SUPPORTING INFORMATION

Title: Nepalese origin of cholera epidemic in Haiti

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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.

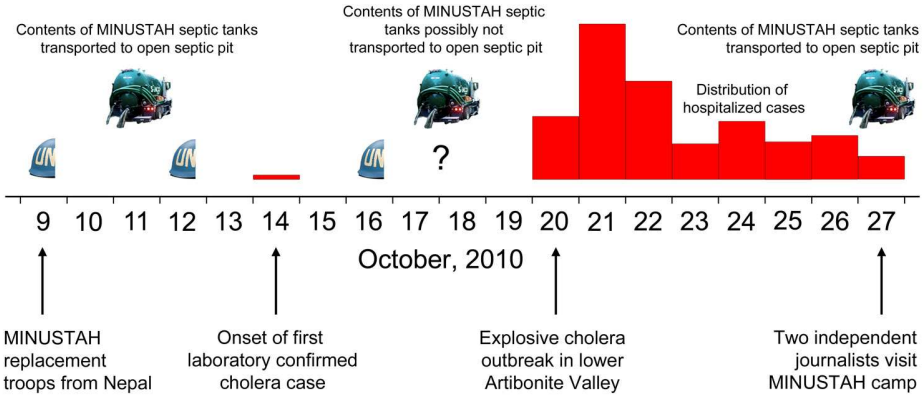

Figure S1. Timeline for Nepalese troop replacement, sewage disposal, and cholera cases, Haiti.

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 time-matched 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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