COMBATING EMERGING EPIDEMICS

Epidemiology's New Weapons for Fighting Disease

By Megan Barlett

History is riddled with the strike of epidemics, which have left such scars in our historical record as the near-collapse of medieval Europe in the wake of the Black Plague and the decimation of the Native Americans after the Europeans' introduction of smallpox to the New World. The sudden and devastating impacts of epidemics have changed religious convictions, inspired new philosophies, and weakened the hold of authority in afflicted societies, serving as powerful forces of selection in cultural evolution. Diseases have even left their mark in human biological evolution, as seen in the overwhelming percentage of useless "junk" DNA in the human genome that is believed to be inserted by viruses, and the preservation of genetic mutations causing such syndromes as cystic fibrosis and sickle cell anemia because of their ability to confer disease resistance.

Our current knowledge of microbes and their role in disease empowers us to fight epidemics as they emerge instead of gradually evolving resistance over generations. While modern medicine has greatly reduced the death toll of infectious diseases in the wealthier areas of the world, epidemics are far from defeated. New outbreaks continually emerge as human expansion brings us into increasingly more frequent contact with new pathogens and their hosts. However, novel methods to examine molecular evolution by peering into the microscopic realm are formidable weapons in discovering and tracking emerging epidemics. Researchers today are far better armed against new diseases than those working only decades ago.

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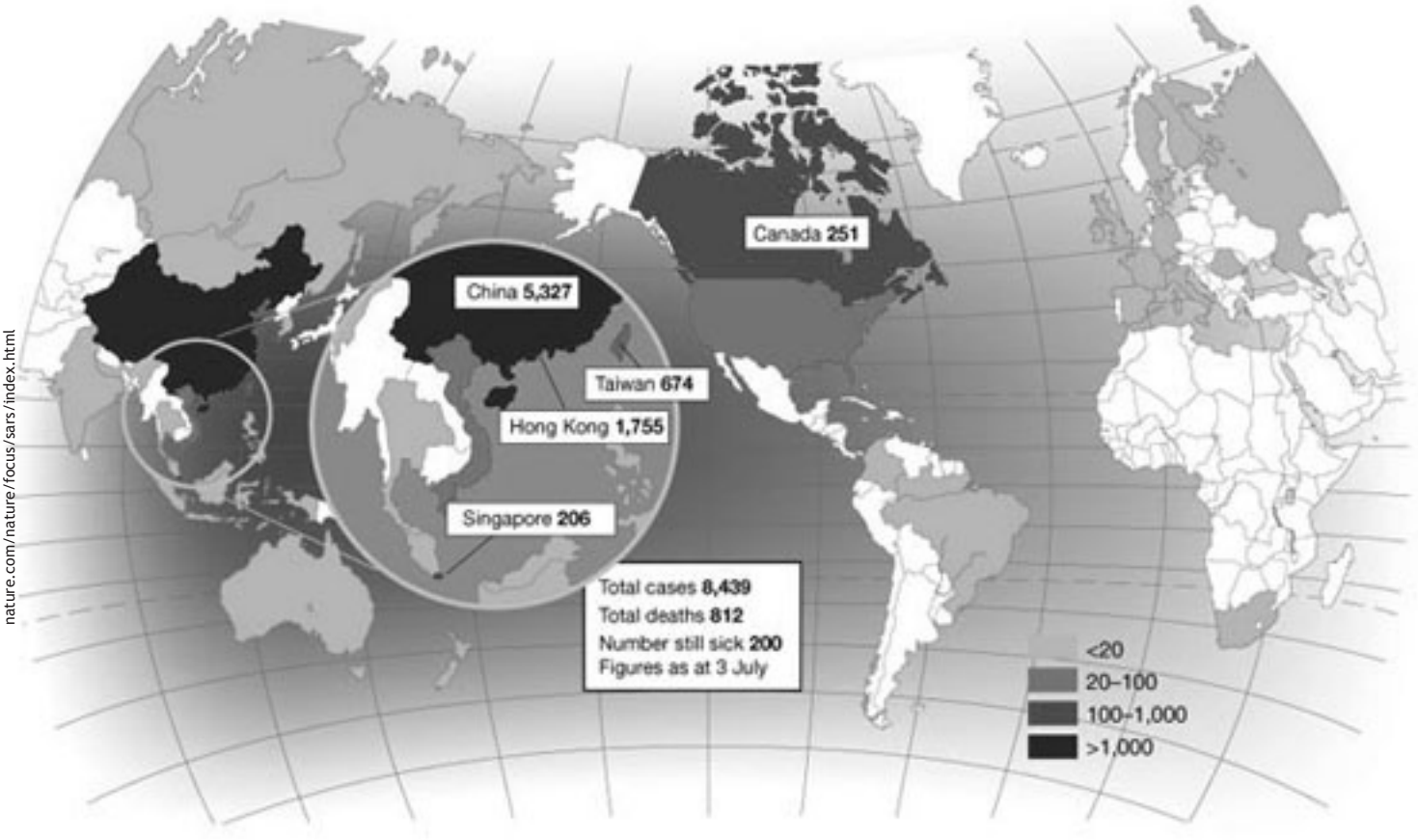
Before Genetics

Early epidemiology lacked all of the tools scientists use today, spanning from knowledge of genetics to recognition of the role of microbes in disease. Constrained to the study of doctors' records and death tolls in their search for disease patterns, handicapped with no real understanding of diseases' causes, the earliest epidemiologists could pinpoint only vague sources of epidemics, such as unclean water or malnutrition. But by the early 20th century, epidemiology assimilated the revolutionary new discipline of bacteriology, forging a new study of disease that linked its symptoms and spread through populations to the harmful effects of bacteria (1). The combination of these two theories allowed for the successful monitoring of several outbreaks of already familiar bacterial diseases, including typhoid and tuberculosis, through the use of light microscopy and bacteria culturing



From Nature Web Focus: SARS, "Pattern of an epidemic." http://www.nature.com/nature/focus/sars/index.html

technology available at the time. In a typical outbreak of salmonella, the suspicious clustering of newly reported cases would alert doctors that something was amiss. Each patient would be questioned to elucidate common environmental exposures or human contacts, and samples of their bodily fluids would be grown in culture to identify specific bacteria. Any overlap in behavior or environmental exposure would be tested (such as tap water from a specific source, or food from a certain market) and a sample would be grown in culture; if the same bacteria was recovered from both the bodily fluid and the environmental source, the source was identified and the contamination removed. This early system of bacterial detection worked fairly well for these simple diseases.

However, the newly evolved field underwent its first test against an emergent epidemic during the 1918 outbreak of the Spanish Flu--a vicious strain of influenza that attacked 33% of the population worldwide, and left only one remote location off the coast of South America unaffected. It began as a seemingly unremarkable influenza outbreak, with the characteristic influenza symptoms of vomiting, fever, weakness, and pain. But it rapidly increased in virulence, with a mortality rate greater than 2.5% (as opposed to the mortality rate of 0.1% observed in other influenza pandemics) (2). By the end of the four month epidemic, 21 million people across the world had died.

While the fledgling Public Health Service in America was able to identify and trace bacterial outbreaks, as they had previously done to successfully counter typhoid infections, they could do nothing to isolate the yet undiscovered viruses. Against the emerging pandemic, they could do little more than heighten hospital staffing and publicize the symptoms. There were even desperate attempts to create a vaccine by deliberately infecting imprisoned soldiers, but the contact with hospitalized patients in these experiments was not sufficient to produce the antiserum needed (3). Although it failed miserably in tracking the origin and evolution of the pathogenic agent itself, the establishment of epidemiology as a study of microbe-induced

▲ Figure 1. Map showing the global distribution of SARS cases- China had the most cases, since the virus originated there, but modern transportation carried it as far away as Canada, showing the ease with which modern epidemics can be spread.

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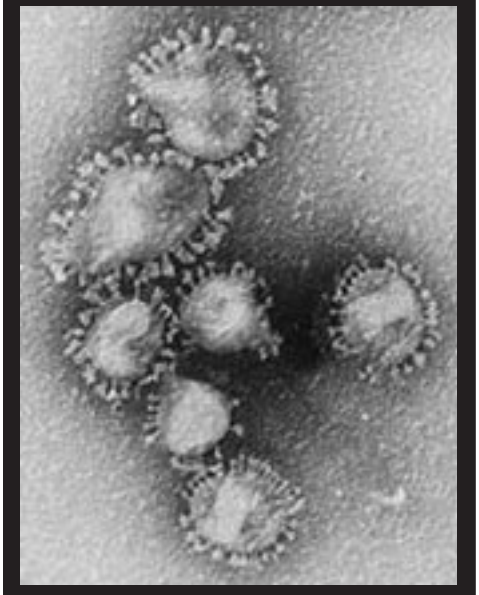

Figure 2. Electron microscope photograph of the SARS virus.

disease did create a framework for collecting and analyzing large amounts of data concerning when and where cases occurred, allowing the origins and spread of disease to be tracked through detailed records. Early epidemiologists also became fairly efficient at isolating, identifying, and tracing bacterial agents in simple infection scenarios (such as linking small outbreaks of typhoid to tainted water or contaminated food), but another revolution in epidemiology theory was required to identify and trace viral outbreaks and any infections where detailed mortality records did not exist.

Finding the Pathogen

In place of the relatively tame outbreaks such as those of salmonella reported in American hospitals at the turn of the 20th century, modern epidemiologists must tackle virulent new diseases that emerge without warning, often in third world countries with poor record keeping and medical care. Early 20th century epidemiology is powerless here- tracking modern epidemics requires knowledge of genetics, molecular evolution, pathogen-and-host relationships, and microbial biology.

On March 12, 2003, the World Health Organization issued a worldwide alert against an abnormal pneumonia that had first struck a traveler in Vietnam, killing him and spreading to the doctors that treated him. The disease had sporadically appeared in hospitals in Hong Kong and the Guangdong Province in China, but it was not brought to global attention until it was found in a Canadian traveler returning from China. By this time, it had infected over 300 people in China and killed at least 10 (5). International travel rapidly spread it to the US, Canada, Europe, Taiwan, and Singapore, with a terrifying mortality rate of 10%, peaking to 50% in patients over 65 years old (6). Frightened by its spread and virulence, scientists immediately attacked the new disease.

As in early 20th century epidemiology, the first step was to isolate the microbe causing the disease. The symptoms- fever, coughing, headache, and pneumonia-like respiratory problems- suggested that the pathogen was related to cold or flu viruses. To search for a similar virus, researchers amplified the DNA in blood samples from SARS patients using the DNA-replicating PCR (polymerase chain reaction) technique, targeting DNA sequences found in different strains of respiratory viruses, including pneumonia viruses, adenoviruses, influenza viruses, and coronaviruses (responsible for colds). None of these target DNA sequences matched the DNA of the unknown microbe, yielding no DNA amplification and proving the SARS microbe was distinct from any known respiratory viruses (7). To glean genetic information from the new virus, mucus samples from SARS patients were treated to isolate RNA (ribonucleic acid), which converts genetic information in the form of DNA to proteins. The RNA was then translated back into DNA with the enzyme reverse transcriptase, producing over 20 fragments of the unknown virus's DNA. These patches of DNA are a very powerful tool- they can be sequenced and used as target sequences to amplify viral DNA, and they can be compared with sequences in other viral genomes to create an evolutionary tree for the new virus. From this comparison, the new virus was shown to be a completely novel type of coronavirus: a nasty cousin of the common cold (8).

However, to prove that a virus is the cause of a disease, that virus must meet four qualifications known as Koch's postulates. It must: 1) be found in all organisms with the disease but not in healthy organisms; 2) be isolated from a sick organism and grown in culture; 3) be shown to cause infection when introduced into a healthy organism; and 4) be re-isolated from the newly infected sick organism. With two of these criteria met, the new virus was injected into healthy macaques, causing 90% of them to develop SARS symptoms. When the macaques' blood was tested, and their DNA was amplified using probes bearing SARS viral sequences, DNA from the SARS virus was detected in each sick macaque, and examination with an electron microscope uncovered the virus (9). The new coronavirus was definitely the cause of SARS.

Tracing Origins

Armed with the knowledge of the virus's DNA sequence and structure, researchers quickly developed diagnostic methods and a successful vaccine, finally halting the virus at 8,000 cases and 700 deaths (6). However, the mystery remained of where the virus originated. The first cases were seemingly unconnected, except for the unexpected link that 7 of the original 11 patients from the Guangdong province worked in restaurants serving wild animal meat (10). Starting with this shared case history, researchers armed with SARS diagnostic tests swarmed into the famous live-animal markets of the province (which boast such delicacies as porcupine and badger) in search of possible animal sources. Their suspicions from the case histories proved to be exactly correct- blood samples from up to 40% of the wild animal traders and 20% of the butchers working in

the market possessed antibodies for the SARS virus (11). In the seven tested animal species themselves, nearly all of the animals that tested positive for SARS were palm civets, a weasel-like animal considered a delicacy by many connoisseurs of exotic meat. With these new findings as a guide, researchers focused on proving that civets were the animals responsible for transmitting SARS to humans.

Definitive proof emerged from a cluster of 4 patients who fell ill in the winter of 2003-2004, whose only connection with each other was that one patient was a waitress at a restaurant that served civet, and another was a doctor who had eaten at the same restaurant. The animals at the restaurant were immediately tested, and all civets were found to harbor SARS DNA. Sequencing the S gene, which was chosen for its usefulness in tracking SARS evolution in earlier infections, showed 4 of the 5 tested civets had identical sequences to the 2 human patients, indicating that- at least in these specific cases-SARS had been directly transferred from civets to humans (12). However, it was unknown whether these civet infections represented an isolated movement of the virus into this species, or a long-standing virus/host interaction, making civets the original source of the human outbreaks of SARS. To test this, the new civet sequences were compared to the sequences of 22 earlier SARS infections, and the sequence changes were mapped into phylogenetic trees, which are diagrams that show evolutionary relationships based on genetic differences. If civets were a new host species for the virus, then these viruses would diverge greatly from the earlier viruses by virtue of having to adapt to the new host biology; however, if civets were truly the original host species, then these viruses should be very closely related to the original viral strands. For all 5 new civet sequences, there were only 5 base pairs that were different from the earlier SARS sequences- an extremely small variation that showed

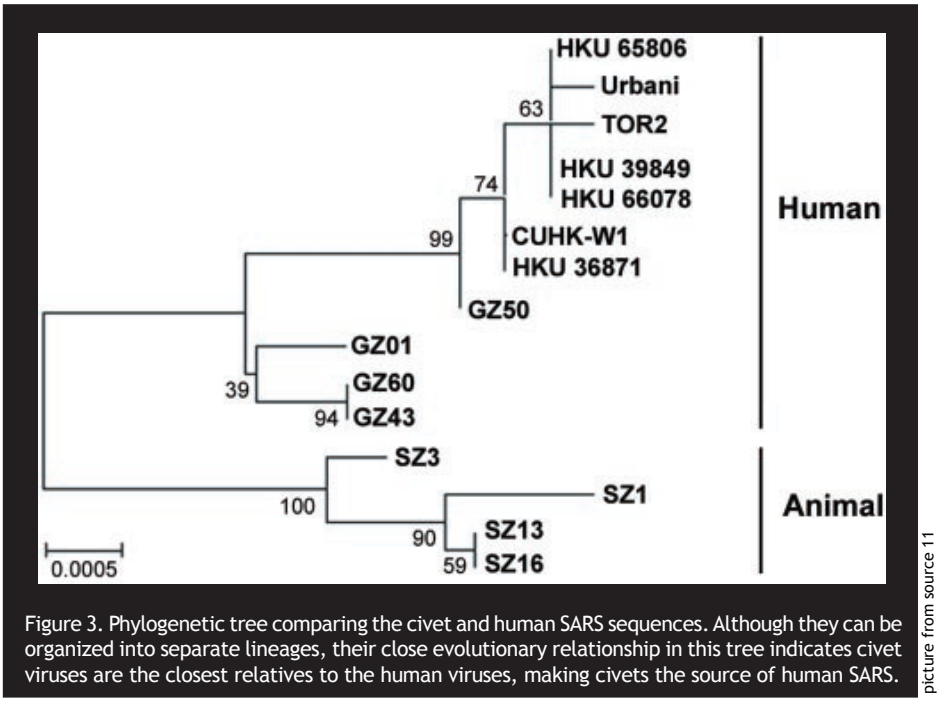

Figure 3. Phylogenetic tree comparing the civet and human SARS sequences. Although they can be organized into separate lineages, their close evolutionary relationship in this tree indicates civet viruses are the closest relatives to the human viruses, making civets the source of human SARS.

picture from source 11

these new civet viruses were indeed closely related to the viruses that caused the initial human outbreaks, thereby implicating civets as the original species that transmitted the deadly SARS virus to humans (12).

Epidemiology's Future

Combining the new techniques of molecular biology with the meticulous detective work of comparative case studies, epidemiology has shown its power to track vicious new diseases and help impede their progression before they achieve their full destructive capabilities. However, there are still mysteries surrounding the emergence of many new diseases, such as the unknown origins of ebola and Marburg fever. Host/virus interactions and interspecies transmission are not well understood even today, despite their vital role in describing how novel epidemics are introduced into humans. Although epidemiology has passed the test presented by SARS, the field still faces the challenge of developing more accurate, more timely, and more powerful techniques that would effect a greater constrain on the diseases' damaging effects. Science shaped by humanitarian goals and policy structured around scientific knowledge must evolve together to release humanity from the stranglehold of infectious disease. H

—Megan Barlett '09 is an Organismic and Evolutionary Biology concentrator in Lowell House.

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