Plague and Trace Metals in Natural Systems

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All pathogenic organisms are exposed to abiotic influences such as the microclimates and chemical constituents of their environments. Even those pathogens that exist primarily within their hosts or vectors can be influenced directly or indirectly. *Yersinia pestis*, the flea-borne bacterium causing plague, is influenced by climate and its survival in soil suggests a potentially strong influence of soil chemistry.

Keywords: disease ; epidemic ; epizootic ; mammal ; plague ; rodent ; soil ; trace metals ; Yersinia pestis

1. Introduction

With the appearance and reappearance of outbreaks of various infectious diseases, there are ongoing efforts to identify ecological factors that play a crucial role in their emergence. The majority of emerging human diseases appear to originate from free-ranging animals. Such diseases are often termed zoonotic. A general descriptor is "diseases in nature communicable to man" [1], a phrase that also identifies a respected international conference for the past 75 years. Although most of the diseases that fall under this category are caused by zoonotic pathogens transmitted by animals, the subtle difference in terminology emphasizes the fact that some of these infectious agents may, at least temporarily, live in an abiotic environment such as soil or water. For example, bacteria causing listeria, leptospirosis, tularemia, yersiniosis, and many others can be carried by mammalian hosts and, in some cases, arthropod vectors but can also live and multiply in either soil or water.

The conditions experienced by these bacteria during their life cycles in the non-host environment are substantially different from the conditions experienced within an animal. Nevertheless, this does not suggest that zoonotic bacteria, which do not persist in soil or water environments for a long period of time, are unaffected by abiotic factors. Rodents (order Rodentia) are important vertebrate hosts of pathogenic bacteria, and many of these animals are evolutionarily adapted to a life in burrows that keep them in close contact with the soil environment, suggesting potential influences of soil chemistry.

Investigations of the influences of climate, especially temperature and precipitation, on disease dynamics seem relatively abundant compared to investigations of influences of soil and water chemistry. However, a potential role of the latter has not been completely ignored. Experiments providing evidence for the effects of chemical elements on the functionality of microorganisms and geochemical influences on the immune status of mammals, including humans, are well documented [2].

A weakness of this area of research is the paucity of reports documenting an association between the concentrations of specific chemical elements in soil or water with dynamics of zoonotic diseases in natural conditions. Existing observations, mostly for plague (caused by the bacterium *Yersinia pestis*), have been conducted in Russia over several decades. The results of these investigations have been published almost exclusively in Russian journals and books and remain quite inaccessible to biologists outside of Russia. This treatise reviews some of those investigations and illustrates a need for a new conceptual approach for investigating the role of environmental factors that may lead to the activation of infectious agents in natural ecosystems.

2. Survival of Plague between Epidemics and Epizootics

The terms epidemic and epizootic can have varied definitions and are often used synonymously. However, epidemic is used more often to refer to severe disease outbreaks in humans, with epizootic referring to similar phenomena in non-human animals. For the purposes of this entry, researchers consider the primary outcome of a plague epizootic (or epidemic) to be the widespread mortality of the host species within a relatively short time period. After centuries of speculation about potential sources for epidemics of plague in humans, Alexander Yersin not only identified the pathogen *Y. pestis* (*Pasteurella pestis* at that time), but also provided evidence that this pathogen is likely carried by rats

[3]. Zabolotnyi demonstrated that non-commensal free-ranging rodents, specifically marmots, can host this bacterium and be a source for human plague [4]. Thus began extensive studies of so-called rodent-borne diseases. Rodent fleas, specifically *Xenopsylla cheopis*, were shown to be an effective vector for the transmission of the plague pathogen among rats [5]. Over the following century, no other zoonotic infection in nature received such intensive attention and research. The Soviet anti-plague system alone heavily invested in supporting the research of thousands of scientists in their investigations of plague in the field and in laboratories.

The commonly accepted view is that *Y. pestis* is hosted by rodents, usually for a limited period of time because of high lethality [6]. Though an enormous amount of information has accumulated as a result of plague investigations, the solutions to many important questions remained obscure. The most intriguing puzzle in the natural history of plague was the question of how *Y. pestis* could be maintained over long periods of only sporadic observable manifestations of this disease in nature. The lack of detection of any visible manifestation of plague can last for many years (even decades) despite the intensive search for *Y. pestis* or its products in animals and arthropods. As an example, plague has reappeared in the northwestern region of Algeria after >50 years without detection [7].

3. Plague Pathogens in Soil and Sapronoses

Although live rodents may host *Y. pestis* for a limited period of time before death, their carcasses (and those of fleas) may provide a reservoir for bacterial maintenance in soils. For instance, septic moribund hosts might harbor $\geq 10^8$ colony-forming units (CFUs) of *Y. pestis* per ml of host blood [8] and total carcasses (with organs) contain additional bacteria. Plague epizootics may kill thousands of rodents (or more) over days to weeks or months, and rodent carcasses accumulate in burrows and runways during outbreaks, thereby depositing huge numbers of *Y. pestis* into the rodent nest soil environment [9], where trace elements may influence *Y. pestis* persistence and dynamics, perhaps even in the absence of continued "rodent-flea" transmission.

An analysis of Soldatkin and his colleagues [10] was especially influential in challenging the paradigm that *Y. pestis* can exist in nature strictly by a series of continuous "rodent-flea-rodent" passages. These investigators analyzed data collected from numerous field investigations over many years and the results of their analysis raised doubts that plague can interminably persist via the "rodent-flea cycle". Many other hypotheses have been proposed to determine how the plague pathogen can survive in nature without a continuous transfer of pathogens from one animal host to another.

One reasonable argument is the hypothesis that *Y. pestis* can persist in soil for an undefinable period without an evident pathogenic manifestation and becomes pathogenic under a specific, though unknown, set of ecological triggers. The argument that *Y. pestis* can not only survive but also multiply in soil has a long history. Alexandre Yersin himself claimed that he was able to isolate this organism from soil [3], cited from [11]. Since that time, this concept has remained controversial. In 1960, Mollaret, while investigating the epidemiology of plague in Iranian Kurdistan, provided experimental evidence of the prolonged survival of the bacilli in soil (17 months in sterile soil and seven months in non-sterile soil) while maintaining its virulence [9]. A similar conclusion, along with coining the term "telluric" plague, was drawn by Karimi in 1962 after detecting virulent strains in soil samples collected from burrows of jirds long after any plague epizootic [9].

Studies have demonstrated that *Y. pestis* not only survived in the nest substrate of infected rodents, but also remained able to infect naïve rodents and their fleas [12]. Soil contaminated with *Y. pestis* for 10, 60, 165, 210, and 280 days has been shown to preserve its infectious properties to kill laboratory mice [13]. The Indian Plague Commission also reported the survival of *Y. pestis* in soils [14]. The survival of *Y. pestis* in soil under natural conditions in southwestern regions of the United States for at least 24 days was reported by Eisen et al. [15] in a follow-up study that demonstrated its rare transmission from soil back into mice [16]. These authors emphasized the uncommonness of the phenomenon, but such rare events could be critically important to re-establish cycles with more common modes of transmission.

Another group of related hypotheses concentrates on the supposition that *Y. pestis* can maintain vitality and virulence not in soil as such, but in a close association with soil protozoan organisms [17]. The association of *Y. pestis* with the soil amoeba *Hartmannella rhysodes* was experimentally demonstrated by Nikul'shin et al. [18]. Electron microscopy revealed that, in another free-living protozoan, *Acanthamoeba castellanii*, *Y. pestis* resided within spacious vacuoles intact, which were characterized as being separated from the lysosomal compartment by using lysosomal trackers [19]. Markman et al. [20] conducted environmental genetic surveys and laboratory co-culture infection experiments to assess whether plague bacteria were resistant to digestion by five environmental soil amoeba species. They demonstrated that *Y. pestis* is resistant or transiently resistant to various amoeba species. Additionally, these authors found that the plague bacterium can reside within amoeba structures similar to those found in infected human macrophages, for which *Y. pestis* is a competent pathogen.

The plague bacterium is not the only pathogen that can presumably live and multiply outside animal hosts, specifically in soil. This phenomenon was recognized by the Russian epidemiologist Terskish [21]. Such infections were termed “sapronoses” to distinguish them from zoonoses, diseases which require animal hosts for their circulation [22]. Multiple sources of evidence suggest that most agents causing such diseases are free-living saprophytic microorganisms that absorb and metabolize decomposed organic matter [23]. Many pathogenic bacteria (*Vibrio*, *Yersinia*, *Salmonella*, *Listeria*, *Escherichia*, etc.) exist autonomously in the external environment and infect humans and animals under particular circumstances [24]. Evidence for soil as a source of human pathogens was rigorously reviewed by [25].

4. Host–Microbe Interactions and Chemical Elements

In an early study [26], Weinberg demonstrated that soil composition can modulate the incidence and severity of infectious diseases. As possible mechanisms for such an influence, he indicated a suppression or strengthening of host defense by minerals contained in soil, the selective inhibition of pathogens by soil minerals, the availability of minerals to selectively suppress saprophytes to permit the growth of pathogens, and the effect of soil minerals on the survival and growth of intermediate hosts and vectors. In another study [27], Rail suggests that the synthesis of specific factors of virulence in *Y. pestis* depends on the level of specific metallic ions. The varied roles of metallic ions in host–plague interactions are quite similar to the structural and catalytic roles of such ions in free-living macro- and microorganisms. The antimicrobial power of mammalian fluids is depressed by low levels of iron and enhanced by an increase in the iron-binding capacity of a system. The bactericidal power of serum and endotoxin components are affected by the levels of calcium and magnesium. Selenium was shown to play a role in the defense mechanism of animals to certain diseases. Yet, there is sufficient uniqueness of the roles of key metallic ions in many specific host systems for the balance to be tipped in favor of either the host or the plague bacillus through the subtle alteration of the metallic ion environment, specifically within an area endemic for the disease.

This entry is not a comprehensive review of investigations of relations between metallic elements and agents of infectious diseases. Researchers leave that task to others, such as Jerome Nriagu and Eric Skaar [28], who edited “Trace Metals and Infectious Diseases”. The main themes represented in this volume are host–microbe interactions from the perspective of the microbe instead of considering these interactions from the host’s perspective (i.e., metals in environments as risk factors for infectious diseases). Stressing gaps in the latter direction, Ackland ([29], p. 300) stated that: “despite considerable research that is taking place separately on trace metals and infectious pathogens, little is currently known about the interactions between these two key determinants of health…”. Direct observations of the dynamics of trace metals in natural populations of animals in connection with the distribution of infectious agents, either manifested in die-offs or in asymptomatic infections, are few. The simple explanation for this is that it is very difficult to make such observations. Here, researchers attempt to highlight the relevant research of a team of Russian investigators whose work has not been widely distributed.

5. Investigation of Association between Microelements in Natural Environment and Plague Activity in Rodent Reservoirs

5.1. Research Methodology Applied by Evgeny Rotshild and Colleagues

Working at the Research Anti-Plague Institute “Microbe” in Saratov, Evgeny Rotshild conducted extensive spatial analyses of the distribution of plague epizootics across main natural foci of this disease in Russia [30]. After continuing his research at the Moscow University Geography Department and later at the Severtsov Institute of Ecology and Evolution, Rotshild was able to access plague research because of his scientific stature and continuous collaboration with scientists of the Russian Anti-plague System. Working closely with scientists investigating plague in the field, Rotshild and his crew identified two kinds of plots: (1) those where plague had been detected either through evidence of rodents dying from the infection or the isolation of *Y. pestis* from animal tissues or their fleas and (2) nearby plots where plague had not been detected over an extended period. Plots were selected on the basis of a shared landscape and ecological characteristics but with varying histories of plague detection. Within each plot, the investigators collected samples of plants commonly consumed by rodents. The main targets for the analysis of environmental sampling were copper (Cu), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), vanadium (V), and zinc (Zn).

To compare the concentrations of chemical elements between plague and control plots, Rotshild and his colleagues [31] operated with a metric that they termed “norm”. Basically, this term corresponds to the term “geochemical background”, which is used to distinguish between a “natural background” and “ambient background”. According to the Dictionary of Geological Terms [32]: “In geochemical prospecting, the range in values representing the normal concentration of a given element in a material under investigation such as rock, soil, plants, and water". Another similar term, "Clarke concentration", representing the relative abundance of a chemical element, was introduced in the 1930s by the Soviet geochemist Alexander Fersman in honor to the American geochemist Frank Wigglesworth Clarke [33]. Measuring the concentration of particular trace elements in plant or insect samples in "norms" (or Clarkes) instead of using weight units, such as grams, the investigators estimated how much an observed concentration differs from the average concentration of this element in plants of this or related species in a specific study area during the period of investigation. The "norm" for each metal was defined as the mean value for the non-plague plots in each study area. The purpose of using this approach was the attempt to avoid absolute concentrations of elements that varied greatly between study areas and were influenced by different techniques used for measuring concentrations of elements during their investigations in each area. Researchers' assessments used these summary data; the original data were no longer available.

5.2. Plague in Ground Squirrels and Trace Metals in Their Putative Plant Diets

The first experience that led Evgeny Rotshild and his colleagues to explore the role of trace metals in the manifestation of plague activity occurred while investigating a plague epizootic among little sousliks (*Spermophilus pygmaeus*). This small ground squirrel belongs to the family Sciuridae and is found from Eastern Europe to Central Asia. The investigation was conducted in the Caspian Depression, which is a flatland region lying much below sea level and encompassing the northern part of the Caspian Sea. The vegetation is relatively sparse and notable for halophytic plants such as sagebrush (genus Artemisia) and some bluegrasses. Another noted geographic pattern specific for this region is a presence of "salt domes" formed by evaporite minerals, mainly sodium chloride. Plague in little susliks within this region has been known since the 1940s, but no plague activity was reported during the following three decades until it was detected again in 1978. Infected little susliks were reported near the middle and lower parts of the Ural River. Sick rodents were found in several clearly separated small areas. The investigators could not find any clear association between the density of rodents and occurrence of plague-infected susliks but assumed a possible connection between the spatial distribution of plague in susliks and presence of the salt domes because such an association had been reported in regard to plague in gerbils in the different parts of the Caspian Depression [34].

The first investigation within this endemic territory was conducted in May 1979. For this investigation, five plots (200–300 m long) were selected where plague was detected during the previous year. Six additional control plots were selected 5–10 km from "plague" plots. As noted above, these control plots were selected based on their landscape and ecological similarity with plots where plague-infected animals were found. Seven species of plants were collected from plague and control plots (*Poa bulbosa*, *Agropyrum desertorum*, *Artemisia lercheana*, *A. pauciflora*, *Ceratocephallus orthoceras*, *Alyssum desertorum*, and *Ceratocarpus turkestanicus*), representing four families (Poaceae, Asteraceae, Ranunculaceae, and Amaranthaceae). The collected plant species are common in this area and frequently used by rodents for food. In total, 28 plant samples from "plague" plots and 43 plant samples from control plots were collected and analyzed to determine the concentrations of five microelements (Mn, Cu, Zn, Mo, and Co) (**Table 1** and **Table 2**). The concentrations of two chemical elements in plants collected from "plague" plots exceeded the "norm" for this area, 2–3 times for manganese and 4–18 times for cobalt [35]. An especially high concentration of cobalt was found in samples of *P. bulbosa* (family Poaceae), a very common food for susliks. In contrast, the concentrations of the other three chemical elements (copper, zinc, and molybdenum) were from 4 to 18 times lower in plants collected from "plague" plots compared to the norm [36]. An especially low concentration of copper was found in plant samples of *P. bulbosa* (**Table 1**).

Table 1. Concentration of trace metals (mg/g dry sample weight; range and mean) in plants col-lected within plots with plague epidemics in little susliks (*Spermophilus pygmaeus*) and in neigh-boring control plots in the Caspian Depression, Kazakhstan, in 1979 (modified from Zhulidov et al., 1981 [35]). *p*-values were derived from repeated-measures comparisons of plague and control mean concentrations with plant families as replicates.

Plant Family		Samples	Mn	Cu	Zn	Mo	Co
Poaceae	**plague**	**12**	**50.8–115.4 (85.5)**	**0.29–0.54 (0.42)**	**2.38–3.89 (3.07)**	**0.48–0.82 (0.63)**	**2.01–4.12 (3.32)**
Poaceae	**control**	**24**	**30.9–45.8 (38.1)**	**3.45–7.85 (5.45)**	**15.34–24.73 (18.9)**	**2.13–3.72 (3.14)**	**0.12–0.34 (0.21)**
Asteraceae	**plague**	**28**	**60.4–108.7 (84.0)**	**0.84–3.05 (1.84)**	**3.71–6.85 (4.68)**	**0.12–0.52 (0.33)**	**1.74–3.21 (2.40)**
Asteraceae	**control**	**43**	**35.1–59.8 (46.6)**	**4.85–22.12 (14.91)**	**20.13–39.75 (28.1)**	**1.75–2.95 (2.17)**	**0.12–0.71 (0.45)**

Plant Family		Samples	Mn	Cu	Zn	Mo	Co
Ranunculaceae	plague	11	77.4–80.9 (79.5)	1.02–1.28 (1.13)	3.21–3.72 (3.39)	0.12–0.24 (0.16)	0.9–1.95 (1.28)
Ranunculaceae	control	10	35.4–37.6 (36.7)	6.21–15.31 (11.56)	17.12–26.84 (22.85)	1.34–1.95 (1.6)	0.1–0.51 (0.24)
Amaranthaceae	plague	8	170.8–197.3 (183.5)	0.84–0.89 (0.87)	2.71–3.1 (2.91)	0.32–0.39 (0.36)	0.88–0.95 (0.92)
Amaranthaceae	control	11	70.2–79.9 (76.3)	3.71–4.32 (3.96)	19.0 0–22.34 (20.40)	1.33–1.54 (1.45)	0.1–0.15 (0.13)
	plague vs. control	*p*-values	0.037	0.042	0.001	0.011	0.046

Table 2. Summary of studies of trace metals in plants associated with plague in animals. Values show relative levels of metals in plots where plague was detected compared to paired areas where plague was not detected (controls). *p*-values reflect exact binomial probability tests to evaluate the frequencies of plague-control plot differences, with the null assumption being that differences in concentrations of the elements within plague-control pairs of sites would be equally positive and negative and excluding pairs that had no data or showed no substantial differences (0).

Main Rodent Host/Region	Year	Plots (*n*)	Plants (*n*)	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Mo
Spermophilus pygmaeus/Caspian Depression, Kazakhstan	1979	11	71	nd	nd	2+	nd	2+	nd	2−	2−	2−
Spermophilus pygmaeus/Caspian Depression, Kazakhstan	1980	7	13	nd	nd	2+	nd	2+	nd	2−	nd	nd
Urocitellus undulatus/Tannu-Ola Mtns., Tuva, Siberia	1988 1989	15	70	1−	1+	0	1+	nd	2+/2−	2−	0	nd
Meriones tamariscinus and *M. meridianus*/Caspian Depression, Kazakhstan	1981	61	61			+		1+		2−	2-	1−
Rhombomys opimus/western Kazakhstan	1980	6	11			+		2+		2−	2-	1−
Rhombomys opimus/Kyzyl-Kum, Uzbekistan	1980	3	6			+		2+		2−	2-	1−
Ochotona pallasi/Altay Mtns., southern Siberia	1981 1982 1985	13	79	1−	0	1+	1+	1+	2−	2−	0	nd
Marmota sibirica/Khangai Mtns., Mongolia	1987	5	14	1−	1−	0	0	nd	2−	2−	0	nd
			p-value	0.13	0.50	0.02	0.25	0.02		0.02	0.06	0.06

0: Concentration of a particular element was not substantially different between plague and control plots (<20%). +: Concentration of a particular element was higher in plague plot than in control plot, but a relative measure was not provided. 1+: Concentration of a particular element was higher in plague plots (20–100%). 2+: Concentration of a particular element was much higher in plague plots (>200%). 1−: Concentration of a particular element was lower in plague plots (20–100%). 2−: Concentration of a particular element was much lower in plague plots (>200%). nd: No data.

In April 1980, plant samples were collected from three additional plots within the Caspian Depression where new cases of plague were reported in little susliks. These samples were investigated along with plants from four control plots selected 4–12 km from the "plague" plots (**Table 2**). The prevalent plants in all areas were *Anabasis salsa*, *Atriplex cana*, and *E. orientale*. Overall, the analysis of the same chemical elements in plants demonstrated a pattern similar to that of the previous year [35]. Specifically, within the "plague" site, the concentrations of cobalt and manganese were 3 to 10 times higher than those of the control plots depending on the plant species, while the concentration of copper was 2–4 times lower in plague plots than in the control plots [36].

A study of the correlation between plague and metal concentration was conducted on a population of another ground squirrel species, the long-tailed ground squirrel (*Urocitellus undulatus*) [36]. This ground squirrel species is distributed across submontane steppes, plains, meadows, and agricultural land in Southern Siberia and Altai. This investigation of plague was conducted in Tuva Republic, which lies at the geographical center of Asia, in southern Siberia. Plague among the long-tailed ground squirrels was frequently reported within the copper, nickel, and molybdenum ore field on the Tannu-Ola mountains at 1800–2200 m elevation. As a part of plague surveillance, ground squirrels were captured annually from each of the 40 spatially isolated plots. In addition, fleas were collected from the squirrels' burrows, and all this material was bacteriologically tested for plague. Within this site, cultures of *Y. pestis* were isolated from the ground squirrels and their fleas each year between 1985 and 1989.

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