

The strange case of a microbiologist who died of a lab acquired infection of Yersinia pestis

Black Plague Claims Researcher

How could this happen with an attenuated strain?



By Christopher Massey

Christopher Massey is the Research and Development Manager at Hardy Diagnostics.

He earned his degree in microbiology at Cal Poly in San Luis Obispo, California.

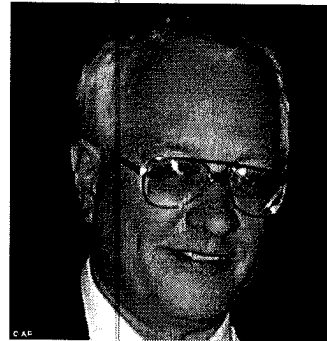
HardyDiagnostics.com



On September 13, 2009 a University of Chicago researcher, Malcom Casadaban, was admitted to a local hospital due to fever, aches, cough and shortness of breath. Blood tests indicated renal failure, severely elevated white blood cell count, and a left shift in the ratio of immature to mature neutrophils. Blood smears indicated bacteremia. The patient was treated with vancomycin and piperacillin/tazobactam, but died of cardiac arrest within 12 hours. Blood cultures later revealed a frightening diagnosis: plague.

PCR analysis of the *Y. pestis* strain concluded that it was similar to the attenuated strain used in Dr. Casadaban's laboratory. Investigation showed the laboratory's safety protocols to be adequate, and the infection of a seemingly healthy person with an

attenuated plague strain was highly unexpected. The *Y. pestis* strain in question had been attenuated through the inactivation of the *pgm* (pigmentation) locus.



Dr. Malcom Casadaban, age 60, died from a laboratory acquired infection of an attenuated strain of Yersinia pestis.

The *pgm* locus is a large and unstable region of the *Y. pestis* genome linked to a siderophore-independent iron uptake gene. *pgm+* strains of *Y. pestis* are able to grow on iron-deficient media and uptake Congo Red, causing a pigmentation of colonies grown in its presence. The attenuated *pgm-* strain is

restricted in its ability to sequester iron from its environment, a necessary survival factor when causing disease, and is, therefore, restricted in its ability to cause disease.

Live vaccinations have been used for years with *pgm*-strains without any reported incidence of death.

So how did Malcolm Casadaban die?

The CDC performed studies to compare the isolated *Y. pestis* with a pathogenic reference strain to see if the bacteria had re-acquired its virulence mechanisms through natural means or genetic manipulation.

Mice were infected with increasing doses of each strain and researchers determined what is known as the strain's LD₅₀, which is the dose at which half the infected mice are expected to die. The pathogenic strain had an LD₅₀ of <100CFUs and the isolated strain had an LD₅₀ of >10⁸CFU.

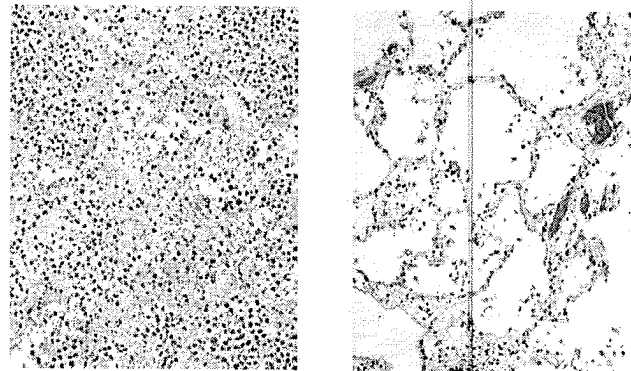
This study indicated that the strain Dr. Casadaban had been infected with was the attenuated *Y. pestis* strain, and it had not acquired (or re-acquired) any virulence mechanisms.



Yersinia pestis, stained red in the gut of a flea and usually associated with flea-borne transmission from rodents, is responsible for three types of plague disease – Bubonic, Pneumonic and Septicemic.

At this point, the investigation began to focus on whether or not Dr. Casadaban had any physiological conditions that may have left him vulnerable to the attenuated plague strain. A pathological investigation found that his alveolar structure was intact without any inflammatory infiltrates which would have been indicative of pneumonic plague. Instead, an immunohistochemical stain using monoclonal antibodies confirmed earlier blood culture tests suspecting septicemic plague.

FIGURE. Pathologic findings indicating that a deceased researcher had septicemic plague, not pneumonic plague* — Chicago, Illinois, 2009



Photos/CDC



* Investigators compared lung pathology images of a patient with primary pneumonic plague (top, left), with that of the deceased researcher (top, right). Lung tissue of the patient with primary pneumonic plague shows extensive inflammation and no open air space. Postmortem lung tissue from the deceased researcher shows open air spaces and preserved alveolar structure, indicating the deceased did not have pneumonic processes consistent with plague. Magnification of postmortem lung tissue (bottom) revealed gram-negative bacteria (red, rod-shaped) in the lumen of a pulmonary blood vessel. Subsequent immunostaining with a *Yersinia pestis* antibody confirmed *Y. pestis* only in blood vessels of numerous tissues, consistent with septicemic plague.

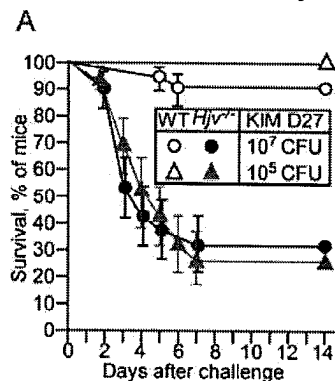
Image sourced from the CDC's Morbidity and Mortality Weekly Report "Fatal Laboratory-Acquired Infection with an Attenuated *Yersinia pestis* Strain — Chicago, Illinois, 2009" February 25, 2011

Additional post-mortem analysis indicated elevated blood serum levels of iron (541 mcg/dL, normal range 40-160 mcg/dL) and blood saturation of iron (83%, normal range 14-50%). A genetic test later confirmed a condition known as hereditary hemochromatosis (HHC). HHC is a rare disorder where the body absorbs more iron than it needs. The prevalence of this disease is one in every 400 people. Since the body lacks a mechanism to deal with this excess iron, it builds up in deposits. If left untreated, this condition can shorten the lifespan of a normal person, but it also goes unnoticed in about half of all who are affected.

It is hypothesized that the buildup of excess iron in Dr. Casadaban's body allowed this attenuated strain of *Y. pestis* to grow and cause disease, even with its deficient ability to acquire iron.

Indeed, research by Jackson and Burrows as early as 1956 showed mouse models with excessive iron supplementation could enhance the virulence of *pgm*-strains of *Y. pestis*. More recently, a study by Quencee et al. researched the virulence of *pgm*-*Y. pestis* on mice genetically modified to display symptoms of HHC. The mice altered to have

hemochromatosis were significantly more likely to

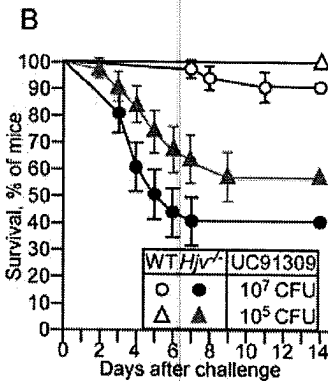


Studies show the increased virulence of two *pgm*- strains (Graphs A and B) of *Y. pestis* in mice altered to have hemochromatosis (shaded circles and triangles) compared to wild type mice (white circles and triangles) having normal iron metabolism. *J Infect Dis.* (2012) 206 (7): 1050-1058.

die from the nonpigmented strains than the unaltered mice.

An unfortunate irony of this case is that hereditary hemochromatosis was heavily selected for in the Dark Ages in response to bubonic plague epidemics. Logic would suggest that with a buildup of iron, victims of hemochromatosis would be more susceptible to the plague. Instead, somehow due to the inability of the host to regulate iron concentrations, the host's macrophages actually have a deficiency in iron compared to a healthy person. In normal cases of bubonic plague, *Y. pestis* can

survive inside a host's lymphatic tissue and is very



good at evading the host immune response. This results in swollen lymph nodes called "buboes," where *Y. pestis* is engulfed by macrophages and hitches a ride to other sites within the body to cause infection. In a subject with HHC, enveloped *Y. pestis* dies within the macrophage due to a paradoxical deficiency of iron, resulting in a reduced ability to cause disease.

Populations of Northern European decent have a disproportionately high incidence of hemochromatosis due to its protective properties during the years of the Black Death.



While it is unclear how Dr. Casadaban was infected with the bacteria and developed septicemic plague, this case underlines the importance of exercising a strict laboratory safety protocol, even with attenuated strains.

Always follow recommended safety precautions when dealing with known pathogens and assume that any bacterial sample is capable of causing disease.

*Chris Massey
Santa Maria, CA*