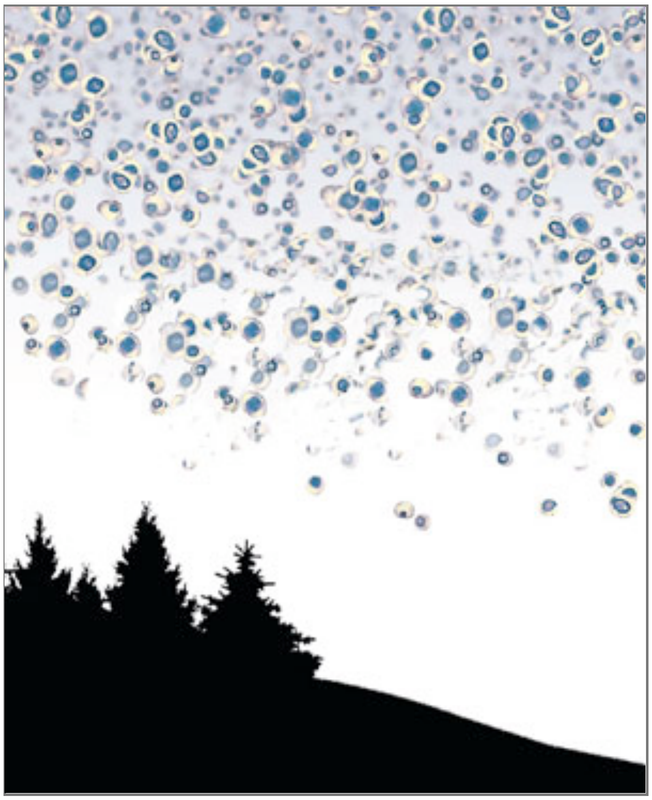
The Scientist

Volume 22 | Issue 12 | Page 36

They came from above



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Opportunistic infections seem to pop up out of nowhere, but new strains are appearing in new places, striking otherwise healthy animals - including humans. A few microbiologists go hunting.

By Brendan Borrell

In the spring of 2000, veterinarian Craig Stephen walked up to the biology department at Vancouver Island University in Nanaimo for what he thought would be a routine autopsy of a dead porpoise. "In my experience of doing stranded marine mammals, the vast majority of them, you don't get anything," says Stephen, who runs the university's Center for Coastal Health, "They've died, they've sunk, they've started to rot, they float back up, they get on the beach and then somebody finds them."

Stretched out in the department's backyard the sleek, four-foot long corpse was remarkably fresh, and Stephen saw nothing unusual as he scanned the eyes, mouth, and blowhole for hints of what led to its demise. Then he slit open the abdomen, removed each organ, and placed pieces into sterile plastic bags for laboratory tests. When he cracked open the chest cavity, Stephen was immediately struck by the lungs. "Usually you feel them and they are spongy, right?" he says. "You feel these and they are like liver." They were dense and heavy, swollen with pneumonia. Under a microscope, the sectioned tissue was dotted with tumor-like cysts. Stephen was shocked to learn the diagnosis: Cryptococcosis, a fungal disease best known for ravaging AIDS patients with weakened immune systems, had killed a seemingly healthy marine mammal.

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That was just the start. By the summer of that year, local veterinarians had recorded 12 cases of cryptococcosis in domestic dogs, cats, and even llamas—up from an average of about five per year. In 2001, scientists noticed that relatively healthy people were getting sick—and even dying from cryptococcal infection.

The infectious yeast looks like a fried egg, a sphere surrounded by a cloudy sheath. By shedding this sheath in a process experts jokingly call "vomitcytosis," *Cryptococcus* absconds from the bacteria in the decaying plant matter, soil, and pigeon droppings where it lives. This tactic works equally well against human and animal phagocytes, thus enabling *Cryptococcus* to evade the immune system. The disease proceeds from shortness of breath to fever and headaches. If it is diagnosed soon enough, several months of intravenous anti-fungal Amphotericin B can knock it out. But not everyone is so lucky: This past April, 45-year-old Sandra Agostini became Vancouver Island's latest fatality due to *Cryptococcus*.

On a recent visit to the British Columbia Center for Disease Control in Vancouver, medical microbiologist Linda Hoang opens a humming incubator to reveal a small sample of the isolates the center has amassed over the last eight years. Today, the reference lab she supervises diagnoses 25-30 human cases of this virulent strain of *Cryptococcus* each year using restriction fragment length polymorphisms (RFLP), and is testing a PCR-based strategy to quicken the turnaround time.

The microbial community may be transforming in ways that scientists are only beginning to notice.

Unlike diseases transmitted strictly via an animal vector or from person-to-person, the ubiquity of opportunistic pathogens like *Cryptococcus* presents a new and daunting set of challenges for scientists and medical professionals. Any organism small enough to be lifted into the air has the potential to achieve a cosmopolitan distribution, provided it can survive where it lands. In the cool climates of a temperate zone, untold numbers of potentially pathogenic bacteria and fungi may subsist—but fail to thrive—just below the level of detection. Microbial ecologists have a saying for this: Everything is everywhere, but the environment selects.[1]

Karen Bartlett holds up a petri dish dotted with *Cryptococcus gattii* colonies.

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Biologists now recognize that this dogma is only partly true, particularly in the face of Earth's warming climate. For example, over 50% of Kazakhstan's croplands have been sucked dry, while the Sahara expands into Nigeria and Ghana at a rate of 3,500 km^2 per year. This global process of desertification is increasing the number of dust storms that ferry microbes across continents and oceans. Meanwhile, in the temperate zone, rising temperatures have rendered some regions more hospitable to colonization by microbial hitchhikers arriving on soils from tropical climes. This new fungal strain cropping up in people and other animals with healthy immune systems may have been a new arrival to Vancouver Island, or it may have always been tucked away in some hidden valley for many years, until one balmy summer triggered its unfortunate bloom. And there are hints that it is steadily furthering its progress. "The question we've been asking over the last 10 years," Hoang says, "Is it going to get to the mainland and will it spread across the Pacific

Northwest?"

A key moment in aeromicrobiology, or the study of airborne microbes, came in 1933, when Fred Meier of the US Department of Agriculture convinced Charles Lindberg to collect samples during an arctic flight from Maine to Denmark. Upon finding everything from fungal spores to algae and diatoms, Meier wrote, "the potentialities of world-wide distribution of spores of fungi and other organisms caught up and carried abroad by transcontinental winds may be of tremendous consequence." We now know that particles of dust, organic matter, and aerosolized water droplets support hardy communities of bacteria, fungi, and viruses—a mere 0.08% of which have ever been cultured.[2]

Some 10,000 bacteria are present in every gram of airborne sediment, and the atmosphere contains at least one billion metric tons of dust.[3] That translates to a quintillion dust-borne bacteria—enough, according to Dale Griffin of the USDA office in St. Petersburg, Fla., "to form a microbial bridge between Earth and Jupiter." Over the course of five days in 2001, NASA tracked a large dust cloud that originated in the Gobi Desert as it moved east across the Pacific, North America, and the Atlantic, before petering out over Europe. Frequently, during African dust storms, a smoke-like strand is visible in satellite photos swirling off the continent, and looming over Italy, Spain, and southern France.

Emilo Casamayor and Maria Vila-Costa collect microbes from the surface of Lake Redo in Pyrenees.

One of the most surprising new findings about airborne microbes is that far from being passive passengers of the wind, some are truly adapted to life in the mesosphere—70 km above the earth's surface—where they must constantly repair their DNA following bombardment by direct UV radiation. Or take a 2008 study that found that airborne microbes haunting Singapore shopping malls are not a random sample of what's outside, but are specialized for survival in the indoor air environment.[2]

One morning this summer, microbial ecologist Emilio Casamayor from the Center for Advanced Studies in Blanes, Spain, and visiting postdoc Maria Vila-Costa hike past hordes of

All photos by Brendan Borrell

tourists and backpackers to Lake Redo, one of 1,200 alpine lakes in the Pyrenees Mountains. After finding a comfortable rock to squat on, the pair pull out metal mesh screens about one meter long and bound by a wooden frame. Vila-Costa presses her screen to the water's mirrored surface and lifts it into the air, a thin film bridging the wire grid. She tilts the screen to the neck of her plastic bottle and fills it one drip at a time. "How long does it take usually?" she asks Casamayor. "One liter, one hour," he responds.

The clock starts ticking, and Casamayor takes time to explain his work, which is aimed at understanding the effect of dustborne microbes on the native communities in the water. Most of the lake's biodiversity, he says, is restricted to this hydrophilic layer on the surface, where native microbes make use of nutrients coming from dust drifting in from Africa. He says that humic acids and other nutrients in atmospheric dust are easier for bacteria to break down than those from local soils, because they have been exposed to intense UV radiation. The dust particles also contain living microbes, and he wants to know how long they survive on the lake's cool surface. In addition to collecting and sequencing samples from 30 lakes in the region, Casamayor has set up a system to collect dust before it hits the lake and sequence organisms within it. These data will help him understand the impact of dust storms on microbial ecology.

Casamayor first noticed the dust in the Pyrenees back in June 2004, when his research focused on microbes living in the water. A late-season snowstorm had dumped fresh snow around Lake Redon near the town of Vielha and which sits at a slightly lower elevation than Redo. But when Casamayor came out to sample under the ice, he noticed the snow was already covered with a fine layer of brown grit. The phenomenon was not new—he had seen it in photos dating back to the 1860s—but with the drying of North Africa over the last 50 years, he reasoned that larger storms may be bringing more foreign microbes.

Indeed, the microbial community may be transforming in ways that scientists are only beginning to notice.

Around the same time the *Cryptococcus* outbreak began in Canada, scientists finally discovered what was killing sea fan coral in the Caribbean. Since the early 1980s, massive numbers of *Gorgonia ventalina* in the West Indies have been smothered by four highly virulent strains of *Aspergillus sydowii*,[4] a common, cosmopolitan fungus that had never caused widespread disease in plants or animals. The virulent *Aspergillus* strains were later shown to have originated in Africa.[5] The dust also brings crop pathogens such as sugar cane rust, *Puccinia melanocephala*, and banana leaf spot, *Mycosphaerella musicola*. During African dust storms, USDA microbiologist Dale Griffin says the number of culturable colonies of microorganisms airborne over the US Virgin Islands rises by a factor of 10. Since scientists began monitoring in 1973, an increase in African dust arriving on the island of Barbados has coincided with a 17-fold increase in asthma levels. In Spain, these dust clouds can have fatal consequences: A study published in the

November issue of *Epidemiology* found that daily mortality in Barcelona increased by 8.4% during periods when African dust clouds were present over the region, possibly due to biological irritants and allergens.[6]

Casamayor began monitoring Pyrenean lakes last year, and one of the first organisms he found was *Acinetobacter*, an opportunistic pathogen that is highly resistant to antibiotics (see "Baghdad hack"). A PhD student is currently developing molecular probes to track what happens when *Acinetobacter* lands in Lake Redo and what conditions would allow it to proliferate. Presently the team is only monitoring the effect of dust on microbial populations, but Casamayor plans to develop collaborations with other biologists to look for effects in plants and wildlife.

For instance, an unknown pestivirus has recently been ravaging populations of the endangered chamois, a goat-like ungulate that lives high in the mountains, and Casamayor wonders whether it could have been brought by the wind. Chamois researcher Emmanuelle Gilot-Fromont at the University of Lyon doubts that the pestivirus could survive over long distances, but agrees that airborne dispersal is a potential threat to humans and wildlife in the region. Q-fever, she notes, is caused by the bacteria *Coxiella burnetii*, and has traveled by wind from rural regions to infect humans in population centers in southern France.

Wandering through Vancouver Island's Rathtrevor Beach Provincial Park this summer, there is little evidence of the panic that gripped this coastal campground in June 2002, when campers cancelled their reservations in droves. Today, children fly kites on the windy beach and young couples pitch their tents beneath stands of towering Douglas Fir trees. No one pays much notice to the turquoise signs posted at every parking lot warning of the ever-present threat in the air.

In late 2001, as *Cryptococcus* infections spiked, Murray Fyfe of the British Columbia Center for Disease Control was just beginning to assemble the province's historic records of cryptococcal disease, when he received results from a Crypto-chek serotyping test. The fungus infecting porpoises and people was not the cosmopolitan strain of *C. neoformans* that infected immunocompromised patients, but was *C. gattii*, a species known primarily from Australian eucalyptus groves and other tropical regions that could infect relatively healthy people. Fyfe's team tested the few groves of eucalyptus present on Vancouver Island, but failed to isolate the fungus. "We were a bit stumped," he says.

So Fyfe contacted Karen Bartlett of the University of British Columbia to find where *C. gattii* was lurking on the island. Bartlett is an expert in environmental health who had spent years studying occupational hygiene, publishing papers on endotoxin exposure in metal shops and the spread of pathogens in schoolrooms. Fyfe believed Bartlett's expertise in airborne pathogens would help the agency pinpoint the fungus in the wild. Bartlett agreed, and in March 2002 took the 90-minute ferry out to the island to join the team as they traveled the east coast from Victoria to Parksville, taking sterile swabs in the cracks, scars, and bark of native trees and transferring it to an agar derived from birdseeds.

Karen Bartlett's recent work demonstrates that *C. gattii* doesn't just hitch rides on air currents, but also inside the wheel wells of vehicles and the bottoms of shoes.

Most of the dishes incubated back in Bartlett's lab turned up nothing, except for one sample from a Douglas fir tree in Rathtrevor Park that was peppered with smooth, brown dots. This melanin-like pigment is a tell-tale sign of *Cryptococcus* and protects it from damaging UV radiation when it is floating in the air. After discovering the positive sample, Bartlett returned to Rathtrevor with her Andersen air sampler in tow. Invented by army bacteriologist Ariel Andersen in the 1950s, the sampler contains six stages with progressively narrower holes to mimic human bronchial passages. Back at Rathtrevor, Bartlett flipped on an air pump for 10 minutes and let it suck 283 liters of outside air through the sampler. Then, she diligently counted dots on petri dishes. One sample contained 1,081 colonies of *Cryptococcus* in every cubic meter of air, practically a pure culture and far higher than any environmental samples Bartlett had cultured in the past. "We don't normally see airborne organisms in that kind of concentration," she says, with characteristic understatement, during an interview at her lab this summer.

On June 6, 2002, BC CDC issued a public advisory, warnings were posted at Rathtrevor, and cryptococcal infection became a reportable disease. By 2003, the infection rate had soared to 37 cases per million residents per year, far higher than the 0.94 cases per million residents in endemic regions of Australia. Over the summer and fall, Bartlett, postdoctoral researcher Sarah Kidd, and collaborators, continued to sample areas where human or animal cases had occurred, taking hundreds of soil, bark, and leaf samples along with additional air samples in the vicinity. *C. gattii*, they found, was turning up in small clusters all along the coast. These patches, they now know, blink on and off like Christmas lights, while others remain permanently colonized. All of these patches fall within two similar biogeoclimatic zones, the Coastal Douglas Fir zone and the Coastal Western Hemlock zone, which are drier than other parts of the Pacific Northwest.

Using PCR-fingerprinting and amplified fragment length polymorphism (AFLP), the team identified most of the fungal samples as the rare VGIIa genotype of *C. gattii*.[7] But the presence of small numbers of the VGIIb subtype suggested that there had been a second introduction of the fungus. Both fungal strains were a single mating type, which later led Duke University researchers to suggest that the outbreak was the result of an unusual same-sex mating event (See sidebar "Same-Sex Mating in *Cryptococcus*?").

A trail at Rathtrevor Beach Provincial Park on Victoria Island, Bristish Columbia, where Karen Bartlett first isolated *Cryptococcus gattii* from an airborne sample in 2002.

All photos by Brendan Borrell

Today, many questions about the ongoing outbreak remain unanswered. The Duke lab, led by Joseph Heitman, believes that *C. gattii* arrived in the Pacific Northwest with shipments of *Eucalyptus camaldulensis* in the early 20th century. But no Eucalyptus trees on Vancouver Island have turned up positive for the fungus, and VGII strains of *C. gattii* have not been found on *E. camaldulensis* in Australia. For her part, Bartlett believes that the hardy fungus, which can endure months in seawater, has probably been around much longer than anyone realizes, but was only been able to propagate after a series of warm summer seasons. Her colleague James Kronstad has been using comparative genome hybridization to try to understand the basis for differences in virulence in strains of *C. gattii* isolated from both the clinic and the environment.

As rainy areas of the Pacific Northwest grow warmer and drier with the warming climate, Bartlett suspects *C. gattii* will continue to show up. Her recent work demonstrates that it doesn't just hitch rides on air currents, but also inside the wheel wells of vehicles and the bottoms of shoes. So there's practically no limit to where it could go from here.

The first animal and human cases of disease have already begun to show up on mainland British Columbia and in Washington and Oregon State. Although Bartlett has obtained positive air samples in the city of Vancouver, she has yet to pinpoint an infected tree. "If it does become colonized on the lower mainland," she says, "That's two million potential cases of exposure."

Have a comment? E-mail us at mail@the-scientist.com

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