



Unassuming. The first time Liu Jikai laid eyes on the small white mushrooms clustered on a tree stump, he didn't believe they could be toxic.

plained and that other environmental factors may abet the little white scoundrel. "It's a long and complicated story," says Chinese CDC epidemiologist Shi Guoqing.

Portrait of a killer

It dawned gradually on Yunnan health authorities that they had a problem on their hands. The unexplained deaths appear to have started in the late 1970s, largely out of sight in remote villages. As cases piled up, some experts began to suspect that the killer was Keshan disease, a rare heart malady linked to Coxsackie virus and low dietary intake of selenium, a trace element (*Science*, 12 June 2009, p. 1378).

Hoping to cast a wider net, a team led by Huang Wen-li, deputy director of the Yunnan Institute of Endemic Diseases Control and Prevention in Dali, in 2002 compiled a long list of risk factors for the syndrome, including enterovirus infection, drinking water from mountain streams, abusing alcohol, and consuming vegetable oil and mushrooms. "But the evidence for any one factor was not convincing," says Shi Wu-Xiang, an epidemiologist at Dali University who is not affiliated with the team. Two other commonalities were that the syndrome struck almost exclusively during the monsoon season, and in villages at altitudes ranging from 1800 to 2400 meters above sea level. A solution eluded the researchers, however, and the death toll mounted. In 2004, Huang and provincial authorities appealed to Beijing for help. The following spring, the science and health ministries held a conference on the syndrome. "There was a lot of political pressure to solve this mystery," says Zeng Guang, Chinese CDC's top epidemiologist.

The central government ordered the Chinese CDC to join the hunt. The task fell to its China Field Epidemiology Training Program (CFETP), an elite unit formed in 2001 with a mission to crack the toughest cases. The disease sleuths did not immediately warm to the idea of chasing a cardiac killer. Heart attacks are usually brought on by years of poor diet

Will a Midsummer's Nightmare Return?

In southwestern China, people of all ages have been dropping dead suddenly and inexplicably. After a 5-year pursuit, scientists have nabbed a surprising culprit

DALI, CHINA—Every summer, a killer stalks the rugged highlands of Yunnan Province in southwest China. Around the time the monsoon rains begin to fall in late June, "people grow afraid," says Li Guanhuai, the sole doctor in Wangjiacun, a village an hour east of the tourist town of Dali in northwestern Yunnan. Li, sitting on a stool in front of a snack shop, cracks a walnut with his bare hand and meticulously picks out the meat. After a while he looks up, brow furrowed. "We wonder," he says, "who will be the first to die? Who will be next?"

For more than 30 years, people of all ages have been dropping dead from sudden cardiac arrest in northern Yunnan. "It's getting everybody across the board: children, adults, older people," says Robert Fontaine, an epidemiologist with the U.S. Centers for Disease Control and Prevention (CDC). The vast majority of deaths occur during the rainy season, from June to August. Yunnan Unknown Cause Sudden Death, as it is called, often strikes in clusters, so the first victim in a village instills dread in the rest of the inhabitants. "It's a fascinating problem," says China's top expert on sud-

den deaths, cardiologist Zhang Shu of Fuwai Hospital in Beijing. Since 1978, more than 400 deaths and several dozen nonfatal cardiac cases have been attributed to the syndrome.

But this summer, people across Yunnan may be able to rest easy. After a 5-year investigation, a team led by the Chinese CDC in Beijing believes it has uncovered the syndrome's chief cause. CDC and Yunnan Provincial Health Department have embarked on a campaign to warn against eating an innocuous-looking mushroom deemed so trifling that most villages don't even have a name for it. If they have fingered the real culprit—known for lack of a better description as the "little white mushroom"—then this summer could be the first in decades without a death from the syndrome.

The case is not closed, however. Some researchers believe that a substantial percentage of syndrome deaths may be from another cause. Hoping to set lingering doubts to rest, the Chinese CDC-led team will test whether the toxins isolated from the fungus, new to science, trigger the heart attacks. But they also acknowledge that some deaths remain unex-

or lack of exercise. “Normally, I would not touch an investigation of sudden death with a barge pole. The deaths are usually from different causes, and the investigation will lead you nowhere,” says Fontaine, who is on assignment as senior adviser to CFETP.

But the case was intriguing, so in June 2005, a team led by Zeng, CFETP’s executive director, arrived in Dali and with Huang’s group set up a surveillance system. Like clockwork, villagers started dying that July—and CFETP started assembling a vivid picture of their last moments. “We heard amazing stories about how people would drop dead in the middle of a conversation,” says Fuwai cardiologist Zhang Jian. But about two-thirds of victims, in the hours before death, experienced symptoms such as heart palpitations, nausea, dizziness, seizures, and fatigue—some of them hard to classify.

At the time, Yunnan investigators were still leaning toward Keshan disease. Genuine Keshan cases had been recorded in areas that reported sudden deaths, and the region’s soil is deficient in selenium. Promoting that idea were researchers from the Institute of Keshan Disease in Harbin who had collaborated with Huang’s group. “They believed it was Keshan, so that’s what they thought they were finding,” says Zeng. In August 2005, the government news channel CCTV aired a report on a hard-hit area, Jingdong County, which had tallied at least 40 sudden-death cases from 1993 to 2005. The TV program pinned the blame on Coxsackie virus.

That indictment quickly unraveled. Yunnan researchers had isolated Coxsackie virus from just four villages, and these were strains that are prevalent across China. There was nothing to suggest a new strain spreading, says Fontaine: “The overall pattern was totally inconsistent with Coxsackie virus.”

The pathology was more revealing. In Keshan victims, Coxsackie virus ravages heart muscle, riddling the organ with lesions. Some hearts of sudden-death victims showed signs of mild infection, and some looked normal. “It’s definitely not Keshan,” says Zhang Jian. About half the autopsies and tissue samples revealed severe underlying heart disease. Often the victims had signs of a genetic disorder called arrhythmogenic right ventricular cardiomyopathy, but that was not the answer either. The chronic condition develops slowly, and it’s never been known to cause clusters,



Lethal locale. The unexplained sudden deaths have occurred in northwest Yunnan.

says Fontaine. Moreover, about two-thirds of cases within clusters were among people who were not related, making a genetic cause exceedingly unlikely.

Then the team caught a lucky break: Local researchers that summer sent the Yunnan investigators pathology slides of heart tissue from three families in which two members of each family died at about the same time. The cardiac lesions were different in each member of each pair, Fontaine says. “And the pathologists said that none of it was enough to kill anybody,” he says, indicating that something like a drug or toxin was clearly throwing hearts off kilter. “All



Shoe-leather epidemiology. After ruling out pathogens as the cause of the sudden deaths, Robert Fontaine (*center*, interviewing villagers) and colleagues at Chinese CDC realized they were on the trail of an unusual toxin.

the evidence was pointing to a fatal arrhythmia.” To probe idea this further, the scientists sought to learn about the electrical activity of victims’ hearts, as measured by electrocardiography (ECG). “We needed ECGs on these people before they died,” says Zhang Jian. The ECGs confirmed their suspicions.

Reviewing all they had learned, the CFETP team kept circling back to the same suspect. “Mushrooms jumped out at us right away,”

Fontaine says. But that didn’t add up. Yunnan is famed for its wild mushrooms, including matsutake that end up on dinner plates in Japan and *Boletus edulis*, or porcini, that are shipped all the way to Europe. Villagers insisted they knew which are poisonous and which are edible. And it seemed that nobody else in the world was dying after dining on Yunnan mushrooms.

Unlikely villain

In the hills east of Dali, villagers lead a hard-scrabble life, says Shi Wu-Xiang, who over the past 5 years has assessed living standards in places where the syndrome has struck, including Wangjiacun. “This disease is related to poverty” and perhaps influenced by local customs, he says. Mushrooms are a key part of everyone’s life. On average, Shi says, one-third of villagers’ income is from tobacco farming, one-third is from other crops such as rice and from handcrafts, and one-third is from wild mushrooms.

Mushrooms are gathered in July and August—the height of the rainy season. “Almost the entire village collects,” says Li Linmei, a farmer in Wangjiacun bedecked in pale-green bracelets made of local jade, a talisman thought to promote longevity. Families fan out into the countryside, she explains, and will often spend several nights at the mushroom grounds. “Mushroom ladies” go village to village buying up the bounty and moving it to middlemen who sell to restaurants or exporters.

Mushroom picking could explain the syndrome’s seasonality and narrow altitude band, says Shi Guoqing. Soon after arriving in Yunnan in 2005, his group had queried villagers about fungi. “We had no idea what kind of mushroom we were looking for. So we asked them what kind they ate,” he says. Most villagers, they learned, refrained from eating mushrooms. “They are very poor; they want to earn money. So they don’t eat the fat and juicy ones; they sell them,” Shi says.

But then in 2006, CFETP began chasing an important new lead. That year, they found curious mushrooms in one home that had experienced sudden deaths. Then a sudden death occurred in another county—and the victim’s family members admitted that they had consumed this kind of mushroom. “The mushroom ladies never buy them,” Shi says. These mushrooms, little and white and fragile-looking, have no commercial value and turn

brown quickly after being picked. The CFETP team learned that 3 years earlier, Huang's group, while investigating a case cluster, had collected the nameless mushroom, diced it, and fed it to mice. The animals suffered no ill effects, so the experiment was filed away as a negative result. Dubious, Chinese CDC toxicologists brought samples back to Beijing and did their own mouse-feeding study. They too did not observe an effect.

But in 2007, the circumstantial case against Little White grew stronger. The CFETP team heard about two more sudden-death clusters and raced to the villages. They showed photos of the mushroom to surviving family members and neighbors, who confirmed that the stricken individuals had eaten it. Could the toxicology tests have been misleading? they wondered. "We thought the mushroom might contain a low-level poison," Shi says. "Some people may eat this, no problem. Other people who eat too much, or who have underlying heart disease—they may have trouble." The mice might not have consumed enough of the fresh mushroom to show an effect, Shi says. They had to try again.

The next summer, CFETP asked Liu Jikai, a medicinal chemist at the Kunming Institute of Botany, in Yunnan's capital, to make preparations of the suspicious mushroom for toxicity testing. Liu had recently extracted several antitumor agents from Yunnan mushrooms, including what may be the priciest variety in all of China: *ganbajun*, or groundwart, which can cost up to \$100 per kilogram. From that species, his group has identified eight new pigments that are 20 times as potent antioxidants as vitamin E.

The first time Liu laid eyes on Little White, "I didn't believe it could be toxic," he says. His institute colleague, taxonomist Yang Zhuliang, deduced that the mushroom is a new kind of *Trogia*. "Not much is known about this genus," Liu says, apart from the fact that it was not thought to include poisonous species. Little White, which sprouts from downed trees, was by no means rare. Yet numerous surveys had missed it.

In 2008, the Institute of Laboratory Animal Science in Beijing tested Liu's extracts in mice—and all the animals died. With evidence mounting against Little White, CFETP and local health officials began to warn villages to steer clear of it. Shi Guoqing's team went to Jingdong, the blighted county in the

CCTV program. Earlier, local investigators had not found the mushroom there. But when CFETP staff showed pictures to villagers, Shi says, "they said, 'Yes, we ate this, but we thought it was safe.'"

Last year, 15 of 16 sudden deaths blamed on the syndrome occurred in areas with no previously reported cases, including 14 in one county. These areas had not been alerted to Little White. Among the deaths were four members of a family: the mother, two daughters, and a son-in-law. Two young children survived. "They could not tell us anything useful," says CFETP's Shen Tao. "But we found dried little white mushrooms in the kitchen. It was quite clear what had happened."

In the meantime, scientists and an army of farmers hit the highlands last summer and



Going door to door. Epidemiologist Shi Wu-Xiang (left) queries a farmer in Wangjiacun about his plans for gathering wild mushrooms this summer. Most villagers can only afford to eat mushrooms that they are unable to sell.

"collected a huge amount" of Little White, Liu says. In the months since, his group has attempted to unmask the toxin. First, they zeroed in on ammonia-based cyclic peptides. These proved benign. Then they isolated three unusual amino acids. Most amino acids are building blocks of proteins, but Little White's trio is not associated with any protein, and one is new to science. "All three are toxic but not extremely so," says Liu. Dissections of mice infused with the amino acids revealed intestinal bleeding and edema—but no cardiac lesions. That makes sense, says Fontaine. "If you find a lesion in the heart, then you've got the wrong poison," he says, because human victims also don't have cardiac lesions from the toxin.

The mechanism remains a riddle. "What's happening in Yunnan isn't expected from any other mushroom toxin," says Fontaine. "What we have here is a toxin that's picking off vulnerable people. Anybody who is susceptible and is pushed over the edge will get a fatal arrhythmia."

An accomplice—or second killer?

Not everybody buys that explanation. "I don't think it's related to mushrooms," says Wangjiacun's doctor, Li Guanhui. He believes that mountain streams are contaminated with a toxin or pathogen that causes the syndrome. In his experience, he says, "most cases are linked to dirty water." Fuwai's Zhang Jian sees merit in that idea. "People in that area like to drink water from the mountain," he says. "To me it has a very strange taste, but villagers don't like to drink purified water because it has no taste!" His Fuwai colleague Zhang Shu also is not convinced that the little white mushroom is the sole culprit. "I don't think this is the last word," he says.

Indeed, not all sudden-death victims ate the mushroom. But CFETP researchers think they have the explanation. From the start of the investigation, they had suspected that heavy metal poisoning may play a role in the syndrome. One element seemed most likely: barium, which is used to induce arrhythmias. "If you want to test a drug for antiarrhythmic properties, you give lab animals barium," Fontaine says.

In 2006, the team rushed to two villages that had reported sudden-death clusters and took blood samples from victims and surviving family members. Many had high barium levels; one victim's was highest. Two years later, examining another cluster, they measured high barium levels in the blood, urine, and hair of victims, as well as in local water. In some Yunnan mushrooms—including Little White—barium readings were off the chart. Also pointing to barium are dozens of ECG readings of ill and healthy villagers. A remarkable 40% of exams revealed an abnormal heart electrical pattern called a long QT interval, a major risk factor for sudden death. Barium is known to trigger a long QT.

Putting the strands together, Zeng's team hypothesizes that the little white mushrooms have caused a large share of sudden deaths in Yunnan, perhaps abetted by barium from local foods or untreated water. If a sudden death occurs this year, CFETP researchers hope to detect Little White's amino acids along with barium in the victim's blood. "That would be strong direct evidence," Liu says.

One question may never be answered. "It's still a puzzle why the villagers didn't figure out themselves that the mushrooms are toxic," Fontaine says. But as a public health threat, he says, Yunnan sudden death syndrome may have been vanquished. **—RICHARD STONE**