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Exhuming Skeletal Remains: How Cholera Deaths of the Past Could Shine a Blue-Light of Hope

Tim Sandle*

School of Pharmacy and Pharmaceutical Sciences, University of Manchester, UK

***Corresponding author:** Sandle Tim, School of Pharmacy and Pharmaceutical Sciences, University of Manchester, UK, Tel: 07808906409; E-mail: timsandle@btinternet.com

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Description

Cholera remains an important cause of morbidity and mortality worldwide. It is currently in its seventh official pandemic, as recognised by the World Health Organization. Whilst the disease remains a scourge of the present, studying its paleopathology could help further with understanding the nature of the disease and its evolution, as well as presenting a potential opportunity for finding a cure or improving current treatment regimes. Cholera is one of a number of diseases that has a presence in the archaeological record [1].

In terms of human osteopathology an important discovery was recently made within the grounds of a cemetery located in an old church in Tuscany, Italy. The exhumation of bodies in this area may provide key information concerning the bacterium responsible for cholera [2]. This article assesses the significance of the on-going excavation.

Cholera is an infection of the small intestine. It is one of the world's oldest diseases and it is caused by the bacterium *Vibrio cholerae*. *V. cholerae* is a Gram-negative, facultative anaerobic, comma-shaped bacterium. The basis of the infection is through *V. cholerae* secreting choleragen (known as "the cholera toxin" [3]). Structurally, the toxin is similar to heat-labile enterotoxins associated with enteric bacteria. Two serogroups of *V. cholerae* termed O1 and O139 cause outbreaks. *V. cholerae* O1 causes the majority of outbreaks, while O139, a more recently discovered serotype identified in Bangladesh in 1992, is, thus far, of less global reach, being confined to South-East Asia [4].

In terms of infectivity it is now thought that the *Vibrio* bacterium senses a shift in temperature as it enters the human body. The bacterium transfers into the host via a mechanism termed the "ribonucleic Acid (RNA) thermometer." An RNA thermometer is a temperature-sensitive non-coding RNA molecule which regulates gene expression [5]. The organism has additionally developed a strategy whereby it is becoming more lethal. Researchers at the École Polytechnique Fédérale de Lausanne (EPFL) that the cholera bacterium can steal other bacteria's DNA. This process will, theoretically, make the bacterium more deadly [6].

Cholera infection caused by drinking contaminated water or eating food that has been in contact with contaminated water. Once a person has become infected, the common symptoms include severe, watery diarrhoea, feeling nauseous and being sick, together with stomach cramps. Without suitable treatment, the combination of diarrhoea and vomiting will cause a person to become dehydrated (and the skin can take on a bluish-grey colour, leading to the colloquialism "blue death" as a substitute term for the disease.) When this occurs, shock invariably follows (accompanied by a large drop in blood pressure). In the most severe cases, the condition can be fatal [7].

One of the reasons cholera has been prevalent for much of human history is because the main reservoirs of *V. cholerae* are people together with aquatic sources such as brackish water and estuaries. The bacterium is invariably found in association with copepods or other zooplankton, shellfish, and aquatic plants, especially in relatively warm water. Given this synergy with people, the relationship between people and the disease is hard to disassociate.

The exact origins of cholera are contestable. One of the earliest recorded cases was in 1563 in an Indian medical report; however, the disease is likely to be considerably older, perhaps by as much a millennia. A key turning point in worldwide infectivity was in 1817 when the disease spread from its probable ancient homeland of the Ganges Delta in India to the rest of the world, propelled by trade and industrial development and abated, in many regions, through poor sanitation [8]. Understanding cholera epidemics and pandemics requires an appreciation of the ecology of the disease; this could be enhanced from fining out more about its origins.

With the current exploratory work, the cemetery of interest is located Badia Pozzeveri, within the grounds of a Pozzeveri church situated near Altopascio, a town located 40 miles from Florence. A millennium ago, San Pietro's church, on the shores of Lake Bientina, was part of a Camaldolese monastery founded in the 11th century. During this period, the monastery flourished; in part this was because its location next to the Via Francigena, a major trade and pilgrimage route, connecting France and northern Europe with Rome. The area continued to be used for burying bodies until the latter part of the twentieth century, thus providing an additional microcosm of for a variety of aetiological historical events.

The excavation site contains the remains of victims of the cholera epidemic that ravaged Europe and much of the rest of the world in the middle of the 19th century. Beginning in 1816, the remainder of the century witnessed continual waves of cholera, claiming the lives of tens of thousands of people across Europe. As an exemplar, during the third cholera pandemic (1852 to 1860), in Spain the disease claimed an estimated 236,000 lives in little over two years (1854 and 1855.) The remains at Badia Pozzeveri are among the best preserved ever to be unearthed. This is a result of the bodies being buried in a manner inconsistent with ritual practices, seemingly in attempt to stop the disease from spreading, and covered with quicklime (CaO) to burn the flesh [9]. The effect of this practice on cadaver decomposition was to harden around the bodies, trapping soil and protecting the bone (skeletonisation.)

The examination of the remains of the cholera victims is being undertaken through Ohio State University, under the watch of anthropologist Clark Spencer Larsen. Around thirty skeletal remains of suspected cholera victims–both male and female, aged between 20 and

60 years-have been identified to date. At a meeting of the American Association for the Advancement of Science, held in 2015 in San Jose, Professor Larsen presented his preliminary findings and research objective. The aim is to find traces of the pathogen that caused cholera among the human remains in the hope that these bacterial remnants could provide clues concerning how people lived and died in this region of northern Italy. The trapped soil around the body may contain trace of the *Vibrio* DNA.

If appropriate bacteria DNA are discovered, then researchers could, using genotypic analysis, possibly determine how cholera has evolved and compare the findings to what the bacteria are like today. This is not only of archaic bio archaeological interest, for the comparator could be the first step towards finding a cure for cholera. Most cholera epidemics have been caused by a strain now described as the classic strain; however, the current seventh pandemic is of a different form: V. cholera biotype eltor [10]. Any DNA from the Badia Pozzeveri site may help explain the emergence of the current genotype.

Infection from cholera remains a matter of global importance, with some 5 million cases of infection per year. The primary at risk areas are sub-Saharan Africa, south and South-East Asia, the Middle East, and Central America and the Caribbean. In these regions, the main reason for transmission is the result of inadequate and inappropriate sanitation and hygiene. Dealing with such outbreaks demonstrates the need for improved understanding of the pathogenesis and epidemiology of cholera [11]. The studies in Italy may one day inform and help shape this mission.

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