can house patients away from their family and community, to prevent the virus from spreading, but that have fewer qualified health-care workers than a standard treatment facility. It is a controversial move, because it risks sending the message that patients are being warehoused to die. But it acknowledges the harsh reality that existing clinics are full, patients are being turned away, and that this is fuelling the disease spread. "Everyone realizes these smaller-level community facilities are not ideal, but it is trying to do something rather than nothing to try to bring transmission down in communities," Hay says.

Another new approach in this epidemic has been the use of experimental therapies and vaccines developed specifically to target Ebola virus. Much attention has focused on ZMapp — a cocktail of antibodies that was first identified using mice vaccinated with Ebola virus proteins, and which has been given to several people in this outbreak. In August, researchers reported that ZMapp protected 18 monkeys from dying of Ebola virus — the first report of a highly successful therapy in animals already showing symptoms of the disease[12]. Researchers are now organizing further tests of this and other medicines, as well as a handful of experimental vaccines.

> "The question in our mind is, how did they survive?"

But even if these therapies are proven to work, the challenge is hardly over. The products that have progressed furthest in development are all focused on *Zaire ebolavirus*, the most lethal of the four species that sicken humans, but it is unlikely that they will be as effective against other filoviruses. Saphire leads an international consortium that is systematically testing combinations of antibodies to find the ones that work best against different filovirus types. Researchers hope one day to produce therapies that work against multiple filoviruses and that could be used immediately when symptoms are detected, rather than spending precious time diagnosing exactly which virus is making someone ill.

All this work has taken on new urgency as predictions about the African outbreak have grown more dire. This year, the US National Institute of Allergy and Infectious Diseases, the Wellcome Trust, the European Union and various drug companies have channelled emergency funding into research on potential drugs and vaccines. And on 3 November, the US Institute of Medicine is convening a meeting in Washington DC to lay out an agenda for research on Ebola virus. That meeting takes place against growing worries that the virus might become endemic in parts of Africa, continuing to sicken and kill people for several years.

Researchers understand that the public-health response to this outbreak is a top priority, but they are also becoming more determined to understand this mysterious family of killer viruses. "What's happening in West Africa is a desperate situation," Saphire says. "It makes people very willing to figure out what they need to do to solve problems." ■

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