Kyasanur Forest Disease: An Inquisitive story – Part 1

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Zeroing in on the genesis of the Kyasanur forest disease was, at one point, called “possibly the most dramatic epidemiological detective story of our time.” This is that story.

All the places where the disease has emerged lie along the Western Ghats. Credit: timjblack/Flickr, CC BY 2.0

Rajagopalan climbed to the top of the tree and brought the carcass of the dead langur down. Placing it on the forest floor, he studied the body and found nothing remarkable on the surface. Then, unaware or unmindful of the dangers, he opened up the dead monkey. It appeared to have suffered a haemorrhage. He removed its kidney, liver, spleen and brain, collected tissue and blood samples and deposited them all in separate containers packed with dry ice, ready to be dispatched to the Virus Research Centre (VRC) in Pune. Elsewhere in the forest, his fellow team members were doing the same.

Only a few days ago, he had been living on a boat on the Godavari river in Andhra Pradesh, collecting mosquitoes and bird blood. There, he and the others were part of a field team from the VRC investigating if migrant birds were responsible for bringing Japanese encephalitis to India. It had been only four years since he had joined the VRC after a master’s degree in zoology, and he was still only a research assistant. But Rajagopalan, twenty-seven and a bachelor, had a thirst to make something of his life and was quite willing to take risks. That was why he had been summoned here to the evergreen forest known as Kyasanur, in the taluks of Sagar and Sorab in Shimoga district, Karnataka, in early April 1957.

The field investigators from VRC were following up on the mysterious deaths of dozens of monkeys in these parts. The first reports had reached Pune on March 23. More alarming was the accompanying news that people in villages near the forests were falling ill with puzzling symptoms – continuous high fever, lasting for up to two weeks, body pain, headache, vomiting and diarrhoea, sometimes with blood in the stool. Tellingly, the villagers had noted that it was those who had gone into the forest and "seen" or "smelt" dead monkeys who were falling sick. There was only one known disease which killed monkeys in the forest and took down humans too: the dreaded yellow fever.

The Rockefeller Foundation, which had established the VRC in 1952, knew all about yellow fever, a disease carried by mosquitoes that had killed tens of thousands, particularly in South America and Africa. Their health programme, aiming for "the well-being of mankind throughout the world", had the eradication of yellow fever as one of its first goals, supporting virus research labs in Brazil, Colombia, Nigeria and Uganda. They pioneered research on yellow fever and developed a vaccine, but at a great cost: five of the 67 staff members of the foundation who participated in their yellow-fever programme had died of the disease.

The foundation pulled out of yellow fever research and eradication efforts in the 1930s but their interest in viruses carried by arthropods – such as mosquitoes – continued. In 1951, the foundation embarked on a worldwide programme to discover and catalogue arthropod-transmitted viruses. For a period of 15 to 20 years, they supported labs around the world – in Trinidad, Brazil, South Africa, Colombia, Nigeria – funding them, sending researchers from their headquarters, training researchers in the host nations. And that was how they were in India, too, setting up the VRC in Pune in 1952. Scientists at the foundation's labs isolated hundreds of viruses by inoculating infant mice with samples that their scientists at field stations around the world had collected. They had wondered why yellow fever appeared to be absent in India. Now, it seemed, they would find out.

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The Kyasanur forest disease "is truly a bhumi dosha, a trouble of the land".

When Rajagopalan and his fellow field investigators arrived in the forest on April 2, they had no trouble finding monkey carcasses, some freshly dead while others were in various stages of decomposition. The villagers told them that monkeys had been dying since January that year and that a similar episode, on a smaller scale, had occurred a year earlier, too. They were able to confirm this from the Mysore Department of Health, which had records of people falling ill with a typhoid-like illness dubbed "enteric fever". Some had experienced drowsiness and "mental confusion". Over 500 fell ill and 70 died of the new disease in 1957.

Locals began calling it the “monkey fever”. 

VRC field investigators on platforms erected around trees. Credit: P.K. Rajagopalan

There was no time to waste. If this was indeed yellow fever, the numbers of forest mosquitoes which carried it could fluctuate rapidly. The very next day, the team of around a dozen people set to work. They started collecting mosquitoes, day and night, from varying heights in the forest by setting up platforms on the trees. However, unlike in South America, they could find no distinctive forest canopy mosquito in Shimoga. Other puzzles soon presented themselves. They couldn’t find many mosquitoes that bit humans during daytime, which was when the villagers would go into the forest and presumably get bitten. “Only a single mosquito,” noted an early paper *[1]* presenting their work, “was collected attacking a quiescent human subject during three hours.”

Yellow fever in South America was more prevalent during the rainy season because that’s when mosquitoes were most abundant. In Shimoga, however, all known human – and most of the monkey – infections were reported after the monsoons. And why did the disease not affect monkeys and humans outside Shimoga? The villages affected in 1957, they learned, were mostly the same that had been affected the previous year. Surely mosquitoes were capable of flying into other districts, too? Yellow fever would have raced a hundred miles in a year.

By the middle of April, they heard back from Pune. The samples they sent had been tested. It was indeed an arthropod-borne virus, classified today in the family *flaviviridae*, to which the yellow-fever, dengue, Japanese-encephalitis and Zika viruses also belong. Human blood samples confirmed the result. The VRC reassigned more insect-collecting teams to Shimoga from Vellore and Pune.

But they could find no virused in the mosquitoes collected. It was clear something else was at work. The samples had been sent to the Rockefeller Foundation’s labs in New York as well. And those results bore a surprise: the virus wasn’t yellow fever. It was, instead, found to be related to the Russian spring-Summer encephalitis, a tick-borne virus prevalent in Russia.

By now, the researchers had noticed that monkey carcasses almost always had large numbers of ticks on them. So they started dragging “flags” of cotton flannel, a square meter across, over the forest floor to collect ticks. They found plenty.

On 17 April, the third day after they had started collecting ticks, two of the team members fell sick. Fearing the worst, work was immediately suspended. Within two weeks, a third man fell ill. The virus was found in all their blood samples.

The great American entomologist Harry Hoogstraal called it “possibly the most dramatic epidemiological detective story of our time.” And it was just beginning.

For half a century, the Kyasanur forest disease (KFD) was confined to five districts of Karnataka: Shimoga, Chikkamagalur, Uttara Kannada, Dakshina Kannada and Udupi. It smouldered quietly most of the time, but there had been major flare-ups in 1982-1984. Over six decades, it has killed more than 500 in Karnataka with a fatality rate of about 5%. In 2006, the first human case outside this endemic area was reported in Gulbarga district that borders Maharashtra. In 2012, six people who handled dead monkeys in the Bandipur National Park, bordering Kerala and Tamil Nadu, fell ill. In 2013, monkeys in neighbouring Nilgiri district, in Tamil Nadu, were found dead and tested positive for the KFD virus.

In the same year, the first human infection was reported from Kerala, in Wayanad district, which borders Bandipur. In 2014, the virus infected adivasis in Nilambur, in Malappuram district in Kerala. In 2015, monkey deaths were followed by more than 100 human infections in an adivasi community in Wayanad, of whom 11 died. It also emerged in Goa, where up to 50 were infected and five died. And this year, it was reported in Sindhudurg, Maharashtra, infecting up to a hundred and killing seven. This caused “a public health alert”, according to the National Institute of Virology, the successor institute to the VRC. In Goa, which has become a new epicentre, 264 cases have been reported this year, with three deaths. Meanwhile, the virus continues to haunt Shimoga, which has had 12 cases and a death this year.

All the places where the disease has emerged lie along the Western Ghats. And therein lies the clue to the case of KFD, which, as an ethnological study of the flare-up in 1982 put it, “is truly a *bhumi dosha*, a trouble of the land.”

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“That was a brilliant example of the prepared mind and the central importance of field biology, but it is also a sterling metaphor for human self-inflicted impact through disturbance of nature.”

In August 2016, I met Payyalore Krishnaier Rajagopalan at his home in Chennai, a few hundred metres from the sea in a quiet neighbourhood. At 86, he keeps himself occupied by writing on a variety of topics and corresponding with scientists in India and around the world. He points me to the desktop wallpaper on his computer – a blood-engorged *Aedes aegypti*mosquito, the vector for many *flaviviruses* such as dengue, chikungunya, Zika and yellow fever. “A beautiful photo,” he tells me.

Rajagopalan shows me other photos as well. “Here’s me with a useless first-class, M.Sc. Zoology degree,” pointing to photos of himself while working as part of the VRC’s team in Shimoga in 1957. He stayed in the KFD area in a field research station for close to 13 years. That is where he says his education really began. One of the tasks he was assigned in the early days was transporting patients, and sometimes dead bodies, from the villages to the hospitals. He recalls how he was chased by a mob on one such occasion and had to run for his life. “Nobody likes their relatives to be cut apart and subjected to post-mortem.”

Later, he himself contracted KFD and began throwing up blood. However, the infection was mild and he recovered. He suspects this is because he had, over the course of his work in Africa and the US where the Rockefeller Foundation sent him, received vaccinations for several related viruses, including yellow fever. But the infection did end up weakening his eyesight. “The Rockefeller Foundation treated my absence as if I was on tour,” he says. “They were great people.”

He also fondly recalls his colleagues at the foundation. Among them was the man he describes as one of his gurus: Jorge Boshell-Manrique, a Colombian physician and epidemiologist. When Boshell arrived in Pune in 1960, Rajagopalan was back from Berkeley where he had been sent for a degree in public health after his initial work in Shimoga.

“For Boshell,” Rajagopalan recalls, “visiting the forest and studying its denizens and their surroundings every day was a must.” That was Boshell’s habit and it was how he had discovered the jungle cycle of yellow fever, a disease that was believed to be spread only by the ubiquitous *Aedes aegypti*, a very urban mosquito. It was thought that humans played an important role in keeping the virus in circulation by inadvertently helping *A. aegypti* thrive. Through the Rockefeller Foundation’s efforts, *A. aegypti*, and thus yellow fever, had been eradicated in South America.

But they did not know that the virus was enzootic, that it was silently circulating in the forests where it was killing howler monkeys. Until, that is, Boshell happened upon woodcutters felling a tree in Villavicencio, Colombia, and watched as they were surrounded by a great number of a hitherto unknown species of mosquito. He had discovered how the virus jumped from mosquitoes, which lived in the forest canopy and infected monkeys, to humans on the forest floor a hundred feet below. “That was a brilliant example of the prepared mind and the central importance of field biology,” a colleague of Boshell wrote later, “but it is also a sterling metaphor for human self-inflicted impact through disturbance of nature.” A virologist visiting Villavicencio in 1963 found a brass plate on a tree commemorating this discovery.

In the Kyasanur forest, Boshell always walked with a machete in his hand to clear the undergrowth, with an eager Rajagopalan behind him. In the five years that they were together at the VRC’s field research station in Shimoga, Rajagopalan scarcely missed an opportunity to listen to Boshell tell stories of his field work all over the world. Boshell too fell ill with KFD, but recovered and spent five years in India before returning to South America. “He was like a walking university,” Rajagopalan says.

Together, they had many mysteries to unravel, now that it had become clear that the virus was not yellow fever. Nevertheless, was it something that had arrived in Shimoga from elsewhere? Migratory birds, for instance, might carry ticks and viruses. But why would they let go of their cargo only in Shimoga? And why now? On the other hand, if the virus was indigenous, why the sudden outbreak? Did this mean the virus had always been enzootic,

silently present in the wild? But that led to the same questions: Why only Shimoga? And why now?

A blood sample being taken from a patient with acute fever in a village in Shimoga, c. 1962. P.K. Rajagopalan is the bespectacled person on the extreme right. Credit NIV

"These questions are far from academic," wrote Boshell in a prescient 1969 paper, collecting the results of years of field work by the VRC. He knew that the way the puzzle unfolded would determine whether KFD would emerge – become epizootic – in new areas in subsequent decades.

Because the Rockefeller Lab in New York had found the KFD virus to be related to that of the Russian spring-summer encephalitis, the possibility arose that this was a virus that had somehow arrived from Russia. As it happened, the ornithologist Salim Ali had a longstanding interest in studying migratory birds by banding them – capturing and attaching small metal tags to the birds – and recapturing them elsewhere, thus tracking their migration. But he hadn't been able to find funds for such a project. He sensed an opportunity and convinced the WHO that he could study the origins of KFD by collecting ticks and blood samples from the birds arriving from Central Asia. The WHO agreed in 1960 and this became the Bird Migration Project of the Bombay Natural History Society.

KFD, however, did not emerge in new areas immediately after the initial outbreak. The WHO stopped funding but the Smithsonian Institution and the Migratory Animals Pathological Survey (MAPS) programme of the US Army Medical Research Unit – a larger bird-banding project in Southeast Asia to study Japanese encephalitis, among other infectious diseases – started funding Ali instead, in 1964. The full MAPS programme, from 1963 to 1975, banded some 1.2 million birds. Ali agreed to share data and samples with the larger project. Soviet scientists were also involved in Ali's project and data and samples were sent to their labs. Despite that, a controversy erupted over the American army funding the project with a possible eye on biological weapons. Knowing bird migration routes, critics felt, could open the door to their usage for the delivery of viruses across international borders.

It was in this period that Rajagopalan did his PhD work with Salim Ali, collecting ticks from migratory birds arriving in Shimoga. He found that the birds did not carry any foreign ticks, much less ones carrying viruses. The only birds which appeared to be capable of distributing ticks, though only locally, were ground-foraging birds such as the jungle fowl.

So, the virus had to be indigenous.

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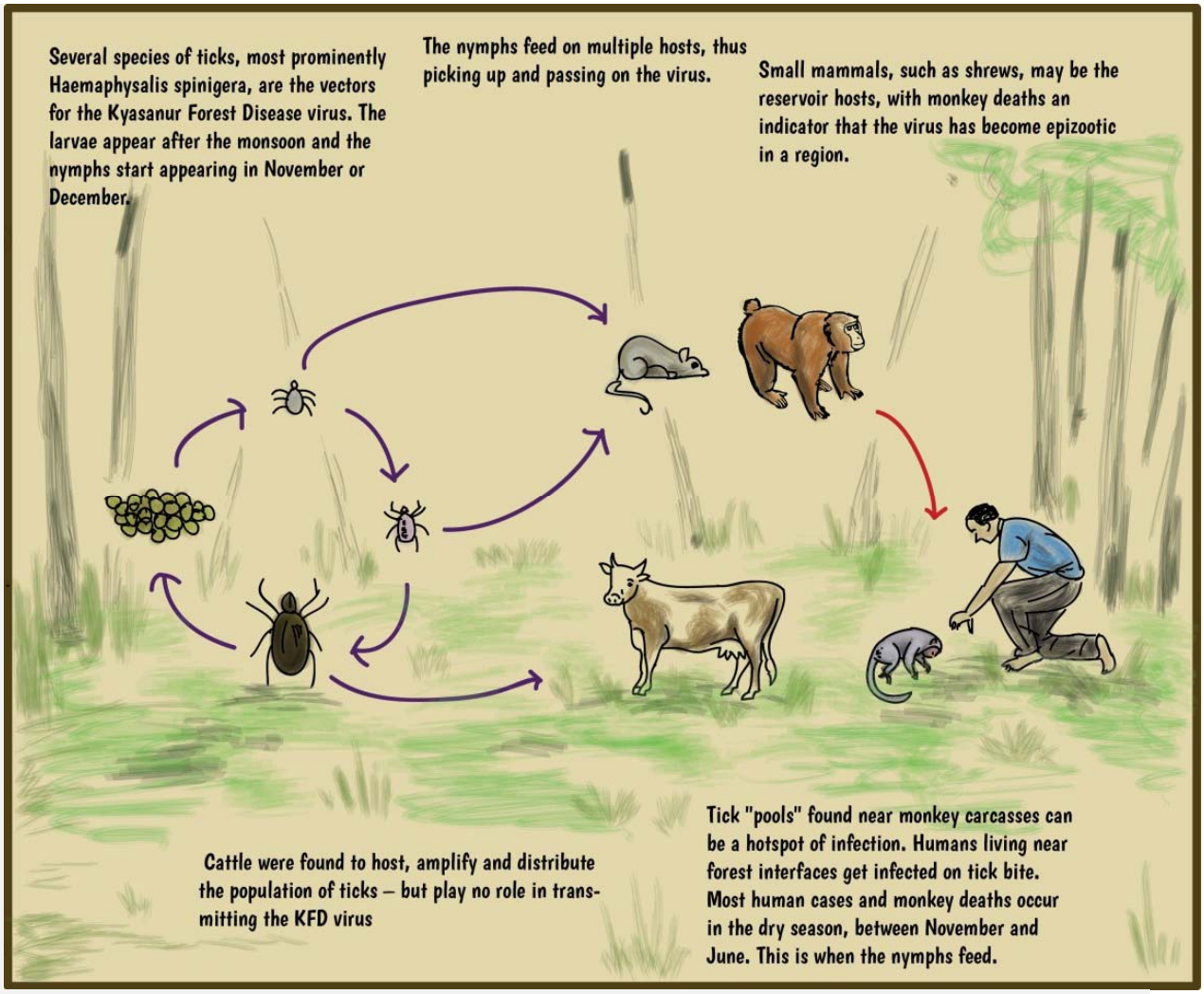
If the VRC researchers wanted to study ticks in the area, they first had to collect them. One way to track down 'pools' of ticks in the forest was to go to a village where a human KFD infection had recently been reported and try to reconstruct where the patient had been in the days before they started showing symptoms of the disease. This was difficult, not least because the villagers tended to travel all over the forest in search of firewood or for grazing their cattle. It was easier with children, especially girls who visited the forest rarely and therefore remembered more clearly. These were girls who had gone into the forest to gather twigs or to find jackfruit or flowers for making garlands and had ended up bitten by ticks.

Once they had identified promising areas, the field investigators dragged flags across the forest floor to collect ticks. They would then pick them off using tweezers. This couldn't be done during the monsoon, so the ticks had to be picked off by hand from under the surfaces of leaves. After sorting the ticks by sex and species, they were put in glass tubes and sent to the VRC lab in Pune for testing. The ticks were indeed carrying the KFD virus.

From the thousands of tick pools collected, a pattern emerged. At the centre was *Haemaphysalis spinigera*, a tick that infested monkeys and bit human beings. Its larvae start building up in numbers in September after the monsoon. They start reaching their adolescent – or nymphal – stage in November or December, feeding on multiple hosts, becoming capable of transmitting the KFD virus. The nymphs peak in numbers in February or March, maturing into adults with the onset of the monsoon in June. It became clear that the period of nymphal activity coincided with the season of monkey and human infections.



Credit: Ita Mehrotra

But there was a puzzle, too: tick pools from which the KFD virus had been isolated were often surrounded by other pools not far away from which no virus was found. Such isolated foci of infected ticks had to mean that there was an amplifying factor acting locally: there must be some animal that could circulate the virus in large quantities in its blood, infecting many ticks.

Monkeys were the obvious suspects, but this had to be confirmed. So the researchers captured some, tied belts to their waists, attached a stick to the belt and walked them around the forest to see if they picked up ticks. “After initials fits of rebellion,” reported a paper *[2]* that resulted, “they submitted and trotted wherever directed.” The monkeys were then taken to a lab in the field research station and placed in cages over water pans. The researchers counted the ticks that dropped from the monkeys after their bloodmeal. In the forest, the ticks drop wherever the monkeys rested or died, explaining the isolated foci.

But were the monkeys really dying of the KFD virus acquired from tick bites? And what was the virus doing to them? To find out, the researchers set up a monitoring system to obtain information about monkey deaths, one that we would today call “crowdsourcing”, complete with rewards. They were not surprised to find that more monkeys died in February and March than in any other month. Over the next seven years, the field team investigated over a thousand monkey deaths and autopsied around 400, the majority of which were langurs. If they found a monkey sick and moribund, it would be transported to the field laboratory or

chloroformed on the spot and autopsied – but not before collecting the ticks it hosted and drawing blood from its femoral vein. If the monkey was found dead, they drew blood straight from its heart. Only a few showed anything unusual externally, such as blood clots on the anal sphincter or a slight swelling in the region of the kidneys.

The researchers collected organs – brain, lung, liver, spleen, kidney – from the monkeys, but soon found only the brain and lung were necessary to isolate the virus. These were dispatched weekly by rail to Pune on wet ice. Or, in later years, to the Virus Diagnostic Laboratory in Shimoga. Examination of the liver revealed an abundance of Kupffer cells, which are immune cells that ingest foreign substances and cellular debris, some of which were huge and in the process of dividing. In the brain, they found dying neurons surrounded by microglial cells, which are the brain's immune cells. "These alterations are not by themselves in any way specific," noted a paper *[3]*, "and could be part of any general toxemic or infective fever."

There were more puzzles: The monkeys were dying in different areas each year, though the total affected area did not expand much. Was the virus moving on after depleting the monkey population in an area? Were the surviving monkeys immune? In any case, monkeys weren't dying throughout the year. And their heavy mortality meant they couldn't be the 'reservoir hosts' for the virus. Besides, the new generation of tick larvae emerging each season weren't infected. Both ticks and monkeys were acquiring the virus from somewhere.