

How the plague bacillus and its transmission through fleas were discovered: Reminiscences from my years at the Pasteur Institute in Paris

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At present, there are only occasional cases of human plague reported in the United States. As an example, there were only 14 nonfatal cases of human plague recorded in 1988 and 5 cases in 1990. Each resulted from exposure to wild rodents, carrying the plague bacillus and fleas, in the western United States: Colorado, New Mexico, California, Arizona, and Texas. The affected individuals were treated with antibiotics (usually streptomycin and tetracycline) and recovered. Local, endemic outbreaks of plague have been reported from Uganda, Kenya, and the island of Madagascar. More recently, in outbreaks of plague, bubonic or pneumonic, in limited local epidemics, a total of over 700 patients have been reported from certain areas of India in 1994.

This manuscript was prepared in order to describe a few interesting details referring to the initial discovery of the plague bacillus and its mode of transmission from the rat carriers to other rats and to humans.

A few centuries ago, plague represented a massive disaster, killing millions of local populations in India, China, Indo-China, Africa, and, particularly, Europe. The cause of this disease was unknown and was attributed to unfavorable constellations of stars, to comets, to the wrath of supernatural powers, and frequently also to poisoning of wells by Jews, or other ethnic groups of people, who paid for this with tortures inflicted on them by the panicked population.

The mystery of plague was solved fairly recently. Alexandre Yersin, who discovered the bacillus of plague, died in 1943, during World War II. In June 1940, Yersin was in Paris, attending a Pasteur Institute meeting, and left Lutetia Hotel, where he was staying (and where I was staying also at the same time), barely a few hours before the German armies entered the French capital; in fact, Yersin left Paris for Saigon, by air, only 6 hours before the airport was closed. Paul-Louis Simond, who discovered that plague is transmitted by fleas, was collecting plants, and living quietly in retirement, in Valence (province of Drome, south of Lyon), in France, and he very kindly replied in writing to my letters, which I wrote to him from the Pasteur Institute in 1938, asking him for details of his fundamental discovery.

Since my childhood years, I have always been interested in finding out how the great medical discoveries were made and how the epidemics of transmissible diseases were prevented. In my early postdoctoral years, I had the opportunity, shortly before World War II, to spend several years, as a young guest investigator, at the Pasteur Institute in Paris.

I was intrigued by an isolated laboratory, located in a separate small building, at 25, rue du Docteur Roux. I was told that the upper floor of this small building has a laboratory dedicated to the problem of plague and that it contains notes and records of Yersin, who recognized that this disease infects predominantly rats, and of Simond. I visited this small laboratory several times and studied the notes of Yersin and

Simond. In fact, I was working in the same laboratory where Simond worked, some 40 years earlier. In 1938, when I was tracing the data leading to the clarification of the plague transmission puzzle, Simond lived at that time, as a retired French Army Medical Corps general, in Valence. I was helped and guided in my task by Edmond Dujardin-Beaumetz, a former friend of Yersin, who was working in the plague laboratory. I was impressed by these fundamental discoveries, which provided the means to control and prevent pandemics of this devastating disease.

Epidemics of plague have devastated for several centuries not only cities but also entire countries. Millions of people died, populations were decimated, and there were not enough people to bury the dead. People panicked. Medical and church authorities thought that these epidemics were caused by supernatural powers, in retaliation for sins committed by some segments of the population. Innocent groups of people were tortured and killed after being accused of spreading the disease. There did not appear to be any remedy or means to prevent this disaster.

The means of a relatively simple solution leading to the prevention of this devastating disease were found just before the turn of this century. These discoveries did not require complicated methods or techniques, except curiosity, common sense, and good observation by a couple of intelligent and persistent investigators, equipped with good will, a microscope, and letters of recommendation signed by Louis Pasteur and his coworker, Emile Roux. These letters facilitated dealing with local authorities in Indo-China and other places where plague epidemics were at that time ravaging the local populations. Both Yersin and Simond were trained at the Pasteur Institute in Paris and were strongly encouraged by Pasteur to follow and to try to solve the problem of plague. The story of this fundamental discovery, very briefly, is as follows.

Yersin, born in Switzerland, descendant of French immigrants, studied in France and worked at the Hotel Dieu Hospital in Paris; at that time Pasteur just introduced his vaccine treatment for those infected with rabies. Yersin was performing an autopsy on the spinal cord of a patient who died following a bite by a rabid wild dog; during the dissection of the spinal cord, Yersin cut his finger; he immediately proceeded to Pasteur's laboratory. Pasteur called his assistant, Emile Roux, and asked him to start vaccinating Yersin against rabies. That was the beginning of a long friendship that developed between Yersin, Pasteur, and Roux. Yersin became interested in bacteriology and frequently spent time in Pasteur's and Roux's laboratories. He was also assisted by Roux in preparation of his French medical doctorate thesis.

When an epidemic of plague developed in and ravaged Hong Kong, Pasteur suggested that Yersin proceed to Hong Kong to study and attempt to isolate the causative microbe of that devastating disease. Yersin accepted this suggestion enthusiastically. Pasteur requested that the French authorities send Yersin, who was a member of the French medical colonial corps, to Hong Kong. The orders came promptly, by telegram, and Yersin proceeded to Hong Kong.

The problem was not as simple as it might have appeared. Yes, the epidemic was in full swing. People died by the hundreds. The city hospital was full of sick and dying patients. But Yersin had no access to the morgue. The hospital director, Dr. Lawson, did not give him permission. After many interventions and appeals, even to the governor, Yersin finally, as a gesture of good will, received permission to have a small table in a corner of a dark corridor, next to the patients' room, where he could leave his microscope, a notebook, and a few cages with guinea pigs, mice, and rats. That was the limit of his allowance. He had no access to the morgue, where he was anticipating piercing the enlarged lymph nodes (bubos) of a patient who died from plague in order to look for the causative bacillus. Frustrated, Yersin in the meantime developed a friendship with an English priest, Father Vigano, who helped him build a small shack outside, but adjoining, the hospital, where Yersin could have a small folding bed and a very small makeshift laboratory. At the advice of Father Vigano, Yersin gave a few dollars to two English sailors who were helping to take care of the morgue at the hospital. Yersin was now able to go with the two sailors into the morgue for a few minutes and have access to the corpse of a patient who just died with plague. Yersin punctured the patient's swollen inguinal lymph node—i.e., bubo—with his sterile pipette and ran to his small laboratory, where one part of the fluid was placed under the microscope, another part was injected into a few guinea pigs, and the rest was prepared for immediate shipment to Roux at the Pasteur Institute in Paris. Yersin was excited after he looked into the microscope; he wrote in his notebook: "June 20, 1894. The specimen is full of microbes, all looking alike, with rounded ends, staining very poorly (Gram-negative); this is without question the microbe of plague." The next note was entered a day or two later: "guinea pigs injected with the plague bubonic fluid all died and their blood and organs were full of the same bacilli." He informed the hospital director of his observations and was now allowed to have access to the morgue. A new, very important observation followed shortly. Yersin, intrigued by the large number of dead rats lying on the streets, around the morgue, and in hospital corridors, decided to examine, under the microscope, the blood, lymph nodes, and other organs of these rats and found that they were full of the same bacilli that he found in patients dying with plague. He now realized that plague affects not only humans but also, and perhaps predominantly, rats. As a matter of fact, Yersin recorded that rats have long been known to be affected not only at the time of plague epidemics but also often preceding such epidemics in humans; ancient people knew about it, and mountain inhabitants in Chinese villages as well as villagers in parts of mountains in India and also on the island of Formosa knew that when hundreds and thousands of rats lie around on the roads and in houses, they precede the outbreak of the fatal epidemic in humans. In fact, plague was designated, in local languages, as a disease of rats. These fundamental, historical observations, made in the summer of 1894, established that plague in humans and rats is caused by the bacillus discovered by Yersin but did not clarify yet how this disease is transmitted from man to man, from rat to rat, or from rat to man. Transmission through air, food, and feces mixed with dust was suspected, but not substantiated.

The fundamental observations of Yersin were published the same year in the *Annales de l'Institut Pasteur* (1), from notes that Yersin mailed to Roux and Albert Calmette.

The mystery of transmission of plague from rat to rat, or from rats to humans, was solved a few years later by Simond, a young French colonial Army physician, who was delegated to Indo-China by Pasteur to take over the research on this devastating disease and to follow and expand the initial observations of Yersin. He worked in the former Metchnikoff's laboratory at the Pasteur Institute in Paris (and where, in fact, I had the privilege of working some 40 years later).

Roux suggested to Simond to go to Indo-China to try to follow up Yersin's work and particularly to try to treat patients suffering from plague with a serum prepared from horses immunized with the bacillus isolated by Yersin. Simond accepted with enthusiasm his new mission with the orders from the French government to proceed to Long-Tcheon in Indo-China where plague was ravaging.

While Simond was busy with the serum treatment most of the time, his thoughts were elsewhere—he was intrigued by the mysterious manner in which plague appeared to be transmitted from sick to healthy persons, individuals frequently separated by relatively substantial distances and apparently not having any contact with each other. People living in houses separated by a distance of half a mile perhaps from those where plague was observed frequently developed symptoms of this disease, even though they did not seem to have had any contact with those suffering from plague. Simond suspected that rats, which carry the disease, transmit it from man to man. He recalled that Chinese in Yunnan ran away from their homes as soon as they saw dead rats. Furthermore, on the island of Formosa (Taiwan) the inhabitants considered any contact with sick or dead rats as a menace leading to infection with plague. He recorded in his notebook that in one house in Bombay, during the plague epidemic, 75 dead rats were found. He even saw some of these animals running on the street dragging their legs and falling down and dying. Another observation recorded in his notes: "one day, in a wool factory, employees arriving in the morning noticed a large number of dead rats on the floor. Twenty laborers were ordered to clean the floor of the dead animals. Within 3 days, 10 of them developed plague, whereas none of the other employees became ill." In another observation, made in Chack-Kalal, in April 1898, a high mortality was noticed among rats but not among the human population. The inhabitants of that locality anticipated, however, that plague may be approaching, because of the dead rats observed all over, and much of the frantic population ran away from the village and settled in an isolated camp. "Two weeks later, a mother and daughter received permission to go back to the village to bring clothing from their house. They found several dead rats on the floor of the house. They picked up the rats by their tails and threw them out on the street and then returned to the camp. Two days later, both developed plague. One more observation: on May 13, 1898, in Bombay, a man walked into a stable to take care of his horse and found there a dead rat on the floor. He picked up the rat by the tail and threw it out. Three days later he developed plague." Before long, Simond began to suspect that people become infected with plague only when they approach rats that have just died from plague within a few minutes. People do not become infected, however, if they approach or even touch rats that are already cold, having died perhaps a day ago, or earlier. "We have to assume," theorized Simond, "that there must be an intermediary between a dead rat and a human. This intermediary might be a flea." At least, thought Simond, let me make an experiment with fleas. My experimental laboratory conditions were very primitive, I had only a tent in Bombay during the plague epidemic in a rainy season. . . (excerpts from letters to the author). In the tent, I had a microscope and a few cages with rats. I could not do too much under such primitive conditions. Nevertheless, I convinced myself and, in fact, before long I demonstrated that it is the flea that transmits plague. Healthy rats have very few fleas or none at all. If they have any fleas, they get rid of them very fast because they are very careful about grooming their skin and hair and try to keep clean at all times. On the other hand, rats that are sick do not take care of their skin hygiene anymore and that allows them to have many fleas. Immediately after the rat dies, when its corpse cools off, the fleas leave the dead rat and jump on other healthy rats or, if no rats are available, they jump on men or women. In this manner, one could explain cases of plague transmitted to people who had

no contact whatever with patients suffering from that disease. A healthy person, finding the corpse of a rat that died recently of plague, picks up the animal by its tail and throws it out and 3 or 4 days later develops the disease.

Simond was convinced that the rat flea (*Xenopsylla cheopis*) transmits the disease, but he had to prove experimentally that this assumption is correct. He realized the danger of having any contact whatsoever with infected fleas and he devised the whole experiment to be carried out in his tent in Cutch-Mandvi, Indo-China, where he was delegated because of the ravaging plague epidemic. His proposed procedure was as follows. He grabbed a rat that had just died because of plague with long forceps and threw it immediately into a paper bag, which in turn would then be dropped into a large container full of warm water saturated with soap. The bag would then be cut open with sharp scissors while it was immersed in water. In this manner, the action of fleas was neutralized. They were attached to the fur of the dead rat. Simond took a few of those fleas from the skin of the rat and placed them under the microscope. He realized at once that their intestines were full of bacilli of plague. In a control study, he checked under the microscope fleas taken from healthy rats and found them free from plague bacilli. He also realized that the danger of an infected flea jumping off the rat is practically limited to the immediate time after the rat dies, when the body of the dead rat is cooling off. The fleas do not tolerate cold and try to leave the dead rats, looking for new hosts with warm skin, preferably for rats, and, if no rats are available, they jump on humans.

A period of excessive heat wave in Cutch-Mandvi led to the temporary abatement of the plague epidemic. Simond moved to Bombay and Kurrachee, where he could carry out a crucial experiment that he had been planning for a long time. Simond brought to Kurrachee from Saigon a very tall glass jar. On the bottom of this jar he put some sand and a rat sick with plague; the rat was carrying fleas. The top of the jar was covered with a fine mesh net. After 24 hours, the rat was dying. At that time, Simond lifted the cover of the jar and inserted carefully into the jar a small cage dangling in the air held by strings attached to the center of the cover of the jar. This small cage contained a young healthy rat that had been kept isolated from infection. This small cage was hanging inside of the glass jar, a few inches above the sick rat crouched on the floor of the jar. The bottom of this hanging cage was made out of a screen, with relatively large openings. The healthy rat inside the suspended cage had no contact whatsoever with the sick rat. It was exposed, however, to the fleas, which could have jumped without difficulty from the sick rat, on the bottom of the jar, to the cage hanging above it. Simond determined in separate experiments, that a flea can jump about 4 inches up, vertically. It could reach, without difficulty, the small cage dangling in the air above the floor of the jar.

When the rat with plague, on the bottom of the jar, died, it was carefully removed. Five days later, the rat inside of the hanging cage, also developed plague. That was on June 2, 1898. The problem of plague transmission was solved. Simond sent the summary of all his observations to Roux in Paris. In October 1898, the article appeared in the *Annales de l'Institut Pasteur* (2).

As a control experiment, Simond placed in the same jar a rat suffering from plague, but carrying no fleas, together with healthy rats. No infection occurred. However, as soon as fleas

were introduced from outside, plague developed in the rats placed in the jar.

In this way, the mystery of the long-observed plague epidemics was solved. It also became clear why, as has long been known, the relatively safest places during plague epidemics were in well-maintained hospitals, where no transmission of plague occurred in doctors, nurses, or service personnel, except in cases of very rare pulmonary plague, transmitted directly from patients to healthy people. The bubonic plague, however, is transmitted only through fleas. Once infected, the fleas remain infected for long periods of time, for several weeks, perhaps even months.

These data are based on information I received in July 1938 from Simond, in handwritten letters addressed to my modest laboratory at the Pasteur Institute, where I was at that time a guest investigator in an unrelated field of cancer transmission in laboratory animals. Other data, particularly those referring to the discovery by Yersin of the plague bacillus in human patients and also in rats, I collected from personal notes and records of Yersin, kept at the Pasteur Institute, and my conversations with Dujardin-Beaumetz. A bacillus similar to that discovered by Yersin, isolated from bubonic plague, was also observed in the same year by Kitasato (3).

Another interesting story. Dujardin-Beaumetz showed me a tube filled with live bacilli of plague. He told me that not only humans and rats but also monkeys, guinea pigs, mice, and many other species are susceptible to the plague bacillus. But not the chicken. Among the species resistant to plague, is the chicken. "Look at this tube full of live bacilli of plague" said Dujardin-Beaumetz to me, taking out of a cabinet a small tube marked with a red pencil B.P. "This small tube contains sufficient quantity of live plague bacilli to infect and kill the population of an entire district of Paris," he continued. "We injected a similar quantity of live plague bacilli into the peritoneal cavity of a young chicken in our laboratory," Dujardin-Beaumetz told me, "and the chicken remained in good health; in fact, the next day she laid an egg. Surprisingly, the chicken got lost, presumably flew out of a small open window in the adjoining laboratory. We were frantic and looked for this animal all over, afraid that it may spread the deadly disease but we could not find the chicken. Only several days later did we learn that the chicken was caught by a house superintendent, residing on a street adjoining the Institute, on rue Falguiere; not realizing the chicken came from our laboratory, he roasted the chicken and consumed it, sharing the unexpected meal with his family. The plague bacilli were presumably destroyed by roasting the chicken. Nothing happened to them. They all remained alive and well."

At present, plague is well controlled. The transmitting rat, predominantly the black rat (*Rattus rattus*), is very seldom around in our climate. The transmitting insect, *Xenopsylla cheopis*, is limited to rats. Other fleas seldom transmit the disease. We have antibiotics to treat plague, if needed; in fact, this is the routine treatment method of unexpected cases of plague recognized in animals and in man. Other antimicrobial agents, such as those of the sulfa group, are efficient, and today we also have preventive vaccines against plague.

1. Yersin, A. (1894) *Ann. Inst. Pasteur* 8, 662-667.
2. Simond, P.-L. (1898) *Ann. Inst. Pasteur* 12, 625-687.
3. Kitasato, S. (1894) *Lancet* ii, 428-430.