# Nepalese origin of cholera epidemic in Haiti

### R. R. Frerichs<sup>1</sup>, P. S. Keim<sup>2,3</sup>, R. Barrais<sup>4</sup> and R. Piarroux<sup>5,6</sup>

1) Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA, USA, 2) Division of Pathogens Genomics, Translational Genomics Research Institute (TGen), Flagstaff, AZ, USA, 3) Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ, USA, 4) Ministry of Public Health and Population, Port-au-Prince, Haiti, 5) UMR-MD3, Aix-Marseille University, Marseille, France and 6) University Hospital Ia Timone, APHM, Marseille, France

# Abstract

Cholera appeared in Haiti in October 2010 for the first time in recorded history. The causative agent was quickly identified by the Haitian National Public Health Laboratory and the United States Centers for Disease Control and Prevention as *Vibrio cholerae* serogroup O1, serotype Ogawa, biotype El Tor. Since then, >500 000 government-acknowledged cholera cases and >7000 deaths have occurred, the largest cholera epidemic in the world, with the real death toll probably much higher. Questions of origin have been widely debated with some attributing the onset of the epidemic to climatic factors and others to human transmission. None of the evidence on origin supports climatic factors. Instead, recent epidemiological and molecular-genetic evidence point to the United Nations peacekeeping troops from Nepal as the source of cholera to Haiti, following their troop rotation in early October 2010. Such findings have important policy implications for shaping future international relief efforts.

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Corresponding author: R. Piarroux, Laboratoire de parasitologie UMR MD3 Aix-Marseille Université, Assistance Publique Hôpitaux de Marseille, 264 Rue Saint-Pierre 13385 Marseille Cedex 5, France E-mail: renaud.piarroux@ap-hm.fr

### Introduction

The cholera epidemic in Haiti has been the world's largest such epidemic during recent decades, with 526 524 suspected cases and 7025 deaths reported by the Haitian government from mid-October 2010 through to January 2012 (Ministry for Public Health and Population (MSPP), Haiti, 20 January 2012). When the outbreak was first observed, immediate focus was on case management, water chlorination and hygiene awareness. Within 2 months, cholera cases were observed throughout the country [1], with dozens of deaths per day, peaking at more than 100 per day by mid-December, 2010 (MSPP, Haiti, 4 January 2011). Cholera had never been reported in Haiti [2] so many wondered where the pathogenic microbe came from.

Some scientists supported a climatic hypothesis for the origin of cholera in Haiti, arguing that Vibrio cholerae is primarily an aquatic bacterium, typically living dormant in coastal waters until an event disturbs the local environment and leads to a disease outbreak [3]. Noting that early cholera cases were reported in the Artibonite Valley near the Haitian coast, Professor David Sacks mentioned the climatic hypothesis to a reporter in late October 2010 [4]. According to the reporter, Sacks opined that the most likely explanation for the Haiti cholera outbreak was a rise in temperature and salinity in the river estuaries around the Bay of Saint Marc in the Artibonite Department of Haiti [2]. The climatic hypothesis was also supported by cholera expert Professor Rita Colwell [3,4]. Other people suspected a human source, the pathogen being brought from country where it was endemic, with early focus on United Nations (UN) peacekeeping troops who had recently settled in the country [5,6], and on non-governmental organizations [7]. The human hypothesis was first circulated by reporters from two different international news organizations who noticed serious sanitary problems in a military camp of MINUSTAH, the French abbreviation for the Haiti UN peacekeeping mission

[5,6]. Although understanding whether the climatic or human hypotheses were true had no impact on the initial management of cholera in Haiti, the consequences of such understanding are vital for future prevention of similar disasters.

### Methods

References were identified through searches of PubMed for articles, regardless of language, from October 2010 to December 2011, using the terms, 'origin of cholera in Haiti', 'cholera, Haiti' and 'vibrio cholera Haiti'. For the news articles, we employed Google search using the terms, 'cholera, origin, Haiti' and 'cholera Haiti Nepal' with equivalent words in English, French, Haitian Creole and Spanish. All internet items, searched on a weekly basis starting in December 2010, were computer-archived for subsequent retrieval in case the original citation was deleted.

The bibliographic research also involved the collection of available reports in the field, e-mail exchanges with reporters and scientists having potentially interesting information, direct discussions with physicians, microbiologists and epidemiologists involved in the treatment, diagnosis and investigation of the first cholera cases in Haiti, and meetings with sanitarian and political authorities in Haiti (including the President of Haiti, the Minister of Health, the special representative of the UN Secretary-General in Haiti, and the staff of MINUSTAH).

Three field investigations were conducted in Haiti by two of us (RB, RP). The first two were joint French–Haitian investigations, one in November 2010 and the second in April 2011. The third investigation was during the first year of the epidemic by the National Public Health Laboratory and the Department of Epidemic Surveillance of MSPP (i.e. following the epidemic and investigating new cholera foci) assisted by one of us (RB). The laboratory investigations of the Haitian isolates alone were performed by other researchers, and on similarly timed Haitian and Nepalese isolates by one of us (PSK).

Previous publications directly related to the assessment of the origin of cholera in Haiti are summarized in Table I. Primary information refers to sources extensively addressed in the article, whereas the secondary information contains sources mentioned in the article but described more completely in the Supplementary material, Data SI section.

## Results

#### **Background information**

Background information is presented in detail in the Supplementary material (Data SI). Briefly, our findings shows that TABLE I. Previous publications on cholera in Haiti

Authors (reference number)	Type of investigation	Location <sup>a</sup>
Primary information		
Cravioto A, Lanata CF et al., (8, S5)	Epidemiological	Haiti
Piarroux R, Barrais R et al., (9, S7)	Epidemiological	Haiti
Hendriksen RS, Price LB et al., (17)	Laboratory	Haiti, Nepal
Secondary information		
LNSP and CDC, (11, S11)	Laboratory	Haiti
Chin C, Sorenson J et al., (12, S12)	Laboratory	Haiti
Ali A, Chen Y et al., (13, S13)	Laboratory	Haiti
Reimer AR, Van Domselaar G, et al. (14, \$15)	Laboratory	Haiti
Ceccarelli D, Spagnoletti M, et al. (15, S14)	Laboratory	Haiti

<sup>a</sup>Location of field investigation or time-similar specimens.

LNSP, National Public Health Laboratory; CDC, Centers for Disease Control.

in early October 2010 a Nepalese contingent of UN peacekeepers with 1280 personnel was exposed to a cholera epidemic in Nepal either during the 3-month training period or, more probably, during a 10-day home visit before leaving for Haiti. Once they returned to their battalion to embark for Haiti, the soldiers were not subjected to a medical examination or stool testing. In Haiti, most of the soldiers were housed in a camp near Mirebalais and the remainder in two smaller settlements in neighbouring towns (Fig. 1). Sanitation conditions were assessed in the MINUSTAH camp near Mirebalais by a panel of scientists assembled by the UN to investigate the origin of the epidemic [8]. They reported many important hygiene deficiencies and concluded that the sanitation conditions were not sufficient to prevent faecal contamination of the neighbouring river, the water source for local cooking and drinking. Although the panel of scientists in their review of the camp's early medical records reported no cases of severe diarrhoea and dehydration, they made no mention of mild or moderate diarrhoea. In addition, no review was made of medical records of Nepalese troops assigned to the two other neighbouring camps. Septic wastewater from all three camps was trucked to and deposited in an open septic pit by the camp near Mirebalais, where the panel of scientists reported that the local river flowed 'a short walk down the hill from the pit' (see Fig. 1).

#### **Epidemiological information**

Several epidemiological field studies of cholera were conducted in Haiti, notably by investigators at CDC, but they did not address the origin issue, or the cause of the explosive outbreak that followed. There have only been two public documents addressing the origin of cholera in Haiti. One is a report by the panel of scientists appointed by the UN [8], and the other is an article published in a scientific peer-reviewed journal that relates the findings of two field investigations [9]. The first of the two field investigations was made in the Mirebalais region by a team of Haitian epidemiologists during



FIG. I. Location of first cholera cases and Nepalese MINUSTAH camp near Mirebalais with villages, rivers and open septic pit (Google earth; Image © 2011 GeoEye; 8 September 2010).

19–24 October 2010. The second field investigation was conducted from 7 to 27 November 2010 by a joint research team from France and Haiti who travelled to the most affected areas of the country. The second team also performed spatiotemporal analyses that identified five significant cholera clusters. The first cluster was near Mirebalais (occurring 16–19 October). The second was along the lower Artibonite River (occurring 20–28 October). The third, fourth and fifth clusters, which will not be discussed here, were some distance north and south of the Artibonite River, caused by the centrifugal epidemic spread during November 2010 [9].

First cluster. On 18 October Cuban doctors working at the Mirebalais community hospital in central Haiti (see Fig. I) reported an increase of acute watery diarrhoea (61 cases treated in Mirebalais during the preceding week) to MSPP. That same day, the situation worsened, with 28 new admissions and two deaths. MSPP immediately sent a Haitian investigation team [9]. This Haitian Investigation team formally identified the first cases, reporting that the epidemic began on 14 October 2010 (i.e. 5 days after the arrival of the first group of Nepalese troops). The first hospitalized patients were members of a family living in Meille (also spelled Mèyé), a small village 2 km south of Mirebalais (Fig. 1). On 19 October the investigators identified ten other cases in the 16 houses near the index family's house. Five of the six samples collected in Meille from these outpatients, who became sick during 14–19 October yielded *V. cholerae* O1, serotype Ogawa, biotype El Tor [9].

The space-time distribution of the early cholera cases is presented in Fig. 2. The first cluster of cases from 16 to 19 October is shown in the top row of Fig. 2, with all cases remaining in the Mirebalais region. During their field investigation, the Haitian epidemiologists also observed sanitary deficiencies at the MINUSTAH camp, including a pipe discharging sewage from the camp into the river. This finding is similar to what was reported by reporter Katz during his 27 October visit to the same location [6]. Both the Haitian epidemiologists and the reporter Katz noted that the villagers regularly used water from the River Meille/Mèyé for cooking and drinking. The Meille/Mèyé becomes the River La Thème and flows north into the Artibonite River at Mirebalais (see Fig. 1).

Following statistical analyses of the spatiotemporal clusters, the French and Haitian researchers wrote, 'Our epidemiologic study provides several additional arguments confirming an importation of cholera in Haiti. There was an exact correlation in time and places between the arrival of a Nepalese battalion from an area experiencing a cholera outbreak and the



FIG. 2. Evolution of cholera cases during the first week of the Haitian cholera epidemic.

appearance of the first cases in Meille a few days after. The remoteness of Meille in central Haiti and the absence of report of other incomers make it unlikely that a cholera strain might have been brought there another way' [9]. The UN Panel, in their later investigation, agreed with the French and Haitian investigators on the epidemic's starting point when they concluded: 'the evidence overwhelmingly supports the conclusion that the source of the Haiti cholera outbreak was the result of contamination of the Meye Tributary of the Artibonite River with a pathogenic strain of current South Asian type *V. cholerae* as a result of human activity [8].'

Second cluster. Regarding the explosion of cases corresponding to the second significant cluster, the investigators reported that there no cholera cases were reported in the lower Artibonite River communes until after 19 October 2010 [9]. Field investigations showed that contamination of the seven communes of the lower Artibonite River occurred simultaneously (see bottom row of Fig. 2, 20-22 October). Their regression model indicated that the spread of cholera was strongly linked to the Artibonite River and not to the proximity to Mirebalais, as would be expected for roaddependent propagation. They also noted that the numbers of new cases and deaths dropped dramatically after only 2 days, suggesting a rapid decrease of the level of cholera contamination in the river [9]. These findings were also confirmed by members of the UN panel who stated 'This contamination [of the Meille/Mèyé tributary of the Artibonite River] initiated an explosive cholera outbreak downstream in the Artibonite River Delta, and eventually, throughout Haiti' [8].

As to what might have caused such a cholera-contaminated sewage plume in the Artibonite River, Piarroux *et al.* [9] were cautious in their interpretation, but noted that an explosive pattern was compatible with rumours that held the dumping of a septic tank responsible for the epidemic [10]. Yet they pointed out that the exact event that provoked the massive contamination of Lower Artibonite could not be definitively deduced from an epidemiological study.

#### Molecular-genetic evidence

Several tools were used to characterize the isolates collected during the Haitian cholera epidemic and to compare them with isolates of other epidemics. These tools were either pulse-field electrophoresis, or analysis of variable-number tandem-repeats also called multiple-locus variable-number tandem-repeat analysis (MLVA), or partial genome sequencing or whole genome sequence typing (WGST). Most of the published analyses were performed only for isolates from Haiti. A detailed review of these Haiti-only analyses is presented in the Supplementary material (Data SI) but briefly summarized here. Overall, the microbial studies of the Haitian isolates exhibited very few polymorphisms, showing that the cholera was likely to have been brought to Haiti by a single source or event [8], probably from South Asia [11–14]. In addition, one study reported that the Haitian isolates were similar, but not totally identical, to an altered El Tor V. cholerae variant which was first isolated during an epidemic in north India in 2007 [15]. Time-similar isolates from both Haiti and Nepal were included in two studies, one published and the other not, and are presented in the following section.

#### Isolates from Nepal and Haiti

Cravioto A et al., Kim DW 2011. In their unpublished report on the 2010 Haiti cholera epidemic, the panel of scientists appointed by the UN included a section entitled 'recent preliminary data from Nepal strains' [8]. They wrote of their correspondence with Dr Dong Wook Kim of the International Vaccine Institute in Korea on the availability in Korea of Nepal strains from 2007 to 2010 of V. cholerae O1. In this group of specimens they mentioned two Ogawa strains isolated in Nepal in 2010, and stated that they were being compared with seven Ogawa strains isolated in Haiti in 2010, but the more definitive whole genome sequence analysis is still 'in process'. The panel of scientists did state, however, that in the interim a simpler genetic typing method was used, namely MLVA. They wrote, 'A careful analysis of the MLVA results and the ctxB gene indicated that the strains isolated in Haiti and Nepal during 2009 were a perfect match.' This information was widely circulated in the news media, as evidence that the cholera organisms in the two countries were genetically linked. One of us (RRF) wrote to Dr Dong Wook Kim to confirm this statement.

Dr Kim clarified in his email that the 'perfect match' was with strains isolated in Haiti and Nepal in 2010, not 2009 as stated in the UN report (R. Frerichs, personal communication, 19 July 2011). Hence this represents the first acknowledged comparative analysis of a 2010 cholera specimen from Nepal time-linked with a 2010 specimen from Haiti, although not published. He further stated that the MLVA method used in the analysis is described by Choi et al. [16].

Hendriksen RS et al., 2011. This is the only analysis of timerelated specimens from both Nepal and Haiti that has appeared in a peer-reviewed scientific publication [17]. The authors analysed 24 V. cholerae isolates from five different districts in Nepal, provided by officials of the Nepalese National Public Health Laboratory. The cases occurred in Nepal from 30 July to 1 November 2010. The investigators compared the 24 genomes of the Nepal isolates with 10 genomes of previously sequenced V. cholerae isolates, including three from the Haitian outbreak. Using WGST, a powerful tool that provides an almost complete picture of genetic polymorphisms, they showed that all 24 V. cholerae isolates from Nepal belonged to a single monophyletic (i.e. sharing a common ancestor) group that also contained isolates from Bangladesh and Haiti. The 24 Nepalese isolates were then phylogenetically divided into four closely related genotypic clusters. One such cluster contained three Nepalese isolates (collected on I and 30 August 2010 in the Banke and Rupandehi districts of south-central Nepal), and three Haitian isolates collected by CDC and provided by National Center

for Biotechnology Information. All six isolates were almost identical, with only one or two base-pair differences [17]. The investigators emphasized that these findings, the first of Haiti–Nepal isolates using the powerful WGST method, are consistent with Nepal as the origin of the Haitian outbreak.

However, the authors offered some words of caution. They stated, 'Attribution of outbreak sources based upon WGST alone requires comprehensive geographic strain collections. The current conclusion that Nepal is the source of the Haitian cholera outbreak can be reached only if both classical epidemiology and highly suggestive WGST are used together' [17]. As presented here, such 'classical epidemiology' now exists [9], along with background information provided by journalists and others, all pointing to Nepalese UN peacekeepers as the initial source of cholera in Haiti.

#### Discussion

The UN Panel assigned to investigate the origin of the Haiti cholera epidemic wrote in their May 2011 report, 'the evidence overwhelmingly supports the conclusion that the source of the Haiti cholera outbreak was due to contamination of the Meye Tributary of the Artibonite River with a pathogenic strain of current South Asian type *V. cholerae* as a result of human activity' [8]. We agree, but feel our findings support more specific conclusions.

The evidence that the Nepalese UN peacekeeping troops brought cholera to Haiti appears particularly strong, based on background events and published epidemiological and molecular genetic investigations. The soldiers came from Nepal where a cholera outbreak had just occurred. The soldiers were not tested for cholera, either before they left Nepal or when arriving in Haiti. A few days after they arrived, cases of cholera appeared in the village next to the UN camp housing the new Nepalese troops. Local people had complained to journalists that pipes from the camp leaked faecal waste into the river, and the Haitian company responsible for waste disposal at the UN camp was seen dumping waste from its truck outside the usual location (see the Supplementary material, Data SI). Finally, a waste septic pit on a hilltop near the UN camp was found to allow waste fluids to seep down into the nearby river.

The laboratory-based analyses comparing specimens obtained from both Nepal and Haiti gave clear results. After correcting the typographic date error, the report from the UN panel reads, 'the strains isolated in Haiti and Nepal during 2010 were a perfect match'. Nevertheless these preliminary results were further confirmed using the 'most powerful molecular approach imaginable ... setting a new standard for infectious disease epidemiology [17].' As stated by Hendriksen RS *et al.*, 'this molecular phylogeny reinforces the previous epidemiological investigation—of Piarroux *et al.* [9]—that pointed towards UN peacekeepers from Nepal as the source of the Haitian cholera epidemic' [17].

At this juncture, there is no more scientific evidence to be gleaned. The only missing findings would have been cholera tests of the Nepalese UN troops at the onset of the epidemic. An attempt to do such testing was made on 21 October 2010, but was unsuccessful. During their initial investigation of the Mirebalais outbreak, the Haitian public health team met the doctor of the UN camp by Meille. He told them that none of the peacekeepers showed symptoms of cholera, but the Haitian public health team was not allowed to examine the soldiers.

In conclusion, the onset of cholera in Haiti was not the result of climatic factors and was not the direct consequence of the January 2010 earthquake. All of the scientific evidence shows that cholera was brought by a contingent of soldiers travelling from a country experiencing a cholera epidemic. Understanding what triggered the epidemic is important for preventing future occurrences, and acknowledges the right of Haitians to understand the events that led to their cholera devastation [18].

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### **Transparency Declaration**

The authors declare no conflict of interest of any nature.

### **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

Data SI. Context and circumstantial evidences.

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### SUPPORTING INFORMATION

Title: Nepalese origin of cholera epidemic in Haiti

Authors: R. R. Frerichs<sup>1</sup>, P.S. Keim<sup>2,3</sup>, R. Barrais<sup>4</sup>, and R. Piarroux<sup>5</sup>

1) Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA, USA, 2) Division of Pathogens Genomics, Translational Genomics Research Institute (TGen), Flagstaff, AZ, USA, 3) Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ, USA, 4) Ministry of Public Health and Population, Port-au-Prince, Haiti, and 5) Department of Parasitology, Aix Marseille University, Marseille, France.

# Introduction

In July 2010, Nepal experienced a cholera epidemic. By the end of September, cholera was reported as epidemic by both the US Embassy in Kathmandu and by a Nepalese newspaper [S1, S2]. Later, the World Health Organization (WHO) listed Nepal as having the third highest number of cholera cases (i.e., 1,790) during 2010 in South Asia [S3]. While this epidemic was occurring, troops were assembled in Kathmandu for a three-month training program, prior to leaving for Haiti as UN peacekeepers. In 2010, Nepal had 1,280 personnel in Haiti, the largest number of police and troops serving in MINUSTAH, among countries in South Asia [S4]. Information concerning these soldiers was made available in a report written by a panel of outside experts, appointed in December 2010 by the UN to determine the source of the 2010 Haiti cholera outbreak. According to this report, a clinical examination was completed before the troops departed Kathmandu for Haiti [S5]. If the clinical examination was negative, it was local policy to not collect and test stool specimens, a point reiterated by the Nepalese Army chief medical officer to BBC News [S6]. Since none of the troops leaving for Haiti exhibited cholera symptoms, no follow-up stool tests were done. In addition, prior to leaving for Haiti the troops took 10 days of home leave with no further medical examination or stool testing.

The first Nepalese troops arrived at the Meille MINUSTAH camp near Mirebalais in the Centre Department of Haiti on October 9, 2010 [S7]. Another Nepalese group arrived on October 12, and still another on October 16 [S7, S8]. Most of the troops from Nepal were stationed at Meille, a small hamlet located in the Centre Department of Haiti, two kilometers south of Mirebalais.

Other Nepalese soldiers were assigned to MINUSTAH camps at nearby Hinche or Terra Rouge [S5]. The UN Panel wrote that they reviewed dispensary clinic data at the Meille MINUSTAH camp and found "no cases of severe diarrhea and dehydration" during this initial period [S5]. No mention was made, however,

of mild or moderate diarrhea cases, nor did they review the medical records of Nepalese troops assigned to either the Hinche or Terra Rouge MINUSTAH camps.

Other than MINUSTAH, no independent testing was done of the Nepalese peacekeeping troops in Haiti, neither by the Haitian LNSP nor by the United States CDC. Instead MINUSTAH informed a reporter that specimens of personnel and water had been sent to a laboratory in Santo Domingo, Dominican Republic and were reported negative [S8]. No independent investigators were given access to the testing procedures or specimen findings.

Sanitation problems at camp with Nepalese troops. On October 27, 2010, two reporters from two different international news organizations travelled to the Meille MINUSTAH camp housing the Nepalese troops and noticed serious sanitary problems. The first reporter stated, "the liquid [sewage from the toilets] seems to be draining into this river just a few meters away, which flows into the nearby town of Mirebalais [S9]." The second international reporter wrote, "A buried septic tank inside the fence was overflowing and the stench of excrement wafted in the air. Broken pipes jutting out from the back spewed liquid. One, positioned directly behind latrines, poured out a reeking black flow from frayed plastic pipe which dribbled down to the river where people were bathing [S10]." As observed and photographed on October 27th by one of the reporters and a photographer, waste from the MINUSTAH septic tanks was transported in a contracted sanitation truck to an open septic pit [S10]. The company owner told the reporter that the septic tanks had also been emptied on Monday, October 11th, two days after the first shift of Nepalese troops had arrived. Finally according to persons living near the MINUSTAH camp, at some mid-way point between October 11th and 27th, the septic tanks had again been emptied, but by an unrecognized new driver and "dumped outside of the usual pits [S8]."

Sanitation problems at the Mirebalais MINUSTAH camp were confirmed by the UN Panel in mid-February 2011 who observed that, "... construction of the water pipes in the main toilet/showering area is haphazard, with significant potential for cross-contamination through leakage from broken pipes and poor pipe connections, especially from pipes that run over an open drainage ditch that runs throughout the camp and flows directly into the Meye Tributary" (shown as "River" in article Figure 1) [S5]. Their report continued, "The black water tanks in the main area and medical area of the camp are emptied on demand by a contracting company approved by MINUSTAH headquarters in Port-au-Prince. MINUSTAH staff reported that the contractor empties the tanks twice per week when called, although earlier in November, 2010 the UN staff had told a reporter that the septic tanks were emptied once a week [S8]. The contracting company dispatches a truck from Port-au-Prince to collect the waste using a pump. The waste is then transported across the street and up a residential dirt road to a location at the top of the hill, where it is deposited in an open septic pit"(see article Figure 1) [S5].

The UN Panel went on to state, "There is no fence around the [open septic pit] site, and children were observed playing and animals roaming in the area around the pit." Additionally, the UN Panel wrote, "The southeast branch of Meye Tributary System (shown in article Figure 1 as "River") is located a short walk down the hill from the pit [S5]."

Finally, the UN Panel noted, "Black water waste for the two other MINUSTAH facilities with Nepalese troops – Hinche and Terre Rouge – is also trucked to and deposited in this pit [S5]." Thus it appears that the open septic pit received fecal material from the Meille MINUSTAH camp, where the UN Panel wrote they had reviewed past medical records (and found them to be negative), and from Hinche and Terre Rouge MINUSTAH camps where there was no mention of such a medical record review.



Figure S1. Timeline for Nepalese troop replacement, sewage disposal, and cholera cases, Haiti.

Figure S1 shows the time-association between the arrival of replacement troops from Nepal, the truck transport schedule from the septic tanks to the open septic pit, and the onset times of cholera. The troops arrived on October 9, 12, and 16 [S8]; the septic tanks were emptied during their weekly schedule on October 11th [S8], sometime in mid-October [S8], and October 27th [10]; the first laboratory-confirmed cholera case occurred on October 14th [S7] and the first large wave of cholera cases in the lower Artibonite Valley occurred after October 20th (also shown in article Figure 2) [S7]. It is plausible that the weekly-scheduled sanitation truck with an unfamiliar replacement driver arrived in the late afternoon on October 17th, did not know the hilltop location of the open septic pit and dumped the septic tank contents either into the river or close to the river. The contaminated water then flowed during the night to the communities of the lower Artibonite Valley and infected the morning- and daytime-water consumers on October 18th. After a short incubation period, cholera cases developed simultaneously in many exposed communities, apparent on October 20th [S7].

### **Molecular-Genetic Evidence**

### Isolates from Haiti but not Nepal

Laboratory studies of Haitian isolates and comparison specimens collected worldwide, but not timematched in Nepal.

**LNSP and CDC, 2010.** The first report of laboratory analyses came from the Haitian MSPP and the United States CDC. In less than a week after the epidemic started, the investigators used rapid tests on eight stool specimens collected on October 19-20, 2010 to identify the organism as *Vibrio cholerae*. Thereafter on October 22, 2010, Haiti's LNSP reported that the organism was *Vibrio cholerae* serogroup O1, serotype Ogawa [S11]. Eventually, CDC did a detailed analysis of 14 isolates associated with the Haiti outbreak, and reported that they were indistinguishable by all laboratory methods including PFGE [S11].

Following their laboratory work, the CDC investigators concluded that, "...as of November 13, data indicated that a single strain caused illness among the 14 persons from Artibonite Department." They went on to state, "if these isolates are representative of those currently circulating in Haiti, the findings suggest that *V. cholerae* was likely introduced into Haiti in one event [S11]."

**Chin C-S et al, 2011.** The next analysis was done by Chin et al, who used a single-molecule DNA sequencing method to determine the genome sequences of two Haitian *V. cholerae* isolates and three additional *V. cholerae* clinical isolates from a 1991 outbreak in Peru, a 2008 outbreak in Bangladesh, and a 1971 outbreak, also in Bangladesh. Their conclusion was that, "the *V. cholerae* strain responsible for the expanding cholera epidemic in Haiti is nearly identical to so-called variant seventh-pandemic El Tor O1 strains that are predominant in South Asia, including Bangladesh [S12]."

Finally, Chin et al. rejected the "climate causal hypothesis" when they wrote, "our data distinguish the Haitian strains from those circulating in Latin America and the U.S. Gulf Coast and thus do not support the hypothesis that the Haitian strain arose from the local aquatic environment. It is therefore unlikely that climatic events led to the Haitian epidemic [S12]."

Ali A et al, 2011. Ali and colleagues presented a third analysis of *V. cholerae* in Haiti derived from 16 patients with severe diarrhea gathered at St. Nicholas Hospital, St. Marc, Artibonite during the first three weeks of the Haiti cholera epidemic [S13]. They used a hypervariable subtyping technique, "variable-number tandem-repeat typing" or multiple-locus VNTR analysis (MLVA) with 187 isolates and found minimal diversity, consistent with a point source emergence for the epidemic, similar to the report of others. Because MLVA is based upon mutable loci it is particularly applicable to young populations. The lack of diversity argues strongly for a very newly emerged pathogen population.

Ali et al. also offered some support in their conclusions for the human origin hypothesis, but did so without mentioning the UN peacekeepers from Nepal. They wrote, "...our findings are consistent with those of others studies implicating southern Asia as the source for these strains..." They appeared to refute the climatic hypothesis when they wrote that their findings..."would support the hypothesis that the epidemic in Haiti was caused by one clone that had little time to undergo diversification of STs (sequence types) expected of strains persistent in an environmental reservoir for extended periods [S13]."

**Ceccarelli D et al, 2011.** Ceccarelli and fellow authors analyzed three Haitian *V. cholerae* genomes that had recently been sequenced and released by CDC [S14]. They reported that the "Haitian strains contain an integrative conjugative element (ICE) of the SXT/R391 family, a major drug-resistance-spreading vector in bacteria, which is 99% identical to ICEVchInd5. This ICE ... was originally identified in strains of *V. cholerae* isolated in India." They go on to state that the Haitian isolates with the designated ICE, "clonally belong to the most prevalent epidemic clade in the Indian subcontinent, represented by the reference strain CIRS101 ... one of the highly virulent Indian *V. cholerae* O1 that are gradually spreading all over the world." They also stated that the Haitian clone …"carries a genotype 7 ctxB gene coding for the cholera toxin subunit B." They then write that this genotype …"was described only in an altered El Tor *V. cholerae* variant isolated during the harsh cholera epidemic in Orissa, India, in 2007 [S14]." No mention was made of Nepal, or that Indian strains might have migrated to bordering Nepal before travelling to Haiti in 2010.

**Reimer AR et al, 2011.** Reimer and collaborators in the fifth analysis studied nine *V. cholerae* isolates directly associated with the outbreak on Hispaniola, seven of which had indistinguishable SfiI and NotI PFGE patterns [S15]. All were *V. cholera* serogroup O1, serotype Ogawa, biotype El Tor and all were collected in 2010. The origin of infection was listed as "Haiti" for four specimens, "Artibonite, Haiti" for two specimens and "South Department, Haiti" for one specimen.

The authors were able to use the extensive CDC database to identify 12 other strains with matched PFGE subtypes to the Haitian isolates. These were then selected for whole genome analysis along with nine Hispaniola isolates. Their strategy, thus, capitalizes upon a much larger PFGE database to identify possible relatives and then employs the highly precise whole genome analysis to understand these very close relationships. The closest relationships to the Haitian isolates proved to be Cameroon and Indian isolates, with the Nepalese 2008 isolate slight more distant. Even in these cases, the isolates were not perfect matches and differed by five or more single-nucleotide polymorphisms (SNPs). The nine Haitian whole genomes were very closely related with only 0-2 SNP from each from one another. Importantly, these SNPs within the Haitian samples define an evolutionary topology resembling a star, which is consistent with a single infective source followed by a rapid population expansion.

While the authors were able to obtain one *V. cholerae* specimen from a 2008 traveler to Nepal, they acknowledged that the evolutionary relationship of the 2008 strain to ones circulating in Nepal in 2010 may differ substantially. Thus other than demonstrating the similarity of the nine Hispaniola isolates, the article does not provide much additional insight as to the origin of cholera in Haiti with similar relationships among widely geographically disparate isolates. They did conclude, however, that their "core genome phylogeny suggests that the Haiti outbreak strain most likely derived from an ancestor related to isolates from within or near the Indian continent [S15]." They also remarked that their study provides "unequivocal genetic evidence for introduction of the outbreak strain into Haiti from an external source as opposed to local aquatic emergence." That is, the organism was brought to Haiti from an outside source, not from a local environmental reservoir.

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