Scientists may have at last cornered their quarry in a half-century-long hunt for the cause of a fatal neurological disorder in eastern Russia. With the disease spreading, unmasking the villain is more urgent than ever

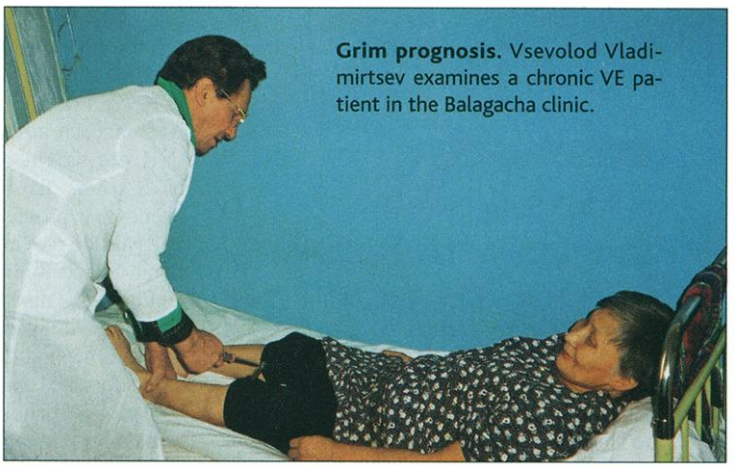
Siberia's Deadly Stalker Emerges From the Shadows

VILIUISK, RUSSIA—A selfless act may have been Ivan Ivanov's* undoing. It was May 1985, and the ice on the Viliui River in eastern Siberia was breaking up. Ivanov, then 24 years old, saw a foal plunge into the icy water and charged in to try to save it. A few days after the failed rescue attempt, medical records show, Ivanov came down with what seemed to be the flu. But the chills and headaches grew more intense, waves of paralysis came and went, and a high fever did not relent for nearly 2 months. To Ivanov and others in his village, it soon became clear that he was suffering from *bokhoror*, or "the stiffness." And they knew he was doomed: Even if he recovered from the initial attack, worse was yet to come.

Since that fateful spring day 17 years ago, Ivanov has lost nearly everything that matters: His mobility and his mind have all but left him, and even his wife and children haven't visited him in years. He now resides in a neurological clinic in Sosnovka, a village several kilometers south of Viliuisk.

On a late afternoon last February, the setting sun cast a reddish hue on a cluster of snow-covered buildings nestled among larch and pine. Outside Ivanov's ward are two snow figures—a horse and a *snegurochka*, or ice maiden—sculpted by a patient. Although the temperature hasn't climbed above freezing for months, the feeble winter sun has managed to dull and glaze the creations. They seem forlorn, like many of the patients abandoned by their community.

Vsevolod Vladimirtsev, a neurologist from the regional capital Yakutsk, has made a special trip to Sosnovka to examine Ivanov and several other victims of *bokhoror*, known more widely as Viliuisk encephalomyelitis (VE). Vladimirtsev watches Ivanov, whom he hasn't seen in more than a year, struggle to rise from a metal cot in an overheated room shared with three other men. Ivanov shuffles stiffly and gingerly, as if the wooden floor were hot coals, and babbles nonsensically. "His gait has improved slightly, but his dementia is worse," says Vladimirtsev, who has devoted his life to a disease that today afflicts only about 200 people, with another 1000 or so showing symptoms that may or may not be VE.



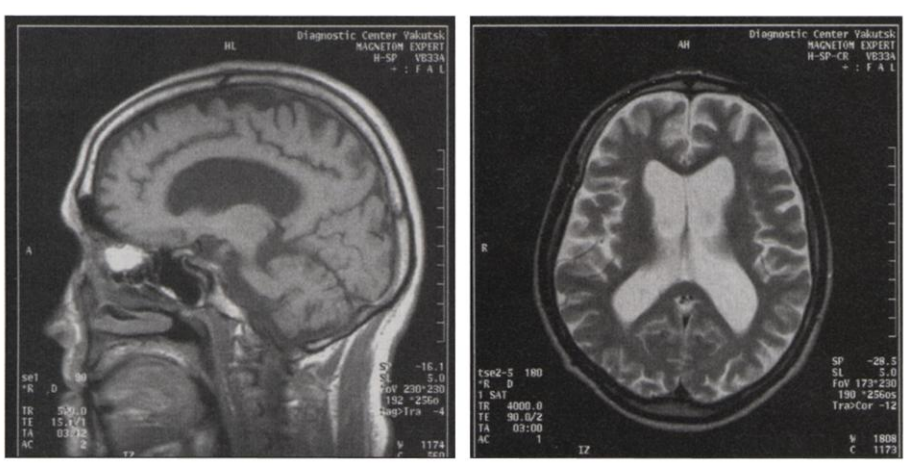
Grim prognosis. Vsevolod Vladimirtsev examines a chronic VE patient in the Balagacha clinic.

This obscure malady is one of medical science's most enduring puzzles. Over the past half-century, some of the finest minds in biology have hunted in vain for VE's cause. Although most researchers believe a virus is to blame, it's extremely difficult to contract the disease: The closest analog in this respect, researchers say, is leprosy. Adding to the intrigue, the disease strikes only the indigenous Sakha and a few other ethnic groups in eastern Siberia—although one Russian woman may have succumbed to VE after injecting herself with cerebrospinal fluid (CSF) from a victim.

To compound the mystery, the disease appears to be altering its guise. In the 1950s, during what is considered to have been a VE epidemic, roughly half the victims died within months of falling ill. These days, nearly all the patients have a long-lasting degenerative form of VE. But surveillance is so poor now that researchers confess they simply don't know if there are people in remote villages dying of the acute form of the disease. "We seriously need to address two things: Are there really fewer of these acute cases, and if so, what is the reason for the decline?" says Ralph Garruto, an anthropologist and neuroscientist at the State University of New York, Binghamton.

"It's a terribly interesting and terribly awful disease. You think that there's a solution at your fingertips, but you can't quite grab it," says Martin Zeidler, a neurologist with the Creutzfeldt-Jakob Disease (CJD) Surveillance Unit in Edinburgh, U.K., who has been to the Sakha Republic, known in Soviet times as Yakutia, to observe VE patients. But a solution to the VE enigma could be close at last. Preliminary findings from a team at the CJD Surveillance Unit link the disease indirectly to a herpesvirus or a close relative. "It

* The patient's name has been changed.



Irreparable damage. These MR images of Ivanov's brain reveal severe atrophy in the cerebral cortex.

CREDITS: (TOP) R. STONE; (BOTTOM) V. VLADIMIRTSEV/INSTITUTE OF HEALTH

could be a new type of herpesvirus that was latent in the Sakha population and which became extremely pathogenic after World War II," speculates Lev Goldfarb of the U.S. National Institute of Neurological Disorders and Stroke in Bethesda, Maryland.

After numerous false leads and prime suspects that later proved to be innocent, researchers are keeping their hopes in check. But if they can unmask the villain, they may be able to answer a pressing question: whether the disease poses a threat to neighboring countries such as China and Japan or beyond. Poor surveillance notwithstanding, the disease does appear to be spreading in the Sakha Republic. "We just don't know enough about the disease to predict its spread," says Vadim Krivoshapkin, director of the Institute of Health in Yakutsk.

"My gut feeling is that VE's not going to be a public health threat," says Richard Knight of the CJD Surveillance Unit. "But there are diseases that started as a regional problem and surprised us. Just think of HIV."

A neurological netherworld

Although an ethnologist named Richard Maak was the first to publish a description of *bokhoror* in 1887, the rare, sporadic disease remained little more than a curiosity for decades. In 1933, on the heels of a bloody 11-year insurrection by the Sakha against Stalinist rule, a Russian epidemiologist described 19 cases of an encephalitic disease clustered around Mastakh Lake, about 100 kilometers northeast of the nearest town, Viliuisk.

It was not until the early 1950s that VE really made its presence felt. Local officials alerted their bosses in Yakutsk that a brain disease was killing dozens of Sakha. The chief of the Yakutian health ministry's neurological department, Afanasiy Vladimirtsev—Vsevolod's father—dispatched a sharp young neurologist to investigate. Prokopiy Petrov, himself a Sakha who had grown up in the Viliuisk region, saw that they had an epidemic on their hands—the toll would reach roughly 500 cases. "Petrov realized it was a new disease and described it," says Goldfarb.

By the late 1950s the VE outbreak subsided, and scientists had a chief suspect. "They thought they had caught the virus," says the Sakha Republic's minister of health, Ivan Egorov. A Russian team had injected mice with blood, serum, and CSF of VE patients; some mice developed fatal encephalitis, and the team isolated a virus. In the mid-1960s, however, another group exposed the "Viliuisk virus" as a contaminant: Theiler's mouse encephalomyelitis virus. Some textbooks still list this picornavirus, the first of many red herrings, as the culprit.

In 1969, a team led by Mikhail Chumakov of the Institute of Poliomyelitis in Moscow banded together with colleagues in Yakutsk to attack the problem with renewed vigor. The researchers scoured Sakha for cases to get a handle on the extent of the disease. The survey uncovered a host of neurological problems in the Sakha Republic. The incidence of one rare disorder—spinocerebellar ataxia type 1—was higher there than anywhere else in the world (see sidebar, p. 644). That got scientists speculating that something in the environment was mounting a wholesale attack on the nervous systems of the inhabitants. Perhaps, they thought, the villain was lurking in the unusual cuisine featuring delicacies such as raw horse, raw fish, and *khumys*, or fermented mare's milk.

Later findings dampened concerns about rampant neurological disorders. For instance, multiple sclerosis (MS) is far less prevalent in Sakha than in other northern lands, says Goldfarb. Nevertheless, VE and its bewildering array of symptoms and courses (see sidebar) remained a scourge. That's why, after a lull in the scientific campaign in the 1980s, Goldfarb's lab and the Institute of Health in Yakutsk revived efforts to understand the baffling disease.

Rural menace

At 8:00 on a frigid February morning, the gray light of dawn is breaking ever so slowly on the taiga as a jeep crawls toward the hamlet of Balagacha. Heading north from Viliuisk, the smooth kilometer-long crossing of the frozen Viliui River gives way to a bone-jarring ride on a snow-packed dirt road. The rugged jeep's heater is no match for the chill penetrating from outside, where the temperature hovers around –46°C. Squat, longhaired Yakutian horses, grazing on hay laid out for them, don't seem to mind the Siberian winter.

Like most of the villages throughout the Viliui Valley, Balagacha has a number of VE patients: eight confirmed or suspected cases out of a mere 700 inhabitants. Vsevolod Vladimirtsev examines patients in Balagacha's clinic, a one-story building that's clean and spartan but filled with tranquil desolation. Vladimirtsev's father diagnosed one of the patients with VE, a 68-year-old woman, in 1952. After the acute onset she went into remission and led a normal life for 12 years, bearing six children. VE came on again when she was 30, and she deteriorated to the point that she had to move into the clinic in the early 1980s. During the course of her long illness, she has held onto her mind but not her family. Many Sakha shun their own relatives with VE. "There are very deep fears of this disease," explains molecular geneticist Irina Brakhfogel, formerly with the Institute of

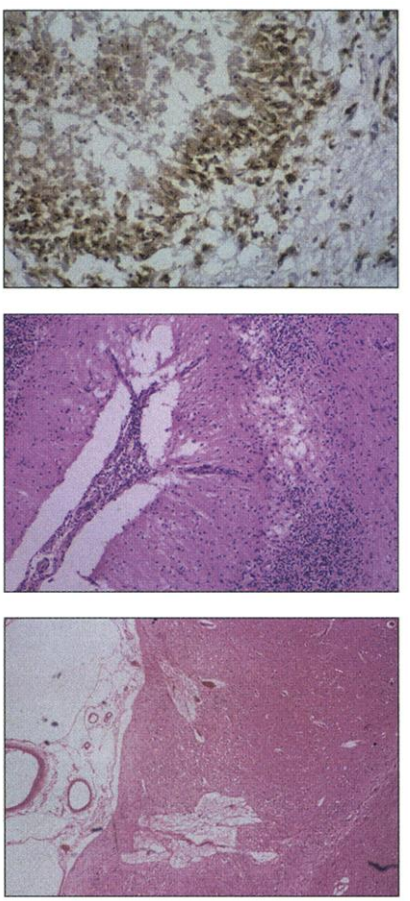
A Many-Headed Medusa

Viliuisk encephalomyelitis (VE) can run any of three courses:

In the **rapid (subacute) form**, the initial attack segues into an assortment of symptoms including the hallmark stiff gait, slurred speech, and rigid, spastic muscles. Victims die within months. Autopsies have revealed severe inflammation in the lining of the brain, with the brain and brainstem riddled with clumps of dead cells encircled by macrophages and lymphocytes (*top image*). "It's a unique pathological picture," says Colin Masters, an expert on VE pathology at the University of Melbourne in Parkville, Australia.

VE's classic manifestation is the **slowly progressive form.** In these cases, patients recover from the acute onset, then suffer bouts of recurrences followed by remissions—much like in multiple sclerosis—before entering a death spiral. Patients with slowly progressive VE have fewer patches of dead cells but clear signs of inflammation suggesting an ongoing infection (*middle image*).

The **chronic form** makes up a rising proportion of VE cases. Many of these victims report never having had an acute attack, or having had one that was mild. These patients deteriorate gradually before stabilizing with varying degrees of impairment for the rest of their lives. Brains of chronic VE victims lack inflammatory cells (*bottom image*), "suggesting the pathogenic process was effectively burnt out," says pathologist Catriona McLean of the University of Melbourne.



CREDIT: C. MCLEAN/UNIVERSITY OF MELBOURNE

Another Disease Blights Families in Siberia's Far North

Lev Goldfarb was instantly suspicious when in 1969 he was shown a subset of patients with, he was told, "a certain form" of Viliuisk encephalomyelitis (VE). Goldfarb, then a young virologist at the Institute of Poliomyelitis in Moscow, was on his first trip to the Sakha Republic to study VE, the mysterious neurological disease that affects indigenous populations there. Like typical VE cases, this subset of people suffered a progressive deterioration in their ability to speak, move, and swallow. But they did not report a flulike onset, nor did they develop dementia. Goldfarb doubted whether these patients had VE at all. His skepticism grew when he learned that many of the atypical cases were clustered above the Arctic Circle—hundreds of kilometers north of the VE epicenter in the Viliui Valley.

VE VIPs. Joining forces in Yakutsk in 1979 were, from left to right, Afanasiy Vladimirtsev, Antonina Struchkova, Lev Goldfarb, D. Carleton Gajdusek, Prokopiy Petrov, Vsevolod Vladimirtsev, Glafira Lyskova, and Anastasiia Ivanova.

Two years later, Goldfarb and his colleagues visited the affected Belaya Gora region on the Indigirka River. "It was June, and the sun was just circling overhead," he recalls. That wasn't the only enlightening part of the trip: All the supposed VE patients, he learned, were related to each other. "We knew then that what we saw was not VE." He discovered that these people were suffering from spinocerebellar ataxia type 1 (SCA1), a disease passed from generation to generation through a faulty dominant gene. Over several years, Goldfarb's group worked up a pedigree spanning 100 SCA1 cases in four regions and traced the disease's origins in Siberia to the Aldan River valley in the early 18th century. They now know that the Sakha Republic has a higher incidence of SCA1 than anywhere else in the world—and it's rising. The number of cases currently stands at 168.

A molecular breakthrough came in 1993, when Huda Zoghbi of Baylor College of Medicine in Houston and Harry Orr of the University of Minnesota, Twin Cities, pinpointed the genetic defect to chromosome 6. The gene, *SCA1*, has a read-write error: a sequence of three nucleotides—cytosine-adenine-guanine (CAG)—that is copied over and over. This notorious, unstable triplet repeat is now known to underlie several other diseases, including Huntington's. For SCA1 victims, more CAG repeats means worse symptoms and an earlier disease onset. People with more than 60 repeats die in their 20s and 30s.

SCA1 appears to be hitting the Sakha population harder now than it did a generation ago, suggesting that the number of CAG repeats in patients is on the rise. Goldfarb has an explanation for this curious phenomenon. An insurrection in the 1920s and 1930s, then World War II, "left a hole in the population structure" in the Sakha Republic, he says. Young men died in droves, so it was primarily older men who were left to father children. To have survived into their 40s and beyond, men with the *SCA1* mutation must have had fewer excess repeats. Likewise, their children "did not have too many repeats," Goldfarb says.

But as the hole in the population structure has mended, subsequent generations have steadily accumulated CAG repeats. By the 1990s, Goldfarb says, "the disease was affecting much younger people and had more terrible complications than I observed in the 1970s." He speculates that the disease's severity today is similar to what it must have been before the insurrection nearly a century ago. And SCA1 will continue to plague the region well into the 21st century—at least until gene therapy comes along or prenatal diagnostics reaches the Siberian outback. **–R.S.**

Health in Yakutsk.

Because VE occurs only in and around villages rather than towns, scientists have continued to search for clues to its origins in the environment or in the Sakha lifestyle. Water is a dominant theme, but efforts to find unusual pathogens, parasites, or mineral imbalances in the region's innumerable lakes (the main source of drinking water) have turned up nothing tangible. Anecdotes abound linking onset of acute VE with falling into water. But such mishaps are common—particularly in springtime, when the frozen rivers break up—and many accident victims never develop VE. One intriguing correlation that has held up in a recent analysis by Garruto and Oleg Broytman is that the acute disease usually strikes in May, particularly among women in their 30s. "The highest priority I see is to track down several of these acute cases within a month or two of onset," says Garruto. "Then we'll have a much better chance of isolating a presumed pathogen."

Goldfarb speculates that the brutal conditions in the Sakha Republic allowed a latent viral infection to roar to life. The worst period, he notes, spanned the insurrection against Stalinism in the 1920s through the famines following World War II. "The people in the Viliuisk region were the poorest in Russia, and life was unbearable," he says. "People were dying and dying." The immune systems of the Sakha may have been weakened to the point that an otherwise manageable virus became a threat. Support for this idea comes from a recent study suggesting that on average Sakha have fewer T cells, an immune fighter, than Russians have.

Tranquil desolation. Balagacha's medical clinic is home to several VE patients.

According to Knight of the CJD Surveillance Unit, a good parallel might be encephalitis lethargica, or sleeping sickness. The disease swept the globe after World War I, afflicting an estimated 5 million people, many of whom developed a chronic form resembling Parkinson's disease. Like VE, cases of sleeping sickness waxed in spring and summer. Within a few years sleeping sickness had nearly vanished, with the presumed virus responsible for the pandemic never identified.

False pretenders

Population studies have only deepened the intrigue surrounding VE. The fact that the disease appears to be confined to three ethnic groups—primarily Sakha, but also Evenks and Evens—points to a genetic

CREDITS: (TOP) L. GOLDFARB; (BOTTOM) R. STONE

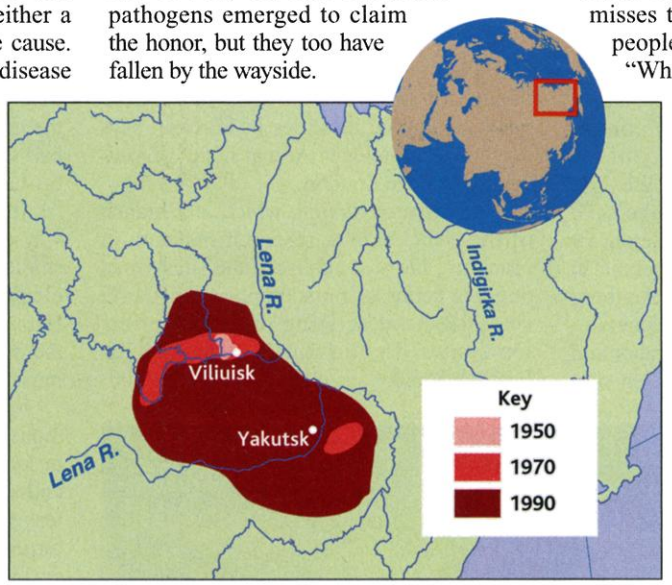
susceptibility to the disease. Goldfarb has collected blood samples from 80 families, including some with more than one case. A team led by Stephen O'Brien at the U.S. National Cancer Institute in Bethesda, Maryland, is now interrogating the DNA, focusing for now on 40 to 50 genes known to confer susceptibility to other diseases.

VE's ethnic nature has led one prominent neuropathologist to propose that neither a virus, nor any other pathogen, is the cause. After examining brain slices from disease victims, Fusahiro Ikuta of the Niigata Neurosurgical Hospital in Japan has noted that VE's pathological picture—including the numerous tiny perivascular lesions in the cerebral cortex—"is much different from any other encephalitis." He sees an uncanny correspondence to neuro-Behçet disease, an autoimmune disorder that occurs disproportionately in Japan and the Mediterranean. He points out that researchers believed for years that a "slow virus" causes MS before the majority of experts concluded that the disease is an autoimmune disorder. Ikuta predicts the same for VE. "I believe it could never be caused by a virus," he says.

The puzzling case of Ludmila Alekseeva suggests, however, that a pathogenic agent is involved—and that the disease is not entirely limited to particular ethnic groups. A Russian lab technician, Alekseeva routinely handled CSF and other tissues from VE patients between 1968 and 1971. In 1972 she developed a progressive disorder that neurologists in Moscow diagnosed as severe MS. "Her illness was very similar to VE but not typical," Goldfarb says. But Alekseeva claimed that in April 1971, during a period of deep depression, she tried to kill herself by injecting into her hand CSF from a man who died from VE. Itinerant neuroscientist D. Carleton Gajdusek, who won a Nobel Prize in 1976 for his work on kuru (a rare brain disorder in New Guinea linked to ritual cannibalism) met Alekseeva in August 1979, a few years before she died. He found her story credible—and of all the cases he saw in Sakha that month, the "most disturbing." As possibly the first victim of Caucasian stock, the Alekseeva case implied that the VE pathogen could escape its ethnic cage.

But exactly how the disease spreads remains a mystery. According to Gajdusek's diary from his trip to Sakha, "leprosy and other slow, long-incubation, intimate-contact diseases come to mind." Goldfarb has tracked down 16 instances in which nonrelated individuals living in the same household got the disease. That frequency is far greater than expected if it were due to chance, Goldfarb says, providing strong evidence that the disease is transmitted from person to person. "The incubation time could be huge," possibly 14 years or more, he says.

But when it came to nabbing the perpetrator, all clues have led to cul-de-sacs. After the so-called "Viliuisk virus" went bust in the 1960s, several candidate pathogens emerged to claim the honor, but they too have fallen by the wayside.



On the march. Although surveillance has dropped off, VE is clearly spreading, as shown by the broadening distribution of known cases over the past 50 years.

SOURCE: L. GOLDFARB

The endgame?

That was the way things stood until a few years ago, when Alison Green, a prion researcher with the CJD Surveillance Unit, obtained CSF from a few VE victims. She thought she might flush out the presumed virus by probing for a kind of immune fingerprint called oligoclonal bands. "What we were looking for was any evidence of inflammation in the brain," says Green. White blood cells in the CSF produce a restricted number of variations of immunoglobulin G (IgG) antibodies. Using a common lab technique called isoelectric focusing, Green tested the CSF and serum against a range of antigens—including ones from cytomegalovirus, varicella, and herpesvirus—to see if any IgG in the samples stuck. The results, she says, were "quite surprising": All three CSF samples showed strong banding for herpes. The initial hit has recently held up in samples from a few dozen more VE patients.

"We thought, 'Oh my God, this is it,' " says Goldfarb. Herpesvirus can infect the brain in AIDS patients and other severely immune-compromised individuals. Further circumstantial evidence came from the other end of the former Soviet empire: Belarus. There, researchers have described a 44-case cluster of chronic herpes encephalitis. People with this extremely rare disease have larger but fewer clumps of dead neurons than VE patients.

As tantalizingly close as they may be, researchers have not yet bagged the VE virus. Green and her colleagues have failed to detect herpesvirus RNA in the CSF samples using the exquisitely sensitive polymerase chain reaction (PCR). That's not necessarily a deal breaker, Green says, as PCR often misses the elusive herpesviruses even in people with a known, active infection. "What Green's work suggests is that we need to look at herpesvirus more carefully," says the CJD Surveillance Unit's Knight. Adds neurologist Albert Ludolph of the University of Ulm in Germany, "In my view, the race is open."

Researchers are gearing up for what could be a final assault. The latest entry is a team led by Alexander Chepurnov, Russia's top expert on filoviruses, a family that includes Ebola and Marburg. His team at the State Research Center of Virology and Biotechnology in Koltsovo is gearing up for a major effort this year to find the virus. They will join forces with Evgeniy Savilov's team at the Institute of Epidemiology and Microbiology in Irkutsk for a door-to-door survey in Sakha to get a better idea of how widely the disease has spread.

The first all-out effort on VE in 30 years might also answer a question that concerns public health agencies throughout the world: Does VE pose a global threat? For now there's only speculation. "We are still in the dark about this," says Vladimirtsev. But after decades of virtual confinement to their territory under czarist and Soviet rule, the Sakha and other indigenous Siberians are now free to travel, he notes: "We can't evaluate the danger."

If a known herpesvirus does prove to be the culprit, that would allay fears that VE could be the next encephalitis lethargica. It might also lead to an explanation for its proclivity for indigenous Siberians. "Maybe different ethnic groups process herpes infections in different ways," Knight says.

Coming up with an explanation—and maybe, someday, a treatment—could also make the stigma of *bokhoror*, like leprosy, a thing of the past. On the grounds of the VE clinic in Sosnovka, a long, wooden building, now boarded up, is all that remains of a 19th century sanctuary for lepers. "Now we have no cases of leprosy," says Vladimirtsev. "I hope one day we can say the same for Viliuisk encephalomyelitis." And perhaps see the VE clinic boarded up as well.

–RICHARD STONE