Kyasanur Forest Disease: An Inquisitive story

- Part 2

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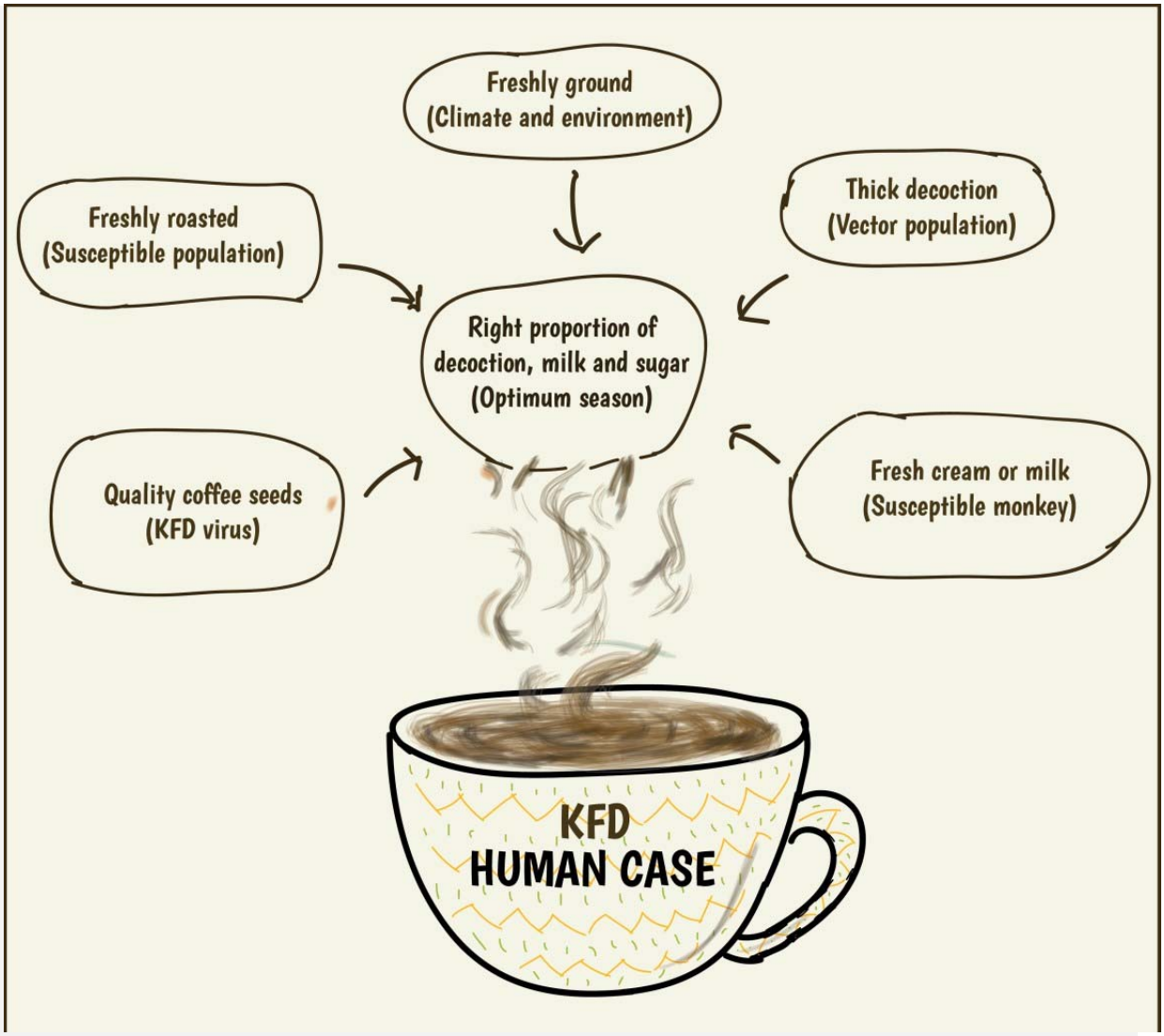
Zoonotic viruses, in order to survive, must find a way go back and forth between a vector and a reservoir organism, a relationship that must have co-evolved over millions of years. “Each virus is a zoological entity unto itself,” wrote the virologist Wilbur Downs, “with its own narrowly defined determinants for its continued successful existence.”

Rajagopalan and his fellow researchers suspected the various smaller mammals roaming about on the forest floor – rodents and shrews. They set up Sherman traps along forest interfaces, in the undergrowth, in the entrances of holes in the ground and outside hollow trunks. Within these traps, they placed an enticing bait: “a spiced split-pea paste mixed with onion and fried in oil”. *[4]* In other words, a *pakoda*. The animals were indeed hosting ticks, of several species, and many were infected with the KFD virus. These small mammals were keeping the virus alive during the monsoon, between infection seasons, hosting both *H. spinigera* and other genera of ticks, mainly *Ixodes*, which are also vectors for the KFD virus. In turn, the quick population turnover of these animals ensures that there’s always a good number of susceptible hosts for the virus. “In three or four months, another batch comes,” Rajagopalan told me, snapping his fingers.

They found, however, that the number of infected animals was a small proportion of the total population. Of those, only some circulated the virus in their blood and became capable of infecting ticks. What had caused the virus to break out of this enzootic cycle and infect humans?

Rajagopalan likes to refer to the confluence of factors responsible as “Boshell’s cup of coffee”. First, one must have quality coffee seeds – the virus. “Then,” he says, “it must be freshly roasted.” A susceptible population of animals. The roasted seeds must be freshly ground; the climate and environment must be suitable. There must be a thick decoction, meaning the presence of large number of vectors, the ticks. Add to this fresh cream or milk – susceptible monkeys. He recalls what Boshell liked to say: “If all these are present in the right proportions,” as in a good cup of Chikkamagalur coffee, “you get a human KFD case.”



An illustration of Boshell's cup of coffee. Credit: Ita Mehrotra

All these factors were at play in Shimoga. The forest was not unbroken; it was interspersed with fields where rice, sugarcane, arecanut and other crops were cultivated. And the villages were on the edges of these forests. Domestic cattle, though not themselves infected, hosted adult ticks, amplified their population and distributed them. The region had witnessed a doubling of its human population in ten years from 1951 to 1961. This was accompanied by an increase in the cultivated area, with clearing of forests.

Boshell reported in his paper that the interface between forest and cultivated area or open grassland, areas where original forests had been destroyed, was often occupied by an invasive weed called *Lantana camara*, which grew in thickets up to three metres high. *Lantana* had made its way to India from Sri Lanka, where it had arrived from Central America. "It is a fact of daily observation, for instance," noted Boshell, "that the last refuge of the hard-pressed jungle fowl is the impenetrable *lantana* thicket." Other invasive weeds linked to abundant tick populations include *Eupatorium* and*Chromolaena odorata*. Small mammals would spend time in this dense undergrowth, shedding ticks that could then be picked up by monkeys forced to spend more time on the ground. As they retreated to the forest, the monkeys would shed ticks wherever they rested. Moreover, monkeys infected with KFD have high viremia, circulating the virus in their blood in large quantities, thus infecting ticks that bite them.

Such transition zones, where animals that normally have different habitats mix, are a known risk factor for spillover of wild pathogens. Lower biodiversity can make this worse because the disease vectors would be forced to more frequently parasitise their primary hosts. Perhaps a species that hosted the vectors, but had low viremia, is now disappearing and the vectors infest high viremia species instead. This has the effect of amplifying the proportion of those hosts and vectors that are infected with a pathogen. A higher biodiversity would mean the vectors can feed on a wider range of hosts, some of which might not be good reservoirs for the pathogen.

A good example is the case of Lyme disease in the US, caused by bacteria and carried by *Ixodes* ticks found on deer. In forests with a smaller diversity of vertebrates, the tick population was found to be larger. Moreover, a larger proportion of the tick population was found to be infected with the bacterium. Another example is the protozoan that causes Chagas disease, whose prevalence in small mammals in fragmented forests was found to be higher than in contiguous forests.

It is notable that no such detailed ecological studies have been carried out for any zoonotic pathogen in India, including the KFD virus, since the 1970s, when the Rockefeller Foundation pulled out and closed their field research stations. This is made worse by poor knowledge of zoonotic pathogens in India's wildlife. Worldwide, an estimated 75% of emerging pathogens are zoonotic.

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After the initial outbreak in 1957, the places where KFD flared-up shifted from year to year but slowly. In 1964, a dam constructed for a hydroelectric power project on the river Sharavathi, which runs through Shimoga and neighbouring districts, caused "submersion of a large area along with loss of biodiversity", according to an impact assessment. Then, in the early 1970s, there were outbreaks in four districts that neighbour Shimoga. There was a major flare-up in 1982-1984 when more than a thousand people were infected, a tenth of whom died. This was preceded by a deforestation for export-oriented cashew plantations financed by the World Bank. The area under cashew cultivation had quadrupled in Dakshina Kannada district in the preceding years. Several papers have noted how KFD has appeared in areas where forests have been cleared for 'monocultures' such as teak, eucalyptus or cashew plantations.

Once a human is infected with the virus, it can take anywhere from two days to a week for the symptoms to show up. Apart from the continuous high fever and body pain, diarrhoea and vomiting is common – as is an extreme sensitivity to light, abnormal red blood cells and a reduction in platelet count. However, there are variations, too. Most cases are not haemorrhagic but of those that are, bleeding has been observed from the nose, gums and intestines. Some people recover after two weeks of persistent symptoms. A few, after a week or so of no symptoms, relapse – this time with neurological symptoms.

This biphasic nature of the illness remains a puzzle. Most patients recover completely but remain weak for months. In these and other aspects, the KFD virus – classified as Biosafety

Level 4, the most dangerous class of pathogens – was found to closely resemble Omsk Haemorrhagic Fever, found in Siberia, and which also belongs to the family of tick-borne flaviviruses.

"KFDV is an interesting virus because it behaves somewhat differently from other related tick-borne flaviviruses," says a researcher who did not want to be named. Unlike for other tick-borne flaviviruses, she says, monkeys play an important part in the transmission. There are more specific questions having to do with what the virus does once inside the body. "What is the immune response following KFD virus infection? Does the virus regulate components of the immune response? What are the target cells for the virus? How does the virus get into the brain? What does the disease process look like in non-human primates?"

According to her, these questions are important because, once someone is infected, "you are often dealing with the immune response or other repercussions of the virus infection rather than the virus itself."

As recently as 2004, India's National Institute of Virology (NIV) had declared KFD a "complete success story from detection of virus to the development of killed vaccine". But a 2016 paper acknowledged that "human infections have reached an alarming level in spite of the availability of a vaccine". The vaccine for KFD was developed in the 1960s based on a strain isolated during the early research. Annual rounds of vaccination have been carried out in the affected areas since 1990. However, this is confined to villages within a five-kilometre radius of areas where infections had been reported in the previous year.

The vaccine also has a cumbersome protocol: two doses spaced one month apart, with a booster after six to nine months. This has to be followed by another booster at the end of one year, repeated yearly for the next four years. The vaccine is limited to those between the ages of 7 and 65 on the grounds that only they are likely to be exposed to the forest – which is another oddity because 17% of infections in a study fell outside this age group. Further, in 2005–2010, 52% of the eligible population did not receive the vaccine. Of those who did receive, some who had received two doses *and* a booster were infected, too.

In 2006, the first human case outside this endemic area was reported in Gulbarga district bordering Maharashtra. After modern diagnostic tests were developed at NIV, unexplained fevers in other places began to be screened for KFD. Since 2012, more than a hundred cases and twenty deaths have been reported from districts that border Karnataka in the neighbouring states of Kerala, Tamil Nadu, Goa and Maharashtra. In Goa, workers at cashew plantations appear to be at greater risk. In both Goa and Wayanad, there is a clamour for monkeys to be declared "vermin". And in both places, tribal communities are the ones affected and have reportedly been refusing vaccination.

This coincides with reports from Goa of a shortage of vaccines. And earlier this year, some people in Wayanad resorted to setting forest undergrowth on fire in an effort to kill ticks, reportedly on the suggestion of health workers.

Ticks being picked off (the person in this image is not P.K. Rajagopalan). Credit: P.K. Rajagopalan

G. Arunkumar of the Manipal Centre for Virus Research acknowledges the need for a better vaccine. He believes KFD has always been misdiagnosed in the new areas because its symptoms resemble that of dengue or that of any other illness causing an acute fever. “There is no reason to believe that it has emerged now. It was always there,” he says. “All our disease surveillance efforts till 2012 were limited only to the Shimoga area. But when we at Manipal Centre for Virus Research started active fever surveillance along Western Ghats, the disease was detected in other areas, too.”

Serological surveys had indeed uncovered the presence of antibodies in animals and humans in places far removed from Shimoga and as far back as 1952. But if modern diagnostic tests and surveillance are the reasons for KFD’s apparent emergence in recent years, it only pushes further back in time the question of when and how it becomes epizootic in these areas. In fact, there are claims that KFD has been infecting people in Goa from the 1980s. But several questions remain: Why are monkeys dying in noticeable numbers now? Why these and not other places along the Western Ghats? And what factors are amplifying the tick population in these new areas?

“Nobody has looked at it. We worked because of the Rockefeller Foundation. Those scientists were dedicated people. If we were purely under the [Indian Council of Medical Research] we would also not have done anything,” says Rajagopalan. The council has been running NIV after the Rockefeller Foundation’s support ended in 1967.

A recent paper on the Kerala outbreaks reported that although people living in the affected areas had been bitten by ticks even in previous years, it was only in 2014-2015 that they fell ill. If this is true, then it suggests that KFD has become epizootic in these areas only now. Arunkumar, however, dismissed this as “baseless”.

Meanwhile, in 1994, a butcher in Makkah, Saudi Arabia fell ill with symptoms similar to KFD. The virus in his blood was found to be closely related to the KFD virus. Cases of the Alkhurma haemorrhagic fever (AHFV) have since turned up in other parts of Saudi Arabia over the years and with a fatality rate similar to KFD. In 2010, it surfaced near the Egypt-Sudan border. It was found that this fever was also carried by ticks of *Ornithodoros* and other genera and which parasitise sheep and camels.

It was initially thought that the KFD virus had been somehow introduced to Saudi Arabia in the recent past. Genetic analysis of the KFD and AHFV viruses, however, revealed that they

diverged around 700 years ago. The Karshi and Farm Royal viruses in Uzbekistan and Afghanistan, respectively, have also been found to be related to the KFD virus. A paper noted that this "raises the possibility of closely related but undiscovered virus variants existing in the regions between Saudi Arabia and India."

There is speculation that the ancestor of all these viruses may have travelled along the Silk Road, between China and Europe, presumably on camels carrying ticks. A related virus was believed to have been found in China but this has since been contested. The history of the virus remains a mystery for now – along with other questions, some more pressing.

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Rajagopalan likes to refer to the pioneering Soviet entomologist and disease ecologist Yevgeny Pavlovsky's concept of the "nidality" of a disease. Just as animals have their natural habitats, Pavlovsky argued, so does a disease. A zoonotic disease exists under certain environmental conditions in which the pathogen, its vectors and its reservoir hosts have a relationship that is defined by the geography they find themselves in. When they penetrate this web of interrelationships, humans can inadvertently cause the nidus, or focus, of the disease to shift.

"There are wheels within wheels," Wilbur Downs had written, referring to the intricate mechanisms a virus has to find over aeons to keep itself alive, flitting from species to species in the wild through various chains of infections that may or may not overlap.

"There is the 'small mammal-*Haemaphysalis*-small mammal' chain and the 'small mammal-*Ixodes*-small mammal' chain," Rajagopalan told me, referring to the two genera to which ticks found to carry the KFD virus belong. "Then there is the 'small mammal-*Haemaphysalis*-monkey' chain. Finally, the 'monkey-tick-man' chain." He also suspects there must be a 'bat-tick-bat' zoonotic cycle in nature, based on work he did just before the field research station in Shimoga was closed. It is not known if such a cycle feeds into the 'small mammal-tick-monkey' chain.

Although ticks have been found to transmit the virus from one generation to the next in the lab, Rajagopalan believes this is not an important mechanism for keeping the virus in circulation between infection seasons. Researchers in the 1960s had found infected *Haemaphysalis* nymphs and adults that survived the dry season preceding the monsoon, overlapping the next generation of ticks that started appearing after the monsoon. Further, shrews are parasitised by both the*Haemaphysalis* and *Ixodes* ticks, perhaps facilitating transfer of infection from one to the other. This is important because*Ixodes* ticks are more numerous during the monsoon.

If ticks are present in unusually large numbers, as was found in Wayanad and Malappuram districts of Kerala, it means that there is an abundance of appropriate hosts. What these hosts are in the new areas where KFD has emerged remains unknown in the absence of detailed field investigations. When they're not attached to a host and feeding, the ticks are

known to require very specific conditions of rainfall, humidity and temperature for their survival.

In Turkey, for example, the risk of occurrence of the Crimean-Congo haemorrhagic fever in new regions has been modelled statistically, based on knowledge of where the relevant tick species was already present, and on climatic conditions and ecological interface zones. Worldwide, a climate-change-induced shift of the abundance and distribution of many tick species has been noted over the last three decades. Increasing mean temperatures have been associated with shorter development cycles in ticks. In fact, the VRC researchers had noted that after an abnormally brief monsoon season in 1965, when it stopped raining abruptly in the middle of August, nymphs had started appearing a month early in October. Whether the emergence of KFD in new areas is in any way linked to climate change is unknown and has also remained unexplored.

There are other unknowns about the transmission of the KFD virus. Review papers have noted how virtually everything that is known about KFD comes from studies in the late 1950s and early 1960s. In this period, over 50 workers at the VRC lab in Pune were infected with the KFD virus. Some spilled infected fluids on their hands, but for others, the route of infection was less clear. Such infections raised the possibility that the virus could enter the body through inhalation of aerosols. After the Rockefeller Foundation pulled out in 1967, and the VRC became the NIV, lab work was discontinued. It only resumed in 2005 when the 'Biosafety Level 4' classification was established.

New studies will require not just money but also expertise in entomology, which Rajagopalan believes India lacks. He has written extensively in the context of vector-borne diseases as to why he believes entomologists are a dying race in India. Rajagopalan, who was awarded the Padma Shri in 1990, has been very critical of the ICMR, a body that he served for more than three decades before rising to become the founding director of the Vector Control Research Centre in Puducherry in 1975. "You ask them to live there in the forest," he says, referring to scientists today. "He's a scientist you know. Without white coat and an A/C room, he cannot work. Without a computer he cannot become a scientist."

Back in 1982, Wilbur Downs had noted in a review of the Rockefeller Foundation's virus research programme that "financing of field epidemiological studies does not receive high priority in today's laboratory-oriented virus research world." Rajagopalan concurs, and refers to field studies today as "safari research". "One thing you must understand in India – as long as people die, everybody will be interested. If people don't die anymore, nobody is interested."

Citations of papers to which permalinks were not available:

[1] Boshell-Manrique, Jorge, P.K. Rajagopalan, M.K. Goverdhan, and K.M. Pavri. "The Isolation of Kyasanur Forest Disease Virus from Small Mammals of the Sagar-Sorab Forests." *Indian Journal of Medical Research* 56.4 (1968): 569-72. Web. 19 Nov. 2016.

[2] Boshell-Manrique, Jorge, and P.K. Rajagopalan. "Observations on the Experimental Exposure of Monkeys, Rodents and Shrews to Infestation of Ticks in Forest in Kyasanur

Forest Disease Area." *Indian Journal of Medical Research* 56.4 (1968): 573-88. Web. 19 Nov. 2016.

[3] Iyer, C.G.S., T.H. Work, D.P. Narasimha Murthy, H. Trapido, and P.K. Rajagopalan. "Pathological Findings in Monkeys, Presbytis Entellus and Macaca Radiata, Found Dead in the Forest." *Indian Journal of Medical Research* 48.3 (1960): 276-86. Web. 19 Nov. 2016.

[4] Boshell-Manrique, Jorge, P.K. Rajagopalan, M.K. Goverdhan, and K.M. Pavri. "The Isolation of Kyasanur Forest Disease Virus from Small Mammals of the Sagar-Sorab Forests, Mysore State, India: 1961-1964." *Indian Journal of Medical Research* 56.4 (1968): 569-72. Web. 19 Nov. 2016.

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