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AN EPIDEMIC OF OROYA FEVER IN THE PERUVIAN ANDES

GREGORY C. GRAY, ALBERTO ANGULO JOHNSON, SCOTT A. THORNTON, WILLIAM ALEXANDER SMITH, JÜRGEN KNOBLOCH, PATRICK W. KELLEY, LUDOVICO OBREGON ESCUDERO, MARIA ARONES HUAYDA, AND F. STEPHEN WIGNALL

Walter Reed Army Institute of Research, Washington, DC; General de Control del Malaria y otras Enfermedades Ministero de Salud, Lima, Peru; Naval Medical Research Institute, Bethesda, MD; Naval Medical Research Institute Detachment, Lima, Peru; Bernhard Nocht-Institut für Tropenmedizen, Hamburg, Federal Republic of Germany; Hospital de Pomabamba, Pomabamba, Peru; and Laboratorio de Bartonella, Instituto National de Salud, Lima, Peru

Abstract. Between February and October 1987, a febrile illness killed 14 persons and seriously affected at least 14 others in Shumpillan, a remote Peruvian mountain village of 353 people. The illness was characterized by fever, headache, chills, and pallor. The fatality rate of untreated cases was 88%. The patients, 71% of whom were male, were 1–75 years of age. Fatal illnesses progressed from lethargy to coma to death in 3–60 days. Patients treated empirically with chloramphenicol survived. *Bartonella bacilliformis* was isolated from the whole blood of 3 patients. A serologic study revealed a high prevalence of antibodies to *B. bacilliformis* in the villagers. It is concluded that the villagers suffered from an epidemic of Oroya fever.

(JES)

Bartonellosis, or Carrion's disease, although described many years ago,[1-3] remains a mysterious disease. It is found in remote mountainous regions with elevations of 600–2,450 m in Columbia, Ecuador, Peru, Chile, Bolivia, and probably Guatemala.[4,5] The severe form of this sand fly-borne disease is called Oroya fever. Symptoms include fever, headache, chills, pallor, and severe hemolysis, which can lead to death in 40–90% of untreated patients.[1,6,7] The incubation period for Oroya fever is about 21 days. A high mortality rate with Oroya fever is most commonly associated with concomitant bacteremic infections, usually with *Salmonella* species.[8] Uncomplicated, less severe *Bartonella* infections have a lower mortality rate and are often manifest by a cutaneous eruption ("verruga peruana," or Peruvian wart). Incubation time for verruga peruana is ≥1 month.

Most bartonellosis studies have dealt with hospitalized patients or hospital records. Endemic areas for the disease are often isolated and difficult to reach. Few field epidemiologic studies have been recorded, and little is known regarding the risk factors for becoming infected with *Bartonella bacilliformis*. We conducted a field investigation in a village where a recent epidemic had occurred.

From February to October 1987, a febrile illness killed 14 persons and seriously affected at least 14 others in a remote Andean village. The village, Shumpillan, is located ~600 km northeast of Lima, Peru near the Marañon River in the Department of Ancash, Pomabamba Province, Parobamba District. The villagers build their homes on the steeply sloping eastern Andean mountainsides at an elevation of ~2,755 m. Village housing is not concentrated, and there is a distance of ~100 m elevation from the home at the highest point to the village square. The villagers live in mud brick houses without plumbing or electricity. Natural springs, the village water supply, originate at the highest elevations of the village and deliver water to the lower elevations via small open canals ~0.5 m in diameter. These canals are not protected from human or animal fecal contamination.

The inhabitants are "mestizos," of Amerindian and Spanish ancestry. They have been living in Shumpillan for >50 years. Several generations of a family live in a typical 3 or 4 room house. Villagers keep numerous domestic animals such as pigs, guinea pigs, chickens, horses, cattle, and goats in connected courtyards and corrals. Guinea pigs regularly inhabit the homes. Often, even large domestic animals are permitted to wander freely in and out of the homes. The villagers have no regular medical care and are largely independent from other villages.

The first recorded serious illness during the outbreak occurred in February 1987. The most frequent clinical signs reported in the outbreak were fever (100%), headache (100%), chills (85%), pallor (78%), icterus (59%), cough (44%), diarrhea (41%), vomiting (40%), nausea (33%), and in some, coma leading to death. Both adults and children were affected. Deaths occurred 3–60 days after the onset of symptoms. According to officials from the Peruvian Ministry of Health (MOH), the area was not known to have been affected by bartonellosis in the past, and the village elders were not familiar with the symptoms of this disease. Coincident with the outbreak, the villagers reported an increase in the rat population of their village, increased flea bites, and increased deaths of pigs and guinea pigs.

In June, the MOH, aware of the rat and flea problem and suspicious of a possible plague outbreak, sent a vector control team to Shumpillan. The team mapped and sprayed the village dwellings with DDT. The epidemic continued. In August, the MOH sent a medical team to the village. The team examined ill villagers, collected sera, trapped rodents, and empirically treated patients with oral chloramphenicol. The physicians confirmed the symptoms of fever, headache, chills, pallor, and mild lymphadenopathy. No dermatologic changes were noted. Sera obtained at that time was evaluated by the U.S. Center for Disease Control Plague Branch and Special Pathogens Branch, which reported the sera negative for antibodies to *Yersinia pestis* and Machupo virus. No other serologic tests were performed.

In October 1987, a team of 4 physicians (2 epidemiologists), 2 microbiologists, an entomologist, a rodent specialist, and various other support staff was assembled to determine the cause of the epidemic. As the team prepared to embark on the 5-day journey to the village, it learned that 2 patients' blood cultures collected in August had grown *B. bacilliformis*.

MATERIALS AND METHODS

Interviews

A case-control house-to-house questionnaire was administered in Spanish to the head of each household. There were 2 interview teams, each with 2 physicians and 1 medical worker who spoke Quechua, the villagers' primary language. Whenever confusion arose, the questionnaire was explained in Quechua. Interviewers were instructed to gather data, especially symptom information, without coaching or suggesting responses. Answers were coded on optical scanner forms. The heads of households were asked about the number of persons living in the dwelling, the ages of the occupants, water sources, signs of illness recently experienced by the occupants, occupations, and recent journeys to the jungle. A case was defined as a patient with fever and headache for > 3 days during February–October 1987.

House selection

The interview teams used information gathered during the preliminary MOH visit in August as a guide to case houses. This was supplemented with disease information obtained from the villagers during the October visit.

The interview teams used a previously existing house numbering system to select control houses. The village was divided into 3 geographic regions (Fig. 1). Region 1 had the highest altitude and contained most of the spring water sources. Region 2 was some 50 m lower in elevation than Region 1. Region 3 was ~100 m lower than Region 1. The open water supply for the village originated in Region 1 and traveled via open canals first to Region 2 and then to Region 3.

Control houses were selected from a list of non-case houses in each region by randomly selecting a sampling interval and then randomly selecting a sampling starting position. The number of control houses in each region was selected in proportion to the number of case houses in that region. Each questionnaire team interviewed 50% of the case households and 50% of the control households in each region. A grid system was designed to aid the investigators in plotting the locations of the houses and water sources. Blood and stool samples were obtained from household members as the questionnaire was administered. If the person identified as a case was not alive or was otherwise unavailable for interview, the head of the household was queried. Demographic and risk factor data from 28 cases (17 households) and 27 control households were collected.

Specimen processing

Blood, tissue, and urine specimens from recently deceased domestic animals and trapped rodents were collected in addition to the human

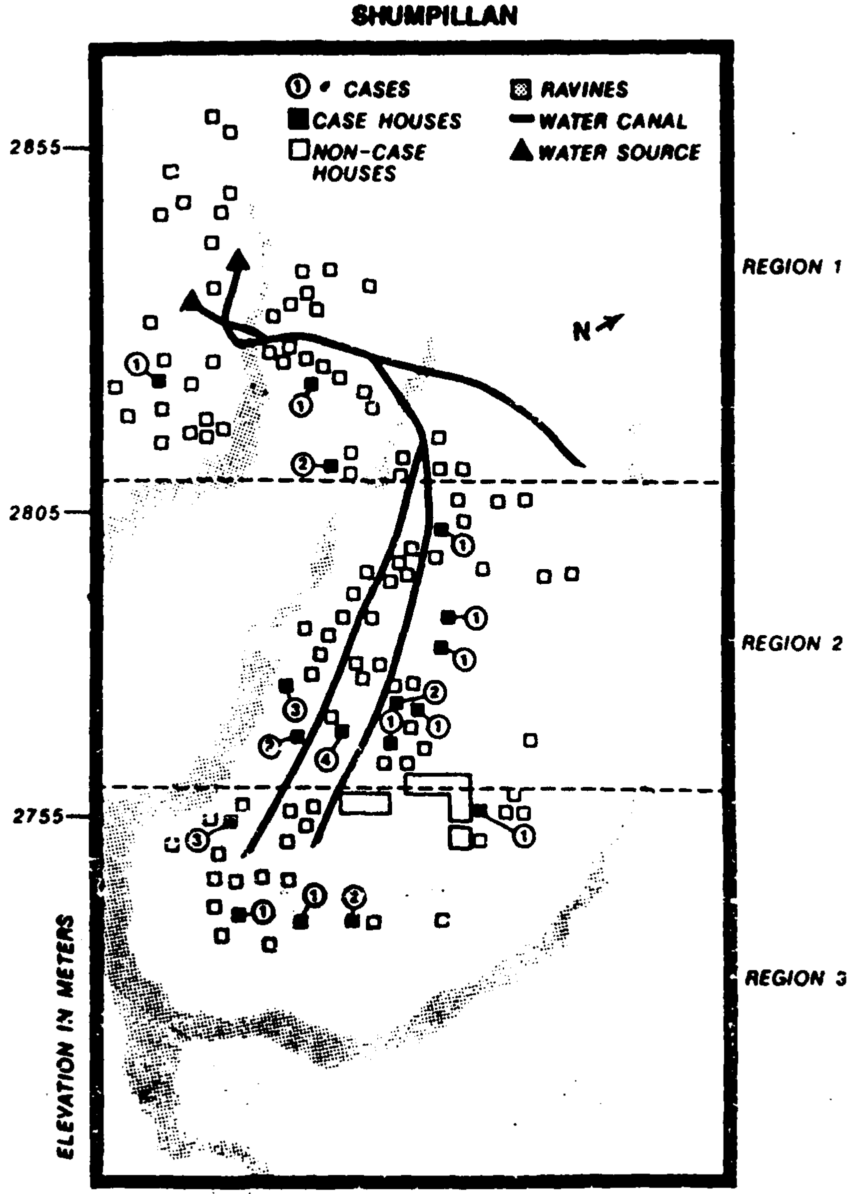

FIGURE 1. Map of Shumpillan, Peru.

blood and stool samples. Thick and thin Giemsa-stained and unstained blood smears were made. Blood was inoculated into paired leptospira media (PLM), Cary-Blair, Fletcher's, phosphate buffered saline, and trypticase soy broth medias. The blood was then allowed to clot. After centrifugation, sera and blood clots were separated under sterile conditions and frozen in liquid nitrogen. The blood clots were cultured for *Bartonella* by MOH microbiologists using the gel-phase agar method in Lima, and were observed for 6 weeks.[9] Stool samples were inoculated into Cary-Blair media and Merthiolate formalin (MF) solution. Urine samples were inoculated into PLM and Cary-Blair medias. Stool and urine specimens in Cary-Blair were later plated on various bacteriologic media. The village water system was traced and 17 water samples were col-

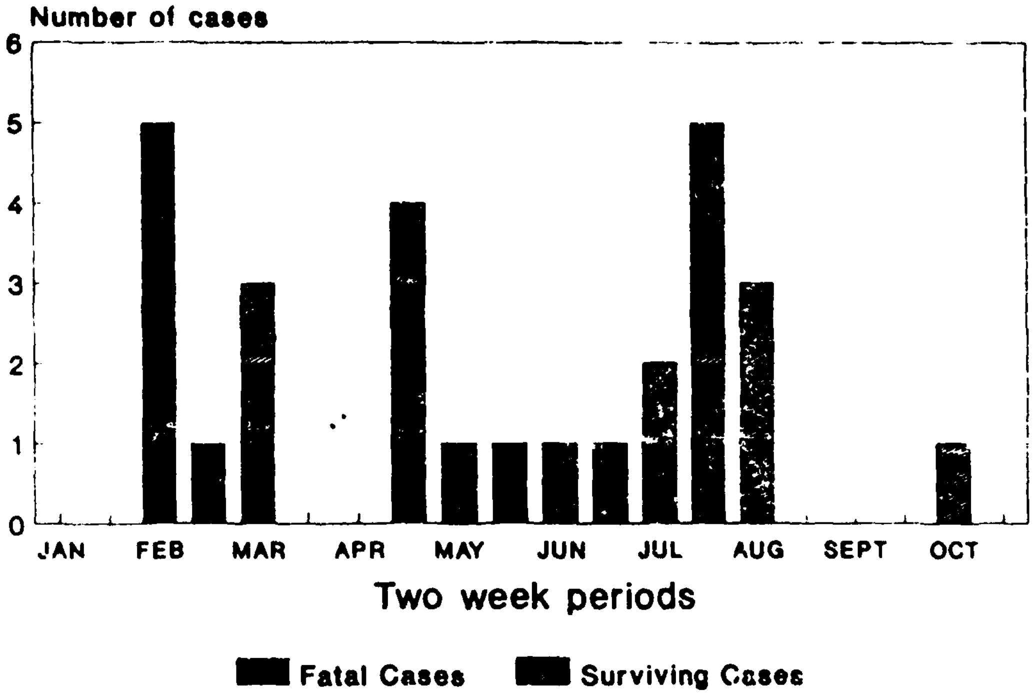

FIGURE 2. Incidence of cases of fever and headache in Shumpillan, Peru, February–October 1987. DDT was sprayed in June. Sick patients were treated with chloramphenicol in August.

lected along the open canals from the uppermost spring to the lowest source immediately below the village. The water was allowed to settle in 15 ml centrifuge tubes for 1 hr. and the sediment was inoculated into a Cary-Blair and MF solution.

IgG antibodies to *Bartonella* antigens were determined by ELISA.[10] The assay antigen consisted of the sonicated Peruvian *B. bacilliformis* strain B13.[10] Sera reactive by ELISA were applied to immunoprecipitation with detergent-soluble extracts of the strain B13 radiolabeled with I^{125}.[11] Only antibodies detected by both ELISA and immunoglobulin were recorded as *Bartonella*-specific.

IgG and IgM antibody levels to *Salmonella typhi* and *S. typhimurium* lipopolysaccharide (Difco laboratories, Detroit, MI) and *Brucella* whole antigen (Fisher Scientific, Springfield, NJ) were determined by indirect ELISA assays.[12,13] Since known positive *Salmonella* specimens from this population were unavailable, positive ELISA values were estimated from previous studies. *Rickettsia prowazekii* serologies for IgG were performed using a Western blot technique and whole cell sonicated antigen.[14] Microscopic agglutination (MA) and indirect hemagglutination tests were used on the sera for the *Leptospira* studies. A Shannon trap with a burro for bait was used to collect sand flies in and around the village.

Statistics

Fisher's exact test and the chi-square test were used for statistical analysis. Cornfield's 95% confidence limits calculations were used for Fisher's exact tests.

RESULTS

The epidemic began during the rainy season, November to May. Many of the first cases resulted in death (Fig. 2).

In addition to fever and headache, patients manifested a broad range of symptoms consistent with Oroya fever. Most cases occurred in the lower regions of the village. The majority of cases were male (71%). There was no fatality rate difference between genders. The disease affected

FIGURE 3. Cases of fever and headache by sex and age in Shumpillan, Peru, February–October 1987.

both the young and the elderly, but was more common in children (Fig. 3), the mean age of patients being 17 years (range 1–75 years). The average duration of illness was 24 days (range 3–65 days).

Chloramphenicol was the only antibiotic used by the villagers and medical teams. The case fatality rate for patients who did not receive antibiotics was 88% (14 of 16). At least 10 acutely ill patients who were treated with chloramphenicol in August survived and were healthy during the survey conducted in October. The overall case fatality rate was 50%.

Data from questionnaires were used to examine risk factors for clinical disease. Individuals were at increased risk of being a case if their households were headed by non-farmers (OR = 3.6, chi-square, 95% CI 1.3, 9.4). About 50% of the villagers interviewed identified their households as non-farming; alternative occupations included teaching, carpentry, and animal-keeping. Housing animals inside the home was also associated with an increased risk of disease as compared to that for villagers who only kept animals outside the home (OR = 3.3, Fisher's exact test, 95% CI 0.9–10.8). There was no increased risk associated with sighting dead rats near the home, visits to the nearby jungle, or changes in the observed insect population.

Over 100 villagers were screened and no eruptions of verruga peruana were observed. Ninety-seven villagers donated sera and 53 villagers donated stool samples (case and control households). The human stool cultures did not yield enteric pathogens. Microscopic stool examination demonstrated various parasitic infections, including ascariasis, giardiasis, trichuriasis, and amebiasis. Numerous stools demonstrated infection with *Endolimax nana*, *Chilomastix mesnili*, *Hymenolepsis* species, *Enterobius vermicularis*, *Balantidium coli*, and hookworm. None of these parasitic infections correlated with being a case.

Human blood and animal urine cultures for *Leptospira* were negative. One culture of human blood collected in October from a clinically asymptomatic 75-year-old man was positive for *B. bacilliformis*. He had recently been hospitalized in Lima with symptoms suggestive of Oroya fever. He had received medications (type unknown) and had fully recovered. He was classified as a case.

Six rats, 5 guinea pigs, and 5 pigs were collected in the village. The guinea pigs and pigs were ill and died. *S. typhimurium* was isolated from the blood of several ill guinea pigs. Guinea pigs were often found living inside family dwellings. Cultures of domestic animal tissue, and blood did not yield pathogens. Human blood smears were negative for pathogens.

Ninety-three of the October sera were available for determination of IgG antibodies to *B. bacilliformis*. The rate of *Bartonella* specific antibodies was higher in cases (50%, 5 of 10) than non-cases (21.7%, 18 of 83) (OR = 3.6, Fisher's exact test, 95% CI 0.8–16.7). One of 2 patients whose blood yielded *B. bacilliformis* at the August visit and who were treated with chloramphenicol was located during the October visit. Her October sera had *Bartonella*-specific antibodies. The October medical team could not locate the other patient nor his family; for the purposes of these analyses, he was not classified as a case. The 75-year-old man whose October blood culture grew *B. bacilliformis* also had *Bartonella*-specific antibodies.

Eighty-four specimens of human sera were available for examination by ELISA for antibodies (IgG and IgM) to *S. typhi* and *S. typhimurium*. Twenty-six of these sera had significant IgG antibodies against *S. typhi* (1 of 5 case sera). Twenty-two (1 of 5 possible cases) of the sera had IgG or IgM to *S. typhimurium*. *Leptospira* serology was positive for 15 of 90 villagers, but such positive serology was not associated with being a case. The assays for brucellosis and epidemic typhus antibodies were negative.

Shannon traps to collect sand fly vectors were set up at dusk for 1 hr periods. Only 1 sand fly was trapped in the village. However, ~200 m below the village, ~50 sand flies were collected. These sand flies were identified as *Lutzomyia verrucarum*.

Fecal coliforms were predominant in 3 of 17 water specimens sampled throughout the village stream water. The remaining water specimens grew predominately *Aeromonas*.

DISCUSSION

The analyses of data collected in this rural village investigation are subject to a number of potential biases. The villagers were questioned retrospectively. The time frame selected for study, February–October, was based on the villagers reports and may have been too narrow. The case definition was broad and may have encompassed other diseases. However, this epidemic caused tremendous stress to Shumpillan, with some families losing as many as 3 children. The villagers were unfamiliar with the illness and had very vivid memories of the outbreak.

There is considerable evidence that Oroya fever is the etiologic diagnosis in this epidemic. *B. bacilliformis* was isolated in 3 patients. None of the non-case participants' whole blood samples grew *Bartonella*. Seventy-eight percent of the cases were described as having pallor, which is uncommon in these dark skinned people and a classic symptom of Oroya fever. The high case fatality rate for untreated patients is also consistent. Additionally, the imputed sand fly vector was found nearby, and the villagers demonstrated a high seroprevalence of IgG to a strain of *Bartonella*.

Other possible etiologies were not consistent with the clinical histories or the laboratory results. All patient sera collected in August were negative when screened for plague, and no buboes were reported previously or found in physical examinations. Untreated leptospirosis case fatality rates rarely reach even 30%, and Leptospira serologic tests could not explain the illnesses. Epidemic typhus is unlikely considering the negative serology, the low frequency of rashes reported in the clinical histories, the long duration of illness in some cases, and the high case fatality rate. Murine and tick typhus are also unlikely considering the high case fatality rate. There are no mosquito vectors for the transmission of malaria at this location. Relapsing fever is not commonly associated with the symptoms of severe hemolysis and pallor. Toxin poisoning and viral hemorrhagic fevers do not resolve with chloramphenicol therapy.

The epidemic may have begun when an infected villager or vectors migrated to Shumpillan. The fluctuations in the epidemic curve amplitude may represent a cofactor which increased the severity of the illness. Contaminated water, associated with early rainy season run-off, might provide such a village-wide risk. The finding of coliforms in the water samples and clusters of cases at the distal ends of the water system suggest that water-borne bacterial infections were a cofactor. A number of authors have observed that Oroya fever associated with salmonellosis causes death.[8,15]

Five of 10 case sera were without *Bartonella*-specific antibodies. All 5 seronegative cases received chloramphenicol, which may have blunted their immune response (3 of these 5 sera had IgG against *Bartonella*; however, their immunoprecipitation verification tests were negative). These epidemiologic and laboratory data suggest that Shumpillan suffered from an outbreak of Oroya fever. The movement of Oroya fever into naive territory, causing many deaths, is not unprecedented.[15]

Spraying residual insecticides inside the village homes is 1 possible preventive public health measure. Maintaining the hygiene of the village water supply is also prudent. The severe financial restrictions and limited resources of the Andean region augur similar epidemics in the future.

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Authors' addresses: Gregory C. Gray and Patrick W. Kelley, Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC. Alberto Angulo Johnson, Direccion General de Control del Malaria y otras Enfermedades Minisiero de Salud, Lima, Peru. Scott A. Thornton, Naval Medical Research Institute, Bethesda, MD. William Alexander Smith and F. Stephen Wignall, Naval Medical Research Institute Detachment, Lima, Peru. Jürgen Knobloch, Bernhard-Nocht-Institut für Tropenmedizen, Hamburg, Federal Republic of Germany. Ludovico Obregon Escudero, Hospital de Pomabamba, Pomabamba, Peru. Maria Arones Huayda, Laboratorio de Bartonella, Instituto National de Salud, Lima, Peru.

Reprint requests: Gregory C. Gray, Navy Environmental Preventive Medicine Unit 5, Naval Station Box 143, San Diego CA 92136-5143.

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