BADIA POZZEVERI CHURCHYARD, ALTOPASCIO, ITALY—On a hot afternoon in July 2012, Giuseppe Vercellotti was digging up bones near the wall of an abandoned medieval church here, thinking about getting a cold drink, when he heard his students call his name. Faces glistening with sweat, they told him that they had found something strange buried half a meter down. Vercellotti took a look and saw a layer of lime, used in ancient times to squelch the stench of rotting corpses. When he tapped the hard layer with his trowel, it sounded hollow. “We immediately thought it was a mass grave,” says Vercellotti, a biological anthropologist at Ohio State University, Columbus, who co-leads a field school here. “We instructors were all excited and hopeful.”

But the students were apprehensive: “They all started talking about possible contagion,” Vercellotti says. Unconcerned, he leaned deep into the trench, where he got a whiff of a pungent odor and spotted an elbow bone poking out of the lime that had sealed it like a cast. The layer spoke of bodies tossed into a pit and hastily covered with lime. Could this trench hold victims of the Black Death, the plague that killed half of Europe in the Middle Ages?

It was the end of the summer field season. So the team carefully covered the trench with tarps and went home, hoping that excavations in 2013 would show that they had struck gold. They had come to expect extraordinary finds in the graveyard of the now decrepit Abbey of St. Peter, where a bountiful store of ancient skeletons was laid to rest in a single place over 1000 years, from the 11th to the 19th centuries. The goal of the ongoing project is to read the history written in these bones: when and where these people were born, what they ate, what diseases they suffered and died from, and how their
Hasty burial. An elderly woman, buried with a gold earring, was entombed in lime, suggesting she died during an epidemic.

Health varied by social class and over time. “This is a superb opportunity to learn about life in the medieval period and how it evolved and changed over that time and into the Renaissance and Industrial era,” says project co-leader Clark Spencer Larsen, a biological anthropologist at Ohio State.

Helped by locals who house and feed 30 students for 6 weeks each summer, the interdisciplinary team of 12 researchers is now in its third year of excavations. Their work is set apart not just by the extraordinary site but also by the variety of tools used to learn the secrets of the bones, from scanning them with 3D computed tomography to extracting isotopes from the teeth. In an unusual collaboration, this year the team brought along an ancient DNA expert to sample for ancient pathogens.

Because the abbey stood beside an ancient pilgrimage route, the results could help track the spread of disease through Europe. DNA from ancient microbes could also help today’s medical researchers keep one step ahead of fast-evolving diseases like cholera and influenza. “What you’re seeing at Badia Pozzeveri is hypothesis testing not only on bones, but also using pathogens and cultural factors,” says bioarchaeologist George Armelagos of Emory University in Atlanta, who is not part of the project. “It’s going to be the poster child for future work in bioarchaeology.”

The Burials Begin
1039 to 1300 C.E.

One afternoon while the students ate lunch, University of Pisa archaeologist Antonio Fornaciari gave a tour of the trenches, pointing to a freshly excavated stone wall beneath an asphalt parking lot in area 4000 (see graphic). In the 12th and 13th centuries, this wall ran along the inner sanctum of the monastery. Monks of the Camaldolese branch of the Benedictine order lived here, surrounded by a tall wall and moat, at the edge of marshes and oak woods, according to the town’s official history.

In this courtyard, the team found two partial skeletons, buried between 1200 and 1300 C.E. in a place of honor that suggests they were monks. The anthropologists are now examining their remains to answer a key question: Did monks have better health than farmers or peasants?

Stature is one clue to health, and most medieval Europeans were short. European men averaged 167 centimeters in the Middle Ages (compared with 178 cm today), and shrank by 5.4 cm by the end of the period. The team thinks that with the rise in population, more people competed for food and resources. The bones at Badia Pozzeveri could confirm a trend toward scarcer food and worse health as the Middle Ages progressed.

The bones could also show whether monks were exceptions. Historical records suggest monks did eat better than peasants—and that both had poorer diets and health than nobles. To begin the analysis, Vercellotti laid out the leg bones of one monk on a table in the makeshift lab inside the church, below a ceiling covered with faded frescoes. He measured the lengths of thighbone and shinbone and made a “very preliminary” height estimate of 165 to 170 cm. A better estimate might give him a clue to the monks’ status: High-status medieval men buried in one churchyard in northern Italy averaged 171 cm, while lower status men averaged 164 cm, according to a study he published in 2011 in the American Journal of Physical Anthropology. The team has also dug up the likely remains of peasants, probably dating from the 11th century—two poorly preserved skeletons found outside the wall—and they’re hoping for more.

Pilgrims also passed right by the church as they followed the main highway of the Middle Ages, the Via Francigena or “road that comes from France.” Knights, clerics, and peasants all traveled this route, leaving traces such as two rare Islamic jugs from North Arabia, found in the cloister this summer. With the travelers came new diseases. Leprosy, for example, may have arrived from the Middle East with the Crusaders. It swept into Tuscany in the 12th century, when four leprosariums sprang up in the area, including one run by the monks.

Those are just the sort of infectious company that Hendrik Poinar seeks. An ancient DNA expert at McMaster University in Hamilton, Canada, he jumped at the rare chance to get DNA from pathogens over time in a single location. He wants to see how many diseases people of each period had to combat and how fast pathogens evolved in different conditions, such as famine and war.
One morning, as Poinar watched, Vercellotti and a graduate student laid out one skull after another on a long table. Poinar looked at the excellently preserved teeth in a freshly excavated jaw. Teeth are a promising source of ancient DNA. “This is it—this is what we came for,” he said.

“Dig in,” said Vercellotti, holding a skull steady. Poinar adjusted his facemask, pulled up his rubber gloves, and yanked a tooth out of a jaw with pliers. “Skilled dentistry,” he joked. If he does get DNA from these teeth, he’ll test it for everything from leprosy to plague to TB.

Online: maps of the Via Francigena and a video reconstruction of the Badia Pozzeveri monastery through the ages.

The Worst Century
1300 to 1400 C.E.

The next day, Poinar knelt head down over a trench in area 2000, trying to excavate a jaw with teeth protruding from the wall of the pit. The team hadn’t yet gotten radiocarbon dates from this trench, but they thought it might be from the 14th century, a time of devastating infections including the Black Death, which killed half of Europe from 1348 to 1350. The teeth glistened in the dirt wall, but the jaw was firmly embedded. “I’ve been drooling over them for 4 days,” Poinar said.

Earlier that week, he had explained why. He gave a presentation in the church and flashed a slide about a news story about a plague-infected squirrel that closed campgrounds near Los Angeles. “Killer squirrels are coming!” he joked.

But it’s no joke to ask if killer strains of plague could return. In 2011, Poinar was part of a team that gathered ancient DNA from people who died in London between 1348 to 1349, apparently of plague. The scientists identified the cause of the Black Death as the bacterium *Yersinia pestis*, rather than anthrax or a mix of pathogens, as some had suspected. This ancient strain was almost identical to a *Y. pestis* strain that still circulates in small rodents in the southwestern United States, Africa, and Asia. But today, *Y. pestis*, although still deadly, infects only about 1000 to 3000 people annually and is transmitted slowly from person to person.

Why is this *Y. pestis* strain so much less virulent today, and why does it only rarely move from rodents to humans? Poinar is one of several geneticists in a neck-to-neck race to find out. They’re trying to learn when and why the Black Death strain jumped from rodents to humans, and what made it spread so rapidly. Was it mutations in the genome of *Y. pestis* or changes in the susceptibility of animal or human hosts—or both? “If we study humans before, during, and after the plague, we should see how the human genome responded to these repeated outbreaks and the response in bacteria,” Poinar says.

That’s why he is seeking the Black Death in Badia Pozzeveri, where cases were recorded in 1348 before the epidemic reached northern Europe. He’ll compare that strain—newly arrived from Asia—with that of the London victims to see if the plague evolved as it tore through Europe. He also can see if plague victims suffered from TB or other infections, to test the idea that 14th century people harbored so many pathogens that they were more susceptible to plague.

Another theory behind the deadliness of plague is that it was hard for anyone in Europe to survive that terrible century. Before the plague hit, the continent had been pounded by bad weather, failing crops, famine, and war. Torrential rains in 1315 and 1316 flooded crops and caused the Great Famine. The Little Ice Age had begun, triggering frigid winters that destroyed more crops. In England, between 1348 and 1375, life expectancy at birth was only 17 years, according to parish records. Overall health, as shown by seven indicators in teeth and bones, plummeted to an all-time low in the 14th century, according to a study of 17,250 individuals from 100 locations in Europe by Ohio State economic historian Richard Steckel, Larsen, and their colleagues in the Global History of Health Project (*Science*, 1 May 2009, p. 588).

Disease may even have influenced the outcome of battles among the Italian city-states, which came right to the doorstep of...
the church at Pozzeveri. In September 1325, Florence’s commander Ramon de Cardona camped at the abbey with most of his 3000 cavalry and 15,000 infantry. The nobles moved into the monastery itself, while the troops probably camped in a field west of the church, near what was then a large lake and swamp. Many got sick.

That may be why Cardona made a move that still puzzles historians: He lingered at Badia Pozzeveri for two long weeks while his rival, the legendary Castruccio Castracani from Lucca, recruited reinforcements. Castracani had far fewer troops at first. But by the time Cardona advanced on 23 September, Castracani’s army outnumbered the Florentines, many of who retreated before the battle had even begun. It was a rout, and Castracani became a hero whose military victories were immortalized by Machiavelli.

Just what sickened Cardona’s men? Fornaciari suspects malaria because they complained of mala ariæ, or bad air. He convinced Poinar to test for that disease, too. “One of our hopes is to discover if malaria is present in the medieval period,” Fornaciari says, because it is not clear when the illness first reached Tuscany.

Another hope is to find the remains of those who died in that famous battle, because relatively few groups of soldiers killed in war have been found in Europe, Larsen says. Three spearheads have turned up so far in area 4000. Human remains would provide the first good physical “record of injuries from the kinds of weapons available in 1325,” Larsen says. “They were doing some really horrible things to each other. They had this square hammer-headed mace for bashing in skulls.”

Commoners’ bones will provide a counterpoint to Fornaciari’s work elsewhere revealing the woeful condition of the well-fed nobility. In Naples, he examined the mummy of Maria d’Aragona, a noblewoman who lived from 1503 to 1568 and was a famed beauty in her youth—but was obese at death. That fits with what he has learned about her fellow nobles’ diet. In 2008, Fornaciari analyzed carbon and nitrogen ratios in bone collagen from other princes of Naples and the Medici of Florence, and found that they had as much nitrogen in their diet as carnivorous mammals. Clearly, Renaissance royalty ate unhealthy quantities of meat at a time when many rural people struggled to get enough calories.

Nor was rank a protection against horrific infectious diseases. When Fornaciari cut off a linen bandage from Maria d’Aragona’s arm, he discovered a large ulcer. He examined the tissue with a scanning electron microscope and rinsed it with antibodies that fluoresce in the presence of the bacteria that cause syphilis, Treponema pallidum. The tissue was so well preserved that he could detect the spiral shape of the bacteria; he sent tissue to Poinar to confirm the diagnosis. Poor Maria also harbored human papillomavirus in a venereal wart—the first diagnosis of this sexually transmitted, cancer-causing disease in the tissue of a mummy, Fornaciari reported in a 2006 paper.

Sexually transmitted diseases were common in Renaissance Italy. Syphilis raced through the country in the 1500s, possibly after Spanish sailors brought a new venereal form from the New World. Fornaciari also examined Maria’s distant relative, Isabella d’Aragona, who was also buried in Naples. She was married to the Duke of Milan and is thought by many to be the model for Leonardo da Vinci’s Mona Lisa. When Fornaciari looked closely at this lady’s teeth, he found that they had been abraded to remove most of the enamel. The remaining enamel traces were black, a sign that she...
had taken mercury, which was then used—ineffectively—to treat syphilis. Lab tests confirmed that the black patina had a high level of mercury and that Isabella d’Aragona was poisoned by her own medicine, dying at age 54 in 1524.

By comparing the teeth and bones of urban nobles with those of Pozzeveri peasants, the team hopes to see how social rank affected health. The teeth of the noblewomen are less worn, because they ate a softer diet with meat, whereas poorer women and children often ate coarse millet. Vercellotti and Larsen expect to see more disruptions in tooth growth, caused by lack of food during childhood, in the peasants. With the graveyard’s large sample sizes, they hope to compare the men and women of Badia Pozzeveri to see who was better fed.

### The Mass Grave

1850s

Almost a year after Vercellotti first tapped his trowel on the bed of lime, he and a crowd of students set to the task of systematically uncovering the entombed skeletons in area 1000. They chipped away the cementlike lime and tried to avoid inhaling the powdery white dust. Once they broke through the shell in early July, they brushed and scooped away the soil, sometimes with teaspoons. They found that each skeleton was buried separately, but all were blanketed in lime.

One skeleton clutched a cross, head to the side, jaw agape. Another had a twisted spine, likely evidence of scoliosis. All had been buried in shrouds and were lying in unusual positions as though they had been dumped hurriedly. Everything fits the hypothesis that they were victims of an epidemic. The researchers carefully uncovered another exceptionally complete skeleton—an older woman, as shown by her frail bones and worn teeth. She was lying on her side, probably in the same position in which she died. In the soil beneath her, they could see the impression of her fingers and ear, and the lines left by her bodice’s laces. “I loved excavating her,” Vercellotti said. “She was beautiful,” Gino Fornaciari agreed. Beneath her skull, they found a single, golden hoop earring, and they began to call her the Lady with the Gold Earring.

That earring was a clue: This was no medieval matron. The hoop style, as well as buttons and fasteners for clothing found with other skeletons, showed that these people died in the mid-1800s—too late to be victims of the Black Death. So what did kill them?

To find out, Poinar and Vercellotti pulled teeth from their skulls and scooped soil from where their stomachs once lay, seeking to sample and identify the pathogen’s DNA. They already have a working hypothesis: cholera.

In 1855, this terrible diarrheal disease, transmitted by the waterborne bacterium *Vibrio cholerae*, swept through Italy, part of a worldwide pandemic. Poinar is just as eager to find DNA from *V. cholerae* as from plague, because tracing the evolution of cholera is still urgent today.

Like other pathogens, *V. cholerae* keeps evolving into new forms, and it continues to erupt into worldwide pandemics. Since the 1960s, the seventh known pandemic has infected 3 million to 4 million people and killed nearly 100,000 every year, with a new strain causing particular devastation in Haiti recently. If researchers can trace the bacterium’s evolutionary history, they might be able to identify the key mutations that trigger virulence or adaptation to different habitats, for example. This could help them design better vaccines or medicines.

Poinar has already sequenced a sample of mid-19th century cholera from the United States. The researchers gathered DNA from a cholera victim’s intestines, which in 1849 were preserved in jars in the Mütter Museum in Philadelphia. Their unpublished results match a cholera victim’s intestines, which in 1849 were preserved in jars in the Mütter Museum in Philadelphia. Their unpublished results match their DNA is being scanned with a new microarray that can detect DNA from 3000 different pathogens, including the microbes that cause plague, TB, malaria, syphilis, Lyme disease, and cholera.

Despite the unexpected bonus of a probable cholera epidemic, Poinar was still intent on finding plague victims. On his last day at the site, he kept going back to the medieval trench, where the teeth peeped tantalizingly from the wall. Vercellotti gently brushed dirt off the jaw and sprayed it with water, hoping to loosen the sediment. But the jaw wouldn’t budge. He finally gave up. “Next year,” he promised. Poinar left Italy still haunted by hopes of a plague sample.

Two weeks later, when he returned to his lab in Canada, he got a tiny package from Vercellotti. It held the tooth he had wanted so badly. Vercellotti had managed to excavate it in the season’s final week, and its DNA is now being analyzed.

---ANNE GIBBONS

Online: medical files on Maria and Isabella d’Aragona and how archaeologists spot disease in skeletons.

Online: video of the excavation of the Lady with the Gold Earring and a slideshow of one skeleton’s story.
The Thousand-Year Graveyard
Ann Gibbons (December 12, 2013)
Science 342 (6164), 1306-1310. [doi: 10.1126/science.342.6164.1306]

Editor's Summary

This copy is for your personal, non-commercial use only.

Article Tools
Visit the online version of this article to access the personalization and article tools:
http://science.sciencemag.org/content/342/6164/1306

Permissions
Obtain information about reproducing this article:
http://www.sciencemag.org/about/permissions.dtl