UNIVERSITÉ D'AIX-MARSEILLE
ÉCOLE DOCTORALE SCIENCE DE LA VIE ET DE LA SANTÉ
UFR DE MÉDECINE
LABORATOIRE : UMR MD3 – Infections parasitaires : transmission et thérapeutique
Laboratoire de Parasitologie-Mycologie – APHM

Thèse présentée pour obtenir le grade universitaire de docteur de l'Université d'Aix-Marseille

Discipline : Pathologie Humaine Spécialité : Maladies Infectieuses

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Né le 29/05/1978 à Ste Foy-les-Lyon

Étude dynamique des épidémies de choléra en Afrique et en Haïti et application à la mise en place de stratégies d'élimination

Soutenue le 08/12/2014 devant le jury :

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RÉSUMÉ

Le choléra est une diarrhée hydrique sévère volontiers épidémique. D'origine bactérienne, elle est causée par des *Vibrio cholerae* O1 fabriquant la toxine cholérique. Ses déterminants environnementaux archétypaux ont donné naissance à un paradigme couramment cité. De nombreuses stratégies récentes de lutte contre cette maladie sont apparues peu efficientes en Afrique comme en Haïti. Elles peuvent cependant être améliorées par une meilleure compréhension de la dynamique des épidémies. Ce travail de thèse comporte donc une synthèse bibliographique des influences de l'environnement sur les épidémies de choléra en Afrique, qui montre les limites du paradigme environnemental du choléra sur ce continent. S'y joint une étude multidisciplinaire suggérant fortement que l'épidémie de choléra en Guinée en 2012 fut importée par voie humaine depuis la Sierra Leone voisine. Une description spatio-temporelle du choléra au Mozambique démontre ensuite l'hétérogénéité de sa transmission et amène à questionner le concept d'endémicité du choléra. Une seconde série de travaux est consacrée à l'épidémie de choléra en Haïti. Celle-ci présente également une répartition spatiale et temporelle très hétérogène. Son importante rétractation en saison sèche et son absence d'enracinement significatif dans l'environnement laissent espérer la possibilité d'une élimination rapide à condition d'apporter une réponse ciblée à tous les foyers épidémiques du pays. En dépit de débats persistants autour de l'existence de réservoirs environnementaux en Haïti, qu'ils aient précédé l'apparition de l'épidémie en octobre 2010 ou se soient constitué depuis, une stratégie d'élimination basée sur nos recommandations est actuellement menée par le Ministère de la Santé d'Haïti, l'UNICEF et leurs partenaires. Après des résultats spectaculaires en 2013 et au premier semestre 2014, les efforts semblent s'essouffler et la situation se détériore à nouveau. L'élimination effective du choléra dans saison sèche à venir demeure cependant réaliste si nous parvenons à convaincre et remobiliser les acteurs de terrain.

Mots clefs : choléra, *Vibrio cholerae*, épidémiologie, environnement, Afrique, Guinée, Mozambique, Haïti, élimination

ABSTRACT

Cholera is an epidemic acute watery diarrhea. It is caused by toxigenic bacteria *Vibrio cholerae* O1, and its environment determinants have been at the source of a popular paradigm. Many recent control strategies have shown little efficiency in Africa or in Haiti. They can however be improved by a better comprehension of the epidemics dynamic. This thesis work includes a bibliographic synthesis about environment influences on cholera in Africa, which highlights the limits of the cholera environmental paradigm on this continent. A multidisciplinary study is presented, which suggests that the 2012 cholera epidemic in Guinea was humanly imported from nearby Sierra Leone. A space-time description of cholera in Mozambique demonstrates heterogeneous transmission patterns and challenges the concept of cholera endemicity. A second part is dedicated to the current cholera epidemic in Haiti. Its transmission also exhibits marked spatio-temporal heterogeneity. Cholera important retraction during the dry season and its absence of significant establishment in the Haitian environment suggest it may be possible to rapidly eliminate cholera in the country, provided that every outbreak focus receives a targeted response. In spite of persisting debates about environmental reservoirs that may have preceded the onset of cholera in October 2010, or may have settled in the meanwhile, an elimination strategy based on our recommendations is currently implemented by Haitian Ministry of Health, UNICEF and their partners. After spectacular results in 2013 and during the first half of 2014, efforts seem to stall and the situation is slowly deteriorating again. Cholera elimination during the coming dry season remains realistic provided that we succeed in persuading and remobilizing the partners present on the field.

Key words: cholera, *Vibrio cholerae*, epidemiology, environment, Africa, Guinea, Mozambique, Haiti, elimination

REMERCIEMENTS

Je tiens en premier lieu à remercier chaleureusement mon directeur de thèse, Renaud Piarroux, pour la confiance qu'il m'a d'emblée témoignée, les conditions de travail humaines et matérielles qu'il a su m'offrir, son honnêteté sans concessions, son engagement sans limites dans ce combat contre le choléra, et les langoustes que nous avons partagées en Haïti pour reprendre cœur à l'ouvrage.

Je tiens aussi à remercier Jean Gaudart, mon co-directeur de thèse, pour sa sympathie et sa patience à m'expliquer et me réexpliquer ses analyses spectrales par ondelettes ou ses modèles additifs généralisés.

Je remercie mes rapporteurs, Éric Caumes et Jean-François Guégan, pour avoir accepté de réviser mon travail. Merci à Christophe Rogier d'avoir accepté de juger une nouvelle fois le fruit de mon labeur. Merci, enfin, à Rony Brauman de me faire l'honneur immense de siéger dans mon jury de thèse.

Je remercie le reste de notre petite équipe choléra pour leur importante participation à ce travail :

Sandy Moore à qui je demande pardon d'avoir gâché les couleurs des couchers de soleil marseillais en lui parlant des usines pétrochimiques de Fos-sur-Mer ;

Martine Piarroux pour son initiation à la cartographie et sa gentillesse ;

Pierre Gazin, Aaruna Abedi, Robert Barrais et Lindsay Osei pour leur compagnonnage sur le terrain en Haïti.

Je remercie toute l'équipe du laboratoire de parasitologie-mycologie pour son accueil, et en particulier :

Stéphane Ranque, pour m'avoir fait une place dans son bureau, offert de belles discussions et fait goûter un magnifique armagnac !

Joëlle Bayle, pour son efficacité à gérer les entorses administratives qu'un tel projet impose à l'APHM ;

Coralie et Fakhri, pour s'être chargés de l'impression de ce manuscrit et des démarches administratives alors que j'étais déjà reparti sur le terrain ;

Carole, Anne-Cécile et les autres pour leurs sourires et leur sympathie.

Je remercie l'ensemble des collaborateurs ayant participé aux différents travaux rassemblés ici, notamment :

Pour les revues : Bertrand Sudre et Benoit Faucher, pour leur connaissance du sujet et leurs apports inestimables ;

Pour la Guinée : Sakoba Keita, Lamine Koivogui, Yacouba Diallo, Seykou Keita, Hafia Diallo ; François Bellet, Alama Keita, Lalit Patra et les autres employés de l'Unicef ; l'équipe du laboratoire de l'HIA Laveran, notamment Éric Garnotel, Hélène Théfenne, Redouane Saidi et Gilles ; Pierre-Edouard Fournier et Adil ; Nick Thomson, Ankur Mutreja et le reste du Sanger Institute ; Billo, Mira et Aliou Diallo qui m'ont offert l'hospitalité ;

Pour le Mozambique : Lorna Gujral, Cynthia Sema, ainsi que Brad Gessner d'Africhol ;

Pour Haïti :

Remerciements

Donald Lafontant, Robert Barrais, Edwige Michel, Katilla Pierre, Roc Magloire, Paul Adrien, Mentor Al-Ber Lucien, Gérard Joseph et les autres membres de la DELR ;

Jacques Boncy, Marie-José Laraque, Emmanuel Rossignol et les autres employés du LNSP ;

La Ministre de la Santé Florence Duperval-Guillaume et ses collaborateurs notamment Donald François et Elie Celestin ;

Edouard Beigbeder, Représentant de l'UNICEF pour sa confiance son audace son activisme son indéfectible enthousiasme et ses « Bonjour bonjour !! », ainsi que ses collaborateurs notamment Stefano Savi, Lieven Desomer, Olivier Thonet, Claudia Evers, Abner Dorvil, Olivier Lamothe, Gregory Bullit, Jérôme Kouachi, Anne-Gaëlle Lebeau et tous les autres ;

Didier Le Bret du Centre de Crise, ainsi que Dominique Delpuech et Jean-François Schemann de l'Ambassade de France pour leur appui ;

Carole Antoine, Paul Jasmin et les autres employés des directions départementales ;

Raoul Toussaint, Paul Christian Namphy et leurs collègues de la DINEPA ;

Samuel Baulieu et tous ses collègues des ONGs s'investissant sur le terrain ;

Sandrine Baron et Jean Lesne de l'ANSES ;

Et bien sûr, tous ceux qui ont contribué à me faire aimer Haïti dont Jean-Hugues Henrys, Marjorie, Franck, Adeline, Erik, Lena, Nadège et les très nombreux autres ;

Je remercie les agents des services administratifs de l'APHM pour la flexibilité dont ils ont su faire preuve dans leur gestion de ces partenariats inhabituels, notamment Bernard Belaigues, Urielle Desalbres, Giovanna Davi et le Directeur Général de l'APHM Jean-Jacques Romatet, dont j'ai eu la honte de faire connaissance vêtu comme un va-nu-pieds !

Je remercie mon père, ma mère et l'un de mes frères, Côme, pour leur relecture attentive de ce manuscrit.

J'en profite pour leur témoigner à nouveau ma plus tendre affection, et les prie de pardonner mes absences et mon parcours d'éternel étudiant.

Je remercie chaleureusement le reste de mes proches, de mes amis, que j'ai du bien saouler avec mes histoires cholériques. Promis, en 2015 je me diversifie !

Je te remercie enfin, Béné, pour ton soutien, ta patience et ton affection. Merci d'avoir eu le courage de m'accompagner en Haïti, puis à Marseille, puis en Haïti, puis à Marseille, enfin on s'y perd ! Il faut croire que le nomadisme est une maladie transmissible !

TABLE DES MATIÈRES

LISTE DES PUBLICATIONS INTÉGRÉES DANS CETTE THÈSE

Les articles sont listés par ordre de publication

Publications parues:

Article 1 : Gaudart J, **Rebaudet S**, Barrais R, Boncy J, Faucher B, Piarroux M, Magloire R, Thimothe G, Piarroux R. Spatio-temporal dynamics of cholera during the first year of the epidemic in Haiti. *PLoS Negl Trop Dis*. **2013** Apr 4;7(4):e2145. doi:10.1371/journal.pntd.0002145.

Article 2 : **Rebaudet S**, Gazin P, Barrais R, Moore S, Rossignol E, Barthelemy N, Gaudart J, Boncy J, Magloire R, Piarroux R. The dry season in haiti: a window of opportunity to eliminate cholera. *PLoS Curr*. **2013** Jun 10;5. doi:10.1371/currents.outbreaks.2193a0ec4401d9526203af12e5024ddc.

Article 3 : Baron S, Lesne J, Moore S, Rossignol E, **Rebaudet S**, Gazin P, Barrais R, Magloire R, Boncy J, Piarroux R. No Evidence of Significant Levels of Toxigenic *V. cholerae* O1 in the Haitian Aquatic Environment During the 2012 Rainy Season. *PLoS Curr*. **2013** Sep 13;5. doi:10.1371/currents.outbreaks.7735b392bdcb749baf5812d2096d331e.

Article 4 : **Rebaudet S**, Sudre B, Faucher B, Piarroux R. Cholera in coastal Africa: a systematic review of its heterogeneous environmental determinants. *J Infect Dis*. **2013** Nov 1;208 Suppl 1:S98-106. doi:10.1093/infdis/jit202. Review.

Article 5 : **Rebaudet S**, Sudre B, Faucher B, Piarroux R. Environmental determinants of cholera outbreaks in inland Africa: a systematic review of main transmission foci and propagation routes. *J Infect Dis*. **2013** Nov 1;208 Suppl 1:S46-54. doi:10.1093/infdis/jit195.

Article 6 : Gujral L, Sema C, **Rebaudet S**, Taibo CL, Manjate AA, Piarroux R, Gessner BD, Jani IV. Cholera epidemiology in Mozambique using national surveillance data. *J Infect Dis*. **2013** Nov 1;208 Suppl 1:S107-14. doi:10.1093/infdis/jit212.

Lettre 1 : Gaudart J, Moore S, **Rebaudet S**, Piarroux M, Barrais R, Boncy J, Piarroux R. Environmental factors influencing epidemic cholera. *Am J Trop Med Hyg*. **2013** Dec;89(6):1228-30. doi:10.4269/ajtmh.13-0499a.

Article 7 : **Rebaudet S**, Mengel MA, Koivogui L, Moore S, Mutreja A, Kande Y, Yattara O, Sarr Keita V, Njanpop-Lafourcade BM, Fournier PE, Garnotel E, Keita S, Piarroux R. Deciphering the origin of the 2012 cholera epidemic in Guinea by integrating epidemiological and molecular analyses. *PLoS Negl Trop Dis.* **2014** Jun 5;8(6):e2898. doi:10.1371/journal.pntd.0002898.

Publications soumises et acceptées:

Lettre 2 : **Rebaudet S**, Piarroux R. Uncertain cholera environmental reservoir in Haiti. *Emerg Infect Dis.* **2015**; ahead of print.

Lettre 3 : **Rebaudet S**, Moore S, Piarroux R. Ebola and cholera : a call to prevent a compound epidemic disaster. *N Engl J Med.* : ahead of print.

Publication en cours de rédaction :

Article 8 : **Rebaudet S** et al. On the laborious way to cholera elimination in Haiti. (*Titre provisoire. Article non achevé*)

À Noa…

1 INTRODUCTION GENERALE

« Grande endémie » causée par un petit bacille en virgule, le choléra est archétypal à plus d'un titre. Cette infection bactérienne causée par des *Vibrio cholerae* de sérogroupe O1 ou O139 producteurs de la toxine cholérique entraîne, dans sa forme classique, une diarrhée aiguë aqueuse si profuse qu'elle tue dans 50% des cas en l'absence de traitement [1]. Son grand potentiel épidémique semble déjà signalé dans des textes des antiquités grecques et indiennes [2]. Source de terreur hier comme aujourd'hui, on ne compte plus ses évocations dans les contes, les chansons populaires [3] et la littérature [4] d'Orient ou d'Occident.

Toutes parties du Golfe du Bengale à l'Est de l'Inde, les cinq pandémies qui secouèrent le monde et notamment l'Europe au 19e siècle furent source d'innombrables avancées médicales. Elles suscitèrent de vastes débats et controverses dont les échos se font encore entendre aujourd'hui. Sur le plan thérapeutique les britanniques William O'Shaughnessy [5] et Thomas Latta [6] furent en 1832 les précurseurs de la réhydratation saline intraveineuse. William Stevens, qui découvrit alors le principe de la réhydratation orale [7], échoua par contre à convaincre ses contemporains et ce traitement révolutionnaire ne finit par s'imposer que plus d'un siècle plus tard [8].

Du point de vue santé publique, des observateurs avaient déjà suspecté dans l'Inde du 17e siècle que les épidémies de choléra se propageaient de manière privilégiée via les transports maritimes [9]. Ces observations furent réitérées deux siècles plus tard sur tous les continents [10], et justifièrent la prescription de quarantaines tentant de freiner la propagation des épidémies en Europe. L'application de ces mesures se heurta souvent aux intérêts commerciaux ainsi qu'à la résistance des partisans de la théorie des miasmes – soutenant que la maladie était causée par un « mauvais air » – face à la

[1] Pollitzer, « Cholera studies- 9. Symptomatology, diagnosis, prognosis and treatment ».
[2] Pollitzer, « Cholera studies- 1. History of the disease ».
[3] Bauduer et Duvert, « Le choléra. . . en chanson! Une épidémie en Pays Basque au milieu du 19e siècle ».
[4] Mann, *La Mort à Venise*; Giono, *Le hussard sur le toit*; Rufin, *Le parfum d'Adam*.
[5] O'Shaughnessy, « Proposal of a New Method of Treating the Blue Epidemic Cholera by the Injection of Highly-Oxygenised Salts into the Venous System ».
[6] Latta, « Saline venous injection in cases of malignant cholera, performed while in the vapour-bath ».
[7] Stevens, *Observations on the Treatment of Cholera*.
[8] Daly et DuPont, « The Controversial and Short-Lived Early Use of Rehydration Therapy for Cholera ».
[9] Wendt et al., *A Treatise on Asiatic Cholera*; Pollitzer, « Cholera studies- 1. History of the disease ».
[10] Wendt et al., *A Treatise on Asiatic Cholera*; Christie, « Notes on the cholera epidemics in East Africa », 28 janvier 1871; Christie, « Notes on the cholera epidemics in East Africa », 11 février 1871; Pollitzer, « Cholera studies- 1. History of the disease ».

progression des thèses contagionistes ou microbiennes. Dès 1949, John Snow à Londres [11] et William Budd à Bristol [12] défendirent en effet l'idée du rôle disséminateur de l'eau de boisson contaminée par les excrétas, que Snow tenta de démontrer en 1854 par une cartographie des décès par choléra à Broad Street restée célèbre [13] et considérée comme l'acte de naissance symbolique de l'épidémiologie moderne [14]. Bien que l'un et l'autre proposaient une brillante synthèse d'un vaste mouvement multidisciplinaire révolutionnant la santé publique [15], leurs adversaires de l'époque surent exploiter les failles méthodologiques de leurs raisonnement pour freiner la diffusion et l'application de ces idées novatrices [16]. En retirant la manivelle d'une pompe à Broad Street pour le premier [17], et pour le second en sécurisant le circuit d'adduction d'eau à Bristol et en promouvant l'ébullition de l'eau, le lavage des mains et la désinfection des excrétas [18], Snow et Budd n'en furent pas moins furent parmi les premiers à proposer des mesures de contrôle efficaces des épidémies de choléra en Europe. En 1854 à Florence, Filippo Pacini [19] fit de son côté la découverte – très vite oubliée – du bacille du choléra… et ainsi l'une des premières descriptions de maladie bactérienne. Longtemps défenseur de la théorie des miasmes, le britannique William Farr finit en 1866 par se ranger aux idées de John Snow devant l'évidence des statistiques médicales, dont il est considéré comme l'un des fondateurs [20].

Entre le début de la première pandémie en 1817 et le reflux – mal expliqué – de la sixième pandémie en 1923, le choléra fut vraisemblablement causé par des *V. cholerae* de biotype Classique [21], et il ôta la vie à des millions de personnes à travers le monde [22]. Son aire de répartition se restreint ensuite à l'Asie du Sud et du Sud-Est [23], jusqu'à ce qu'une nouvelle souche, *V. cholerae* O1 El Tor, émerge en Indonésie et se propage mondialement à partir de 1961 en une septième pandémie qui se poursuit actuellement

[11] Snow, *On the Mode of Communication of Cholera*, 1849.
[12] Budd, « Malignant Cholera ».
[13] Snow, *On the Mode of Communication of Cholera*, 1855.
[14] Brody et al., « Map-Making and Myth-Making in Broad Street »; Newsom, « Pioneers in Infection Control »; Koch, « Commentary ».
[15] Morens, « Snow and the Broad Street Pump ».
[16] Brody et al., « Map-Making and Myth-Making in Broad Street »; Newsom, « Pioneers in Infection Control »; Koch, « Commentary ».
[17] Brody et al., « Map-Making and Myth-Making in Broad Street ».
[18] Dunnill, « Commentary ».
[19] Pacini, *Gazzetta medica italiana*.
[20] Halliday, « William Farr, The Lancet, and Epidemic Cholera ».
[21] Devault et al., « Second-Pandemic Strain of Vibrio Cholerae from the Philadelphia Cholera Outbreak of 1849 ».
[22] Pollitzer, « Cholera studies- 1. History of the disease ».
[23] Swaroop et Pollitzer, « Cholera studies- 2. World Incidence ».

[24]. A cette époque, une somme impressionnante de connaissances avait été accumulée et synthétisée de manière remarquablement cohérente par Robert Pollitzer pour le compte de l'OMS, intéressant tous les aspects de cette maladie, notamment les mécanismes de genèse, de propagation et de déclin du choléra en situation épidémique comme endémique [25], ainsi que les stratégies de prévention et de contrôle les plus pertinentes à promouvoir [26]. Dans la vision que l'OMS donnait alors, le réservoir du choléra se limitait à l'intestin des sujets malades ou convalescents. Les épidémies étaient importées par voie humaine. La transmission se faisait essentiellement par contact ou par la contamination transitoire des réserves d'eau de boisson (puits, citernes, rivières, estuaires…) ou de la nourriture, par les déjections des malades, soit directement soit par l'intermédiaire des mouches. Les poissons et les fruits de mer apparaissaient potentiellement à risque si pêchés dans des eaux contaminées par des excrétas humains. L'influence des facteurs climatiques sur la transmission saisonnière du choléra était clairement établie et mise sur le compte : (1) pour le vent, des modifications des routes commerciales maritimes ; (2) pour la température, des changements d'habitudes alimentaires, de la multiplication des mouches et des variations d'incidence d'autres infections gastro-intestinales favorisant le choléra ; et (3) pour la pluviométrie, de la contamination des réserves d'eau par l'écoulement des selles cholériques, tout en notant que de très fortes pluies pouvaient diluer les *V. cholerae*, ou qu'au contraire la concentration des matières organiques dans l'eau en saison sèche pouvait parfois favoriser la survie des *V. cholerae*. Les éléments en faveur d'une périodicité à long terme apparaissaient discordants, mais dans les situations où elle semblait présente, elle était attribuée à l'acquisition d'une immunité de groupe. Le même phénomène d'immunité de groupe, associé aux facteurs climatiques et aux politiques de contrôle mises en place, était considéré comme suffisant pour expliquer l'arrêt des épidémies. Le choléra n'apparaissait endémique que dans les vastes deltas et basses vallées des grands fleuves de l'Est indien se jetant dans la Baie du Bengale. Dans ces vastes zones au climat tropical chaud et humide, il était impossible de creuser des puits satisfaisants et de mettre en place des systèmes d'adduction d'eau. Les populations y étaient déjà très denses et la pratique des règles d'hygiène élémentaires quasi inexistante. Enfin, des inondations

[24] Organisation Mondiale de la Santé, « Choléra, 1969 »; Organisation Mondiale de la Santé et Section D'hygiène Du Secrétariat De La Société Des Nations, « Choléra, 2013 ».
[25] Pollitzer, « Cholera studies- 10. Epidemiology ».
[26] Pollitzer, « Cholera studies- 11. Prevention and control ».

saisonnières rendaient difficile la détection et l'isolement rapide des malades. Selon Pollitzer et ses coauteurs, ces facteurs favorisaient une transmission continue mais probablement tempérée par une immunité de groupe que des disettes et l'afflux de travailleurs saisonniers venaient périodiquement déséquilibrer, occasionnant ainsi des recrudescences épidémiques [27].

Cette vision de la dynamique des épidémies de choléra fut progressivement mise à mal à partir du milieu des années 1970, près de vingt ans après le début de la septième pandémie. Des océanographes travaillant sur les populations bactériennes aquatiques de la côte Est des USA, parmi lesquels Rita Colwell de l'Université du Maryland, commencèrent en effet à montrer en laboratoire [28] et en milieu estuarien [29], qu'à l'instar des *V. parahaemolyticus*, les *V. cholerae*, dont ceux du sérogroupe O1, étaient bien adaptés à la vie en milieu saumâtre. Une chitinase notamment leur permettait de se multiplier à la surface des copépodes, microscopiques crustacés qui constituent en partie le zooplancton [30]. À la même époque, des médecins rapportèrent la survenue de cas autochtones de choléra par consommation de fruits de mer le long de la Côte du Golfe aux USA [31], mais aussi en Sardaigne [32] et possiblement en Australie [33]. Certains experts se demandèrent à nouveau pourquoi la transmission du choléra apparaissait régulière, parfois sans évidence d'importation et avec une composante saisonnière, non seulement au Bangladesh mais aussi dans de nombreux pays asiatiques ou africains. Ils en vinrent à faire l'hypothèse d'une responsabilité des réservoirs environnementaux de *V. cholerae* O1 dans ces endémies de choléra [34]. S'en suivirent plus de trois décennies d'intenses recherches sur les réservoirs environnementaux de *Vibrio cholerae,* ses liens avec l'incidence du choléra, et ses déterminants climatiques. Elle furent principalement

[27] Pollitzer, « Cholera studies- 10. Epidemiology ».
[28] Singleton et al., « Effects of Temperature and Salinity on Vibrio Cholerae Growth »; Hood et Ness, « Survival of Vibrio Cholerae and Escherichia Coli in Estuarine Waters and Sediments »; Singleton et al., « Influence of Salinity and Organic Nutrient Concentration on Survival and Growth of Vibrio Cholerae in Aquatic Microcosms »; Baker, Singleton, et Hood, « Effects of Nutrient Deprivation on Vibrio Cholerae »; Miller, Drasar, et Feachem, « Response of toxigenic Vibrio cholerae 01 to physico-chemical stresses in aquatic environments ».
[29] Kaper et al., « Ecology, Serology, and Enterotoxin Production of Vibrio Cholerae in Chesapeake Bay »; Colwell et al., « Occurrence of Vibrio Cholerae Serotype O1 in Maryland and Louisiana Estuaries »; Hood, Ness, et Rodrick, « Isolation of Vibrio Cholerae Serotype O1 from the Eastern Oyster, Crassostrea Virginica »; Hood et al., « Distribution of Vibrio Cholerae in Two Florida Estuaries ».
[30] Nalin et al., « Adsorption and Growth of Vibrio Cholerae on Chitin ».
[31] Dastidar et Narayanaswami, « The Occurrence of Chitinase in Vibrios »; Blake et al., « Cholera--a Possible Endemic Focus in the United States ».
[32] Salmaso et al., « Recurrence of Pelecypod-Associated Cholera in Sardinia ».
[33] Rao et Stockwell, « The Queensland Cholera Incident of 1977. 1. The Index Case »; Rogers et al., « The Queensland Cholera Incident of 1977. 2. The Epidemiological Investigation ».
[34] Nalin, « Cholera, Copepods, and Chitinase »; Miller, Feachem, et Drasar, « Cholera epidemiology in developed and developing countries ».

menées par l'équipe du Professeur Rita Colwell [35] et l'International Center for Diarrheal Disease Research (ICDDR,B) au Bangladesh [36], à la fois en laboratoire et en milieu côtier, aux USA et dans le Golfe du Bengale. En 1996, le Professeur Colwell publia dans la revue Science une première synthèse de ces travaux sur les liens entre l'environnement, le climat et cette maladie hydrique emblématique, dont la portée, à visée universelle, justifiait d'être élevée au rang d'un « Paradigme du choléra » [37], ou d'un « Modèle du choléra » [38].

A l'appui de ce paradigme, des *Vibrio cholerae* pathogènes et autochtones ont en effet été isolés dans les eaux saumâtres de certains estuaires d'Asie et des USA. Ils se sont aussi montrés capables de se développer dans les eaux côtières et de tolérer l'eau douce des rivières, canaux, étangs ou lacs, si le niveau trop faible ou trop élevé de salinité est compensé par la chaleur et l'apport de nutriments organiques [39]. Dans cette phase aquatique, les *V. cholerae* ont été retrouvés de manière planctonique sous la forme de bacilles libres ou de manière benthique sous la forme de biofilms attachés aux surfaces. Lorsque les conditions environnementales ne sont pas favorables, plusieurs études ont suggéré que les vibrios pouvaient entrer dans un état viable mais non cultivable (*viable but nonculturable*, VBNC), et survivre pendant les périodes inter-épidémiques en conservant intact leur potentiel pathogène [40]. Des *V. cholerae* ont été identifiés associés à de nombreux composants de la chaîne alimentaire marine [41] : cyanobactéries ; phytoplancton ; plantes ; amibes libres ; copépodes et autres crustacés, dont ils se nourrissent de la chitine grâce à leur chitinase [42] ; bivalves ; intestins de certains poissons, de dauphins ou d'oiseaux aquatiques ; sédiments aquatiques... Parmi ces réservoirs potentiels, les copépodes demeurent ceux dont l'importance a été la mieux démontrée pour les souches pathogènes *V. cholerae* O1 et O139 [43]. Chaque copépode pourrait en effet porter jusqu'à 10^4 *V. cholerae*, approchant ainsi l'inoculum

[35] « Rita Colwell | UMIACS ».
[36] « icddr,b- Knowledge for global lifesaving solutions ».
[37] Colwell, « Global climate and infectious disease ».
[38] Lipp, Huq, et Colwell, « Effects of global climate on infectious disease ».
[39] Colwell, « Global climate and infectious disease »; Lipp, Huq, et Colwell, « Effects of global climate on infectious disease », 2002.
[40] Colwell, « Global climate and infectious disease »; Faruque et al., « Transmissibility of cholera »; Alam et al., « Viable but nonculturable Vibrio cholerae O1 in biofilms in the aquatic environment and their role in cholera transmission ».
[41] Senderovich, Izhaki, et Halpern, « Fish as reservoirs and vectors of Vibrio cholerae »; Vezzulli et al., « Environmental Reservoirs of Vibrio Cholerae and Their Role in Cholera ».
[42] Pruzzo, Vezzulli, et Colwell, « Global impact of Vibrio cholerae interactions with chitin »; Nahar et al., « Role of Shrimp Chitin in the Ecology of Toxigenic Vibrio cholerae and Cholera Transmission ».
[43] Vezzulli et al., « Environmental Reservoirs of Vibrio Cholerae and Their Role in Cholera ».

requis chez les sujets susceptibles [44]. Le long de la Baie du Bengale, des associations significatives ont été établies entre d'une part l'incidence des cas de choléra, et d'autre part la concentration des copépodes dans l'eau [45] ou celle du phytoplancton dont ils se nourrissent, mesurée directement dans des échantillons d'eau ou indirectement par estimation satellite de la chlorophylle-a [46]. La température des eaux de surfaces (*sea surface temperature*, SST) et les nutriments charriés par les fleuves lors de la saison des pluies apparaissent influer fortement sur la prolifération du phytoplancton dont se nourrit le zooplancton sur lequel vivent les *V. cholerae* [47]. Au Bangladesh, celle-ci a de fait pu être corrélée à diverses variables climatiques comme les précipitations, le débit des fleuves, la hauteur des mers, la SST [48], ainsi que les variations interannuelles de la SST sous l'influence du phénomène d'oscillation australe El-Niño (*El Niño-Southern Oscillation*, ENSO) [49].

Ce paradigme environnemental du choléra a depuis été largement accepté par le monde scientifique et les acteurs de santé publique. L'émergence des épidémies de choléra est en effet souvent attribuée à la contamination des populations locales par des réservoirs environnementaux de *V. cholerae*, lorsque les conditions climatiques sont favorables et que l'accès à l'eau, à l'hygiène et à l'assainissement (EHA) n'est pas assuré [50]. Le caractère *a priori* ubiquitaire de ces réservoirs environnementaux et l'importance des populations vulnérables a ainsi conduit à considérer : (1) de nombreux pays comme endémiques pour le choléra (i.e. ayant rapporté des cas suspects de choléra au cours d'au moins 3 des 5 dernières années selon l'OMS) ; (2) les niveaux d'incidence comme homogènes dans ces territoires ; et (3) une sous-déclaration massive des cas à l'OMS. Ces 3 postulats sont couramment cités et servent de base à des estimations du poids mondial du choléra tablant sur 3 millions de cas et 100 000 décès annuels. Promues par l'OMS, ces valeurs représentent 10 à 20 fois le volume des données qui lui sont rapportées [51].

[44] Cash et al., « Response of man to infection with Vibrio cholerae. I. Clinical, serologic, and bacteriologic responses to a known inoculum »; Colwell, « Global climate and infectious disease »; Nelson et al., « Cholera transmission ».
[45] Huq et al., « Critical factors influencing the occurrence of Vibrio cholerae in the environment of Bangladesh ».
[46] Constantin de Magny et al., « Environmental signatures associated with cholera epidemics ».
[47] Colwell, « Global climate and infectious disease »; Jutla et al., « Warming oceans, phytoplankton, and river discharge ».
[48] Lobitz et al., « Climate and infectious disease »; Huq et al., « Critical factors influencing the occurrence of Vibrio cholerae in the environment of Bangladesh »; Constantin de Magny et al., « Environmental signatures associated with cholera epidemics »; Koelle, « The impact of climate on the disease dynamics of cholera ».
[49] Colwell, « Global climate and infectious disease »; Pascual et al., « Cholera dynamics and El Niño-Southern Oscillation ».
[50] Nelson et al., « Cholera transmission »; Morris, « Cholera-modern pandemic disease of ancient lineage ».
[51] « WHO | Cholera surveillance and number of cases ».

Une série de résultats issus de l'épidémiologie de terrain, de l'analyse spatiotemporelle des épidémies de choléra ou de la comparaison génétique des souches de *V. cholerae* est cependant venu apporter des éléments discordants avec ce paradigme environnemental du choléra.

L'arrivée puis la progression de la septième pandémie en Afrique à partir de 1970 apparaît en effet avoir été la conséquence d'événements d'importation impliquant parfois des voyageurs clairement identifiés [52].

Madagascar, affectée en 1999-2000 par une intense épidémie arrivée des Comores voisines, est depuis 2002 demeurée indemne du choléra alors que l'accès à l'eau et à l'assainissement y reste très mauvais [53] et que les estuaires y sont nombreux.

Une série de travaux dirigés par Renaud Piarroux sur la problématique du choléra dans la Région des Grands Lacs à l'Est de la République Démocratique du Congo [54], l'une des zones les plus touchées d'Afrique [55], a montré qu'en utilisant des échelles spatiales et temporelles plus fines, la vision d'une endémicité globale du choléra dans la région laissait la place à celle d'une transmission hétérogène associant des clusters asynchrones communiquant entre eux selon un schéma métastable. Divers facteurs environnementaux – proximité et concentration phytoplanctonique des lacs, pluviométrie… – apparaissent significativement associés à la transmission, sans pour autant pouvoir prétendre en être la cause.

Récemment également, une équipe du Sanger Institute de Cambridge a publié dans Nature une étude comparant le génome total de 154 souches de *V. cholerae* isolées à travers le monde depuis 1937, après l'avoir débarrassé de ses éléments mobiles [56]. Selon ce travail spectaculaire, toutes les épidémies de la sixième pandémie apparaissent causées par des *V. cholerae* O1 El Tor appartenant tous à un même complexe clonal dont l'ancêtre commun remonte aux années 1950. La septième pandémie semble ainsi s'être répandue, via un portage humain, en trois vagues successives mais partiellement

[52] Carme et al., « L'implantation et l'extension du choléra en Afrique Noire : 1970 -1986 »; World Health Organization, « Cholera in Africa »; Osei et Duker, « Spatial and demographic patterns of cholera in Ashanti region - Ghana »; Echenberg, *Africa in the Time of Cholera*.
[53] World Health Organization, « Cholera [every year since 1968] »; World Health Organization (WHO) et UNICEF, *Progress on drinking water and sanitation. 2012 update*.
[54] Bompangue et al., « Lakes as source of cholera outbreaks, Democratic Republic of Congo »; Bompangue et al., « Cholera epidemics, war and disasters around Goma and Lake Kivu »; Bompangue Nkoko et al., « Dynamics of cholera outbreaks in great lakes region of Africa, 1978-2008 ».
[55] Griffith, Kelly-Hope, et Miller, « Review of reported cholera outbreaks worldwide, 1995-2005 »; Gaffga, Tauxe, et Mintz, « Cholera ».
[56] Mutreja et al., « Evidence for several waves of global transmission in the seventh cholera pandemic ».

superposées à partir de la Baie du Bengale où a persisté une évolution continue de *V. cholerae*.

Enfin, en octobre 2010, Haïti a été frappé pour la première fois de son histoire par une épidémie de choléra qui, avec 706 291 cas et 8584 morts recensés au 27 août 2014 [57], représente la plus vaste épidémie de ces dernières décennies. Son origine environnementale apparaissait initialement évidente à de nombreux observateurs [58]. Des enquêtes de terrain, des éléments statistiques et une étude de biologie moléculaire ont cependant vite établi que cette épidémie avait été importée par un contingent de casques bleus fraîchement arrivé du Népal, alors en proie à une épidémie de choléra. L'épidémie haïtienne n'était donc pas une conséquence du séisme du 12 janvier 2010 [59], mais directement celle du déversement du contenu des latrines de ce camp militaire népalais dans la rivière toute proche. Plusieurs dizaines de milliers de personnes vivant en aval ont ainsi été contaminées au cours des jours suivants.

Cette tragédie haïtienne relance donc le débat sur l'origine environnementale ou importée des épidémies de choléra, notamment pour l'Afrique qui, jusqu'en 2010, était le continent rapportant le plus de cas dans le monde [60]. La résolution de cette question apparaît pourtant un préalable indispensable à la mise en place de stratégies de lutte adaptées.

La conception environnementaliste du choléra a en effet conduit à proposer la filtration des copépodes de l'eau de boisson par un simple tissu, comme une mesure efficace de prévention des épidémies de choléra dans le Bengale [61]. Plus fréquemment, face à une incidence du choléra perçue comme largement sous-estimée, face à l'impossibilité actuelle d'améliorer rapidement l'hygiène et l'accès aux services d'eau et d'assainissement à une échelle aussi globale, et face à la menace des changements climatiques et environnementaux à venir, nombreux sont les acteurs de santé publique plaçant de grands espoirs dans la vaccination orale contre le choléra [62].

[57] « Ministère de la Santé Publique et de la Population de la République d'Haïti. Centre de Documentation ».
[58] Parker et Circle of Blue, « Cholera in Haiti - The Climate Connection ».
[59] Piarroux et al., « Understanding the cholera epidemic, Haiti »; Hendriksen et al., « Population genetics of Vibrio cholerae from Nepal in 2010 ».
[60] World Health Organization, « Cholera [every year since 1968] ».
[61] Colwell et al., « Reduction of cholera in Bangladeshi villages by simple filtration »; Sack, Sack, et Chaignat, « Getting serious about cholera »; Huq et al., « Simple sari cloth filtration of water is sustainable and continues to protect villagers from cholera in Matlab, Bangladesh ».
[62] Sack, Sack, et Chaignat, « Getting serious about cholera »; Zuckerman, Rombo, et Fisch, « The true burden and risk of cholera »; Ali et al., « The global burden of cholera ».

A l'inverse, l'identification des nœuds de transmission dans l'Est de la RDC a conduit les autorités sanitaires de ce pays à intégrer dans leur plan national de lutte contre le choléra, des interventions ciblées sur ces endroits et ces populations les plus à risque portant sur l'eau, l'hygiène et l'assainissement, puis la vaccination [63]. Une approche épidémiologique similaire menée par la même équipe en République de Guinée a également permis au Ministère de la Santé d'adapter son système d'alerte précoce aux épidémies de choléra [64], et de travailler à un plan de lutte ciblée.

Le présent travail de thèse s'intègre donc dans ce débat. En me proposant de rejoindre en novembre 2011 sa petite équipe de recherche interventionnelle sur les épidémies de choléra, Renaud Piarroux me confiait ainsi la tâche de participer à compléter ce grand puzzle de la compréhension des épidémies de choléra, à la fois en Afrique et en Haïti. Nos travaux devaient se dérouler au gré des partenariats mis en place avec les autorités nationales, les agences onusiennes, la Coopération française, ou tout autre bailleur s'intéressant à lutter efficacement contre les épidémies de choléra sur ces territoires.

[63] Piarroux et al., « From research to field action: example of the fight against cholera in the Democratic Republic of Congo »; Muyembe et al., « Elimination of Cholera in the Democratic Republic of the Congo ».
[64] Sudre et Bompangue, *Epidémiologie du choléra et Evaluation du Système d'Alerte Précoce en République de Guinée. Rapport de mission*; Sudre, *Projet de préparation aux épidémies de choléra à Conakry, Guinée. Épidémiologie du choléra dans la ville de Conakry (République de Guinée). Étude rétrospective des facteurs de risque dans la ville de Conakry*.

2 TRAVAUX SUR LE CHOLÉRA EN AFRIQUE

2.1 Déterminants environnementaux du choléra en Afrique

> « The First Epidemic of Cholera in East Africa to which any definite date can be fixed occurred in December, 1835, and January, 1836, during the prevalence of the north-east monsoon, and its line of progress was in the direction of the monsoon from north to south. »
>
> James Christie,
> médecin à Zanzibar, 1871 [65]

2.1.1 Le paradigme environnemental du choléra est-il applicable à l'Afrique ?

Le premier travail effectué dans le cadre de cette thèse d'université fut la rédaction d'une revue synthétisant les déterminants environnementaux du choléra en Afrique. Cette zone de transmission majeure du choléra depuis les années 70 faisait paradoxalement l'objet de connaissances très fragmentaires n'ayant pas été synthétisées depuis de nombreuses années. Cette nécessité se trouva en résonnance avec le projet d'Africhol [66] – un consortium lancé par l'Agence de Médecine Préventive (AMP) avec le financement de la Bill & Melinda Gates Fondation dans le but d'appuyer la surveillance du choléra dans 11 pays africains – de coordonner la rédaction d'un numéro spécial dédié au choléra en Afrique dans l'un des grands journaux de maladies infectieuses et tropicales. L'université d'Aix-Marseille faisant partie du consortium depuis sa création, il nous fut donc proposé d'y intégrer ce travail.

Cette revue fut réalisée avec le concours de deux anciens collaborateurs de Renaud Piarroux : Benoit Faucher qui avait travaillé sur le choléra an Haïti en 2010-2011 ; et Bertrand Sudre qui avait travaillé sur le choléra en Guinée en 2009-2010 puis dans le Bassin du Lac Tchad en 2011. Trop vaste pour être synthétisée en un seul manuscrit, elle fut scindée en 2 articles, l'un portant sur l'Afrique côtière [67], l'autre sur l'Afrique continentale [68], publiés en 2013 dans un numéro spécial du Journal of Infectious Diseases après avoir été examinés par des pairs indépendants.

Toutes deux sont présentées l'une après l'autre ci-après.

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[65] Christie, « Notes on the cholera epidemics in East Africa », 28 janvier 1871.
[66] « Africhol. The African Cholera Surveillance Network ».
[67] Rebaudet et al., « Cholera in Coastal Africa ».
[68] Rebaudet et al., « Environmental Determinants of Cholera Outbreaks in Inland Africa ».

2.1.2 Article 4 : « Cholera in coastal Africa: a systematic review of its heterogeneous environmental determinants. »

2.1.3 Article 5 : « Environmental determinants of cholera outbreaks in inland Africa: a systematic review of main transmission foci and propagation routes. »

SUPPLEMENT ARTICLE

Cholera in Coastal Africa: A Systematic Review of Its Heterogeneous Environmental Determinants

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According to the "cholera paradigm," epidemiology of this prototypical waterborne disease is considered to be driven directly by climate-induced variations in coastal aquatic reservoirs of *Vibrio cholerae*. This systematic review on environmental determinants of cholera in coastal Africa shows that instead coastal epidemics constitute a minor part of the continental cholera burden. Most of coastal cholera foci are located near estuaries, lagoons, mangrove forests, and on islands. Yet outbreaks often originate in coastal cities, where cholera is more likely to be imported from distant areas. Cholera outbreaks also may intensify in densely populated slum quarters before spreading to adjacent regions. Frequent seasonality of cholera incidence appears driven by the rainfall-induced contamination of unprotected water sources through latrine overflow and sewage, as well as by the periodicity of human activities like fishing or traveling. Lulls in transmission periods of several years are repeatedly recorded even in high-risk coastal areas. To date, environmental studies have failed to demonstrate a perennial aquatic reservoir of toxigenic *V. cholerae* around the continent. Finally, applicability of the cholera paradigm therefore appears questionable in Africa, although available data remain limited. Thorough surveys with microbiological analyses of water samples and prospective genotyping of environmental and clinical strains of *V. cholerae* are needed to understand determinants of cholera in coastal Africa and better target prevention and control measures.

Keywords. cholera; *Vibrio cholerae*; Africa; epidemiology; environment; climate; cities; seasons; reservoirs; molecular epidemiology.

Every year, cholera affects several hundred thousand people globally, with a case fatality rate over 2% [1]. Africa has reported most cases during the current seventh cholera pandemic. Yet the understanding of cholera epidemiology in Africa and notably in its coastal countries still heavily relies on findings from studies performed in Asia and especially around the Bay of Bengal, cholera's historical place of origin. There, autochthonous pathogenic *Vibrio cholerae* have been isolated in the brackish waters of certain estuaries but also thrive in coastal seawaters and tolerate freshwater in rivers, canals, ponds, or lakes, if saline levels are compensated by warmth and organic nutrients [2, 3]. In this aquatic phase, *V. cholerae* can be found as free swimming bacilli or attached to various surfaces as biofilm. In unfavorable environmental conditions, several studies have suggested that *Vibrio* spp could enter a viable but nonculturablecoccoid state and survive during interepidemic periods with intact pathogenic potential [2, 4, 5].

V. cholerae has been associated with numerous environmental components of the marine food chain [6, 7]: cyanobacteria; phytoplankton; plants; free-living amoebae; crustaceans such as copepods (main microscopic and ubiquitous constituents of zooplankton) or blue crabs, whose chitin may feed chitinase-equipped *Vibrio* [8, 9]; bivalves; gut of certain fish, dolphins or aquatic birds; and aquatic sediments. Among these putative reservoirs, copepods remain one of the most demonstrated importance for pathogenic *V. cholerae* O1 and O139

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The Journal of Infectious Diseases 2013;208(S1):S98–106

DOI: 10.1093/infdis/jit202

strains [7]. Each copepod may carry up to 10^4 *V. cholerae*, approaching the infectious dose in susceptible individuals [2, 10]. Along the Bay of Bengal, significant associations have been established between cholera cases and both copepod counts in water samples [11] and phytoplankton blooms directly measured or indirectly estimated by remote satellite sensing of chlorophyll—a concentration [12]. These blooms precede copepod production [2], and their driving by sea surface temperature (SST) or rainfall-induced river discharges of terrestrial nutrients [2, 13] has thus been considered as the source of the frequently observed seasonal patterns of cholera incidence [2, 14, 15]. Cholera incidence in Bangladesh has been correlated with various climatic variables such as rainfall, river discharge, sea level, or SST [11, 12, 16, 17] and SST interannual variability driven by El Niño-Southern Oscillation (ENSO) events [2, 18]. First proposed almost 3 decades ago [19], these relations between this prototypical water-borne disease, the aquatic environment and climate parameters have been called the "cholera paradigm" by Colwell [2, 3].

Apart from Latin America in 1991 through 1993, and again in 2010 and 2011, and Asia in 1994 (because of *Vibrio cholerae* O139), sub-Saharan Africa has been the most affected region with regard to cholera over the past 2 decades [1, 20, 21]. Between 2002 and 2011, Africa reported over two-thirds of the 2.2 million worldwide cases. Along African coasts, several areas have suffered from recurrent cholera outbreaks, such as the estuarine cities of Bissau in Guinea-Bissau, Calabar in Nigeria, Douala in Cameroon, Beira in Mozambique, or Dar es Salam in Tanzania. But unlike in the Bay of Bengal, no comprehensive overview of cholera transmission dynamic has ever been proposed for these coastal African hotspots. The present review thus aims at searching for evidence supporting environmental cholera determinants in coastal Africa and at assessing the degree to which this evidence fits the cholera paradigm established for the Bay of Bengal.

MATERIALS AND METHODS

A systematic Pubmed query was conducted using the terms "cholera OR *Vibrio*" AND ("Africa" OR the current or past names of all sub-Saharan African countries) between 1970 and September 2012. Retrieved citations were selected for articles published in English or French, whose title or abstract addressed cholera outbreaks or epidemiology in Africa or *Vibrio* detection in the environment (Supplementary Figure 1). Other articles from nonindexed journals and reports from several agencies were searched using Google, Google Scholar, and reference lists from key textbooks and searched articles (Supplementary Figure 1). ProMED-mail alerts were also investigated using the website's (www.promedmail.org) search archives function with the term "cholera" and the country names. Selected full texts were assessed as eligible provided that they gave information on cholera morbidity or outbreak processes. Data describing cholera outbreaks were extracted, including: exact location and local environmental characteristics; year and season of outbreak start, peak, and end; population affected; epidemic dynamics; suspected origin and/or underlying risk factors; local environmental isolation of *V. cholerae* and other *Vibrio* species; and genotyping of epidemic strains. In this review, only reports relevant for countries having access to the sea (defined as "coastal countries") and, if available, for regions of seaside countries located on the coast or along an estuary (defined as "coastal regions") were included (Supplementary Figure 1). Links between cholera and environment in inland African countries and inland regions of seaside countries have been addressed in a different review [22].

RESULTS

Cholera Burden in Coastal Africa

Nearly three-quarters of the 1.5 million cholera cases reported in Africa during the past 10 years were located in countries with access to the sea [1] (Table 1). Nevertheless, most of the major outbreaks affecting countries like Nigeria, Cameroon, Democratic Republic of Congo, Kenya, or Sudan actually occurred in their inland part (locations cited in the text are mapped in Figure 2). According to the available subnational data, it has thus been estimated in a separate review dedicated to inland Africa [22] that only less than one-quarter of all cholera cases reported by Sub-Saharan Africa in 2009–2011 actually affected its coastal regions.

Cholera in Estuaries, Lagoons, and Mangrove Areas

Like in the Bay of Bengal, numerous cholera foci in Africa have been located in estuarine areas. For instance, in Guinea-Bissau, cholera epidemics recorded during the past 20 years mainly affected the capital Bissau and the adjacent Biombo region, 2 areas bordering estuaries [43]. In the neighboring Guinea, cholera between 2003 and 2008 frequently struck Kamsar, a city located on the Rio Nunez estuary [23]. In Sierra Leone, Freetown, the capital built at the mouth of a vast estuary, has been among the mostly affected districts of the country [44]. In Nigeria, although most cases have been notified in the Lake Chad Basin and other northern states [45, 46], cholera also has repeatedly stricken coastal southern areas like the estuarine city of Calabar, in the Southeast corner of the country [47]. Similarly in Cameroon, cholera has regularly affected the maritime regions that notified 37% of the 22 762 national cases in 2011, especially around Douala, a port city built on the swampy plain of the Wouri delta [45, 48]. In Mozambique on the east coast of the continent, cholera has recurrently stricken estuarine cities like Maputo, Beira, or Quelimane. In 1997–1998, for instance, a major outbreak of >50 000 cases originated in Maputo City before spreading to Beira City, on the Pungwe River [32, 33].

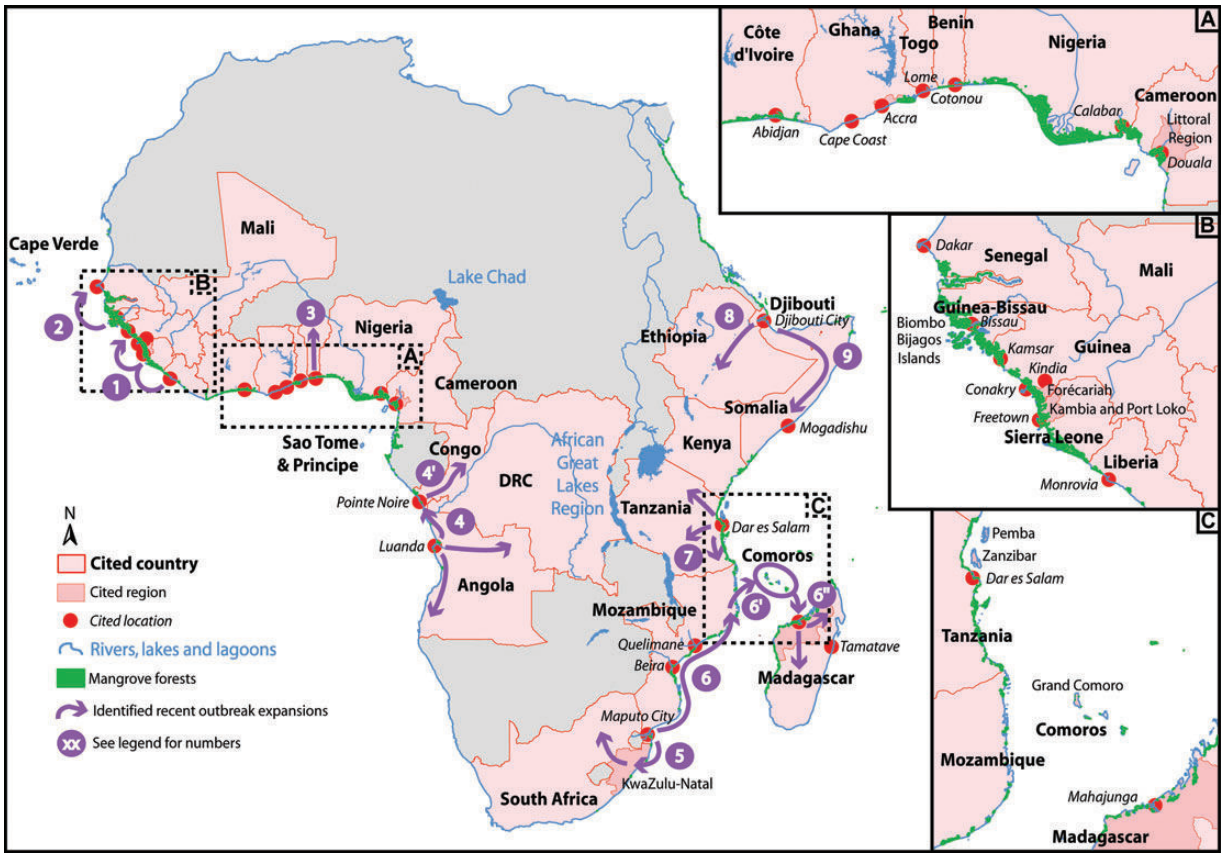

Figure 1. Cholera in coastal Africa. Places cited in the text and identified outbreaks expansions. (DRC, Democratic Republic of the Congo). Global distribution of Mangroves (V3.0, 1997) compiled by UNEP World Conservation Monitoring Centre (UNEP-WCMC) in collaboration with the International Society for Mangrove Ecosystems (ISME). Available at: www.unep-wcmc.org. Information on outbreak expansions (numbers on figure): 1: in 1994, 2003–2007 and 2012, transboundary epidemics from Liberia (uncertain for 2012) and Sierra Leone to Guinea [23–25]. 2: in 1994–1995, from Guinea-Bissau to Senegal [26]. Genetically confirmed (see Table 2). 3: in 1991, from Cotonou to northern Benin [27]. 4: in 2006–2007, from Luanda to whole Angola and probably Congo [28, 29]. 4′: in 2006–2007, from Pointe-Noire (Congo) to Brazzaville [30]. 5: in 1980–1981 and 1997, from Mozambique to KwaZulu-Natal and the rest of South Africa [31]. 6: in 1997, from Maputo to Beira and the rest of Mozambique [32, 33]. 6′: in 1997, from Mozambique to Comoros [34, 35]. 6″: in 1999, from Comoros to Mahajunga and the rest of Madagascar [36–38]. Genetically confirmed (see Table 2). 7: in 1997, from Dar es Salam to the rest of Tanzania [39]. 8: in 1993 and 1998, from Djibouti to Ethiopia [40]. 9: in 1994 and 1999, from Djibouti to Somalia [41, 42].

Besides estuaries, cholera has repeatedly affected lagoon areas like Abidjan in Côte d'Ivoire. Between 2001 and 2005, 60% of the 11 874 Ivorian cases were reported from this 6-million-inhabitant port city built on either side of the brackish Ebrié lagoon [49, 50]. In Benin, the 1991 outbreak was reported to originate close to the lagoonal Lake Nokoué before spreading northward from ponds and rivers to wells and cisterns [27]. Located on its southern shores, the capital Cotonou and its surroundings recorded over half of national cases in 2008 [51], and over one-third in 2011 [52].

Remarkably, almost all these cholera-affected areas are lowlands forested with more or less saline mangrove swamps (Figure 2). They often form complex networks of channels and islets with important fishing-related activities and population movements, as observed between Liberia and Guinea-Bissau. Hence, all cholera epidemics in Guinea except in 1994 [25] have started in such ecosystems in Forecariah or Boke prefectures close to the Sierra Leone and Guinea-Bissau borders, respectively [23, 53]. Epidemics have affected mainly these mangrove lowland areas, especially Conakry, the 2-million-inhabitant capital that lies on a peninsula, and they have shown a limited inland spread. During late 2011 and early 2012, an important outbreak emerged in Kambia and Port Loko, 2 coastal districts of Sierra Leone, before spreading through fishing activities northward across the Guinean border to the Forecariah Prefecture, then Conakry, and southward to the capital Freetown [24, 54, 55].

Cholera on Islands

Cholera outbreaks, often related to fishing activities, have also repeatedly affected wider groups of islands such as the Bijagos Islands in Guinea-Bissau [43, 56] or the Tanzanian

Table 1. Cholera Cases Notified by African Coastal Countries

Coastal country[a]	Total cases[b] 1970–2011	Total cases[b] 2002–2011	Main location of cholera burden	Environment type in coastal cholera foci
Algeria	12 729	0		
Angola	182 875	101 503	Coastal > inland	Urban
Benin	28 835	4983	Coastal > inland	Lagoonal, urban
Cameroon	72 551	46 053	Inland > coastal	Estuarine, urban
Cape Verde	14 144	0		
Comoros	17 866	3183	Coastal	Insular
Congo	17 385	9010	Coastal > inland	Urban
Côte d'Ivoire	23 389	7093	Coastal > inland	Lagoonal, urban
Democratic Republic of the Congo	391 524	217 569	Inland	
Djibouti	19 553	3384	Coastal	Urban
Egypt				
Equatorial Guinea	6962	6450		
Eritrea	120	120		
Gabon	649	637		
Gambia	252	227		
Ghana	128 525	24 510	Coastal > inland	Urban
Guinea	62 635	17 750	Coastal > inland	Estuarine, insular, urban
Guinea-Bissau	91 609	40 916	Coastal	Estuarine, insular, urban
Kenya	99 022	21 831	Inland > coastal	
Liberia	84 999	55 454	Coastal and inland	
Libya				
Madagascar	46 531	32	Coastal and inland	Insular, urban
Mauritania	17 765	4320		
Mauritius	0	0		
Mayotte	12	0		
Morocco	63	0		
Mozambique	315 295	106 842	Coastal > inland	Estuarine, urban
Namibia	3854	3854		
Nigeria	264 119	105 648	Inland > coastal	Estuarine, urban
Sao Tome and Principe	7861	3101		
Senegal	69 841	38 590	Coastal > inland	Urban
Seychelles	178	178		
Sierra Leone	38 343	5360	Coastal	
Somalia	255 788	142 563	Coastal and inland	Urban, refugee camps
South Africa	186 462	34 602	Coastal and inland	
Sudan + South Sudan	80 634	75 315	Inland	
Togo	15 820	4956		
Tunisia	60	0		
United Republic of Tanzania	204 569	57 822	Coastal and inland	Estuarine, urban, island
Total cases in Africa	3 589 002	1 568 701		
Total cases in coastal countries	2 762 819	1 143 856		
(%)	(77%)	(73%)		

Abbreviation: ND, no data.

[a] Any African country with access to the sea.

[b] Notification data extracted from the WHO yearly cholera overviews [1].

archipelago of Zanzibar [57]. African islands more distant from the continent have intermittently been affected. Since 1971, Sao Tome and Principe has experienced only 2 major epidemics: in 1989–1990 and 2005–2006 with 4 757 and 2 892 reported cases, respectively [1, 58]. Cape Verde had remained cholera-free since 1976, until a major epidemic occurred in late 1994. On this archipelago where the volcanic soil makes water supply inadequate and latrine construction difficult, the

epidemic produced nearly 13 000 cases in 1995 [1, 59]. Similarly, a severe outbreak hit the volcanic Comoros archipelago in 1998 after a 2-decades lull period. The highest attack rates were observed in lowland areas of Grand Comoro, a rocky island with chronic and severe water scarceness, where many people still have to rely on unprotected collective wells or watering places close to the sea and filled with brackish water [34, 35]. From Comoros, cholera crossed over to Madagascar where it landed in March 1999 in Mahajunga, an estuarine and port city with close commercial exchange with Comoros; from there it spread throughout the Red Island within a few months [36, 37].

Cholera in Coastal Cities

Whether estuarine, lagoonal, insular, or not (like Accra and Cape Coast in Ghana [60], Pointe Noire in Congo [30], or Luanda in Angola [28, 29, 61]), main cholera transmission foci along African coasts have often been localized to densely populated urban settings. More precisely, cases within these coastal cities have been commonly clustered in slum quarters with limited safe water access and low sanitation standards, like in the ancient inner-city neighborhoods of Calabar in 1989 [47], in Luanda in 2006 [61, 62], or in Dar es Salam in 2006 and 2008 [63]. In Douala in 2004, cholera spared quarters well connected to the water distribution network and mostly struck those relying on traditional shallow and poorly protected wells [64]. In Quelimane, Mozambique, during the civil war in the early 1990s, the outbreak mostly affected quarters overcrowded with recently arrived displaced people [65, 66]. In chronically war-torn Somalia, suspected cholera outbreaks have also recurrently affected congested displaced persons camps, especially in the Mogadishu area. The country declared over 41 000 cases in 2007, nearly 78 000 in 2011, and 11 478 cases had been recorded in 2012 by late April [1, 67, 68].

Associated lowland location and particular hydroecological characteristics have been pointed out in many of these highly affected overcrowded quarters. Affected areas of Lome [69], Douala [64, 70, 71], Djibouti [72–74], Beira [75], and Tamatave [76] have all been situated in floodable areas prone to contamination of surface waters, unprotected wells, or shallow boreholes. In Conakry in 2007 [23, 77], in Abidjan during the 1996 outbreak [78], or in Cotonou in 2008 [51], cholera mostly affected areas neighboring inlets, backwaters, or lagoons. A high and sometimes brackish groundwater table feeding the wells and marshes was identified in several high-risk urban neighborhoods like Bandin in Bissau [79], Bépanda in Douala [70, 71], in Beira [75], or in Quelimane [65, 66].

Overall, certain African coastal cities, especially ports of estuarine or lagoonal locations, may thus constitute favorable repositories and amplifiers for cholera. Large population movements—by sea, road, rail, or air—can favor cholera importation. The combination of factors such as high human density, lack of adequate safe water supplies due to urban expansion, and vulnerability of surface and ground water resources to fecal contamination can favor onset and propagation of outbreaks. These outbreaks may subsequently spread to surrounding regions and countries.

Cholera Spreading from Coastal Urban Foci

All documented cholera pandemics have spread inland into the African continent from its coastal belt, sometimes through identified importation events [80–83]. Notably, the current Seventh Pandemic (1961-) first reached Guinea in August 1970 probably through a flight coming from Crimea [83]. Spreading southeast along the coast, cholera arrived in Ghana a few months later possibly with a Togolese person coming from Conakry who collapsed in the transit area of the Accra airport, and with the corpse of a Ghanaian person who died from cholera while fishing in Togo, Liberia, and Guinea [84].

Since then, numerous inward and cross-border epidemics have been identified that followed terrestrial, maritime, and aerial routes from coastal urban transmission foci (Figure 2). Often suspected by epidemiology but rarely confirmed by biology, some epidemic routes have been traced by molecular comparison of *V. cholerae* strains (Table 2), like from Guinea-Bissau to Senegal in 1995 [26], or from Comoros to Madagascar in 1999 [36]. Similarly, genetic comparisons of cholera strains from Africa and different countries worldwide [85–88] have demonstrated several waves of cholera importation into Africa (Table 2). In recent years, epidemics in coastal African countries all proved to be caused by new and atypical strains of *V. cholerae* El Tor, secreting the classical toxin, which obviously emerged in the early 1990s in the Bay of Bengal [95] (Table 2).

Seasonality of Cholera in Coastal Africa

Like in Asia, influence of the rainy season on cholera epidemics has been repeatedly suggested by observations, temporal correlations, or time-series analyses along West, East, and Austral African coasts. For example, in Conakry, the earlier the first cases recorded within the rainy season, the larger the epidemics that followed [77]. Increased spread of cholera during the rainy season was also observed in Guinea-Bissau [43]; in Sierra Leone, and Liberia [44, 100, 101]; in Côte d'Ivoire [49]; in Angola [61, 102]; in KwaZulu-Natal, a South African province [103]; on the Mozambican coast [32, 65, 66, 104]; in the Tanzanian archipelago of Zanzibar and Pemba [57, 105]; and in Somalia [106]. Conversely, some other cholera epidemics have emerged during the dry season, as observed in Guinea in 1986 [53], 2007 [23], and 2012 [24], in Benin in 1991 [27], in Côte d'Ivoire in 2001, 2003, and 2004 [49], in Calabar in 1989, concomitantly with an increase of estuary's salinity [47], in Grand Comoro Island in 1998 [34, 35], or in Madagascar during the first phase of the 1999 epidemic [37]. In Douala, outbreaks usually start during the dry season [71] and may recede with the onset of

Table 2. Genetic comparisons of *Vibrio cholerae* strains sampled in Africa

Sampling country (year)	Strains' origins[a] (no.)	Genotyping method	Conclusions	References
Guinea-Bissau (1987) Guinea-Bissau (1994–1995)	C (5) C (14)	Ribotyping	Distinct origin for the two consecutive epidemics	[89]
Senegal (1978; 1988) Senegal (1995–1996) Guinea-Bissau (1994)	C (2) C & E (117) C (7)	Ribotyping	The last Senegalese epidemic originated in Guinea-Bissau	[26]
Comoros (1998–1999) Madagascar (1999)	C (N/A) C (N/A)	Ribotyping	Epidemic spread from Comoros to Madagascar	[36]
South Africa (1980) South-Africa (2001–2002)	C (15) C (112)	PFGE	Second epidemic due to the introduction of a new strain, likely from Mozambique	[31]
Djibouti, Kenya, Mozambique, Sudan and Tanzania (1968–2009) Worldwide (1910–2010)	C (30) C (127)	Genome-wide SNP analysis	Cholera importation in Africa through 3 independent waves arisen from the Bay of Bengal	[88]
Algeria, Chad, Comoros, Guinea, Kenya, Malawi, Morocco, Mozambique, Senegal and Sierra Leone (1970–2004) Worldwide (1937–2002)	C (13) C (56)	SNPs analysis, MLVA	African strains dispatched in several groups without clear geographical and temporal systematization	[86, 87]
Goma in DRC (1994) Equatorial-Guinea (N/A) Worldwide (1910–2005)	C (9) C(3) C & E (130)	MLVA	Separate clustering of congolese and guinean strains	[85]
Angola (2006), Mozambique (2004–2005) Zambia (1996–2004) Nigeria & Cameroon (2009) Zimbabwe (2008) Ghana (2006) Kenya (2009–2010)	C	Classic PCR +/- Southern blot, gene sequencing, MAMA PCR, MLSA	Recent importation in Africa of two types of atypical/variant El Tor strains secreting the classical toxin: hybrid El Tor (Mozambique, Zimbabwe, Zambia . . .) and altered El Tor (Angola, Zimbabwe, Ghana . . .)	[90–99]
Kenya (2009–2010)	C (170)	MLVA	Multiple simultaneous outbreaks: numerous importations or local reemergences?	[99]

Abbreviations: MAMA, Mismatch Amplification Mutation Assay; MLSA, MultiLocus Sequence Analysis; MLVA, MultiLocus-Variable no. of tandem repeats (VNTRs) Analysis; PFGE, Pulse Field Gel Electrophoresis; SNP, Single-Nucleotide Polymorphisms.

[a] Clinical (C) or environmental (E)

heavy rains, whose collection may provide a safer source of water to the population [64].

Climate Influences on Cholera Transmission

Beside its seasonal variations, the burden of cholera has exhibited important interannual fluctuations in numerous costal African countries. According to the cholera paradigm, these fluctuations could be attributed to global climate interannual variability. Indeed, rainfalls in East and West Africa appear deeply influenced by Pacific ENSO, and African climates may be even more impacted by the Atlantic ocean's SST variations [107]. For instance, early 1990s cholera epidemics in Ghana, Togo, Benin, and Nigeria showed a significant synchrony with rainfall and Indian Oscillation Index [108]. Cholera incidence between 1971 and 2006 in southeastern African countries appeared significantly impacted by SST anomalies at hemispheric scales [109]. Finally, the 2001–2002 outbreaks in the KwaZulu-Natal province of South Africa presented a strong temporal association with local SST, and a 6-month lagged association with marine chlorophyll-a concentration estimated by satellite-sensing [103].

Sometimes, these global climatic forces have provoked local hydrometeorologic disasters, which have been contemporaneous with several cholera epidemics. Examples include floods

and the 2005 outbreak in Dakar [110, 111], floods and the 1994 and 1997 outbreaks in Djibouti city [72, 73]; or cyclones, which were associated with the 1998 Mozambican [32] and with the 2000 Madagascan [112] epidemics. However, according to these reports, these natural disasters did not initiate the cholera outbreak but rather contributed to outbreak expansion particularly in densely populated areas.

Lull Transmission Periods

Cholera around coastal Africa has exhibited repeated lull transmission periods of several years [1], even in high-risk and often designated "endemic" countries like Guinea-Bissau [56], Guinea [113], Côte d'Ivoire [50], Benin [114], Cameroon [115], or Angola [29, 61]. For reasons yet unexplained by the cholera paradigm as described in Southeast Asia, cholera has failed to settle in Cape Verde, Sao Tome and Principe, Comoros [34, 35], Madagascar [37], or Djibouti City [72] in the aftermaths of explosive outbreaks. Cholera has also spared numerous impoverished and overcrowded areas near estuaries or lagoons, including Gambia, Casamance in Senegal, or Gabon. WHO reports and various articles have repeatedly deplored a widespread underreporting of cholera cases [112, 116, 117], notably in Africa where observed lull periods may thus be the consequence of poorly functioning surveillance systems. However, the systematic national surveillance program implemented in Guinea after the 2004–2007 major epidemics [1, 23] identified only 42 cholera cases in 2009 and none in 2010 and 2011. Similarly, Beira City in Mozambique barely recorded cholera cases in 2010 and 2011 despite an enhanced surveillance scheme [104] and irrespective of the vaccination campaign that in late 2003 vaccinated only 10% of its population [118]. On a finer timescale, no study has ever described a continuous cholera transmission in a given area, whatever its location along the African coasts. Conversely, multiyear time-series available for Guinea-Bissau, Guinea [23], Côte d'Ivoire [49], or Mozambique [104] all exhibit numerous periods apparently free from cholera cases.

Environmental Reservoirs of *Vibrio cholerae*

To further explore determinants of cholera transmission along African coasts, several microbiological investigations, summarized in Supplementary Table 1, have searched for evidence of environmental reservoirs of *V. cholerae* or other *Vibrio* species. Most of these 36 identified studies focused on water, aquatic sediment, plankton, or shellfish sampled from brackish estuaries or lagoons in Calabar, Douala, or Côte d'Ivoire. A few others targeted lagoonal waters in Ghana, Togo, and Benin, estuarine waters in Ghana, Luanda, and Beira, city effluents (Douala), fresh water from water tanks, wells, lakes, dams, or rivers (Yaounde, Kenya, Djibouti City), sea water (Ghana, Togo, Benin, Kenya), or marine fish or shellfish (Senegal, Monrovia, Togo, Cameroun, Kenya). Although only 2 studies aimed at identifying viable but nonculturable *Vibrio* through adequate techniques (sensitive membrane antigen rapid test [SMART], cholera direct fluorescent antibody [DFA], polymerase chain reaction [PCR]), all but 2 articles reported *Vibrio* detection in the environment, reaching sometimes high concentrations. *V. cholerae* was isolated in 25 studies, including *V. cholerae* O1 strains in 11 cases. Three studies reported results of El Tor biotyping, and/or Ogawa/Inaba serotyping. Four studies reported the capacity to produce cholera toxin, 3 of which exclusively isolated non-O1 non-O139 *V. cholerae* strains in Côte d'Ivoire. When performed, genotyping always exhibited a clonal similarity with concomitant clinical strains [26, 133, 137]. In most cases, environmental *V. cholerae* O1 strains were either isolated during cholera epidemics, like in Monrovia in 2007–2008 [120], Abidjan in 1996 [78], Calabar in 2006 [128], Douala in 2005–2007 [133], or Luanda in 1992 [102, 137], or during unspecified periods. Sampling organized during lull periods either were negative for *Vibrio* like in Cameroon in 2007 [135], or, like in Côte d'Ivoire, isolated only non-*cholerae Vibrio* [122], unserotyped *V. cholerae* [123], or non-O1/non-O139 V. *cholerae* [126]. *V. cholerae* O1 identified from Beira's estuary water in 2005–2007 [140], and *V. cholerae* O1 Ogawa cultured from an Ebrié Lagoon's alevin in 1991 (cited by [123]), both during interepidemic periods, were not tested for toxin production. In most cases, it was therefore impossible for us to determine if the presence of *Vibrio* in the environment preceded and thus potentially caused a cholera outbreak or if it was the consequence of a human waste contamination secondary to the outbreak. Consequently, the perennial presence of pathogenic *V. cholerae* in coastal African environments seems to have never been confirmed until now.

DISCUSSION AND CONCLUSION

Numerous locations along the African coasts are repeatedly affected by cholera outbreaks. Like in the Bay of Bengal, this coastal cholera exhibits strong links with environmental factors. Main foci are located along estuaries, lagoons, close to mangrove forests, and in island areas where people often neighbor brackish water expanses used for drinking, cooking, and washing. In various areas, cholera incidence appears related to the rainy season and may be influenced by global climatic trends and concomitant hydrometeorologic disasters. These links with marine ecosystems and rainfall are often highlighted to express the role of the environment as an initiator of cholera in African coastal areas. Toxigenic *V. cholerae* has been cultured from water and seafood samples in several occurrences.

Conversely, links between cholera and coastal environments in Africa appear highly heterogeneous. Some epidemics occur during both the dry and cool seasons, and cholera's frequent concurrence with rainfall may reflect processes independent from planktonic reservoirs of *V. cholerae*. Most cholera foci are

located in densely urbanized areas deprived of clean water and proper sanitation, where seasonal rains can periodically favor contamination of wells and surface water resources by washing out waste and excrements from open defecation or by overflowing latrines. This phenomenon was observed in Kindia (Guinea) [23] but also in various areas distant from the coasts like eastern DRC [145] or Lusaka in Zambia [146]. Periodicity of human activities has also been pointed out to explain cholera seasonality, like fishing in Calabar estuary [47] or, in the 19th century, the sailing trade driven by the monsoon winds along the eastern African coast [80–82].

Interepidemic transmission periods are repeatedly observed, even in high-risk coastal locations of endemic African countries. No cholera serosurveys have been published to question the hypothesis of periodic waning of immunity. Evidence remains also insufficient to attest the perennial presence of toxigenic *V. cholerae* in aquatic ecosystems and its implication in sustaining cholera over the long term in a given place and in the emergence of outbreaks in coastal Africa. Available data have thus not allowed definitive determination of whether cholera outbreaks are the consequence of a proliferation of environmental *V. cholerae* in brackish water expanses or if they are due to the importation of strains by travelers. Indeed, coasts, especially port cities and fishing areas, constitute intense exchange zones prone to cholera importations, which are sometimes genetically confirmed. Along coastal Africa, new atypical El Tor strains likely originating in the Bay of Bengal have replaced previous strains in a matter of a few years. This phenomenon is still not completely understood [95] and raises public health concern because those strains have been associated with more severe outcome [94, 147].

Overall, the temporo-spatial distribution and environmental determinants of cholera outbreaks in coastal Africa exhibit complex particularities, which so far remain incompletely explained by the cholera environmental paradigm. Currently, the hypothesis of an environment-to-human genesis of cholera epidemics in coastal Africa lacks the demonstration of perennial aquatic reservoirs of toxigenic strains. Therefore, the understanding of cholera dynamics in this part of the world would highly benefit from interdisciplinary surveys combining prospective ecological and microbiological analyses of water bodies, dynamic temporo-spatial descriptions of epidemics, and genotyping of clinical and environmental *V. cholerae* isolates. Such data would be invaluable to improve cholera preparedness and response plans implemented in Africa. The evidence of an environmental origin of cholera would indeed justify a careful monitoring of *V. cholerae* aquatic reservoirs and prevention policies specifically targeting their related human activities such as fishing. In contrast, human-borne cholera outbreaks related to imported strains would urge to develop cross-border epidemiological collaboration and to target control efforts on transport facilities and highly mobile populations. It is all the more important to address these key issues in the near future as the spread of recent epidemics has demonstrated the ongoing threat of cholera in Africa, in which the economic context renders it crucial to focus limited resources on the most relevant strategies.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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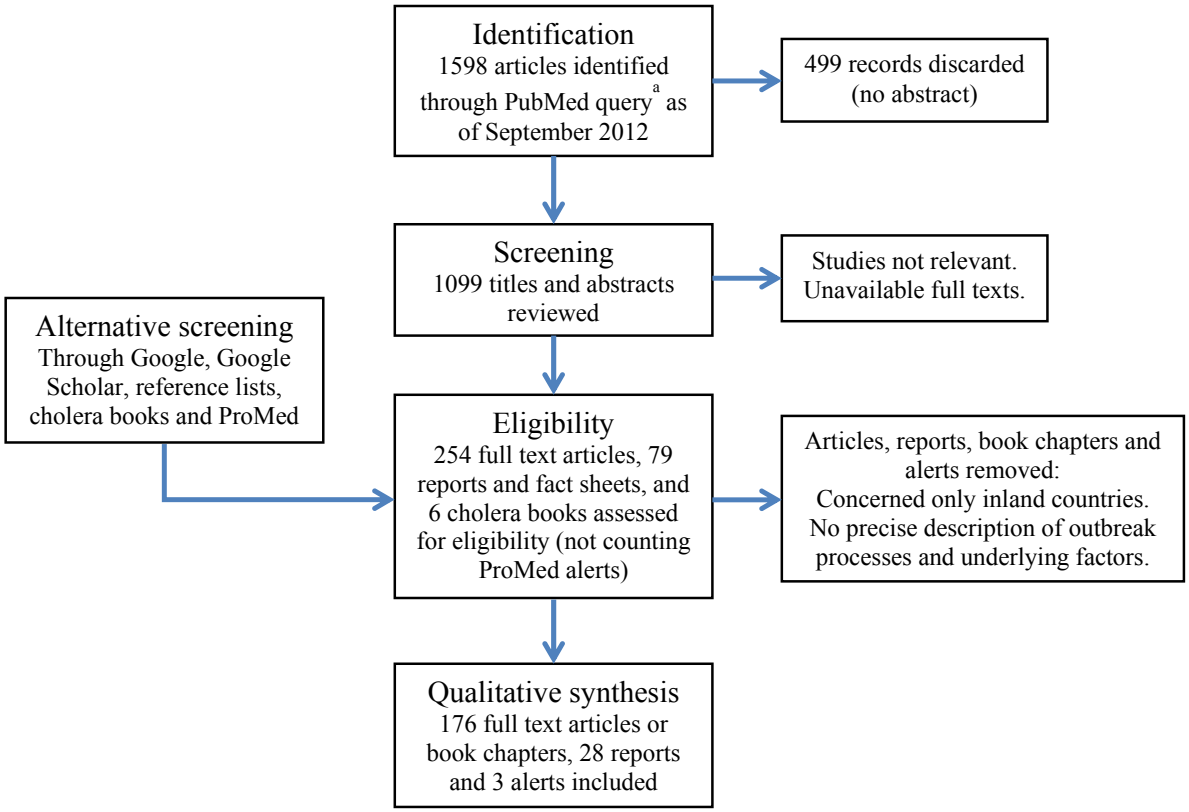
51–147. See the Supplementary data online.

Supplementary Figure 1 – Flow diagram on articles and reports selection for systematic review concerning cholera and environment in coastal Africa

[a] PubMed query : "(cholera OR Vibrio) AND (Africa OR algeria OR angola OR benin OR dahomey OR botswana OR burkina faso OR haute volta OR burundi OR cameroon OR cape verde OR central african republic OR chad OR comoros OR congo OR cote d'ivoire OR ivory coast OR democratic republic of the congo OR zaire OR djibouti OR afar OR equatorial guinea OR eritrea OR ethiopia OR gabon OR ghana OR gambia OR guinea OR guinea-bissau OR kenya OR lesotho OR liberia OR madagascar OR malawi OR mali OR mauritania OR mauritius OR mayotte OR morocco OR mozambique OR namibia OR niger OR nigeria OR rwanda OR sao tome principe OR senegal OR seychelles OR sierra leone OR somalia OR somaliland OR "south africa" OR sudan OR swaziland OR togo OR tunisia OR uganda OR tanzania OR zambia OR zimbabwe OR rhodesia)"



Supplementary Table 1 – Environmental research of *Vibrio cholerae* around coastal Africa

Country	Site	Sampling period	Concomitant status of cholera transmission	Type of samples (detection method)	Isolated strains (no. positive samples)	Comments (Toxigenicity)	References
West Africa							
Senegal	?	July - October 1982	Lull	Sea fish (culture)	non-*cholerae* vibrio (52 strains / 34 fish)	(N/A)	[119]
	?	1995 - 1997	Local epidemic	Water (culture)	*V. cholerae* O1 (?)	Same ribotype as concomitant clinical strains (ND)	[26]
Liberia	Monrovia	2007 - 2008 ?	National epidemic	Sea fish (culture)	*V. cholerae* O1 (2/15)	(ND)	[120]
Côte d'Ivoire	Ebrié Lagoon	1985	Lull	Surface & deep water (culture)	*V. cholerae* (7/378)	(ND)	[121]
	Ebrié Lagoon	July 1987 - July 1988	Lull	Surface & deep water (culture)	*V. parahaemolyticus* and *alginolyticus* (164/501)	No mention of *V. cholerae* (N/A)	[122]
	Ebrié Lagoon (aquaculture)	1991	Lull	Alevin (culture)	*V. cholerae* O1 Ogawa (1/?)	(ND)	[123]
	Ebrié Lagoon (aquaculture)	1991-1992	Lull	Water, plankton, sediment (culture)	*V. cholerae* (between 0% and near 60%)	Vibrio concentration up to 10^8 CFU/L (ND)	[123]
	Ebrié Lagoon (aquaculture)	Between 1992 and 1997	N/A	Water, plankton, sediment (culture)	*V. cholerae* O1 El Tor Ogawa (?)	(ND)	Cited by [123]
	Ebrié Lagoon in Abidjan	Summer 1996	National epidemic	Plankton (SMART, DFA)	*V. cholerae* O1 (3/12)	Concomitant water samples negative (ND)	[78]
	Grand-Lahou Lagoon	2003-2004	End of national epidemic	Water (culture)	*V. cholerae* non-O1 non-139 (up to 20%)	PCR negative for *ctxA* and *tcpA* genes (ABSENT)	[124]
	Abidjan (well, lagoon, drainage ditches)	February - March 2012	Post-epidemic	Water (culture)	*V. cholerae* non-O1 non-139 (9 strains / 362 samples)	PCR negative for ctxA and tcpA genes (ABSENT)	[125]
	Ebrié Lagoon	June 2009 - December 2010	Lull	Shrimps and crabs (culture)	non-*cholerae* vibrio and *V. cholerae* non-O1 non-139 (25/322)	Mean concentration > 6 Log CFU/g. PCR negative for ctxA and ctxB genes (ABSENT)	[126]
Ghana	Atlantic Ocean ; lagoon of Keta and mouth of the Volta River	1972	Low national epidemic	Water (culture)	*V. parahaemolyticus* (4/8 from ocean and lagoonn of Keta)	No mention of *V. cholerae* (N/A)	[127]
Togo	Atlantic Ocean ; Lake Togo, Anécho and Lomé lagoons	February 1972 - April 1973	End of low epidemic	Water (culture)	*V. parahaemolyticus and alginolyticus*	No mention of *V. cholerae*	[127]

					(from 5% to 57% of water samples)	(N/A)	
	Atlantic Ocean ; Lake Togo and Lomé lagoons ; markets of Lomé	February 1972 - April 1973	End of low epidemic	Fish (culture)	*V. parahaemolyticus* (from 1% to 68% of fish samples)	No mention of *V. cholerae* (N/A)	[127]
Benin	Atlantic coast ; coastal lagoon and Lake Nokoué	1972	Low national epidemic	Water (culture)	*V. parahaemolyticus* (4/12 from lagoons and Lake Nokoué)	No mention of *V. cholerae* (N/A)	[127]
Nigeria	Calabar estuary	1989	Local low epidemic	Water sediment (culture)	*V. cholerae* (?)	Shellfish negative (ND)	[47]
	Calabar estuary	2006	Local low epidemic	Shellfish (culture)	*V. cholerae* O1 El Tor (1/308 [shrimp])	Rest of shellfish heavily contaminated by other vibrio (ND)	[128]
	Calabar estuary	?	?	Shrimp (culture)	*V. cholerae* (?)	(ND)	[129]
	Calabar estuary	?	?	Clams (culture)	non-*cholerae* vibrio (64/110)	(N/A)	[130]
	Calabar, restaurants' freezers	?	?	Stagnant water (culture)	*V. cholerae* 01 (3/8)	(ND)	[131]
Central Africa							
Cameroon	Douala slum	2004	Local epidemic	Water from swamps, wells (culture)	*V. cholerae* (?)	(ND)	[71]
	Douala estuary	2005	Local low epidemic	Water (culture)	None 0	Culture media non specified (N/A)	[132]
	Douala, major drains	2005-2007	Local low epidemic	Water (culture)	*V. cholerae* (48/767) *V. cholerae* O1 (2 strains)	O1 strains, genetically similar to concomitant clinical strains (ND)	[133]
	Yaounde (river, wells)	2006	Local low epidemic	Surface water (culture)	*V. cholerae* (Up to 79%)	All wells negative (ND) (ND)	[134]
	Coast of South-West Region	2007	Lull	Fish (culture)	None 0	Culture media non specified (N/A)	[135]
	Coast of South-West Region	?	?	Shrimps (culture)	Vibrio spp. (73/236) *V. cholerae* (1/4 of strains)	(ND)	[136]
Angola	Luanda, Bengo River estuary	April 1988	Local epidemic	Water (culture)	Vibrio spp. (?)	(ND)	(MSF, unpublished, cited by [102])
	Luanda, Bengo River estuary	1992	Local epidemic	Water (culture)	*V. cholerae* O1 toxigenic (100%)	Water supplying the city. Vibrio concentration > 100 CFU/L. Same clone as concomitant clinical strains **(PRESENT)**	[102,137]
Austral Africa							

South Africa	Alice Town, Eastern Cape Province (Wastewater treatment plant)	?	?	Wastewater treated effluents (culture and PCR)	*V. fluvialis*, *V. vulnificus*, *V. parahaemolyticus, V. metschnikovii* (52 strains)	Vibrio concentration up to 1.9 x10^5 CFU/mL ; no *V. cholerae* found (N/A)	[138]
	Dimbaza and East London, Eastern Cape Province (Wastewater treatment plant)	August 2007 - July 2008	Lull	Wastewater treated effluents (culture and PCR)	*V. fluvialis*, *V. vulnificus*, *V. parahaemolyticus* (*V. metschnikovii* ?) (108 strains)	Vibrio concentration up to 1.8 x10^5 CFU/mL ; no *V. cholerae* found (N/A)	[139]
Mozambique	Beira estuary	2005 - 2007	Local epidemic and interepidemic	Water (DFA)	*V. cholerae* O1 (48/99) *V. cholerae* O139 (6/99)	Also detected in fish and surrounding lakes, PCR and culture all negative (ND)	[140]
East Africa							
Kenya	Markets at coast and up country	?	National epidemic	Sea and lake fish, crustacean, molluscan (culture)	*V. parahaemolyticus* (53/584) *V. alginolyticus* (100%)	No *Vibrio cholerae* found (N/A)	[141]
	Nairobi and Mombasa markets	?	?	Fresh marine fish, fresh and dry lake fish, crustaceans, molluscs ; sea water and sediments (culture)	*V. parahaemolyticus* (74/912)	Pretreatment of samples by GSTB +/- copper sulphate. No mention of *V. cholerae* (N/A)	[142]
	Lakes, river, dam and coastal sea	?	?	Fresh fish, water	*V. parahaemolyticus* (29/666)	No mention of *V. cholerae* (N/A)	[143]
Djibouti City	Water tank, well	1993	Local epidemic	Water (culture)	V. cholerae O1 (?)	Tap water negative (ND)	[72,144]

The Seventh cholera Pandemic has been mainly caused by *Vibrio cholerae* serogroup O1, biotype El Tor, serotype Ogawa or Inaba, and, to a lesser extent and only in Asia, by *Vibrio cholerae* O139
PCR, Polymerase Chain Reaction
SMART, Sensitive Membrane Antigen Rapid Test
DFA, Cholera Direct Fluorescent Antibody
GSTB, glucose salt teepol broth
ND, no data
N/A, not applicable

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SUPPLEMENT ARTICLE

Environmental Determinants of Cholera Outbreaks in Inland Africa: A Systematic Review of Main Transmission Foci and Propagation Routes

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Cholera is generally regarded as the prototypical waterborne and environmental disease. In Africa, available studies are scarce, and the relevance of this disease paradigm is questionable. Cholera outbreaks have been repeatedly reported far from the coasts: from 2009 through 2011, three-quarters of all cholera cases in Africa occurred in inland regions. Such outbreaks are either influenced by rainfall and subsequent floods or by drought- and water-induced stress. Their concurrence with global climatic events has also been observed. In lakes and rivers, aquatic reservoirs of *Vibrio cholerae* have been evocated. However, the role of these reservoirs in cholera epidemiology has not been established. Starting from inland cholera-endemic areas, epidemics burst and spread to various environments, including crowded slums and refugee camps. Human displacements constitute a major determinant of this spread. Further studies are urgently needed to better understand these complex dynamics, improve water and sanitation efforts, and eliminate cholera from Africa.

Keywords. cholera; *Vibrio cholerae*; Africa; epidemiology; environment; lakes; cities; seasons; reservoirs.

During the past decade, except for the current Haitian outbreak, which is linked to the importation of a cholera strain from Asia [1, 2], most cholera epidemics, cases, and deaths have been reported in sub-Saharan Africa [3–5]. In 2009, for instance, 98% of the 221 226 notified cases worldwide were from Africa [6]. Yet, most studies assessing the determinants of cholera outbreaks have been conducted in the Bay of Bengal estuaries, its traditional home, or in other coastal areas all over the world. They have revealed strong links between this prototypical waterborne disease, aquatic environments, and climate. Designated as the cholera paradigm by Colwell [7], these links have been assumed to be relevant worldwide.

Nevertheless in recent years, remarkable epidemics struck various African regions located far from the coast. For instance, in 2008–2009, Zimbabwe experienced the largest cholera outbreak ever recorded in Africa, with >100 000 cases and 4000 deaths [8]. In 2010–2011, the Lake Chad Basin, a Sahelian region between Nigeria, Niger, Chad, and Cameroon, was also severely affected [9]. These examples stress the need to better characterize cholera outbreaks in noncoastal regions of Africa and their links with coastal cholera outbreaks. They also question the coastal origin of the recent cholera epidemics in Africa. Although many inland outbreaks have been described by specific reports, no thorough review has compiled these data and specifically addressed this problem. The present article aims more particularly at exploring the main environmental determinants of cholera epidemics in inland Africa.

MATERIALS AND METHODS

A systematic PubMed query was conducted using the terms ["cholera OR Vibrio cholerae"] AND ["Africa" OR the current or past names of all sub-Saharan African countries] between 1970 and September 2012. Given the extent of the issue, citations were selected for articles published in English or French, whose title or abstract addressed cholera or *Vibrio cholerae* infection outbreaks or epidemiology in Africa. Complementary

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The Journal of Infectious Diseases 2013;208(S1):S46–54

DOI: 10.1093/infdis/jit195

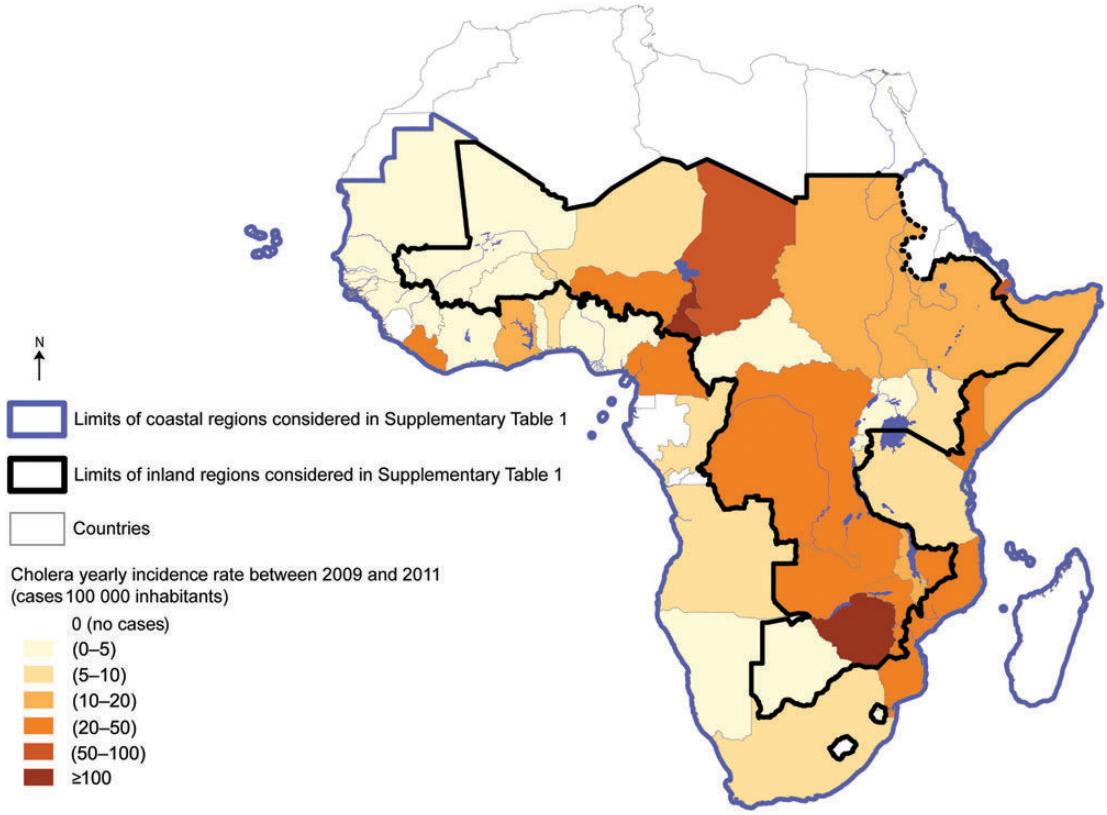

Figure 1. Cholera yearly incidence rates in inland and coastal Africa. Incidence rates were computed using data in Supplementary Table 1. The bold line denotes the borderline between inland and coastal regions, according to available subnational cholera and population data. The dotted black line is used for Sudan because no precise cholera data at the state scale could be obtained. Nevertheless, various reports indicate that cases were almost exclusively located in southern Sudan, around Khartoum, and in Kordofan and Darfur states.

articles from nonindexed journals and reports from various agencies were additionally searched using Google, Google Scholar, and reference lists from key textbooks and other articles. ProMED-mail alerts were also investigated by searching the archives (available at: http://www.promedmail.org) for the term "cholera" and relevant country names. This screening process was performed independently by 2 of the authors (S. R. and B. S.). Selected full texts were assessed as eligible for inclusion if they gave information on cholera morbidity or outbreak processes. Data describing cholera outbreaks were extracted, including exact location and local environmental characteristics; year and season of beginning, peak, and ending; population affected; epidemic dynamics; suspected origin and underlying factors; local environmental isolation of *V. cholerae*; and genotyping of epidemic strains. In the present review, only reports relevant for countries having no access to the sea (defined as "inland countries") and, if available, for regions of seaside countries located >100 km from the coast or from an estuary (defined as "inland regions"), were included (Supplementary Figure 1). Links between cholera and the environment in coastal African regions have been addressed in a distinct review [10].

To assess the relative importance of both coastal and inland regions in the overall cholera burden in Africa, cases reported to the World Health Organization (WHO) between 2009 and 2011 were analyzed (Supplementary Table 1). When morbidity and population data were available at subnational levels, countries with access to the sea were divided into 2 coastal and inland regions. Their respective cholera cases numbers and incidence rates were then calculated and mapped using Quantum GIS software (QGIS), version 1.7.3 (available at: http://www.qgis.org/), and ESRI shape files from the Map Library (available at: http://www.maplibrary.org; Figure 2). After aggregation of these regions and exclusion of North African countries (which have barely notified cholera cases for the past 2 decades), case numbers and yearly incidence rates were computed for both coastal and inland Africa.

RESULTS

Predominance of Cholera in Inland Africa

According to the yearly cholera global surveillance summaries of the WHO [11], one third of the 1.5 million cases reported in Africa between 2001–2010 were located in inland countries.

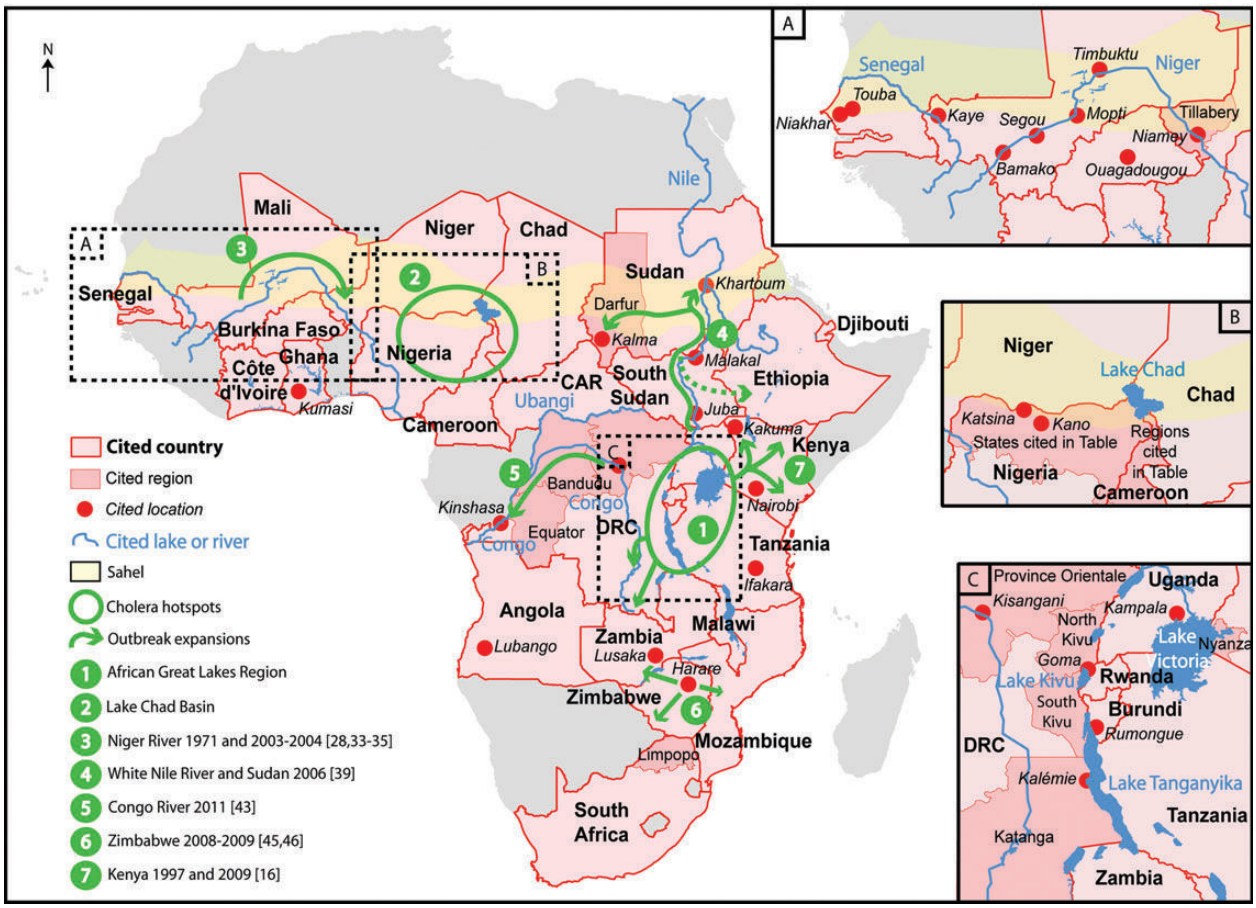

Figure 2. Places cited in the text, main cholera hotspots and outbreak expansions. Abbreviations: DRC, Democratic Republic of the Congo; CAR, Central African Republic

Furthermore, according to ProMED-mail [3, 12], a few national reports from the WHO [13–16], and a transborder epidemiological assessment in the Lake Chad Basin [9], many major outbreaks affecting countries having access to the sea actually occurred in their inland areas (Figure 2).

Thus, taking into account subnational morbidity and population data available for Nigeria, Cameroon, Democratic Republic of the Congo, Mozambique, Kenya, and Sudan, as well as national data for the other countries (Table 1, Figure 1, and Supplementary Table 1), it can be estimated that a minimum of 76% of all reported cholera cases in sub-Saharan Africa actually affected noncoastal regions in 2009–2011. During this period, the yearly incidence rates in inland and coastal Africa were 72.86 and 26.75 cases/100 000 inhabitants, respectively.

Geographical Determinants: The Role of Lakes and Rivers

For the past 2 decades, most cases reported in Africa have indeed clustered in 2 lakeside locations: the African Great Lakes Region [17] and the Lake Chad Basin [9]. The African Great Lakes Region spreads along the Albertine Rift and comprises parts or totality of the Democratic Republic of the Congo (formerly Zaire), Uganda, Kenya, Rwanda, Burundi, and Tanzania. Sprinkled with lakes, this overpopulated and repeatedly war-torn area has hosted many refugee camps and concentrated many cholera cases. Since its likely importation from Tanzania in 1978, cholera has annually been encountered in eastern Democratic Republic of the Congo, especially along the shores of Lake Kivu and Lake Tanganyika. Exhibiting a meta-stable pattern, in which cholera stability on a regional scale originates from interactions between asynchronous local foci prone to extinction [17], this area has accounted for most of the 370 000 cases reported by the country during this period [11, 17]. Further south and north along the Rift, cholera has also repeatedly affected other lakeside regions, in Zambia [18, 19] and Ethiopia [20, 21]. Proximity to the lakes was a significant risk factor for cholera in several ecological studies conducted at various geographical scales in Kenya [22], Democratic Republic of the Congo [23, 24], and Rumonge, a

Table 1. Estimates of the Burden of Cholera in Inland and Coastal Regions of Sub-Saharan Africa During 2009–2011

Variable	Total Sub-Saharan Africa[a]	Inland Africa[b]	Coastal Africa[b]
Population in 2010[b]	850 274 779	451 945 491	398 329 288
2009			
Reported cholera cases[b]	219 601	176 181 (80)	43 420 (20)
Estimated incidence rate[c]	25.83	38.98	10.90
2010			
Reported cholera cases[b]	110 480	87 403 (79)	23 077 (21)
Estimated incidence rate[c]	12.99	19.34	5.79
2011			
Reported cholera cases[b]	105 786	65 714 (62)	40 072 (38)
Estimated incidence rate[c]	12.44	14.54	10.06
2009–2011			
Reported cholera cases[b]	435 867	329 298 (76)	106 569 (24)
Estimated yearly incidence rate[c]	51.26	72.86	26.75

Data are no. or no. (%) of cases, unless otherwise indicated.

[a] Morocco, Algeria, Tunisia, Libya, and Egypt were excluded from analysis.

[b] For definitions and references, see Supplementary Table 1.

[c] Data are cases/100 000 inhabitants.

Burundian town on the shore of Lake Tanganyika [25]. On these lakesides, cities like Goma and Kampala have experienced particularly severe outbreaks. In 1994, during the weeks following the genocide in Rwanda, almost a million persons sprawled into the neighboring North Kivu province of Zaire [26]. In the huge refugee camps that burgeoned around Goma on the northern shore of Lake Kivu, *V. cholerae* infected virtually everyone and caused at least 50 000 cholera cases. Together with dysentery, it claimed almost 50 000 lives during July alone. With less intensity, cholera also struck Uganda's capital, Kampala, in 1997–1998. In only 4 months, this lakeside city counted >6000 cases scattered around most of its parishes [27]. On the Kenyan side of Lake Victoria, Nyanza Province has been repeatedly affected and appeared as the origin of important national outbreaks both in 1997 and 2009 [16].

The seventh cholera pandemic hit the Lake Chad Basin in mid-1971 [9] and was imported by patients traveling north from coastal Nigeria and west along the Sahelian belt. While the following 2 decades were marked by occasional flares in the region, there have been several thousand cases of cholera annually since the mid-1990s. Areas surrounding Lake Chad have thus become the most affected regions in its 4 bordering countries (Figure 3). The situation recently worsened, with a severe outbreak affecting >100 000 people in the region during 2010–2011 [13, 14, 28–31].

Spread of cholera across the African continent has also benefited from human traffic along its main rivers, notably in Sahel. Following the Niger River downstream from Mopti, Mali, cholera struck Niger heavily in 1971, causing almost 10 000 cases in towns and fishing villages of the riverbanks [32]. In 2003–2004 and 2011, new outbreaks spread along the Niger River in Mali (in Bamako, Segou, Mopti, and Timbuktu) [33–35] and Niger (in Tillabery and Niamey provinces) [28]. And in 2005 cholera was also reported in the Senegal River valley in Mali (in Kayes). Further east in Sudan, traffic along the White Nile River obviously contributed to the spread of the 2006 cholera outbreak from the Juba area up to Malakal and Khartoum [36–39], before it moved west and reached Darfur by a passenger train [40]. The same phenomenon has recently been observed in the vast equatorial forest of Central Africa, along the Oubangui River in the Central African Republic, in 1997 [41], and along the Congo River in the Democratic Republic of the Congo. A cholera epidemic started in February 2011 in Kisangani, a major river port within Province Orientale. Descending the Congo River, cholera crossed the downstream provinces of Equateur and Bandundu and eventually traveled 2000 km to the megacity of Kinshasa in only 130 days. On its cruise, it affected >7000 people, including 10% in Kinshasa, and killed >300 individuals [42, 43]. Between 1996 and 2001, Kinshasa had previously been affected by a double-peaked epidemic involving >5000 notified cases and about 300 deaths [43]. In 2011, like a decade earlier, the most affected quarters of the capital were located along the Congo River and one of its tributaries [43, 44].

In 2008–2009, Zimbabwe was struck by a 100 000-case epidemic [8], whose dynamic did not appear to be associated with any lake or river. It originated in Harare and Chitungwiza, 2 urban centers that remained the most affected areas, and then spread to the other provinces and neighboring countries [45, 46]. In Harare, cases clustered in very poor and lowly elevated suburbs, such as areas where people from Chitungwiza arrive daily to work in adjacent informal markets [46, 47].

Seasonal and Climatic Determinants

As in many coastal regions, precipitation patterns have had an important influence on temporal patterns of cholera epidemics in Uganda [48], Zambia [18, 49, 50], and Malawi [51]. In Kinshasa, cases between December 1998 and March 2001 were significantly correlated with rainfalls, with a 7-week time lag [43]. Between 2002 and 2008 in eastern Democratic Republic of the Congo, the impact of rainfall on cholera appeared more profound as the distance from the equator increased [17, 24]. Yet, these seasonal patterns may reflect the seasonal variations of human exposure to contaminated water, as in temporary fishing settlements on the Congolese [17] and Zambian [18]

shores of Lake Tanganyika, and the seasonal variations of human movements, such as those occurring between these fishing camps and the markets of surrounding cities. With regard to the Lake Chad Basin, past observations and field studies conducted during the recent and major 2010 and 2011 epidemics suggest a correlation with the rainy season in this lakeside region, too [9, 29]. In the rest of Sahel, cholera seasonality appears to be complex. Whereas epidemics repeatedly struck drought- and famine-affected areas of Mali during the 1970s and in 1984–1986 [35, 52], the major outbreaks of the last 20 years mainly started during the rainy season [34, 35]. Cholera in Niger was also associated with severe droughts in 2004, but its resurgence in 2006 followed excessive rains [32]. In 2005, cholera affected the Ouagadougou area of Burkina Faso during the rainy season [53]. And while it usually affects Sokoto and other northern states of Nigeria during the rainy season [54], cholera was reported in Katsina at the end of the 1982 dry season in the context of acute water shortage [55] and hit Kano state at the end of several dry seasons, too [56]. Further east, in 2006, 30 000 people from the area that is now Sudan and South Sudan experienced a severe cholera outbreak that started during the dry season and extended throughout the year [57]. Floods have also favored extension of cholera outbreaks in both countries [58, 59]. Conversely, cholera struck Kakuma Camp in 2005 and 2009, despite the very dry climate of this northwestern region of Kenya [60, 61]. Finally, the 2008 outbreak in Zimbabwe was reported long before the beginning of the rainy season [62].

Cholera incidence fluctuations in inland Africa are also linked to interannual climate variability. In East and West Africa, rainfall levels are deeply influenced by El Niño–Southern Oscillation (ENSO) events, the periodic warming of surface waters across the central equatorial Pacific Ocean [63]. At the continental scale, the strong 1997–1998 ENSO event and its associated higher temperatures and flooding coincided with an increased number of outbreaks reported to ProMED [3]. Between 1978 and 2008 in the African Great Lakes Region, years with a large increase in cholera incidence significantly correlated with abnormally warm ENSO events [17]. In addition, cholera outbreaks over a 30-year period in the Lake Victoria Basin also seemed to coincide with peaks of high river flow during ENSO events [64]. Yet on the Kenyan side of the lake and on a finer time scale, the 1997 outbreak in Nyanza province actually started before the floods, and the 2008 outbreak began almost 1 year after the ENSO rains [65].

Human-Related Determinants

Apart from spatial and temporal determinants, several human-related factors have been associated with cholera transmission in inland Africa. Numerous outbreaks have affected slums of inland cities, such as Ouagadougou [53]; Kumasi, in Ghana [66]; Bauchi and other northern Nigerian urban centers [9]; Kinshasa [43]; Addis Ababa, in Ethiopia [67, 68]; Nairobi, in Kenya [69]; and Lusaka, in Zambia [70, 71]. Markets and other trading areas have been pointed out as possible sources in Kumasi [72], northern Central African Republic [41], Kinshasa [43], the Lubango area in Angola [73], Harare [46], and a refugee camp in Malawi [74]. Several such camps populated by refugees and internally displaced persons have been stricken [75], not only in Goma [26], Malawi [76, 77], and Zimbabwe [78] during the Mozambican civil war, but also in Kenya, in 2005 [60] and 2009 [61], and in Darfur, in 2006 [39]. Cholera has sometimes taken advantage of other social and geopolitical events, like the Touba Pilgrimage in Senegal in 2005 [79, 80], the economical collapse of Zimbabwe [45, 81], and conflicts in Rwanda [26] or Sudan [39]. The severity of outbreaks may sometimes have been exacerbated by impaired nutrition and immunity among affected populations, as suggested during the 1970s and 1980s Malian famines [52] or in the Goma camps [26]. Yet cholera transmission in these situations has mainly benefited from promiscuity, a significant risk factor reported in a rural Senegalese area in 1996 [82]; in Kumasi, between 1999 and 2005 [66, 72, 83]; in the Lake Chad Basin, between 2003 and 2010 [9]; or in Kinshasa, in 1998–1999 [43].

As suggested by the drought-associated epidemics in Sahel in the early 1970s and in 1984, when refugees had to crowd around limited water supplies [52], deprivation of clean water is another key factor of cholera transmission, which has been frequently incriminated in various report and in ecological, case-control, and microbiological studies (Supplementary Table 2). In Zimbabwe, for instance, a comparison between 2 outbreaks in 1992 suggested that access to a protected water supply and a population density per borehole were both important factors in explaining the spatiotemporal distribution of cholera cases [78]. More recently, in 2008, the outbreak in Chitungwiza was clearly enhanced by a breakdown in the water supply, which compelled people to rely on shallow wells for drinking water [45, 81]. Drinking from or bathing in lakes or rivers contaminated with *V. cholerae* have been associated with cholera transmission in several places, including Burundi [25, 84], Tanzania [85], and South Africa [86]. In this latter country, some people have been reported to prefer drinking fresh river water instead of water from safer sources [87].

Associated impaired sanitation has also been involved in numerous contexts (Supplementary Table 2). Cholera has repeatedly benefited from the scarcity of pit latrines in areas with very poor living conditions, such as slums and camps, locations where rocky volcanic soils make digging difficult, and regions where there is a cultural preference for open defecation. In the over crowded Goma camps, for instance, most refugees had to defecate in open spaces. And because overwhelmed relief organizations could only provide a mere liter of purified water to each person per day, cholera was related to the practice of drinking untreated water from subsequently contaminated ponds

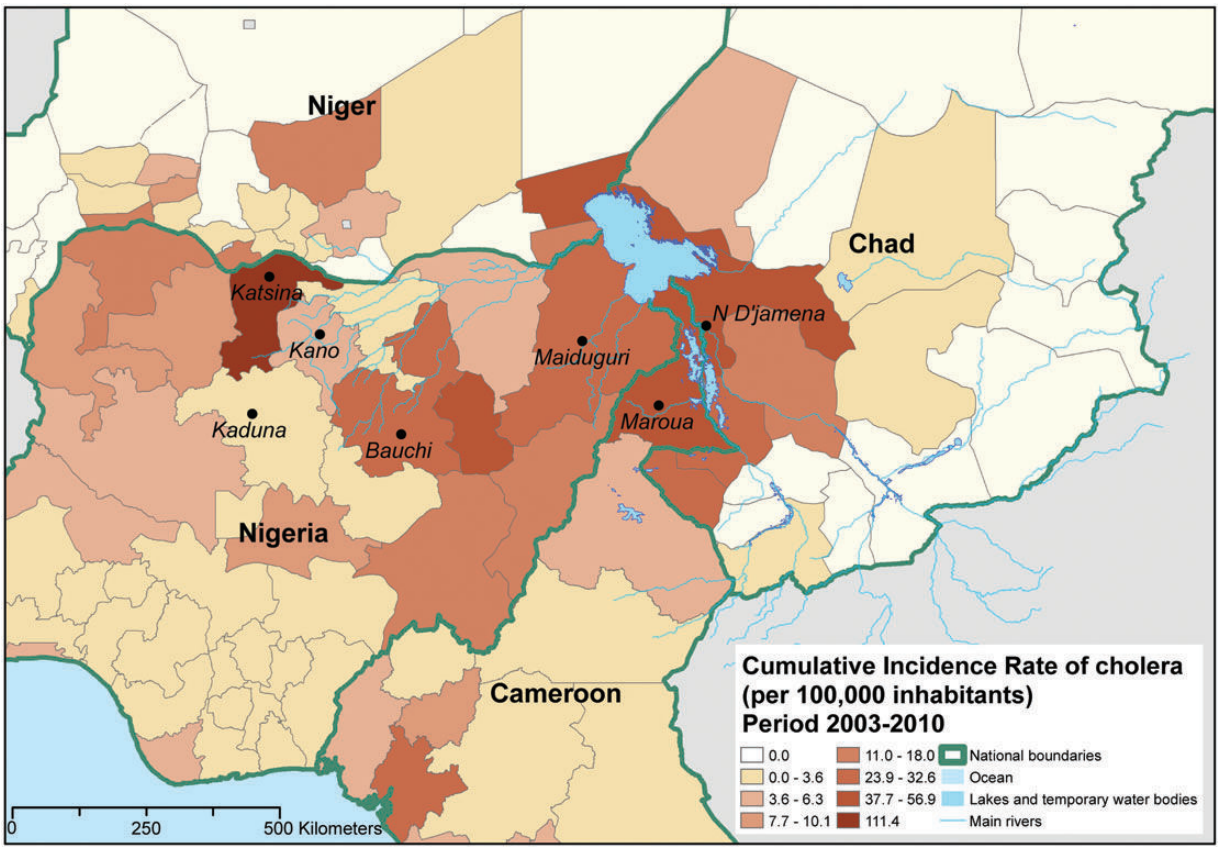

Figure 3. Incidence of cholera in countries of the Lake Chad Basin between 2003 and 2010.

and Lake Kivu [26, 88]. In addition to the lack of latrines [70], the cholera incidence in Lusaka was also statistically associated with insufficient drainage networks [50]. In Kumasi, it was significantly correlated with the concentration of and proximity to refusal dumps [66, 83]. Together, the frequently observed influence of rainfall levels may thus be related to latrine overflow and flushing of human waste, as proposed in urban areas like Kumasi [66], Juba [89], Lusaka [50], and Harare [47] or rural areas like southern Malawi [90, 91].

Cholera transmission in Africa has also been exacerbated by bad domestic storage conditions and lack of efficient treatment of water (Supplementary Table 2). Numerous epidemics have been related to unhygienic practices, including the lack of soap use after defecation and before meals and hand eating from common plates (Supplementary Table 2). Attendance at funeral feasts has been repeatedly described as risk factor, especially when individuals used bowel enemas to clean the body of cholera victims prior to preparing the meal as reported in Tanzania for example [92].

Various foods been associated with cholera transmission in Africa (Supplementary Table 2) and around the world [93]. Consumption of cooked but nonreheated leftover food was a significant risk factor in several case-control studies. Conversely, cooking with acidic ingredients, such as lemon, tomato, or curled milk, seems protective (Supplementary Table 2). The impact of Ramadan is equivocal and barely studied: whereas collective meals with raw vegetables and fruits have been suspected to favor cholera transmission in Nigeria [56], fasting may limit the consumption of leftover food and the number of water sources accessed.

According to Mintz et al [93], direct person-to-person transmission of cholera is "not expected." Except for limited documented outbreaks in a gold mine and several pediatric wards (Supplementary Table 2), this transmission route does not seem to have played a prominent role in the spread of cholera in Africa.

V. cholerae–Related Determinants and Environmental Reservoirs

Whatever its route, transmission of cholera implies sufficient survival or multiplication of toxigenic *V. cholerae* outside the human gut, as reviewed elsewhere [93, 94]. In particular, vibrios proved able to grow in a few African foods, like millet gruel or peanut sauce (Supplementary Table 2). The role of at-risk lakes

and rivers has been explored by only a few environmental microbiological studies, and toxigenic *V. cholerae* has scarcely been isolated from water samples in inland African settings (Supplementary Table 2): rivers, canals, and a trough in South Africa [86, 87]; a well in Mali [52]; Lake Tanganyika, in Burundi [25]; a river in Tanzania [85]; and several previously identified but nonspecified foci in Sudan [95]. In South Africa, Mali, Burundi, and Tanzania the positive specimens were obtained only during epidemic periods; in Lake Tanganyika *V. cholerae* could not be isolated 2 months later; the time since the previous outbreak was not mentioned in the Sudanese study. Overall, a perennial presence of *V. cholerae* in inland African environments has therefore never been expressly confirmed so far.

Several putative environmental reservoirs of *V. cholerae* have been proposed [96], such as copepods (ie, ubiquitous microscopic crustaceans and main constituents of zooplankton), chironomids (ie, nonbiting midges abundant in freshwater habitats such as African lakes), and water hyacinths. But caution is required in interpreting these data. On the Congolese shores of Lake Tanganyika, the temporal correlation between cholera incidence and satellite-estimated concentration of chlorophyll *a*, a remote surrogate marker of copepod population, may only be a synchronous consequence of rainfall: a plankton bloom induced by an increase in terrestrial nutrients and a fecal contamination of lake waters induced by the overflow of latrines [17]. Despite interesting experiments, chironomid egg masses and flying adults have to date been found to harbor and carry only nonpathogenic strains of *V. cholerae* [97, 98], and the rough correspondence between the directions of dominant winds and cholera spread in Africa during 1970–1971 and 2005–2006 remains weak support for the iconoclastic hypothesis of anaeroplanktonic transport of *V. cholerae* [99]. A positive association between the number of cholera cases and the yearly water-hyacinth coverage has been exhibited only in Nyanza Province in the Kenyan section of Lake Victoria [65]. Finally, *V. cholerae* has been associated with *Acanthamoeba* in Sudanese samples [95], suggesting that this free-living amoebae may enhance the survival of vibrios, as previously described [96]. Yet these hypotheses have not been confirmed anywhere else in Africa.

Spread of Cholera Epidemics

Rather than emerging from such putative environmental reservoirs, several cholera epidemics in inland Africa have been historically described as the spread of distant epidemics, as illustrated by the initial course of the seventh pandemic in Africa during 1970–1971 [100, 101], when cholera traveled from Abidjan to Mopti and then to Niger, and from coastal Nigeria and Sahel to the Lake Chad Basin. Other diffusions of cholera in inland Africa more recently described in the literature are illustrated in Figure 2.

In several instances, molecular typing of strains indicated that outbreaks corresponded to the importation of new *V. cholerae* strains. Ribotyping suggested that the 1997 epidemic in the Central African Republic corresponded to simultaneous cholera importation from Chad in the north and the Democratic Republic of the Congo in the south [41]. Similarly, multilocus variable tandem repeat analysis of Kenyan strains sampled in 2009–2010 identified 5 concurrent clonal complexes, which were not randomly distributed [102] and may have been imported from surrounding foci. Emergence of new atypical El Tor strains secreting the classical cholera toxin has been recently confirmed in Zambia [103] and Kenya [102, 104]. This may explain the severe epidemics that affected Zimbabwe in 2008–2009 [105] and the Lake Chad Basin in 2010 [9, 106]. Conversely, some other inland outbreaks have been characterized as locoregional resurgences of previous outbreaks. In the Lake Chad Basin, the 2010 epidemic emerged, after an interepidemic period of several months, from residual epicenters in northeastern Nigeria [9]. In the African Great Lakes Region between 2002 and 2008, all hotspots exhibited noncompletely synchronous lull periods, after which they were likely recolonized from other lakeside areas still undergoing cholera outbreaks [17].

DISCUSSION AND CONCLUSION

The major finding of our review is that most of the cholera cases recently recorded in Africa concerned inland areas, while maritime and estuarine locations represented only a minority of the total recorded cases. Moreover, these inland African cholera foci appear to a great extent to be unrelated to coastal affected areas. Such a development of cholera in regions distant from the coasts was previously notified in the Americas during the 1990s. Indeed, even if cholera mostly affected the coastal regions of Peru [107, 108], Brazil [109, 110], Ecuador [107, 111], or Mexico [112], epidemics also deeply struck inland in areas such as Bolivia, the Peruvian Amazon basin [107], northern parts of Argentina [113], and central Mexico [112]. Yet, unlike in Africa, cholera did not took root on this continent. In Asia, it has also recurrently attacked inland countries, such as Nepal and Afghanistan [11].

In inland Africa, the cholera distribution exhibits an important spatial heterogeneity. It particularly affects the African Great Lakes Region and the Lake Chad Basin, 2 hotspots that have notified cases every year during the past decade. Its spread generally follows natural communication routes, such as the Niger, the Congo, and the White Nile rivers, as well as major roads or railways [23, 101]. Currently, only a few inland cholera epidemics seemed to originate in coastal areas, and such inward diffusions have rarely been reported, even in Cameroon and Nigeria, where cholera regularly affects both coastal and Lake Chad Basin areas [9]. Conversely, numerous outbreaks in the Lake Chad Basin and the African Great Lakes Region obviously emerged locally before spreading within the continent.

Despite the remoteness from potential coastal and estuarine environmental reservoirs, cholera outbreaks in inland Africa frequently concur with the rainy season [17, 24], a phenomenon that was also observed at the worldwide scale [114]. In addition to the seasonal variability in cholera incidence, our review shows that interannual variations also occur in inland Africa and have partly been correlated with climate variability, being sometimes exacerbated by ENSO-related floods and severe droughts. By analogy with South Asian estuaries, cholera flares in lakeside African regions during the rainy season have been proposed as the consequence of plankton blooms induced by the increase in levels of terrestrial nutrients. Yet they could instead reflect the fecal pollution of shallow wells and surface waters induced by the overflow of latrines and the washing of waste. Conversely, epidemics during the dry season may be enhanced by scarce and easily contaminated water sources and by prolonged storage of domestic water in unsafe conditions.

Overall, cholera in inland Africa appears to be a highly dynamic process in which the strains probably move from one area to another, through a fecal-oral route, following trade and human population displacements. Unfortunately, very few studies have genotyped and compared *V. cholerae* strains to confirm this hypothesis on the basis of epidemiological observations. Similarly, very few environmental microbiological studies have addressed the potential role of perennial aquatic reservoirs of *V. cholerae* in inland Africa. Consequently, the source of outbreak recurrences in inland Africa is still debatable. In accordance with the cholera paradigm, they could be due to the persistence of toxigenic *V. cholerae* strains in some lakes' ecosystems. More likely, they may be due to asynchronous local outbreaks circulating between neighboring areas, as described in the African Great Lakes Region [17].

In conclusion, inland cholera is emerging as a relevant and major epidemiological entity in Africa. Proximity to possible coastal environmental reservoirs appears less important than other environmental determinants, such as proximity to lakes, as well as social determinants, like population density and movements and access to safe water and sanitation. Although inland cholera represents most of the cases currently reported in Africa, this fact is rather unrecognized. Thorough dynamic reports of outbreaks; ecological studies of water bodies; systematic collecting, genotyping, and comparison of environmental and clinical strains; as well as social and economical studies should be implemented. Their achievement could help direct efforts in surveillance, prevention, and control to reservoirs of *V. cholerae* or to population-movement-associated facilities in order to get rid of this persisting scourge.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We are indebted to the Cameroonian, Chadian, Congolese, Mozambican, Nigerian and Nigerien ministries of health, the WHO national offices, and the UNICEF (the United Nations Children's Fund) national offices, for providing data used in Figure 1, Figure 3, and Supplementary Table 1.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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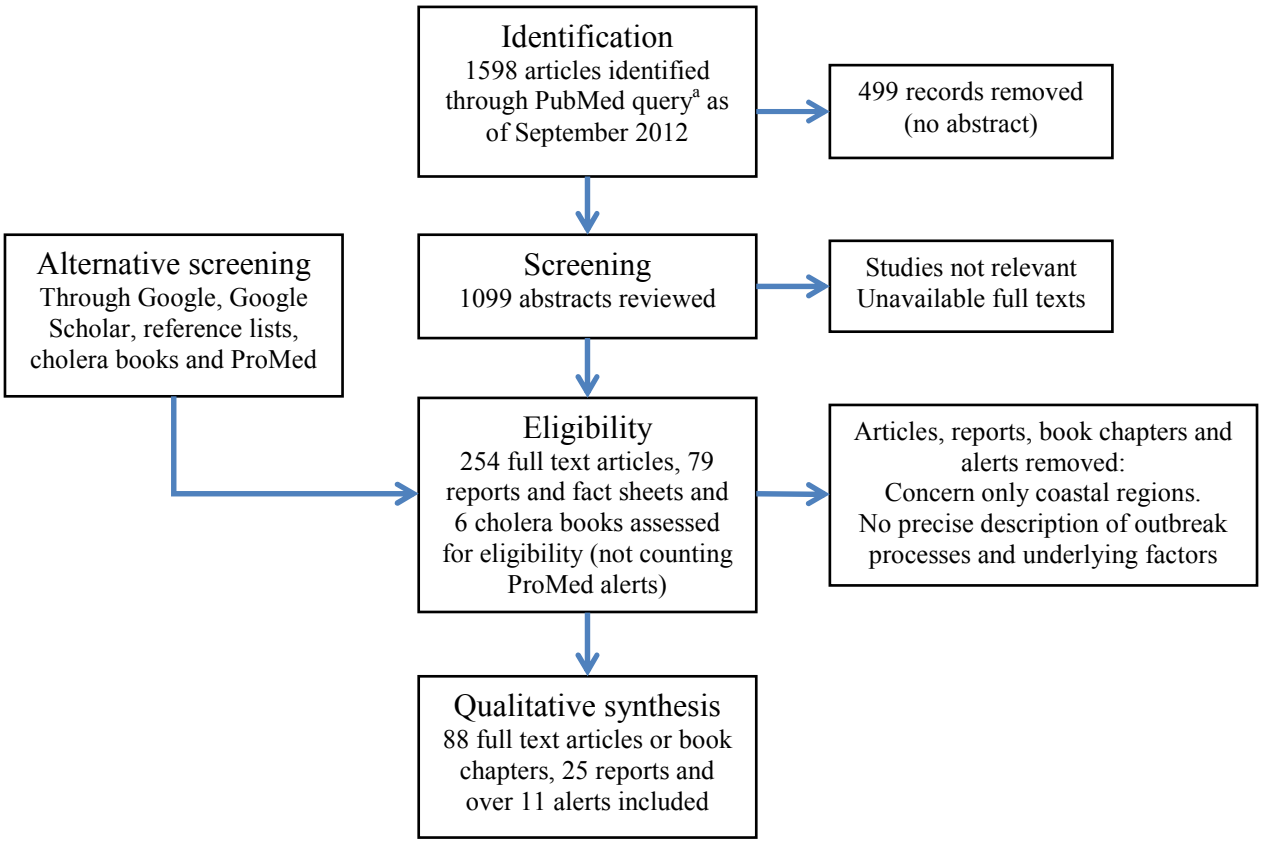
Supplementary Figure 1 – Flow diagram on articles and reports selection for systematic review concerning cholera and environment in inland Africa

[a] PubMed query : "(cholera OR Vibrio) AND (Africa OR algeria OR angola OR benin OR dahomey OR botswana OR "burkinafaso" OR "haute volta" OR burundi OR cameroon OR "cape verde" OR "central african republic" OR chad OR comoros OR congo OR "cote d'ivoire" OR "ivory coast" OR "democratic republic of the congo" OR zaire OR djibouti OR afar OR "equatorial guinea" OR eritrea OR ethiopia OR gabon OR ghana OR gambia OR guinea OR "guineabissau" OR kenya OR lesotho OR liberia OR madagascar OR malawi OR mali OR mauritania OR mauritius OR mayotte OR morocco OR mozambique OR namibia OR niger OR nigeria OR rwanda OR "sao tome e principe" OR senegal OR seychelles OR "sierra leone" OR somalia OR somaliland OR "south africa" OR sudan OR swaziland OR togo OR tunisia OR uganda OR tanzania OR zambia OR zimbabwe OR rhodesia)"


Identification
1598 articles identified through PubMed query[a] as of September 2012
499 records removed (no abstract)
Alternative screening
Through Google, Google Scholar, reference lists, cholera books and ProMed
Screening
1099 abstracts reviewed
Studies not relevant
Unavailable full texts
Eligibility
254 full text articles, 79 reports and fact sheets and 6 cholera books assessed for eligibility (not counting ProMed alerts)
Articles, reports, book chapters and alerts removed:
Concern only coastal regions.
No precise description of outbreak processes and underlying factors
Qualitative synthesis
88 full text articles or book chapters, 25 reports and over 11 alerts included

Supplementary Table 1 - Cholera cases reported in 2009-2011 in Subsaharan Africa. Estimation of the respective burden of inland and coastal regions.

	Estimated population in 2010			Cholera burden reported to the WHO in 2009						Cholera burden reported to the WHO in 2010						Cholera burden reported to the WHO in 2011						Cholera burden reported to the WHO in 2009-2011								
				whole country		inland regions		coastal regions		whole country		inland regions		coastal regions		whole country		inland regions		coastal regions		whole country			inland regions			coastal regions		
	whole country[a]	inland regions	coastal regions	cases[a]	incidence	cases	incidence	cases	incidence	cases[a]	incidence	cases	incidence	cases	incidence	cases[b]	incidence	cases	incidence	cases	incidence	cases	incidence	yearly_incidence	cases	incidence	yearly_incidence	cases	incidence	yearly_incidence
Coastal countries																														
Angola	19 081 912		19 081 912	2 019	10,58			2 019	10,58	1 484	7,78			1 484	7,78	1 730	9,07			1 730	9,07	5 233	27,42	9,14				5 233	27,42	9,14
Benin	8 849 892		8 849 892	74	0,84			74	0,84	983	11,11			983	11,11	755	8,53			755	8,53	1 812	20,47	6,82				1 812	20,47	6,82
Cameroon[c]	19 406 100	5 530 643	13 875 457	666	3,43	666	12,04	0	0,00	10 572	54,48	9 893	178,88	679	4,89	23 150	119,29	9 206	166,45	13 944	100,49	34 388	177,20	59,07	19 765	357,37	119,12	14 623	105,39	35,13
Cape Verde	495 999		495 999	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Comoros	734 750		734 750	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Congo	4 042 899		4 042 899	93	2,30			93	2,30	0	0,00			0	0,00	762	18,85			762	18,85	855	21,15	7,05				855	21,15	7,05
Cote d'Ivoire	19 737 800		19 737 800	5	0,03			5	0,03	32	0,16			32	0,16	1 261	6,39			1 261	6,39	1 298	6,58	2,19				1 298	6,58	2,19
Democratic Republic of the Congo[d]	64 420 000	61 688 711	2 731 289	22 899	35,55	22 899	37,12	0	0,00	13 884	21,55	13 884	22,51	0	0,00	18 044	28,01	18 044	29,25	0	0,00	54 827	85,11	28,37	54 827	88,88	29,63	0	0,00	0,00
Djibouti	888 716		888 716	0	0,00			0	0,00	2 047	230,33			2 047	230,33	0	0,00			0	0,00	2 047	230,33	76,78				2 047	230,33	76,78
Equatorial Guinea	700 401		700 401	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Eritrea	5 253 676		5 253 676	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Gabon	1 505 463		1 505 463	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Gambia	1 728 394		1 728 394	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Ghana	24 391 823		24 391 823	1 294	5,31			1 294	5,31	438	1,80			438	1,80	10 628	43,57			10 628	43,57	12 360	50,67	16,89				12 360	50,67	16,89
Guinea	9 981 590		9 981 590	42	0,42			42	0,42	0	0,00			0	0,00	3	0,03			3	0,03	45	0,45	0,15				45	0,45	0,15
Guinea-Bissau	1 515 224		1 515 224	5	0,33			5	0,33	0	0,00			0	0,00	0	0,00			0	0,00	5	0,33	0,11				5	0,33	0,11
Kenya[e]	40 025 338	34 182 686	5 842 652	11 425	28,54	8 125	23,77	3 300	56,48	3 188	7,96	1 000	2,93	2 188	37,45	74	0,18	0	0,00	74	1,27	14 687	36,69	12,23	9 125	26,69	8,90	5 562	95,20	31,73
Liberia	3 994 122		3 994 122	1 070	26,79			1 070	26,79	1 546	38,71			1 546	38,71	277	6,94			277	6,94	2 893	72,43	24,14				2 893	72,43	24,14
Madagascar	20 713 819		20 713 819	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Mauritania	3 459 773		3 459 773	0	0,00			0	0,00	0	0,00			0	0,00	46	1,33			46	1,33	46	1,33	0,44				46	1,33	0,44
Mozambique[f]	22 307 982	5 097 458	17 210 524	18 863	84,56	3 972	77,92	14 891	86,52	5 433	24,35	719	14,11	4 714	27,39	1 135	5,09	57	1,12	1 078	6,26	25 431	114,00	38,00	4 748	93,14	31,05	20 683	120,18	40,06
Namibia	2 283 289		2 283 289	159	6,96			159	6,96	0	0,00			0	0,00	0	0,00			0	0,00	159	6,96	2,32				159	6,96	2,32
Nigeria[g]	158 423 183	59 349 364	99 073 819	16 913	10,68	*15 095*	25,43	1 818	1,83	42 014	26,52	41 102	69,25	912	0,92	23 307	14,71	14 744	24,84	8 563	8,64	82 234	51,91	17,30	70 941	119,53	39,84	11 293	11,40	3,80
Senegal	12 433 728		12 433 728	4	0,03			4	0,03	3	0,02			3	0,02	5	0,04			5	0,04	12	0,10	0,03				12	0,10	0,03
Sao Tome and Principe	165 397		165 397	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Sierra Leone	5 867 536		5 867 536	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00	0,00				0	0,00	0,00
Somalia	9 330 872		9 330 872	208	2,23			208	2,23	3 510	37,62			3 510	37,62	0	0,00			0	0,00	3 718	39,85	13,28				3 718	39,85	13,28
South Africa	49 991 300		49 991 300	10 520	21,04			10 520	21,04	0	0,00			0	0,00	0	0,00			0	0,00	10 520	21,04	7,01				10 520	21,04	7,01
Sudan[h]	43 551 941	41 973 793	1 578 148	13 681	31,41	13 681	34,20	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	13 681	31,41	10,47	13 681	34,20	11,40	0	0,00	0,00
Togo	6 027 798		6 027 798	218	3,62			218	3,62	72	1,19			72	1,19	4	0,07			4	0,07	294	4,88	1,63				294	4,88	1,63
Tanzania	44 841 226		44 841 226	7 700	17,17			7 700	17,17	4 469	9,97			4 469	9,97	942	2,10			942	2,10	13 111	29,24	9,75				13 111	29,24	9,75
Inland countries																														
Botswana	2 006 945	2 006 945		15	0,75	15	0,75			0	0,00	0	0,00									15	0,75	0,25	15	0,75	0,25			
Burkina Faso	16 468 714	16 468 714		0	0,00	0	0,00			0	0,00	0	0,00			20	0,12	20	0,12			20	0,12	0,04	20	0,12	0,04			
Burundi	8 382 849	8 382 849		355	4,23	355	4,23			333	3,97	333	3,97			1 072	12,79	1 072	12,79			1 760	21,00	7,00	1 760	21,00	7,00			
Central African Republic	4 401 051	4 401 051		0	0,00	0	0,00			0	0,00	0	0,00			117	2,66	117	2,66			117	2,66	0,89	117	2,66	0,89			
Chad	11 227 208	11 227 208		67	0,60	67	0,60			6 395	56,96	6 395	56,96			17 267	153,80	17 267	153,80			23 729	211,35	70,45	23 729	211,35	70,45			
Ethiopia[i]	82 949 541	82 949 541		31 509	37,99	31 509	37,99			1 682	2,03	1 682	2,03			0	0,00	0	0,00			33 191	40,01	13,34	33 191	40,01	13,34			
Lesotho	2 171 318	2 171 318		0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0	0,00			0	0,00	0,00	0	0,00	0,00			
Malawi	14 900 841	14 900 841		5 751	38,60	5 751	38,60			1 155	7,75	1 155	7,75			120	0,81	120	0,81			7 026	47,15	15,72	7 026	47,15	15,72			
Mali	15 369 809	15 369 809		0	0,00	0	0,00			0	0,00	0	0,00			1 193	7,76	1 193	7,76			1 193	7,76	2,59	1 193	7,76	2,59			
Niger	15 511 953	15 511 953		0	0,00	0	0,00			1 154	7,44	1 154	7,44			2 324	14,98	2 324	14,98			3 478	22,42	7,47	3 478	22,42	7,47			
Rwanda	10 624 005	10 624 005		67	0,63	67	0,63			0	0,00	0	0,00			0	0,00	0	0,00			67	0,63	0,21	67	0,63	0,21			
Swaziland	1 186 056	1 186 056		19	1,60	19	1,60			0	0,00	0	0,00			0	0,00	0	0,00			19	1,60	0,53	19	1,60	0,53			
Uganda	33 424 683	33 424 683		1 095	3,28	1 095	3,28			2 341	7,00	2 341	7,00			0	0,00	0	0,00			3 436	10,28	3,43	3 436	10,28	3,43			
Zambia	12 926 409	12 926 409		4 712	36,45	4 712	36,45			6 794	52,56	6 794	52,56			330	2,55	330	2,55			11 836	91,56	30,52	11 836	91,56	30,52			
Zimbabwe	12 571 454	12 571 454		68 153	542,13	68 153	542,13			951	7,56	951	7,56			1 220	9,70	1 220	9,70			70 324	559,39	186,46	70 324	559,39	186,46			
Total	**850 274 779**	**451 945 491**	**398 329 288**	**219 601**	25,83	**176 181**	38,98	**43 420**	10,90	**110 480**	12,99	**87 403**	19,34	**23 077**	5,79	**105 786**	12,44	**65 714**	14,54	**40 072**	10,06	**435 867**	51,26	17,09	**329 298**	72,86	24,29	**106 569**	26,75	8,92
		53%	**47%**			**80%**		**20%**				**79%**		**21%**				**62%**		**38%**					**76%**			**24%**		
Incidence (/100,000 inhab)				**25,83**		**38,98**		**10,90**		**12,99**		**19,34**		**5,79**		**12,44**		**14,54**		**10,06**		**51,26**			**72,86**			**26,75**		

Notes

[a] National population estimates in 2010 from the World dataBank [1]; National cholera cases reported in 2009-2011 from the WHO [2]

[b] Provisional notification data from January to November 2011 [3]

[c] Inland Cameroon consisting of Region Nord and Region Extreme-Nord. Regional population estimates in 2010 from [4]. Regional cholera data from the WHO National Office for Cameroon

[d] Inland Democratic Republic of the Congo consisting of all provinces except Bas-Congo. Province population estimates in 2010 from [5]. Province cholera data from the RDC Ministry of Public Health, except for 2011 [3]

[e] Inland Kenya consisting of all provinces except Coast Province and North-Eastern Province. Province population in 2010 estimated from the Kenya 2009 census [6] with a growth rate from the World dataBank [1]. Province cholera data estimated for 2009 from [7] and for 2010 from [8]

[f] Inland Mozambique consisting of Manica, Tete and Niassa Provinces. Province population estimates in 2010 from the Mozambique National Ministry of Health. Province cholera data from the Mozambique Ministry of Health. Data for 2011 only until week #19

[g] Inland Nigeria consisting of Adamawa, Bauchi, Borno, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto, Yobe and Zamfara states. State populations in 2010 estimated from the 2006 census [9] with a growth rate from the World dataBank [1]. State cholera data for 2009-2010 from WHO National Office for Nigeria, and for 2011 from [10]

[h] Inland Sudan consisting of all Sudan and South Sudan states except Red Sea and Kasala States. State populations in 2010 estimated from the Sudan 2008 census [11] with a growth rate from the World dataBank [1]. No precise cholera data at the state scale could be obtained for 2009, but various reports indicate that this epidemic was mostly located in Southern Sudan, around Khartoum, and in Kordofan and Darfur states.

[i] Very likely cholera outbreak in 2009 reported as acute watery diarrhoea by Ethiopian authorities

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Supplementary Table 2 - Implicated source of cholera transmission reported during epidemics in Africa

Implicated contamination sources and associated factors	Country (region)	Year	Criteria	Comment	Reference(s)
Unsafe water sources					
Sea, lagoons, estuaries	Summarized in a separate review dedicated to cholera in coastal Africa				[1]
Lakes	Burundi (Lake Tankanyika)	1992	*V. cholerae* culture; ecological and case-control studies	in Rumongue; samples negative two months later	[2,3]
	DRC (Goma camps with Lake Kivu)	1994	Reports		[4,5]
	Guinea-Bissau	1994	Case-control study		[6]
	Kenya (Nyanza Province with Lake Victoria)	1997-1998	Case-control study		[7]
	Eastern DRC	2000-2007	Ecological studies		[8,9]
	DRC (Lake Tanganyika)		Reports	in Kalemie, where many people draw up the fecally contaminated water of the lake as it flows out in the Lukuga River	(RP, personnal data)
	Sudan	ND	*V. cholerae* PCR	in lakes from previously affected regions	[10]
Rivers and canals	South Africa (Mpumalanga)	1980	*V. cholerae* culture ; report	culture from various rivers, canals and streams ; fresh river water prefered by the population	[11]
	South Africa (Limpopo)	1981	*V. cholerae* culture; case-control study	culture from a river; drinking from this river was a significant risk factor	[12]
	Malawi (refugee camp)	1990	Case-control study	no multivariate analysis	[13]
	Tanzania	1997	*V. cholerae* culture; case-control study	bathing in the river	[14]
	DRC (Kinshasa and Congo River)	1999	Ecological study		[15]
	DRC (Kinshasa and Congo River)	1996-2001; 2011	Ecological study		[16]
	Ethiopia (Oromiya Region)	2006	Report	drinking from the river	[17]
Wells, reservoirs, tanks, troughs	South Africa (Mpumalanga)	1980	*V. cholerae* culture	in troughs receiving borehole water subsequently drawn up by scooping	[11]
	Mali (Mopti Region)	1984	*V. cholerae* culture; case-control study	traditional well	[18]
	Zimbabwe (refugee camps)	1992	Ecological study	access to protected water supply and density per borehole	[19]
	Guinea-Bissau	1994	Case-control study		[6]
	Zambia (Lusaka)	2003-2004	Ecological and case-control study	drinking from shallow well	[20]
	Lake Chad Basin (Cameroon and Nigeria)	2003-2010	Ecological study	open wells	[21]
	Zimbabwe (Chitungwiza and parts of Harare)	2008-2009	Reports	shallow wells in place of the running water absent for 2 years	[22,23]
	Sudan	ND	*V. cholerae* PCR	in reservoirs and tanks from previously affected regions	[10]
Street vendors water	Nigeria (Kano State)	1996	Case-control study		[24]
Impaired sanitation					
Pit-latrines					
scarceness	Kenya (Kakuma refugee camp)	2005	Case-control study	sharing of latrine by ≥3 households	[25]
	Zambia (Kusaka)	2003-2004	Ecological ans case-control study	lackand sharing of latrines	[20]
obstacle to digging	RDC (Goma camps)	1994	Report		[4]
	Cape Verde	1994	Report	rocky volcanic soil	[26]
	Comoros	1998	Report		[5]
	DRC (Eastern Kasai Province)	2002	Report	reluctance to dig latrines in the diamantifeous soil	[5]
unsafety	Nigeria (Katsina)	1982	Report	latrines close to the water table and wells with uncemented walls	[27]
	DRC (Kinshasa)	1999	Case-control study	usage of latrine with no water	[15]
Open defecation	Malawi (refugee camp)	1990	Case-control study	no multivariate analysis	[13]
	DRC (Goma camps)	1994	Report		[5]
	South Sudan	2007	Report	near the Nile River	[28]
Drainage network	Zambia (Kusaka)	2003-2004, 2003-2006	Ecological study	insufficient coverage of drainage network	[29]
Refusal dumps	Ghana (Kumasi)	2005	Ecological studies	concentration and proximity of refusal dumps	[30,31]
Water handling					
Domestic water storage	South Africa (Mpumalanga)	1980	*V. cholerae* culture	rainwater barrel	[11]
	Malawi (refugee camp)	1990	*V. cholerae* culture ; case-control study	placing hands in water storage container	[13]
	Guinea-Bissau	1994; 1996	Case-control studies	storage in bucket or basin	[6,32]
	Kenya (Kibwezi, Malindi)	2005	Case-control study	storage in bucket, open container	[33]
	Kenya (Kakuma camp)	2005	Case-control study	unsealed or uncovered container	[25]
	Kenya (Kakuma camp)	2009	Case-control study	dirty containers	[34]
	Sudan	ND	*V. cholerae* PCR	in home pots from previously affected regions	[10]
	N/A		Lab experiments	survival varying according to the type of storage container and the delay before inoculation	[35]
Lack of water treatment	DRC (Kinshasa)	1999	Case-control study	unboiled water	[15]
	Kenya (Kibwezi)	2005	Case-control study	untreated water	[33]
	Zambia (Lusaka)	2003-2004	Case-control study	chlorination of drinking water ; no multivariate analysis	[20]
Hygiene habits					
Usage of soap	Guinea	1986	Case-control study	for routine hand washing before meals	[36]
	Guinea-Bissau	1994	Case-control study	presence in the house; significant for women only	[6]
	Zambia (Lusaka)	2003-2004	Case-control study	presence at home	[37]
	Zambia (Lusaka)	2003-2004	Case-control study	no multivariate analysis	[20]
	Kenya (Kakuma camp)	2009	Case-control study	no use for hand washing	[34]
Washing of patients clothes	Tanzania	1997	Report	in the river used for drinking	[14]
Collective eating	Guinea-Bissau	1994	Case-control study	hand eating in the same plate	[6]
	Kenya (Nyanza Province)	1997-1998	Case-control study	sharing of food with a person with watery diarrhea	[7]
	Comoros	1998	Report	feast of weddings	[5,38]
Diarrheic person in the family	DRC (Kinshasa)	1999	Case-control study		[15]
Funerals and related factors					
Attending funerals (feasts)	Guinea	1986	Case-control study	attending funerals and eating at the feast	[36]
	Guinea-Bissau	1994	Case-control study	eating at a funeral with a non-disinfected corpse ; touching the body. No multivariate analysis	[39]
	Guinea-Bissau	1996	Case-control study	especially funerals with consumption of drinks and food	[6]
	Kenya (Nyanza Province)	1997-1998	Case-control study	attending funeral feasts	[7]
	Kenya (Malindi)	2005	Case-control study		[33]
Dead body ritual cleaning	Tanzania	1977	Report	epidemics after funeral feasts whose women cookers had just cleaned the bed sheets and body of the cholera victim (including evacuation of the bowel contents with enemas)	[40]
	Guinea	1986	Report		[36]
	Comoros	1975; 1998	Report		[5,41]
	Guinea-Bissau (Biombo Region)	1994	Ecological study	lower incidence in villages disinfecting the corpse	[39]
	Central African Republic (North)	1997	report	implication of the cleaning of the bodies by the families	[42]
Death-related ritual	DRC (Eastern Kasai Province)	2002	Report	drinking the water in which clothes of the deceased have been soaking	[5]
Food sources and associated factors					
Cold left-over food	Mali (Segou Region)	1984	Case-control study	millet gruel	[18]
	Guinea	1986	Case-control study	cold peanut sauce	[36]
	Malawi (refugee camp)	1990	Case-control study	cooked pigeon peas. No multivariate analysis	[13]
Seafood (fish and shellfish)	Summarized in a separate review dedicated to cholera in coastal Africa				[1]
Other at risk food					
Dried fish	Tanzania	1997	Case-control study		[14]
street grilled meat	DRC (Kinshasa)	1999	Case-control study		[15]
ugali outside home	Kenya (Malindi)	2005	Case-control study	i.e. a maize meal	[33]
Raw vegetables	Zambia (Lusaka)	2003-2004	Case-control study		[37]
	Zambia (Lusaka)	2003-2004	Case-control study	Absence of consumption of kapenta (i.e. a local sardine-like fish)	[37]
Protective acid ingredients					
Curled goat-milk	Mali (Segou Region)	1984	Report ; lab experiments	survival of epidemic *V. cholerae* O1 in millet gruel is impaired by addition of curled goat-milk	[18]
Tomato	Guinea	1986	Case-control study	consumption of tomato sauce	[36]
Tomato	Guinea	1986	Lab experiment	*V. cholerae* rapid growth in peanut sauce but not in tomato sauce. Killing of bacterias at 65°C	[36]
Mahewu	Zimbabwe	1993	Case-control study	i.e. a fermented porridge-like drink	[2]
Lime; tomato	Guinea-Bissau	1994; 1996	Case-control studies	using lime or tomato sauce	[6,32]
Lime; curled milk	Guinea-Bissau	1996	Lab experiment	growth of *V. cholerae* in peanut sauce is impaired by addition of lime juice, and impossible in curled milk	[32]
Direct person-to-person transmission					
During a goldmine outbreak	South Africa (Limpopo)	1974	Report with V. cholerae culture of sewage and rectal swabs	possible transmission in the acclimatation room. Possible alternative transmission routes	[43]
During hospital outbreaks	Tanzania (Dar es Salaam)	1977-78; 1981; 1983	Report	three outbreaks in children's infectious diseases ward	[44]
	Mozambique (Maputo)	1980; 1981	Report	two outbreaks in chikdren's wards	[45]
During visiting patients at hospital	CAR (North)	1997	Report		[42]

Abreviations

DRC, Democratic Republic of the Congo
CAR, Central African Republic

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2.1.4 Des éléments très limités en faveur du paradigme environnemental en Afrique

Ces deux revues synthétisent donc une somme importante de données relatives à l'épidémiologie du choléra en Afrique. L'ensemble dresse un tableau apparaissant peu compatible avec le paradigme environnemental du choléra, dont la validité sur ce continent doit être mise en doute. En effet, le choléra y affecte surtout des zones situées à distance des côtes. La propagation des épidémies apparaît liée aux déplacements humains. Divers facteurs environnementaux jouent un rôle important dans la dynamique des épidémies, mais leur impact sur la transmission peut être compris sans recourir à des réservoirs aquatiques de *V. cholerae* O1 toxinogènes, dont aucun ne semble à ce jour avoir été mis en évidence de manière *pérenne* et indépendante d'une épidémie de choléra en cours.

A l'issue de ce travail bibliographique, de nombreux aspects concernant les liens entre le choléra et l'environnement en Afrique demeurent néanmoins mal compris. Des études complémentaires intégrant épidémiologie de terrain, analyses spatio-temporelles des épidémies, comparaisons génétiques des souches isolées, microbiologie environnementale, etc… s'avèrent indispensables.

2.2 Origine de l'épidémie de choléra en Guinée en 2012

« Ce 'choléra des plages' a déjà fait près de 250 morts »

Michel Van Herp,
épidémiologiste chez MSF, 2012 [69]

2.2.1 Quelle a été l'origine de l'épidémie de choléra en 2012 en Guinée ?

Une première opportunité de mener ce type d'étude pluridisciplinaire nous fut donnée à l'occasion de l'épidémie de choléra qui a frappé la Guinée entre février et novembre 2012, après 3 années d'accalmie, et qui occasionna 7350 cas dont 133 décès selon l'OMS [70]. La section Eau-Hygiène-Assainissement du Bureau Régional de l'Unicef pour l'Afrique de l'Ouest et l'Afrique Centrale, en pointe sur la lute contre les épidémies de choléra, et la Division Prévention et Lutte contre la Maladie de la République de Guinée, très satisfaite des études menées par l'Université de Franche-Comté sous la supervision de Renaud Piarroux en 2009-2010 [71], nous sollicitèrent alors pour enquêter sur l'origine de cette épidémie. Il convenait de tester 2 hypothèses alternatives : (1) celle d'une origine environnementale, souvent présentée comme évidente dans ce pays habituellement qualifié d'endémique pour le choléra [72] ; ou au contraire (2) une origine importée depuis la Sierra Leone voisine, que les investigations préliminaires des autorités sanitaires guinéennes semblaient soutenir.

A la demande officielle du Ministère de la Santé guinéen, l'Assistance Publique-Hôpitaux de Marseille fut donc contractualisée par l'Unicef-Guinée, afin de me détacher 1 mois sur place en août-septembre 2012 et d'analyser les données recueillies au cours des semaines suivantes. Des investigations de terrain furent conduites, des rapports d'investigation et des bases de données furent rassemblés, et des isolats cliniques de *V. cholerae* O1 furent sélectionnés puis acheminés à Marseille. Le génotypage de ces derniers fut effectué par une technique de microsatellite en collaboration avec le Laboratoire de Biologie de l'HIA Laveran à Marseille sous la direction du Pr Éric Garnotel. Je me chargeai pour ma part des analyses spatiotemporelles et de l'analyse des génotypes identifiés. Un séquençage total de l'une de ces souches fut effectué dans le

[69] Médecins Sans Frontières, « MSF ».
[70] Organisation Mondiale de la Santé et Section D'hygiène Du Secrétariat De La Société Des Nations, « Choléra, 2012 ».
[71] Sudre et Bompangue, *Epidémiologie du choléra et Evaluation du Système d'Alerte Précoce en République de Guinée. Rapport de mission*; Sudre, *Projet de préparation aux épidémies de choléra à Conakry, Guinée. Épidémiologie du choléra dans la ville de Conakry (République de Guinée). Étude rétrospective des facteurs de risque dans la ville de Conakry*.
[72] Gaffga, Tauxe, et Mintz, « Cholera »; Ali et al., « The global burden of cholera ».

laboratoire de bactériologie de la Timone, dont je participai activement à l'analyse. Un second séquençage fut effectué et analysé par le Sanger Institute de Cambridge, afin d'intégrer cette souche dans l'arbre phylogénétique de la septième pandémie.

Les résultats de cette étude furent synthétisés dans un rapport rendu à l'Unicef début 2013 [73], puis finalement publiés en Juin 2014 dans PLoS Neglected Tropical Diseases [74].

2.2.2 Article 7 : « Deciphering the origin of the 2012 cholera epidemic in Guinea by integrating epidemiological and molecular analyses. »

A lire ci-après.

[73] Rebaudet et Piarroux, *Origine et déterminants de l'épidémie de choléra 2012 en République de Guinée*.
[74] Rebaudet et al., « Deciphering the Origin of the 2012 Cholera Epidemic in Guinea by Integrating Epidemiological and Molecular Analyses ».

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PLOS | NEGLECTED TROPICAL DISEASES

Deciphering the Origin of the 2012 Cholera Epidemic in Guinea by Integrating Epidemiological and Molecular Analyses

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Abstract

Cholera is typically considered endemic in West Africa, especially in the Republic of Guinea. However, a three-year lull period was observed from 2009 to 2011, before a new epidemic struck the country in 2012, which was officially responsible for 7,350 suspected cases and 133 deaths. To determine whether cholera re-emerged from the aquatic environment or was rather imported due to human migration, a comprehensive epidemiological and molecular survey was conducted. A spatiotemporal analysis of the national case databases established Kaback Island, located off the southern coast of Guinea, as the initial focus of the epidemic in early February. According to the field investigations, the index case was found to be a fisherman who had recently arrived from a coastal district of neighboring Sierra Leone, where a cholera outbreak had recently occurred. MLVA-based genotype mapping of 38 clinical *Vibrio cholerae* O1 El Tor isolates sampled throughout the epidemic demonstrated a progressive genetic diversification of the strains from a single genotype isolated on Kaback Island in February, which correlated with spatial epidemic spread. Whole-genome sequencing characterized this strain as an ''atypical'' El Tor variant. Furthermore, genome-wide SNP-based phylogeny analysis grouped the Guinean strain into a new clade of the third wave of the seventh pandemic, distinct from previously analyzed African strains and directly related to a Bangladeshi isolate. Overall, these results highly suggest that the Guinean 2012 epidemic was caused by a *V. cholerae* clone that was likely imported from Sierra Leone by an infected individual. These results indicate the importance of promoting the cross-border identification and surveillance of mobile and vulnerable populations, including fishermen, to prevent, detect and control future epidemics in the region. Comprehensive epidemiological investigations should be expanded to better understand cholera dynamics and improve disease control strategies throughout the African continent.

Citation: Rebaudet S, Mengel MA, Koivogui L, Moore S, Mutreja A, et al. (2014) Deciphering the Origin of the 2012 Cholera Epidemic in Guinea by Integrating Epidemiological and Molecular Analyses. PLoS Negl Trop Dis 8(6): e2898. doi:10.1371/journal.pntd.0002898

Editor: Edward T. Ryan, Massachusetts General Hospital, United States of America

Received December 14, 2013; **Accepted** April 13, 2014; **Published** June 5, 2014

Funding: This work was co-financed by UNICEF-Guinea, Assistance Publique – Hôpitaux de Marseille (APHM). A portion of the field investigations and all sampling and isolation of clinical cholera isolates were financed by the African Cholera Surveillance Network - Africhol (http://www.africhol.org/), which is supported by a grant from the Bill & Melinda Gates Foundation. APHM is a non-profit French university hospital employing author S Rebaudet as a researcher on epidemiology. Africhol is a consortium dedicated to cholera surveillance in Africa launched by the non-profit NGO Agence de Médecine Préventive (AMP), and integrating African Ministries of Health such as Guinean's. UNICEF and the Bill & Melinda Gates Foundation had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Cholera is generally considered endemic in West Africa [1], especially in countries such as Nigeria, Benin, Togo, Ghana, Liberia and the Republic of Guinea [2]. In 2004–2008, Guinea was struck by a succession of regional cholera outbreaks responsible for 17,638 reported cases and 786 deaths [3]. In 2009, the country established an early cholera alert system including cholera microbiological surveillance to quickly detect emerging epidemics [4]. However, the following years in Guinea were marked by a lull in cholera transmission until new cases were reported between February and April 2012 in several maritime prefectures spanning 200 km [5]. Between April and June, a reactive oral cholera vaccination campaign was implemented by the Guinean Ministry of Health and Médecins Sans Frontières (Doctors Without Borders) in two prefectures, Forecariah and Boffa [6]. However, during the rainy season in July and August, the epidemic exploded in the capital Conakry and then spread to inland areas. By the time the end of the epidemic was declared in December 2012, 7,350 cases and 133 deaths had been officially reported to the World Health Organization (WHO), from 11 out of 34 prefectures [7].

To provide a scientific foundation for the control and prevention of future outbreaks, it is critical to understand the origin of cholera epidemics in coastal areas, which has remained subject to debate. In Peru and Bangladesh, a similar near

Author Summary

Cholera is a potentially deadly diarrheic disease caused by the toxin-secreting bacterium *Vibrio cholerae*. In many poor countries, this prototypical waterborne disease is considered endemic and linked to the climate-driven proliferation of environmental reservoirs of the pathogen. Although such a statement implies radical public health consequences, it has never been proven in Africa. The present study aimed to elucidate the origin of the cholera epidemic that struck the Republic of Guinea in 2012 following a three-year lull period. This investigation integrated a spatiotemporal analysis of the national case databases, field investigations and thorough genetic analyses of 38 clinical bacterial isolates sampled throughout the Guinean epidemic. The Guinean *V. cholerae* DNA sequence results were aligned and compared with the sequences of nearly 200 strains isolated throughout the world over the past 60 years. Overall, these results suggest that the 2012 cholera epidemic strain was likely imported from Sierra Leone to Guinea by traveling fishermen. The emergence of cholera epidemics due to human-driven activity may be widespread throughout Africa. This highlights the importance of transborder collaborative public health strategies targeting highly mobile and high-risk populations. Similar integrated studies should be conducted in other countries impacted by the disease to better understand the spread of recent epidemics and thus better intercept future outbreaks.

simultaneous appearance of cholera at different locales along coastal or estuarine areas has been considered a key argument in favor of the "cholera paradigm" [8]. According to this general model for cholera transmission, coastal waters in these regions represent reservoirs of multiclonal epidemic-provoking *Vibrio cholerae* strains whose growth is directly associated with plankton blooms driven by climatic and environmental conditions [8–12]. Conversely, whole-genome-based phylogenetic analyses of Peruvian and other South American isolates from the 1990s have found that the strains form a clonal and independent lineage within the seventh pandemic [13,14]. Such molecular approaches have recently highlighted the function of human-to-human transmission of the disease [12], which could be the main driver of clonal outbreak diffusion, even along coastal areas.

To assess whether the 2012 Guinean cholera epidemic was caused by local environment-to-human transmission or was rather initiated by the human-driven importation of a single toxigenic clone we used a multidisciplinary approach involving spatiotemporal analyses, field investigations and several complementary *V. cholerae* genotyping methods.

Materials and Methods

Cholera cases and deaths, rainfall, population and geographical data

The Republic of Guinea spans 245,857 km^2 and is administratively divided into 33 prefectures plus the capital Conakry. In 2012, the country had an estimated population of 12 million inhabitants. At that time, the Guinean national health surveillance system prospectively reported all suspected cholera cases based on the WHO definition of the disease [15]. Each Prefectural Health Directorate (DPS – *Direction Préfectorale de la Santé*) tallied new cases recorded at the various health structures of the prefecture on a weekly basis. Aggregated morbidity and mortality cholera data were then transmitted to the Directorate of Prevention and Disease Control (DPLM – *Direction de la Prévention et de la Lutte contre la Maladie*), which compiled the information in a national database of 7,350 cases. DPLM also retrospectively compiled a line list of 6,568 patients, which included the date of consultation and geographical origin down to the village level and was anonymized prior to analysis. To limit notification bias, both databases were subsequently compared and merged, which enabled the retrieval of 393 additional cases. The use of these data for epidemiological, research and publication purposes was approved by the Guinean Ministry of Health (*Ministère de la Santé Publique et de l'Hygiène Publique*). Daily-accumulated rainfall data were obtained from satellite estimates (TMPA-RT 3B42RT derived) provided by the National Aeronautics and Space Administration (available at: http://disc2.nascom.nasa.gov/Giovanni/tovas/realtime.3B42RT_daily.2.shtml). As most cases were recorded in Maritime Guinea and, to a lesser extent, Middle Guinea, daily rainfall data were averaged on the position 9.00N-12.00N/15.00-11.75W, which excluded the eastern two-thirds of the country where precipitation levels were lower and much fewer cholera cases were reported. Population estimates for 2012 were obtained from the Guinean Expanded Program for Immunization at both the prefectural and sub-prefectural levels. Their estimates were based on the general population census of 1996 considering prefecture-specific annual population growth rates, which were provided by the Guinean Statistics National Institute (INS – *Institut National de la Statistique de Guinée*) and ranged from 0.71% to 6.51%.

Field investigations

Field investigations of index cases and local conditions that supported cholera emergence and transmission were prospectively conducted in affected areas throughout the epidemic by epidemiologists of the Guinean Health Ministry and the country team of the African Cholera Surveillance Network (Africhol; http://www.africhol.org) to organize the public health response. They included basic interviews among affected communities identified by the hospital- and community-based surveillance system (including rumors) and followed routine procedures of the Integrated Disease Surveillance and Response System of the Guinean Ministry of Health. Retrospective field investigations were also conducted in August and September 2012 mainly to review the register books of treatment facilities, but also to interview local health authorities and staff regarding the 2012 outbreak as well as to observe ecological, social, water and sanitation conditions in affected areas.

Sampling of clinical *V. cholerae* isolates and DNA extraction

With the support of the Africhol Consortium and following standard procedures [16], the reference laboratory of the Public Health National Institute (INSP – *Institut National de Santé Publique*) tested 236 clinical samples positive for *V. cholerae* O1 throughout the duration of the 2012 epidemic, out of which 212 isolates were prospectively stored in a biobank created for that purpose. In September 2012, 50 of these isolates were selected for genotyping, subcultured and then transported in glycerol tubes at room temperature to Marseille, France. Isolates were selected in a manner in which the samples were temporally and spatially representative of outbreak diffusion during the first 8 months of the epidemic and included early and later isolates from all 7 prefectures available in the biobank. Upon arrival in Marseille, the strains were recultivated on non-selective trypticase soy agar (TSA) medium (Difco Laboratories/BD) for 24 hours at 37°C. Suspected *V. cholerae* colonies were identified via Gram-staining, oxidase reaction and agglutination assessment with *V. cholerae* O1

polyvalent antisera (Bio-Rad). For DNA extraction, an aliquot of cultured cells was suspended in 500 µL deionized water, incubated for 10 min at 100°C and centrifuged for 10 min at 1500× g. The pellet was then resuspended in 250 µL deionized water and incubated for 5 min at 100°C. The supernatant (containing DNA) was subsequently stored at −20°C. DNA was directly extracted from the glycerol transport tubes for the isolates that failed to grow upon culture.

MLVA-based genotyping of sampled strains

Genotyping of the *V. cholerae* strains was performed via MLVA (Multiple Loci VNTR (Variable Number Tandem Repeat) Analysis) of 6 VNTRs (Table 1), including 4 previously described assays [17,18] and 2 assays specifically designed for this study to improve the discriminating power of the analysis. The novel VNTR assays were designed based on the reference strain El Tor N16961 (GenBank accession numbers AE003852.1 and AE003853.1) using Perfect Microsatellite Repeat Finder webserver (currently unavailable). Specific primer pairs were subsequently designed using the Primer3 program (http://simgene.com/Primer3) (Table 1). Fluorescent-labeled primers were purchased from Applied Biosystems.

For each PCR assay, DNA amplification was carried out by mixing 0.375 µL of each primer (20 µM), 1 X LightCycler 480 Probes Master (Roche Diagnostics) and approximately 100 ng of template DNA in a total volume of 30 µL. PCR was performed using a LightCycler 480 System (Roche Diagnostics) with the thermal cycling conditions described in Table 1. PCR amplicons were subsequently verified via agarose gel (2%) electrophoresis.

VNTR PCR product size was determined via capillary electrophoresis. Aliquots of the PCR products were first diluted 1:100 in sterile water, which was further diluted 1:100 in a solution containing 25 µL Hi-Di Formamide 3500 Dx Series (Applied Biosystems) and 0.5 µL GeneScan 500 LIZ Size Standard (Applied Biosystems). The fluorescent end-labeled amplicons were analyzed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) with POP-7 Polymer (Applied Biosystems). Finally, amplicon size was determined using GeneMapper v.3.0 software (Applied Biosystems).

Whole-genome sequencing

To better characterize the *V. cholerae* strains responsible for the epidemic, whole-genome sequencing was performed on a strain isolated at the onset of the epidemic (strain G298_Guinea) using a GS FLX+ System (454 Life Science, a Roche company). The DNA sequence was assembled using Newbler, from GS *De novo* Assembler (http://454.com/products/analysis-software/index.asp).

To perform a phylogenetic assessment of the core *V. cholerae* genome based on genome-wide SNPs (single nucleotide polymorphisms), strain G298_Guinea DNA was re-sequenced using a HiSeq Illumina System (Illumina).

Statistical and analytical methods

For the spatiotemporal description of the epidemic, rainfall data were aggregated weekly and graphically represented in parallel with cholera morbidity. Cholera attack rates were calculated and mapped, by prefecture and sub-prefecture, for various time periods using shapefiles of administrative divisions obtained from the HealthMapper application (WHO, Geneva, Switzerland) and Quantum GIS v1.8.0 (QGIS Geographic Information System, Open Source Geospatial Foundation Project, available at: http://qgis.osgeo.org).

MLVA-based genotypes were compared at each of the 6 VNTR loci. Genetic relatedness between the strains was first assessed using eBURSTv3 (http://eburst.mlst.net/), which aims to identify the founding genotype. A simple network of all possible links between genotypes was also assembled using Gephi.0.8.1 beta software (https://gephi.org/). Molecular epidemiology analyses were completed via the sequential mapping of each genotype by month at the prefecture level.

After the first whole-genome sequence was obtained with the GS FLX+ System, proteins were predicted using Prodigal software (http://prodigal.ornl.gov/). Data was then annotated employing the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) and the Clusters of Orthologous Groups database using BLASTP with an E-value of 10^{-5}. Allelic polymorphism of the cholera toxin B subunit and other virulence factors was characterized by comparing the obtained sequence with the genome description of *V. cholerae* strains available in GenBank and recent literature.

Table 1. Characteristics and primer sequences of the 6 genotyped VNTRs.

Locus name	Repeated pattern	Chr.[1]	Position[2]	Primer sequence (5′→3′)	Ref
VC1	AACAGA	1	137106	fw: CGGATACTCAAACGCAGGAT	[17,18]
				rv: 6FAM*-CTTTCGGTCGGTTTCTCTTG	
VC4	TGCTGT	2	187759	fw: TGTTTGAGAGCTCGCCTCTT	[17,18]
				rv: PET*-TCATCAAGATGCACGACACA	
VC5	GATAATCCA	1	1915539	fw: AGTGGGCACAGAGTGTCAAA	[17,18]
				rv: VIC*-AATTGGCCGCTAACTGAGTG	
VC9	GACCCTA	1	467111	fw: CGTTAGCATCGAAACTGCTG	[17,18]
				rv: NED*-AGAAAACAATCGCCTGCTTG	
VCLAV6	ACCAGA	2	303939	fw: NED*-GCCTCCTCAGAAGTTGAGAATC	Present study
				rv: CCGATGAACTCTCTGAACTGG	
VCLAV8	TTGTCGA	1	532253	fw: VIC*-CTCGCTTAAGTTGCCTTACCC	Present study
				rv: GCGAACCAGACGTACTTTCAG	

[1]Chr.: chromosome.
[2]Based on the reference strain El Tor N16961 (GenBank accession numbers: AE003852.1 and AE003853.1).
PCR thermal cycling conditions for all assays: 95°C for 5 min; followed by 30 cycles of 95°C for 30 sec, 58°C for 30 sec and 72°C for 45 sec; 72°C for 5 min.
doi:10.1371/journal.pntd.0002898.t001

For phylogenetic analyses, the paired-end read data obtained with the HiSeq Illumina System and sequence data from 198 previously sequenced strains available in the NCBI SRA database were mapped to the reference N16961 El Tor strain (NCBI accession numbers AE003852 and AE003853) using SMALT software (http://www.sanger.ac.uk/resources/software/smalt). A whole-genome alignment was obtained for each strain in this analysis, and SNPs were called using the approach described by Harris *et al.* [19]. The reads that did not map to the N16961 genome were filtered out during SNP calling, and any SNP with a quality score less than 30 was excluded. A true SNP was only called if there were at least 75% of the reads at any heterogeneously mapped ambiguous sites. High-density SNP clusters indicating possible recombination sites were excluded using the methodology previously described by Croucher *et al.* [20]. Maximum Likelihood phylogenetic trees were estimated using the default settings of RAxML v0.7.4 [21] based on all the SNPs called in the manner explained above. M66 (accession numbers CP001233 and CP001234), a pre-seventh pandemic strain, was used to root the final phylogenetic tree of the seventh pandemic strains [13]. FigTree (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize and order the nodes of the phylogenetic tree.

Results

Spatiotemporal epidemic progression

Taking into account both the national database and patient line list, this epidemic was responsible for an estimated 7,743 suspected cases (global attack rate: 6.3 cases/10,000 inhabitants) and 138 deaths (case fatality ratio: 1.8%). The initial case was reported on February 2, 2012 (epidemiological week 5) in the midst of the dry season (Figure 1). The weekly number of new cases remained below 100 until July. The epidemic then peaked in August, 5 months after the onset of the rainy season, with nearly 1,188 new cases recorded during week 34. Cholera incidence began to markedly decline in September. The final case was recorded on December 11, 2012, and the Minister of Health officially declared the end of the epidemic on February 6, 2013.

Overall, the capital of Conakry reported 4,642 cases (25.9 cases/10,000 inhab.), which represented more than half of the national case total, but only 24 deaths (case fatality ratio: 0.5%). Moreover, 2,178 additional patients were located in the 5 other prefectures that border the Atlantic Ocean, with the highest attack rate observed in Coyah (55.1 cases/10,000 inhab.) (Figure 2). Twelve other prefectures were also affected, including distant inland prefectures such as Kerouane (Figure 2).

The initial cholera cases in Guinea emerged on February 2, 2012 on Kaback Island (Prefecture of Forecariah) (Figure 3), which is located in a remote mangrove zone close to the border with Sierra Leone, where an epidemic of acute diarrhea and vomiting had been reported in January. The Guinean index case was a fisherman who had just traveled by boat from Sierra Leone (a village on Yeliboyah Island, Kambia District) and arrived in the fishing village of Khounyi, on a land strip of the southern tip of Kaback Island. During the first month of the epidemic, this small village, which lacked safe water and improved sanitation facilities, recorded over 100 cases and represented the most affected community in the prefecture.

The cholera epidemic then progressively diffused northwestward along the Guinean coast, striking the prefectures of Boffa on February 23 and Boke on April 22 (Figure 3). As observed in Forecariah Prefecture, the initial cases in Boffa and Boke were also reported in fishing camps, namely Sakama and Yongosale,

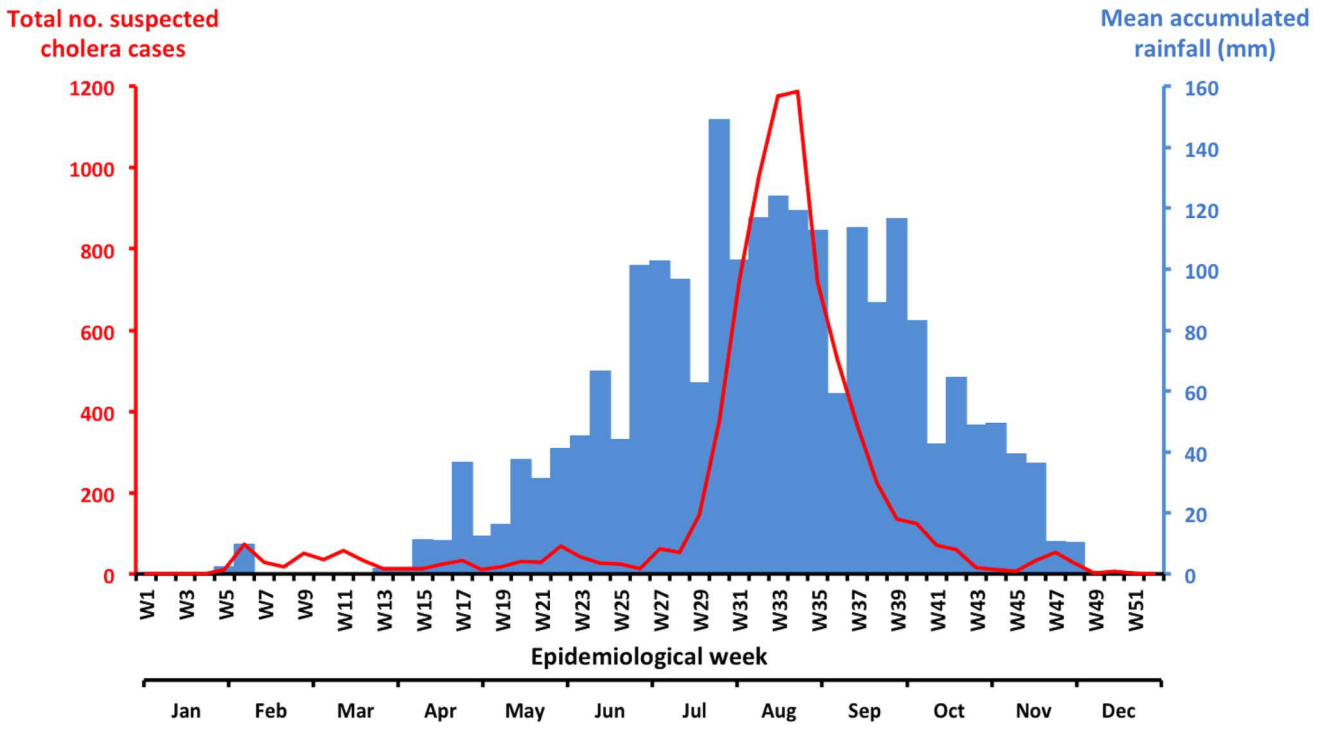

Figure 1. Evolution of the weekly cholera cases and rainfall in Guinea in 2012. Accumulated rainfall data for the most affected areas of the country (Maritime and Middle Guinea) were obtained from satellite estimates (TMPA-RT 3B42RT derived), which was averaged on the position 9.00N-12.00N/15.00-11.75W and is available at: http://disc2.nascom.nasa.gov/Giovanni/tovas/realtime.3B42RT_daily.2.shtml. The blue bars indicate the weekly rainfall levels, and the red line indicates the number of suspected cholera cases per week.
doi:10.1371/journal.pntd.0002898.g001

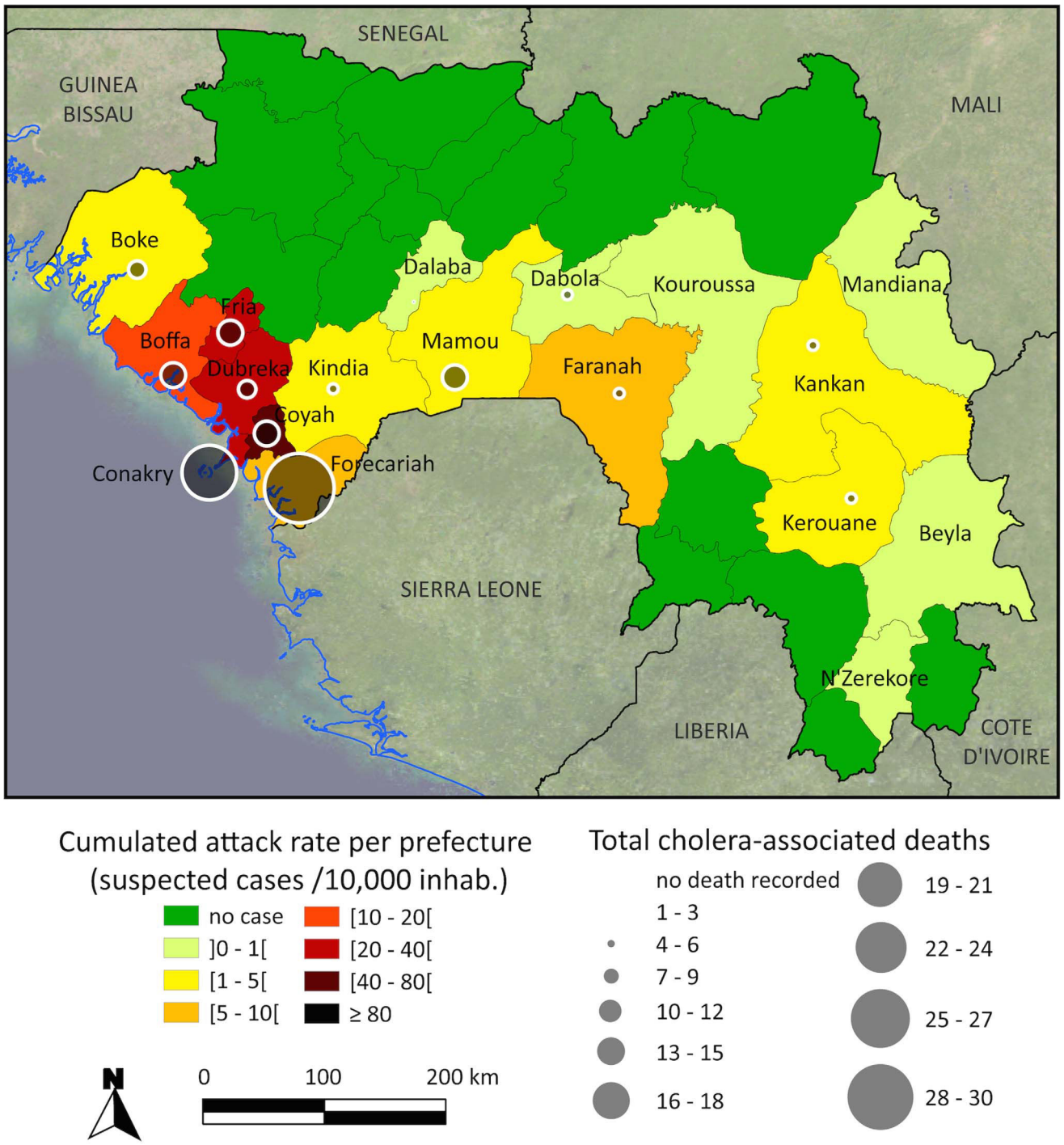

Figure 2. Cumulated cholera attack rates and deaths per prefecture during the 2012 Guinean epidemic.
doi:10.1371/journal.pntd.0002898.g002

respectively. At each of these lowland fishing locales, the index case was a fisherman who had recently returned from an already affected area (i.e., travelling from Kaback to Sakama and from Koukoude (Boffa prefecture, Douprou sub-prefecture) to Yongosale). Concomitant with the expansion of the epidemic along the coast, cholera had also begun to spread inland. However, the inland prefectures were not significantly affected until the onset of the rainy season. Likewise, although Conakry is situated on a peninsula between the early affected regions of Kaback and Boffa, cholera did not strike the capital until a month after the inception of the rainy season. The first case in Conakry was officially recorded on May 29, who appeared to be a merchant returning from the Kaback market. Conakry subsequently acted as an amplifier of epidemic spread, especially towards the interior portions of the country, where several identified index cases were found to be drivers, merchants or students recently returning from the capital.

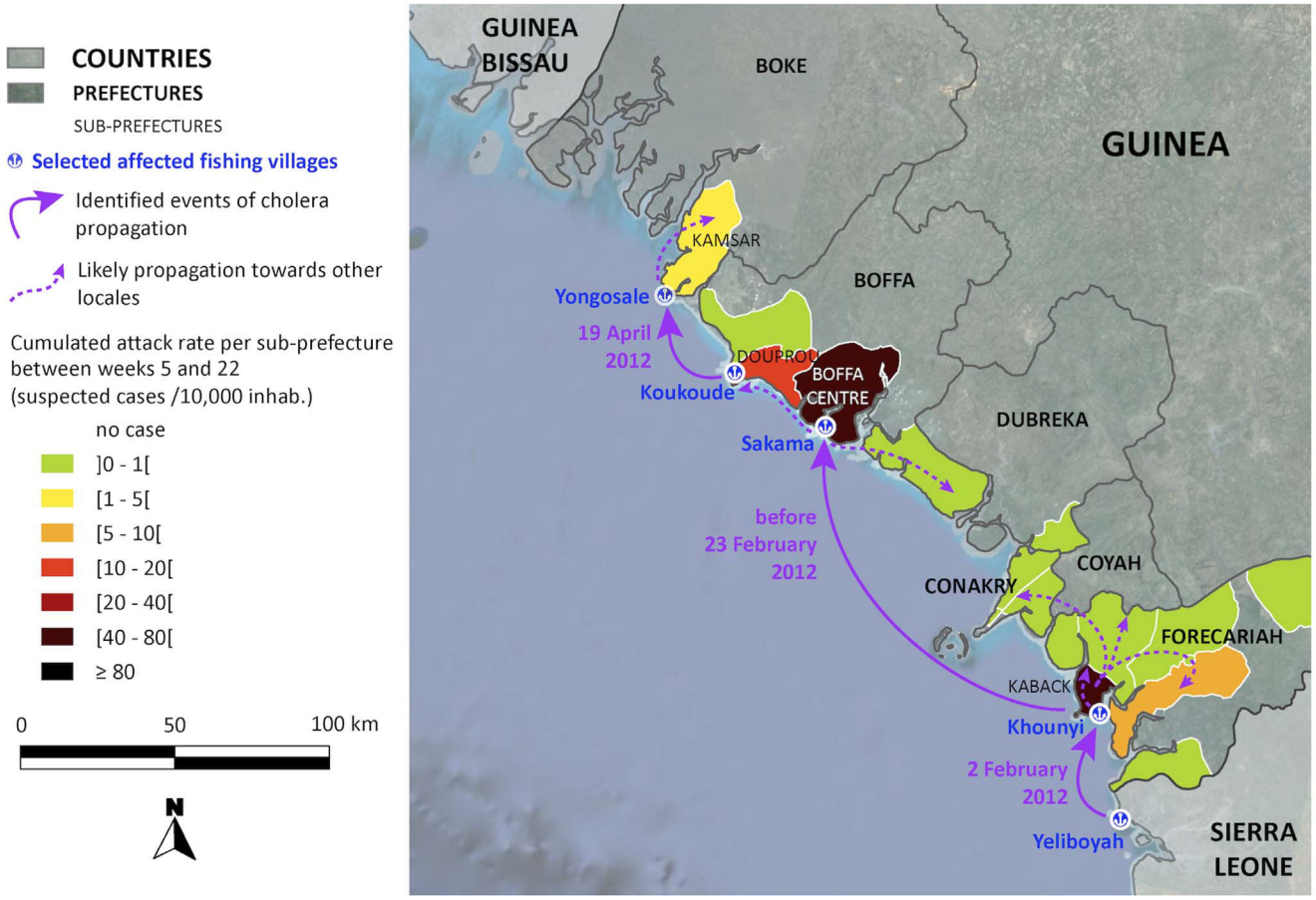

Figure 3. Cholera in Maritime Guinea between February and May 2012. The map illustrates the early propagation of the outbreak along the coast and the cumulated attack rate per sub-prefecture. Village positions are available on the Index Mundi website (http://www.indexmundi.com/zp/gv/).
doi:10.1371/journal.pntd.0002898.g003

MLVA-based genotyping and strain relatedness analysis

Fourteen samples out of 50 were not positive by culture and 2 additional samples were heavily contaminated. However, direct DNA extraction from transport tubes was successful for 4 culture-negative isolates. Genotype analysis with the 6-VNTR panel was thus performed on 38 *V. cholerae* isolates. All strains displayed constant results for the VC1, VC5 and LAV8 assays, while the VC4, VC9 and LAV6 assays revealed 4, 3 and 6 allelic variants, respectively. Based on the MLVA results, the strains were grouped into 12 different genotypic profiles, all of which were very closely related (Figure 4). All strains seemed to have arisen from genotype #1, which was identified as the founder genotype using the eBURST algorithm. Genotype #1 represented the earliest genotype isolated during the 2012 epidemic (on Kaback Island in February 2012) as well as the most frequent genotype identified (Figure 4). Subsequent diversification of this clone occurred via 1 or 2 mutational events during its propagation across the country (Figure 4).

Whole-genome sequencing of the *V. cholerae* founder clone

The genome of a genotype #1 strain isolated on February 28, 2012 in Kaback was examined via whole-genome sequencing. The cluster composition of the virulence genes displayed one "hybrid" CTXϕ prophage on chromosome 1 but no RS1 fragment. Sequence results showed that this "hybrid" CTXϕ harbors a majority of El Tor allele genes (e.g., *zot*, *ace* and *cep*) with a classical *ctxB* gene (encoding the B subunit of the cholera toxin) and a classical *rstR* gene. Strain phylogeny based on genome-wide SNP analysis situated this Guinean "atypical" El Tor variant within a new clade of the third and most recent wave of the seventh pandemic (Figure 5). This strain was thus distinct from both strains isolated in Mozambique in 2004–2005 (second wave) and strains isolated between 2005 and 2010 in Eastern Africa (i.e., the Kenyan clade within the third wave, indicated in purple on Figure 5). The Guinean 2012 strain was also found to be clearly separated from two South Asian clades (indicated in sky blue on Figure 5), which includes the Haitian clone. The closest relative of the Guinean strain was a strain isolated in 1994 in Bangladesh.

Discussion

While tracking the origin of the 2012 Guinean cholera epidemic, this multidisciplinary study demonstrates the monoclonal nature of the epidemic, as clinical *V. cholerae* strains exhibited a progressive genetic diversification that paralleled outbreak diffusion from Kaback Island. Molecular results confirmed the epidemiological findings, as the single ancestral and most abundant genotype was the sole *V. cholerae* strain isolated during the onset of the epidemic in February, at the initial focus of Kaback. According to field investigations, the index case was a

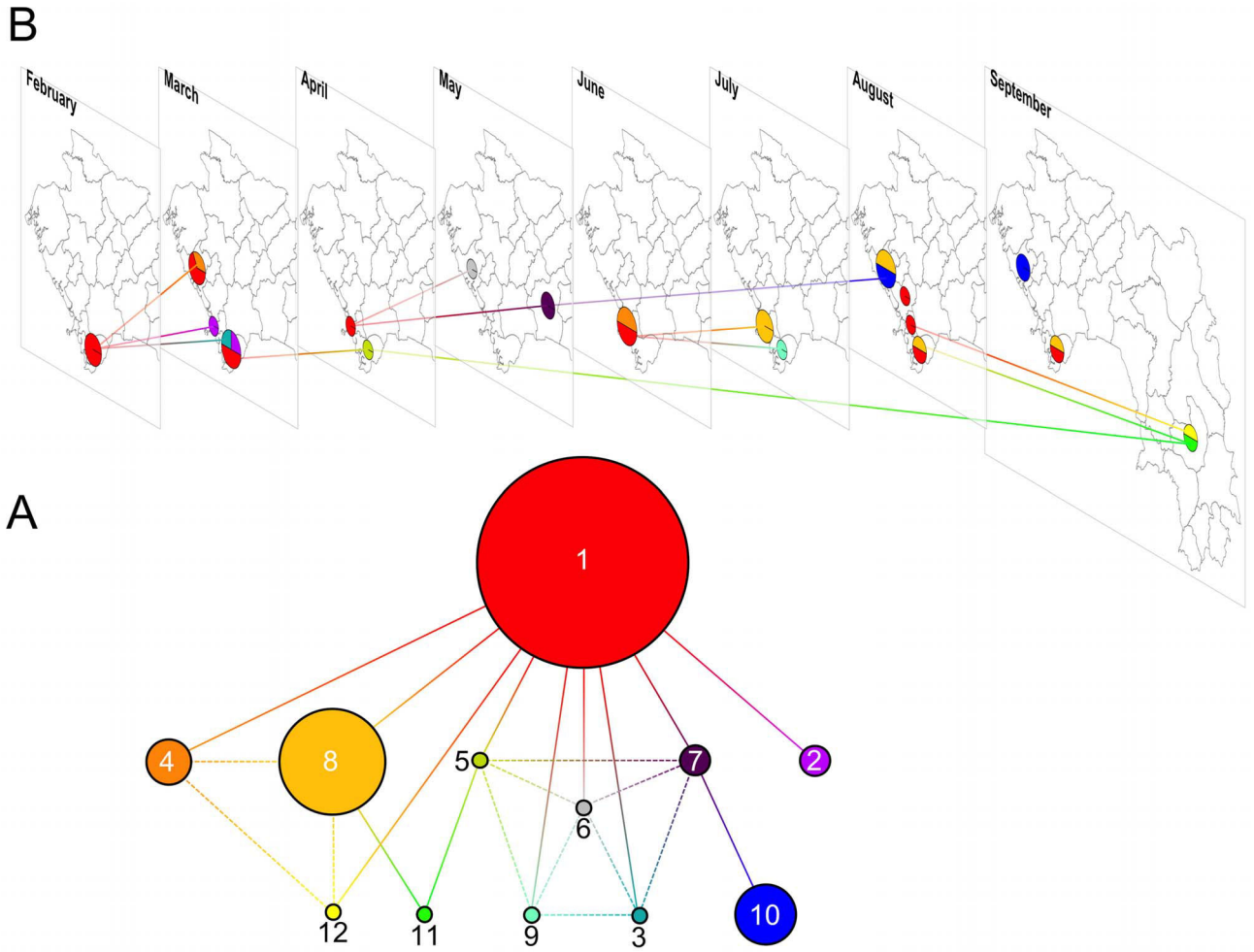

Figure 4. MLVA-based genotypes and relatedness of 38 clinical *V. cholerae* strains isolated in Guinea in 2012. (A) Network of *V. cholerae* strain relatedness based on MLVA genotype. Following the genotype analysis of 38 *V. cholerae* strains using 6 different microsatellite loci, the strains were grouped according to the resulting MLVA genotype profiles. Each colored circle corresponds to a different genotype. The numbers indicate the sequential order when the first strain of the corresponding genotype was isolated. Circle diameter is relatively proportional to the number of isolates represented by each genotype (e.g., 14 strains displayed genotype #1, 7 isolates displayed genotype #8 and 1 strain displayed genotype #3). Each segment corresponds to a single mutation at 1 of the 6 assessed VNTRs. Bold segments represent primary and likely relatedness links between genotypes, while the dashed segments represent secondary and less likely genetic relationships. Genotype #1 was identified as the founder genotype using the eBURST algorithm. (B) Spatiotemporal repartition of genotyped *V. cholerae* strains. Prefecture-level maps of Guinea are displayed for each month from February to September 2012. The genotype color code described in Figure 4A was applied to spatially and temporarily localize the isolated strains. Therefore, the pie charts reflect the month and prefecture of strain isolation (represented by strain genotype) as well as the relative proportion of each genotype among them. Segments between different months spatially and temporarily illustrate the genetic relatedness displayed in Figure 4A. Only primary and sequentially earliest links between genotypes are represented.
doi:10.1371/journal.pntd.0002898.g004

fisherman arriving from a nearby cholera-affected district of Sierra Leone. Cholera then bounced along the Guinean coast, likely carried by other infected fishermen, before exploding during the rainy season in the capital Conakry and subsequently spreading inland. This clone was found to be an "atypical" El Tor variant of *V. cholerae*, as determined via whole-genome sequencing. Furthermore, this Guinean strain phylogenetically grouped into a new clade of the third wave of the current pandemic, and the closest known relative was a strain isolated in Bangladesh in 1994. This study represents the first such molecular analysis of a cholera epidemic conducted in West Africa.

Overall, these results strongly suggest that cholera spread along the coast of Guinea due to human-driven diffusion of the bacterium. According to the molecular analyses, this epidemic was caused by a single clone, which rapidly evolved in parallel with the spatiotemporal spread of the epidemic. A few weeks after identification of the founder clone in Kaback, the same genotype was identified in new outbreak foci further along the coast, where it was likely transported by infected traveling fishermen. Likewise, isolates characterized by descendant genotypes were found to have spread across the country throughout the year, with strains of the most distant genotypes primarily identified in distant prefectures, such as Kerouane, several months later (e.g., August and September). Such genotype analysis has rarely been conducted to assess cholera epidemic diffusion from the onset. However, similar genetic diversification from an initial *V. cholerae* clone has been recently observed throughout the current epidemic in Haiti [22], where the human-associated importation of cholera is largely undoubted [23,24]. Furthermore, the diffusion of cholera by traveling fishermen has already been documented in West Africa.

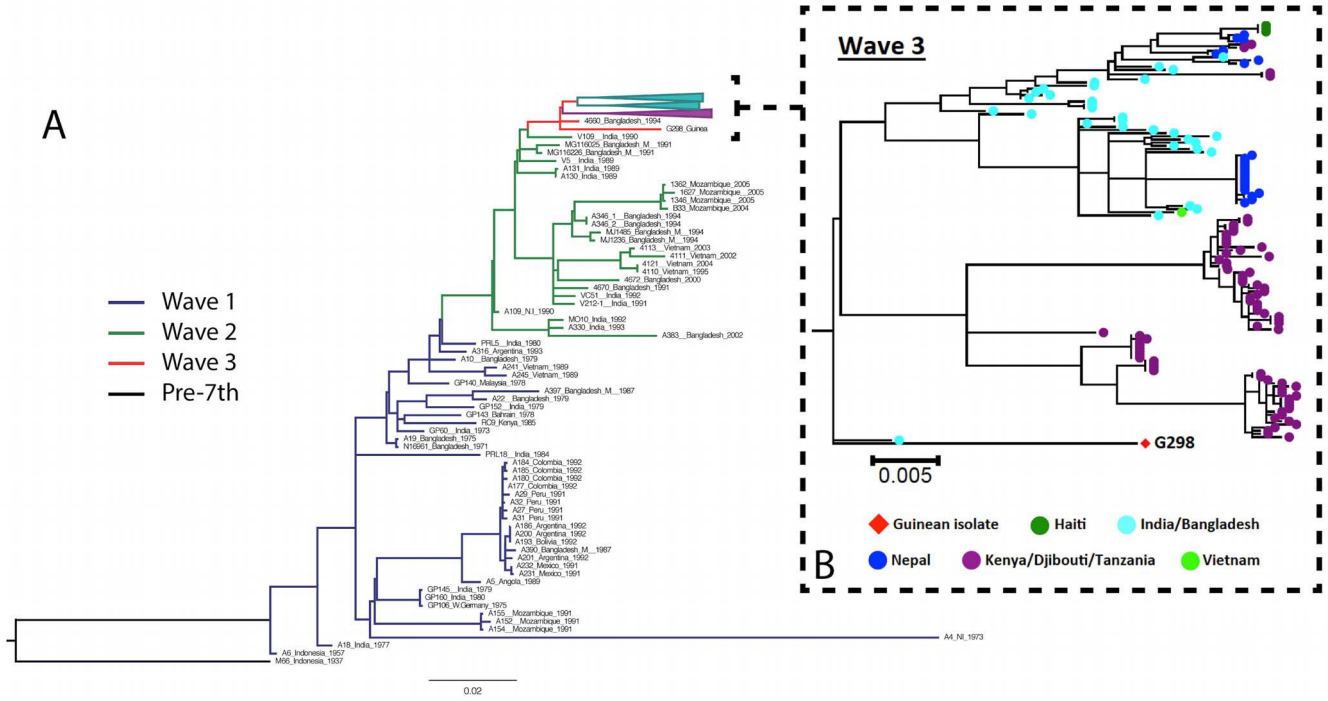

Figure 5. (A) Maximum likelihood phylogenetic tree of the seventh pandemic lineage of *V. cholerae* based on the SNP differences across the whole core genome and including a strain isolated during the onset of the Guinean 2012 outbreak. The pre-seventh pandemic isolate M66 was used as an outgroup to root the tree. Blue, green and red branches represent waves 1, 2 and 3, respectively. Purple and sky blue clade lineages represent the Kenyan clade and two South Asian clades within the third wave, respectively. Scale is provided as the number of substitutions per variable site. (B) Greater resolution of wave 3 of the seventh pandemic, in which the Guinean strain clustered distinctly from the two South Asian clades and the dominant Kenyan clade. Guinean isolate G298 is represented by the square while each colored circle indicates a spatially different isolate (as shown in the key). Scale is provided as the number of substitutions per variable site.
doi:10.1371/journal.pntd.0002898.g005

For example, the arrival of the seventh cholera pandemic in Ghana in 1971 was linked to the repatriation of a man who had succumbed to the disease while fishing in the waters of Togo, Liberia and Guinea [25].

Conversely, had the 2012 cholera epidemic originated from a local aquatic reservoir of proliferating vibrios, the diversity of *V. cholerae* strains found in the environment would have resulted in the early identification of several distinct clones [8,26]. Therefore, the emergence of a unique *V. cholerae* genotype in clinical samples isolated on Kaback Island in February does not correlate with environment-to-human transmission of the disease. Furthermore, this period was not characterized by the wet and warm climatic conditions that are considered to be a favorable to *V. cholerae* proliferation in water bodies [8–12]. Finally, a recent review addressing cholera epidemics in African coastal areas has indicated that no perennial environmental reservoir of toxigenic *V. cholerae* O1 has yet been identified in West Africa, which may be attributed to the lack of appropriate studies [27].

The epidemiological data rather suggest that cholera was imported to Guinea from Sierra Leone. Indeed, Kaback is situated less than 30 km away from this neighboring country. Nearby districts of Sierra Leone, including Kambia and Port Loko, were already affected by the disease in early January 2012 [28]. Furthermore, the index case identified in Kaback was a travelling fisherman who had just arrived from a fishing village in Kambia. Unlike Guinea, where an efficient early alert system [4] enabled the detection, report, investigation, laboratory-confirmation and official declaration of the outbreak within 8 days after the appearance of the first cholera case observed in the past 3 years, health authorities in Sierra Leone did not perform similar investigations. Thus, the origin of this cholera epidemic in Sierra Leone remains unclear, although possible importation events by fishermen travelling from Liberia and Ghana have been reported [29].

Finally, according to whole-genome sequence analysis, this epidemic was caused by an "atypical" El Tor variant of *V. cholerae* O1, a type of strain that harbors both El Tor biotype genetic elements and the Classical biotype *ctxB* gene [30]. Such "atypical" El Tor strains initially emerged in Asia in 1991 and were first detected on the African continent in 2004 [31]. This may also present major public health implications as these strains have been suggested to be associated with more severe clinical symptoms compared with conventional El Tor strains [32,33]. Furthermore, genome-wide SNP-based phylogeny analysis grouped the Guinean 2012 clone into a recent clade within the third wave of the seventh pandemic. Several studies have shown that this monophyletic radiation is largely distinct from the vast diversity of *V. cholerae* environmental strains [14,34], which suggests that cholera epidemics are clonal and caused by a specific subset of related *V. cholerae* strains often spread via human-to-human transmission [14,35].

Nevertheless, to confirm the origin of the *V. cholerae* clone responsible for this epidemic, it would have been ideal to analyze pre-epidemic environmental isolates as well as isolates from previous epidemics in Guinea, isolates from Sierra Leone and strains from other countries the region. However, earlier Guinean isolates were not stored and we did not have access to strains from

Sierra Leone. Furthermore, no study of environmental *V. cholerae* strains had previously been performed in the region.

In conclusion, by tracking the origin of the 2012 cholera epidemic in the Republic of Guinea, this study identified fishermen as cholera victims and vectors during the early phase of epidemic propagation. Improving water and sanitation infrastructures, implementing enhanced hygiene education programs and targeting oral cholera vaccination campaigns in high-risk coastal areas could thus benefit these vulnerable populations and prevent the spread of future cholera outbreaks. The likely Sierra Leonean origin of this Guinean epidemic highlights the importance of encouraging transborder collaboration in the surveillance and control of highly mobile populations and main communication routes so as to rapidly identify emerging foci and organize coordinated targeted responses. These results also support the implementation of biobanks dedicated to prospective clinical and environmental *V. cholerae* isolates, to perform molecular epidemiological analyses, which have become essential to interpret field investigation data. Such an integrated approach would provide valuable insights concerning cholera in other African regions, where the key determinants of all too frequent epidemics still remain poorly understood and prevention or control strategies are not always accurately oriented.

Acknowledgments

The authors would like to thank all the staff who took part in patient care, field investigations, data reporting as well as sample collection, transport, processing and analysis. In particular, the authors are indebted to Leonard Heyerdahl (AMP, France), who took part in organizing the prospective sampling of clinical cholera samples. This study wouldn't have been possible without H Diallo (INSP, Guinea) who performed initial vibrio cultures, the staff of HIA Laveran who cultured and performed the MLVA in Marseille and the staff of Aix-Marseille Université who sequenced and analyzed the *V. cholerae* clone. The authors are also grateful to Bertrand Sudre, who provided critical comments on the manuscript.

Author Contributions

Conceived and designed the experiments: SR MAM BMNL SK RP. Performed the experiments: SR LK SM AM YK OY VSK PEF EG SK. Analyzed the data: SR AM PEF SK RP. Contributed reagents/materials/analysis tools: SR MAM LK AM BMNL PEF EG RP. Wrote the paper: SR MAM LK SM AM YK OY VSK BMNL PEF EG SK RP.

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2.2.3 L'importation humaine d'une épidémie clonale depuis la Sierra Leone

Ces analyses sur l'origine du choléra en Guinée en février 2012 suggèrent donc très fortement que l'épidémie fut importée par un pêcheur arrivé de la Sierra Leone voisine, alors touchée par une épidémie de choléra non contrôlée. Le clone de *V. cholerae* O1 impliqué est l'un de ces nouveaux variants El Tor apparus dans les années 1990 et vraisemblablement associés à des épidémies plus sévères qu'auparavant. De fait, il appartient à la troisième vague de la septième pandémie, au sein de laquelle la souche la plus proche a été isolée au Bangladesh en 1994. Ce travail démontre également que l'utilisation intégrée de différentes méthodes incluant épidémiologie de terrain, analyses spatio-temporelles et génotypage des souches constitue une approche pertinente pour comprendre l'origine des épidémies en Afrique. Afin d'améliorer la prévention des épidémies futures, ces résultats incitent à renforcer la coopération transfrontalière en matière de surveillance des épidémies de choléra, et à maximiser les interventions sur les populations à haut risque comme les pêcheurs. A la fois premières victimes et vecteurs inauguraux de cette épidémie, ceux-ci furent ainsi ciblés par des campagnes de vaccination orale contre le choléra menées en 2012 avec le soutien de Médecins-Sans-Frontières [75], puis en 2013 [76]. Enfin, ce travail confirme la bonne sensibilité du système de surveillance du choléra mis en place en Guinée.

En 2013, le pays connut une reprise épidémique partie de Mamou, une préfecture de l'intérieur, avant de gagner la capitale Conakry. Au total, 319 cas dont 32 décès furent comptabilisés [77]. Depuis le début de l'année 2014, seuls 2 cas suspects ont été recensés dans une zone côtière du pays [78], ce qui est bien heureux compte-tenu de l'épidémie d'Ebola sans précédent qui frappe la Guinée, la Sierra Leone et le Liberia depuis fin 2013 [79]. La survenue concomitante d'une épidémie de choléra compliquerait certainement cette situation déjà dramatique. Non loin de là, le Ghana est pourtant touché actuellement par une épidémie importante de choléra. Le risque de superposition des aires de répartition de ces 2 épidémies doit être pris en compte et nous avons ainsi soumis une lettre au New England Journal of Medicine pour sensibiliser

[75] Luquero et al., « First Outbreak Response Using an Oral Cholera Vaccine in Africa »; Luquero et al., « Use of Vibrio cholerae Vaccine in an Outbreak in Guinea ».
[76] Ministère de la Santé et de l'Hygiène Publique de la République de Guinée et Division Prévention et Lutte contre la Maladie, *Infochol Guinée 2013*.
[77] Organisation Mondiale de la Santé et Section D'hygiène Du Secrétariat De La Société Des Nations, « Choléra, 2013 ».
[78] UNICEF WCARO, *Cholera outbreak in the West and Central Africa: Regional Update, 2014*.
[79] Baize et al., « Emergence of Zaire Ebola Virus Disease in Guinea »; « Ebola Virus Disease in West Africa — The First 9 Months of the Epidemic and Forward Projections ».

la communauté internationale sur le sujet. Elle a été récemment acceptée, pour une publication dont la date n'est pour l'instant pas connue.

2.2.4 Lettre 3 : « Ebola and cholera : a call to prevent a coumpound epidemic disaster »

A lire ci-après.

The NEW ENGLAND JOURNAL of MEDICINE

Ebola and cholera: a call to prevent a compound epidemic disaster

Journal:	*New England Journal of Medicine*
Manuscript ID:	Draft
Article Type:	Letter about NEJM Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Rebaudet, Stanislas; Aix-Marseille University/Asisitance-Publique Hôpitaux de Marseille, Parasitology and Mycology Moore, Sandra; Aix-Marseille University, Piarroux, Renaud; Aix-Marseille University/Asisitance-Publique Hôpitaux de Marseille, Parasitology and Mycology
Abstract:	

The WHO Ebola Response Team recently predicted that the current Ebola epidemic will claim a dreadful 20,000 combined cases by early November 2014, assuming no change in the control measures applied in West Africa (1). The threat that Ebola poses to national public health, social, economic and security foundations may worsen if a secondary epidemic eventually exploded in the region. Since June 2014, nearby Ghana has been affected by a serious cholera epidemic already responsible for 12,622 cases as of September 6 (2). Current cholera and Ebola zones are separated by Ivory Coast, a frequented crossing point for commuters traversing West Africa. Cholera control requires specialized treatment centers as well as community water, sanitation and hygiene prevention activities. However, in Ebola-affected areas, quarantine units are overwhelmed, many health facilities are dysfunctional following desertion by staff fearing viral contamination and conducting awareness campaigns has become increasingly dangerous due to violence against health and humanitarian workers accused of spreading Ebola. Likewise, neglecting to rapidly control this cholera epidemic in Ghana could have unpredictable yet potentially devastating consequences.

2.3 Épidémiologie dynamique du choléra au Mozambique de 2009 à 2011

2.3.1 Comment se caractérise l'endémicité du choléra au Mozambique ?

De l'autre côté du continent africain, le Mozambique représente un second pays où le choléra est classiquement présenté comme endémique [80]. De fait, les autorités sanitaires y ont recensé des cas sans discontinuer depuis 1997 [81]. En 2003-2004, une campagne de vaccination de masse par un vaccin anticholérique oral fut donc conduite dans un quartier pauvre du centre de Beira, une ville inondable construite au bord d'un estuaire [82].

Dans le cadre du consortium Africhol, nous fûmes mis à contribution afin d'aider méthodologiquement l'équipe mozambicaine à analyser ses données de surveillance du choléra entre 2009 et début 2011. Nous nous chargeâmes des analyses spatiales, incluant la recherche de clusters spatiaux, et participâmes à l'écriture d'un article également publié en 2013 dans le supplément du JID [83].

2.3.2 Article 6 : « Cholera epidemiology in Mozambique using national surveillance data »

A lire ci-après.

[80] Gaffga, Tauxe, et Mintz, « Cholera »; Ali et al., « The global burden of cholera ».
[81] World Health Organization, « Cholera [every year since 1968] ».
[82] Lucas et al., « Effectiveness of mass oral cholera vaccination in Beira, Mozambique ».
[83] Gujral et al., « Cholera Epidemiology in Mozambique Using National Surveillance Data ».

SUPPLEMENT ARTICLE

Cholera Epidemiology in Mozambique Using National Surveillance Data

Lorna Gujral,[1] Cynthia Sema,[2] Stanislas Rebaudet,[3] Catia Luciana Abulfattahe Taibo,[4] Alice Afonso Manjate,[4] Renaud Piarroux,[3] Bradford D. Gessner,[5] and Ilesh V. Jani[2]

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Background. Mozambique has experienced cholera for several decades. This study was undertaken to evaluate epidemiologic patterns to assist in guiding public health interventions.

Methods. We evaluated district-level Ministry of Health data for 123 consecutive weeks starting 1 January 2009. Cholera cases reported to the national level were based on clinical suspicion rather than microbiological confirmation. Time and space analyses with mapping and spatial statistics were undertaken.

Results. During 2009–2011, Mozambique identified 220 deaths among the 25 431 reported suspected cholera cases (case fatality ratio [CFR], 0.87%). There were 108 outbreaks that occurred in 73 (50%) of Mozambique's 145 districts. Five distinct spatial clusters were identified involving inland and coastal as well as rural and urban populations. Among 78 outbreaks whose duration was known, average duration was 7.2 weeks (median, 6; range, 1–25). During weeks 1–3, 4–6, 7–9, and ≥10 after an outbreak, CFRs were 1.6%, 0.66%, 0.33%, and 0.25%, respectively. During 2010, districts that experienced an outbreak during 2009 had a CFR of 0.2% compared with 4.3% among other districts.

Discussion. Mozambique continues to experience widespread cholera outbreaks of short duration involving distinct spatial clusters. These findings will influence choice of public health strategies.

Keywords. Africa; cholera; epidemiology; mapping; Mozambique; spatial mapping; *Vibrio cholerae*.

Cholera is endemic currently in sub–Saharan Africa and causes substantial outbreaks in many countries [1, 2]. Despite relatively straightforward medical management, such as the administration of oral rehydration solution, case fatality ratios (CFRs) exceed 5% in several countries [1, 2]. The World Health Organization (WHO) has noted that epidemics in Africa are becoming "more frequent, larger and longer lasting" and the new variant strain of *Vibrio cholerae* O1 El Tor may cause a more severe disease [3].

Mozambique is one of the sub–Saharan African countries that has experienced endemic and epidemic cholera for decades. Annual incidences reported in the literature have ranged from 0–211 per 100 000 population; CFRs before the mid-1980s frequently exceeded 10% but more recently have decreased substantially [4]. This study was designed to review and update data on cholera in Mozambique with an objective of guiding public health interventions.

METHODS

Historical Data Reported to the World Health Organization

We reviewed data reported by the Mozambique Ministry of Health (MOH) to the WHO reportable disease surveillance database [5] to identify cholera cases and deaths in Mozambique for the years 2000–2009. For calculation of incidence, we assumed a national population of 18.3 million during 2000 and an annual 2.5% growth rate (http://siteresources.worldbank.org/INTPROSPECTS/Resources/334934-1199807908806/Mozambique.pdf).

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The Journal of Infectious Diseases 2013;208(S1):S107–14

DOI: 10.1093/infdis/jit212

Current Data Housed at the Ministry of Health

We reviewed data collected by the National MOH for the most recent period available, namely 2009 through week 19 of 2011. In Mozambique, epidemiological data are used to identify districts at cholera risk and to target evaluation of outcomes such as insufficient supply of drinking water, sanitation practices harmful to health (such as defecating in the open, using dirty or contaminated water, and handling and sale of unhygienic food), and natural disasters (drought or flood).

Data are recorded in record books and reporting forms, which are then transferred to daily summary sheets and collected and sent weekly from a health facility to the District Health Office. The District Health Office sends information to the Provincial Health Office, which sends the information to the Department of Epidemiology at the National MOH.

Historically, diarrheal disease incidence and case reporting have been higher during the rainy season, which occurs from approximately November to April with a peak during January to March. An increase in diarrhea cases raises suspicion of cholera if (1) the proportion of adults with diarrhea is rising, (2) there is an increase in the registration of cases of diarrhea with dehydration in adults, or (3) there are deaths from diarrhea in adults. If one of these events occurs, laboratory confirmation is sought, regardless of whether or not symptoms are typical of cholera.

The initial case of suspected cholera in a neighborhood is tested for microbiological confirmation if the case involves severe diarrhea and dehydration and stool with a cholera appearance (rice water possibly with a distinct smell). Confirmation is performed at the local level and specimens and results are rarely sent to the National Reference Laboratory. If these conditions are met, a rectal swab is collected and sent to the provincial, district, or, in rare cases, private laboratories. These laboratories perform culture and routine microbiology but generally no serotyping or antibiotic resistance testing. If the rectal swab is positive on culture for *V. cholerae*, the case is considered confirmed. All persons with clinical cholera (severe diarrhea, dehydration, and characteristic appearance of stool) from the same neighborhood as the confirmed case are then considered cholera cases. Depending on local practices, additional cases may have culture performed, but this is not necessary for the national surveillance system to consider additional cases to be cholera if they meet the clinical definition. From the point of confirmation of cholera in a neighborhood, clinically appropriate illness is considered cholera for a varying period of time depending on the district, ranging from 2–6 months. Sites use the same criteria for cholera in children aged <5 years.

Once cholera is confirmed, the affected area is considered to be experiencing a cholera epidemic or outbreak (ie, an outbreak is defined as identification of ≥1 confirmed cholera cases in a neighborhood regardless of previous experience of cholera). During the outbreak period, case notification occurs daily to weekly by radio, telephone, or fax. Data reported to the national infectious disease surveillance system include new cases, total number of cases, district, and outcome (death or survival). Outside of special investigations, data are not available currently on age, sex, residence at the subdistrict level, or other epidemiological features; laboratory data are also not available.

Data Analyses and Mapping

We used national surveillance data to calculate incidence and mortality rates at provincial and district levels using 2007 census data for the appropriate administrative level (ie, by country, province, or district) provided by the National MOH. We evaluated individual outbreaks at the smallest geographical unit available (ie, the district level). An outbreak was considered to have begun during the first week when any case was reported and ended during the last week in which cases occurred and that was followed by at least 4 weeks with no cases. We conducted a separate analysis based on defining individual outbreaks as those with at least 8 weeks with no cases. However, in all but 2 instances where there was a gap of at least 4 weeks there was also a gap of at least 8 weeks; consequently results did not change substantially, and data are not presented. Because our data started on 1 January 2009, for outbreaks that were already underway, we could not estimate outbreak duration.

For the studied period, cholera cases at district level were mapped on ESRI shapefiles from the Map Library (http://www.maplibrary.org) and using Quantum GIS software version 1.7.3 (http://www.qgis.org/). To investigate for spatial clustering of cases on the studied period, we used SaTScan software version 9.1.1 (http://www.satscan.org) [6]. This purely spatial scan statistic systematically moves a circular scanning window of increasing diameter over the district centroids and compares observed case numbers inside the window with the numbers that would be expected under the null hypothesis (ie, a random distribution of cases). Because of their small size and their semicircular location, districts of Cidade de Maputo were aggregated. The maximum spatial cluster size was 50% of the at-risk mean 2009–2011 population. The significance for each cluster was obtained through Monte Carlo hypothesis testing with 999 random replications.

To investigate the possible link between cholera and rainfall for each cluster, we obtained estimated daily precipitation from the National Oceanic and Atmospheric Administration Climate Prediction Center provided by the International Research Institute for Climate and Society IRI/LDEO Climate Data Library (http://iridl.ldeo.columbia.edu/SOURCES/.NOAA/.NCEP/.CPC/.FEWS/.Africa/.DAILY/.RFEv2). Daily rainfall was extracted at each cluster centroid, aggregated on a weekly basis, and plotted together with weekly cases within clusters.

Laboratory Analysis

Laboratory surveillance and national epidemiological surveillance exist separately in Mozambique. We evaluated data collected between 2007 and 2011 for stool samples analyzed at the National Reference Laboratory in Maputo. Stool samples were cultured in tubes containing peptone water and incubated for 2 hours at 37°C. After this, the content was transferred to plates with thiosulfate-citrate-bile salts-sucrose (TCBS) solid-selective media and incubated again at 37°C for 18–24 hours. Colonies were initially identified based on morphologic characteristics. Preliminary speciation of suspected cholera was performed using appropriate biochemical tests, whereas final identification was performed using agglutination with specific antiserum. Antimicrobial susceptibility testing was determined by disc diffusion.

Ethics

The current evaluation used only aggregated public health surveillance data. Consequently, no institutional review board approval was sought or obtained.

RESULTS

World Health Organization Reports 2000–2009

During 2000–2009, suspected cholera cases reported annually to the WHO varied from a low of 2226 during 2005 to a high of 24 375 during 2002 (Table 1). Cholera deaths varied from a low of 22 during 2007 to a high of 342 during 2002. CFRs varied from 0.46%–1.4% during 2000–2009. Neither case counts nor CFRs showed any consistent decline during the evaluation period. The national annual cholera incidence varied 12.0–126.5 per 100 000 population during 2000–2009.

Table 1. Annual National Cholera Incidence and Case Fatality Ratios Based on Data Reported to the World Health Organization by the Mozambique Ministry of Health

Year	Cases	Deaths	Case Fatality Ratio	Estimated Population (millions)	Annual Incidence (per 100 000)
2000	17 649	238	1.4%	18.3	96.2
2001	8794	102	1.2%	18.8	46.8
2002	24 375	342	1.4%	19.3	126.5
2003	13 758	102	0.7%	19.7	69.7
2004	20 080	110	0.6%	20.2	99.2
2005	2226	24	1.1%	20.7	10.7
2006	6306	29	0.5%	21.3	29.7
2007	2622	22	0.8%	21.8	12.0
2008	9087	102	1.1%	22.3	40.7
2009	19 679	155	0.8%	22.9	85.9

Data from [16].

National Surveillance Data 2009–2011

Cases and Incidence

From 2009 through week 19 of 2011, there were 25 431 suspected cholera cases reported, and the average annual incidence during 2009–2010 was 55 per 100 000 population. Cases mostly occurred in early 2009 (Figure 1), and their distribution exhibited a marked heterogeneity at province and district levels. Total cases within Mozambique's 11 provinces ranged from 37 in Inhambane Province and 128 in Gaza Province to 5473 in Cabo Delgado Province and 6806 in Zambezia Province; annual incidences in these 4 provinces were, respectively, 1.4, 5.6, 143, and 82 per 100 000 population during 2009–2010.

Case count and incidence variations within Mozambique's 145 districts were even more extreme (Figures 2 and 3). Seventy-two districts (50%) reported no cholera cases during 2009–2011, whereas 16 (11%) reported >500 cases and 8 (6%) reported >1000 cases (maximum, 2553). Twenty districts (14%) reported incidences of >200 per 100 000 population during at least 1 year, with an annual peak of 1163 per 100 000 population. Most affected districts were not systematically located along the coast, including Montepuez, Gurue, Mocuba, Machaze or Mossurize.

Spatial cluster analysis over the study period identified 5 areas with an increase in the observed to expected number of cases (O/E) (Supplementary Figure 1). The first cluster (O/E = 4.50; $P < 10^{-17}$) was located in northeastern Mozambique and included 7 districts around the coastal Pemba City, which was severely stricken in early 2009. The second cluster (O/E = 11.63; $P < 10^{-17}$) was in Quelimane City, an estuarine city repeatedly affected in the past. Tete City, a riverside town in Eastern Mozambique, represented the third cluster (O/E = 5.06; $P < 10^{-17}$). Cluster 4 (O/E = 2.61; $P < 10^{-17}$) consisted of 3 inland districts—Gurue, Namarroi, and Cuamba—affected in early 2010. Cluster 5 (O/E = 2.21; $P < 10^{-17}$) was located in Machaze and Mossurize, 2 inland districts along the border with Zimbabwe. Despite a high number of cases, Beira City, Mocuba district, and Maputo City were not detected as spatial clusters because of their large populations.

Seasonal and Other Temporal Variations

Time distribution of cholera exhibited a marked heterogeneity as well, with outbreaks separated by sometimes prolonged lull periods (Figures 1, 2, and 4). Cases at provincial (Figure 1) and district (Figure 2) levels exhibited seasonality with cases reported from November to January, peaking during the period from January to April and subsiding to nearly none from July through October. As observed in the 5 spatial clusters (Supplementary Figure 1), this periodicity coincided with the rainy season.

Mortality

During 2009–2011, Mozambique identified 220 deaths among the 25 431 reported suspected cholera cases for a CFR of 0.87%.

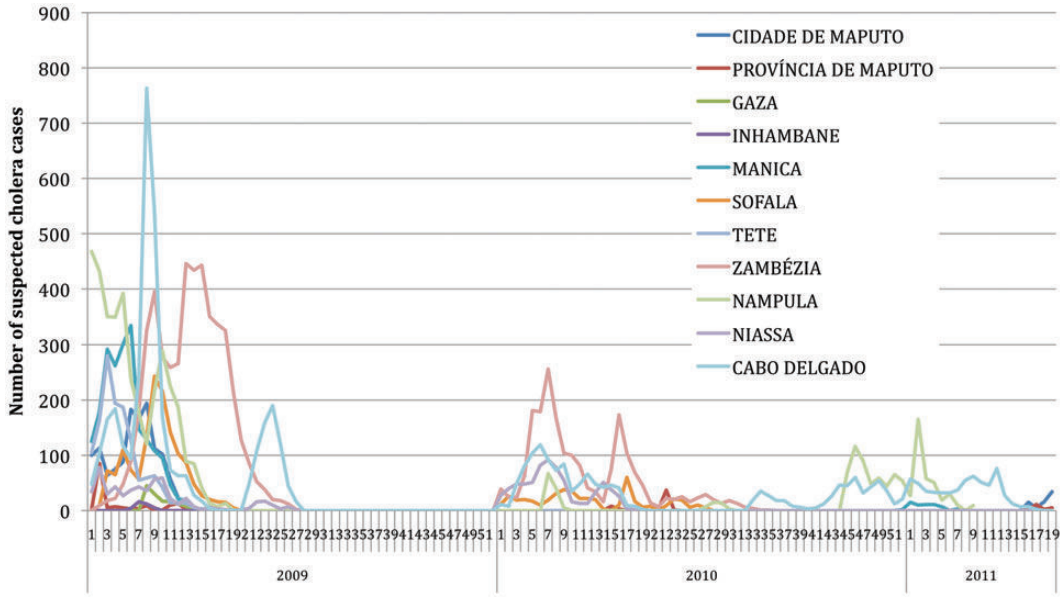

Figure 1. Number of suspected cholera cases reported to the Mozambique Ministry of Health by week for January 2009 through week 19 of 2011.

The CFR was 0.78% during 2009, 1.3% during 2010, and 0.35% through the first 19 weeks of 2011. Among the 11 provinces, CFR varied 0%–11% but the latter was based on only 4 deaths among 37 cases. Among 16 districts with at least 500 cases during the study period, the average CFR was 0.69% (median, 0.6%; range, 0–1.8%).

Outbreak Evaluation

We identified 108 outbreaks in 73 districts. Forty-seven districts (64%) reported 1 outbreak, 21 (29%) reported 2 outbreaks, 4 (5%) reported 3 outbreaks, and 1 (1%) reported 4 outbreaks. Of the 108 outbreaks, 30 were underway during the first 4 weeks of 2009, and thus their duration was unknown; for 11 districts,

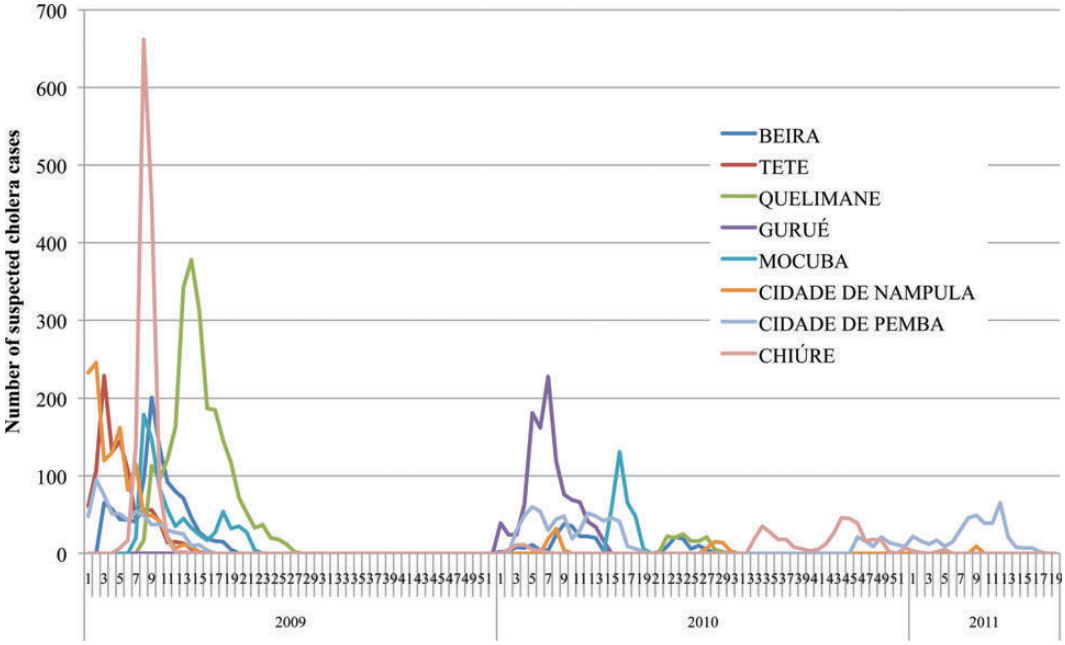

Figure 2. Weekly numbers of suspected cholera cases in the 8 districts with >1000 suspected cholera cases reported to the Ministry of Health from week 1 of 2009 through week 19 of 2011.

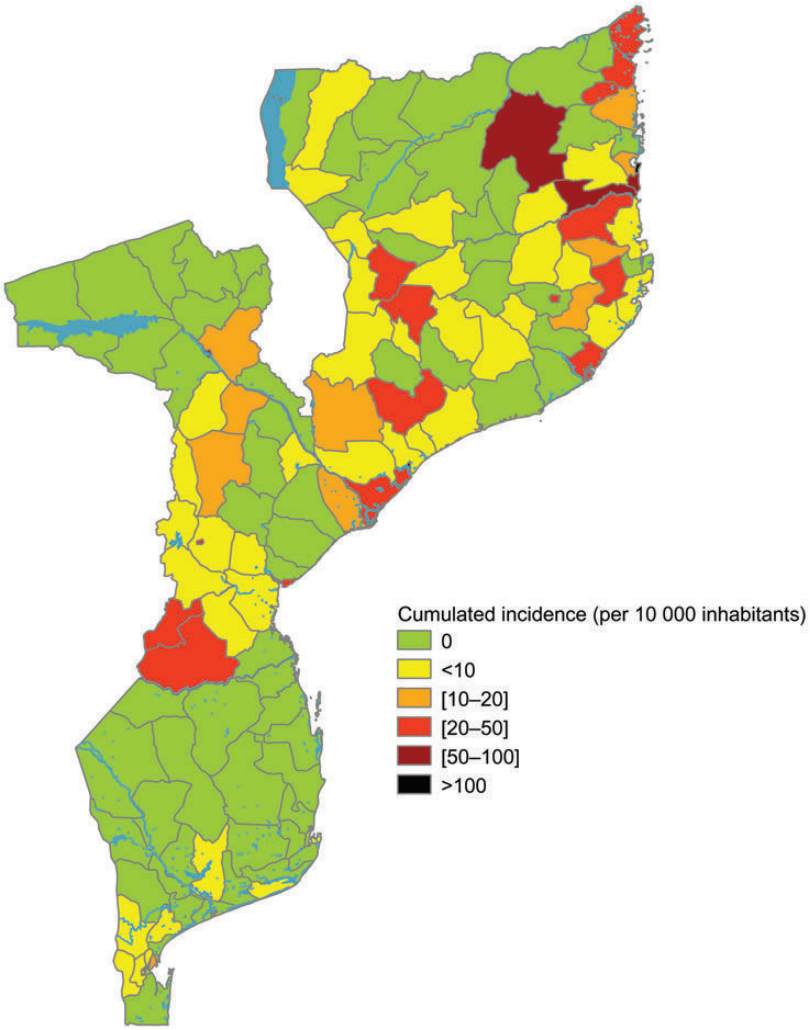

Figure 3. Cumulated cholera incidence per district from 2009 through week 19 of 2011.

this was the only outbreak reported. Among the 78 outbreaks in 62 districts for which duration was known, the average duration was 7.2 weeks (median, 6; range, 1–25), and 19 outbreaks (24%) lasted ≥10 weeks. Among the 30 outbreaks that were underway during January 2009, the average minimum duration was 10.7 weeks (mean, 10; range, 2–27), and 17 outbreaks lasted ≥10 weeks.

Among outbreaks at the district level whose duration was known, we determined the distribution of cases and deaths by outbreak week. Of the 15 197 cases reported during these 78 outbreaks, 5580 (36%) occurred during the first 3 weeks, 4830 (32%) during weeks 4–6, 2396 (17%) during weeks 7–9, and 2391 (16%) ≥10 weeks after the start of the outbreak (Figure 4). Among 132 reported deaths, 91 (66%) occurred during weeks 1–3, 32 (23%) during weeks 4–6, 8 (6%) during weeks 7–9, and 6 (4%) ≥10 weeks after the start of the outbreak. The CFRs during these 4 periods were 1.6%, 0.66%, 0.33%, and 0.25% ($\chi^2 = 56.3$; $P < .000001$).

We evaluated whether the presence of any outbreak during 2009 predicted CFR during the following year (2010). Among 23 districts with outbreaks in both 2009 and 2010, there were 3775 cases and 7 deaths (CFR, 0.2%), whereas among 11 districts with outbreaks reported during 2010 but none during 2009 there were 1658 cases and 71 deaths (CFR, 4.3%) ($\chi^2 = 137$; $P < .000001$).

All 11 provinces had ongoing outbreaks during the study period. In 3 provinces, 1 district in each experienced a single outbreak. At the extreme, 1 province (Cabo Delgado) had cases reported during 79 (64%) of the 123 study weeks.

Laboratory

During 2007–2011, 459 stool samples were evaluated, of which residence was known for 428; of these, 332 (78%) came from Maputo City and 49 (11%) from Maputo Province. Of the 459 samples, 204 (44%) had cholera identified, all serotype Ogawa. Among those with cholera identified, 106 (52%) were female;

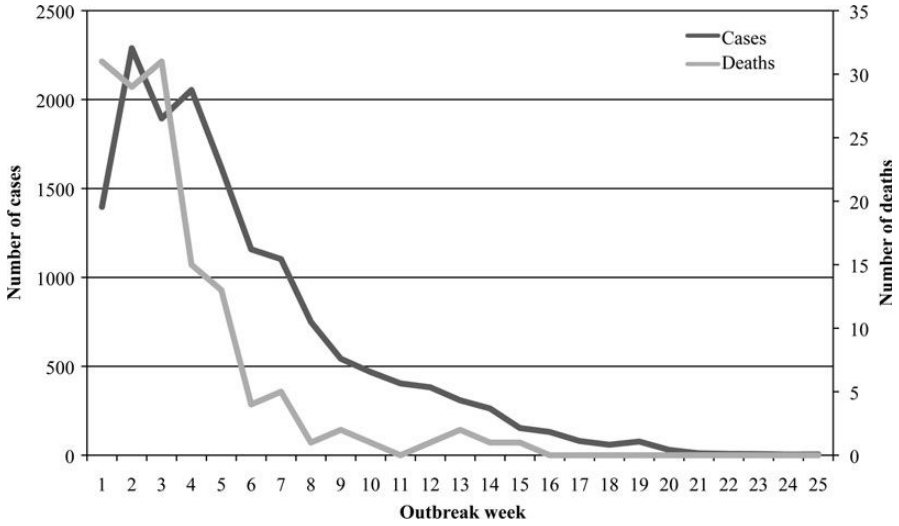

Figure 4. Summary number of reported suspected cholera cases and deaths for 78 district-level outbreaks for which duration was known by week after the outbreak commenced, Mozambique, 2009–2011.

4 (2%) were aged <1 year, 25 (12%) were aged 1–4 years, 21 (10%) were aged 5–9 years, 40 (20%) were aged 10–19 years, 64 (31%) were aged 20–29 years, 33 (16%) were aged 30–39 years, 10 (5%) were aged 40–49 years, and 7 (3%) were aged ≥50 years.

Sensitivity testing was done for 6 antibiotics but inconsistently based on availability of testing material. For ciprofloxacin, 200 isolates were tested, and 11 (6%) were intermediate or fully resistant (range, 0–21% by year). For gentamicin, 132 of 193 isolates (68%) were intermediate of fully resistant (range, 42%–93%). For erythromycin, 93 of 183 (51%) were intermediate of fully resistant (range, 24%–93%). For nalidixic acid, 167 of 193 (87%) were intermediate of fully resistant (range, 76%–86%). For tetracycline, 112 of 175 (64%) were intermediate of fully resistant (range, 42%–93%). For cotrimoxazole, 165 of 174 (95%) were intermediate of fully resistant (range, 61%–100%). For chloramphenicol, 106 of 146 (73%) were intermediate of fully resistant (range 51%–82%). From 2007–2009 to 2010–2011, statistically significant increases in intermediate of fully resistant status were observed for gentamicin (60%–100%; prevalence ratio 1.7; 95% confidence interval [CI], 1.5–1.9]), erythromycin (43%–93%; prevalence ratio 2.1; 95% CI, 1.7–2.6), and tetracycline (59%–93%; prevalence ratio 1.6; 95% CI, 1.3–1.9).

DISCUSSION

According to national surveillance data from 2009 through week 19 of 2011, cholera in Mozambique exhibited a marked spatial heterogeneity. Although all provinces were affected, outbreaks were distributed in only half of the districts. Cases clustered in both coastal and inland districts and in both rural and urban districts. Yet, Beira City and Maputo City, 2 estuarine cities with a historically high number of cases, were moderately affected during the study period, perhaps because of water chlorination and health education preventive activities, population awareness, or population immunity (including the effects of a previous vaccine demonstration project in Beira several years earlier [7]). Similarly, lakeside areas such as Lake Malawi or Lake Cahora Bassa were spared or very little affected, unlike previous observations in the African Great Lakes Region [8].

Moreover, we found that cholera in Mozambique was highly seasonal, with a marked concentration of cases during the rainy period, as observed in the past on a national level [4] or in other African regions distant from the equator, such as the Democratic Republic of Congo [9]. In such areas with inadequate water and sanitation, rainfall-induced overflow of latrines may indeed repeatedly contaminate wells and surface waters as suggested previously for the Democratic Republic of Congo [9] and Guinea [10]. Interannual variations of cholera morbidity also were observed, with 2009 more affected than 2010 and 2011. Previous studies have attributed this to variations in climate and precipitation [11, 12], although in Mozambique, areas experiencing clusters did not have more rainfall during 2009 than subsequent years.

During the study period, cholera cases were concentrated during relatively short outbreaks, with almost 70% of cases occurring during the first 6 weeks after an outbreak was notified. Between outbreaks, both inland and coastal districts often experienced prolonged periods with no reported cases. This provides evidence against a coastal endemicity of cholera in Mozambique, although more definitive microbiological studies are required.

Cholera outbreak identification and case management are important because mortality can be rapidly reduced by early and aggressive intervention. The majority of areas in the world have achieved CFRs of <1% [2]; even with limited resources, African countries have reduced CFRs from 10% to just under 2%. Mozambique in particular has made great strides, with a national CFR of 0.87% and no districts that reported a substantial number of cases having a CFR >2%. By comparison, a recent manuscript from the Democratic Republic of Congo reported a CFR of 2.2% over a 7-year period in the Kivu Provinces, a CFR likely accentuated by war [13].

We found that the great majority of cholera deaths occurred during the first weeks after the beginning of an outbreak. This result may have occurred because of delay in care-seeking or accessing traditional healers instead of health clinics at the beginning of an outbreak. It also may reflect differing case severity or improved case management as an outbreak progresses. Interestingly, districts that reported an outbreak during the previous year had much lower CFRs than other districts, suggesting that response time and quality are linked to local staff experience, the existence of streamlined outbreak response processes, or more recent training and intervention from provincial- or national-level staff.

Our study had several limitations. Foremost among these is that we relied on national surveillance data, and the sensitivity and specificity of this system are unknown, either over time or between sites. We also were limited in the type of information available to us, with no data on factors such as patient age, sex, and other individual risk factors or environmental factors that might influence cholera risk. Lastly, we found that 44% of tested stool specimens were positive for cholera. Data were not collected systematically on other potential etiologies; failure to detect cholera also may have occurred because of problems during shipment of processing of specimens.

Nevertheless, we think our findings have implications for the design of public health strategies in Mozambique, particularly vaccine intervention. Effective cholera vaccines now exist, with some of the early trials conducted in Mozambique [7, 14–16], and the WHO has endorsed vaccine use [3]. However, it is not clear how this would be implemented in Mozambique. Reactive campaigns targeting specific districts would have to be implemented extremely rapidly to impact the course of an epidemic and particularly cholera mortality. However, the experience of meningococcal meningitis in the African meningitis belt, which also occurs at a highly focused geographic and temporal location, shows that even with a vaccine stockpile, accepted procedures in place, and high staff motivation, it is difficult to get vaccine into the field rapidly [17, 18]. Preventive immunization thus might be a better strategy. However, during the almost 2.5 years of the study, 26 districts reported >1 outbreak, and no particular characteristic identified these districts. These issues are likely to be even more problematic at subdistrict levels. Lastly, the progression of cholera outbreaks from 1 site to another will make vaccine impact assessment difficult.

Consequently, the best strategies for Mozambique may be to implement broad-based national measures that impact cholera and other causes of diarrheal illness among all populations. In the short term, this could include epidemic response and targeted water chlorination, cholera education for the public and healthcare providers, and improving response time to epidemics, for example, through streamlining information flow and ongoing training of district staff. However, these interventions may be difficult to sustain indefinitely. The main long-term solution will be improvements in water and sanitation, a major public health goal in Mozambique [19, 20], and one whose achievement will require substantial effort. For example, the percentage of the population with access to sanitation during 2008 varied from nearly 100% in Maputo City to 22% in Nampula Province and 19% in Zambezia Province and was <50% in 6 of 11 provinces [19]. Similarly, clean water access among rural communities varied from 80% in Maputo Province to 36% in Nampula and 38% in Zambezia Provinces [20]. Targeted preventive immunization may have a role in specific high-risk districts that demonstrate repeated outbreaks, but this will require a robust risk assessment to identify the appropriate target groups.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Financial support. The Africhol project and this manuscript were funded through a grant from the Bill & Melinda Gates Foundation.

Potential conflicts of interest. B. D. Gessner works for AMP, which receives unrestricted support from Sanofi Pasteur and grant specific support from Sanofi Pasteur, Merck, Glaxo-Smith-Kline, and Pfizer. All other authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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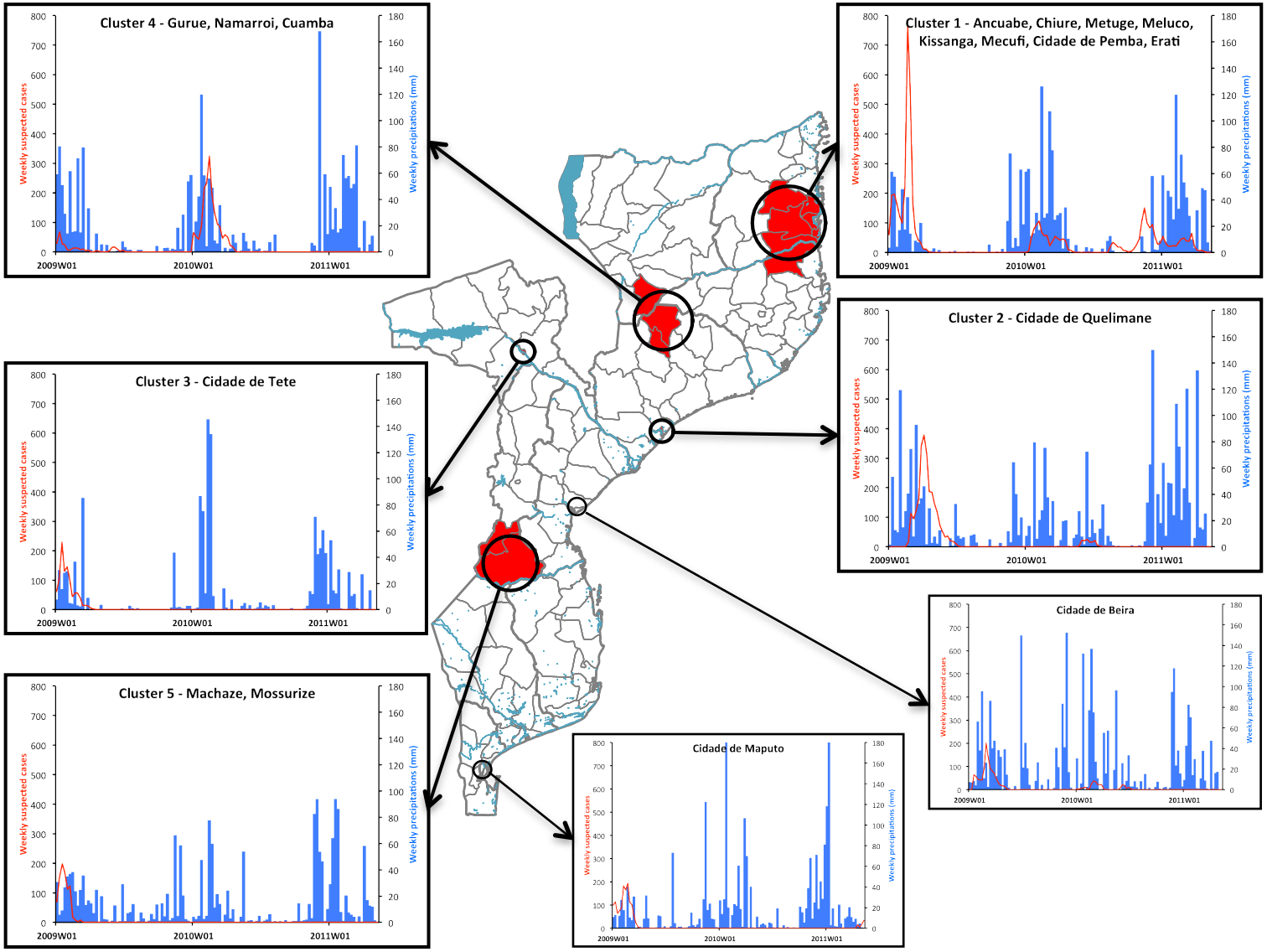
Supplementary Figure 1. Spatial clusters of cholera from 2009 through week 19 of 2011, as detected by SaTScan™ (*P*-values for all five clusters, $<10^{-17}$), and their weekly cases and rainfalls. Cidade de Maputo and Cidade de Beira were also represented because of their past epidemics.



2.3.3 Une transmission hétérogène dans le temps et dans l'espace

Comme en RDC et en Guinée, la description spatio-temporelle du choléra au Mozambique montra une répartition hétérogène sur cette période d'étude de 123 semaines, à condition d'utiliser des échelles suffisamment fines. La transmission s'y déroula sous la forme d'épidémies de courte durée, et se concentra dans des clusters spatiaux côtiers et non côtiers. Comme le reste du pays, ces clusters connurent des phases d'accalmie qui dépassèrent souvent les limites des saisons sèches et ne furent pas toujours synchrones. La ville de Beira elle-même ne fut pas identifiée comme un cluster à haut risque, plus de cinq années après la campagne de vaccination.

Ces observations conduisent à questionner le concept d'endémicité défini selon un critère aussi grossier que celui d'une notification de cas de choléra au cours d'au moins 3 des 5 dernières années à l'échelle d'un pays. Au Mozambique comme dans l'Est de la RDC, le choléra pourrait fonctionner selon un schéma métastable, les épidémies tournant entre des nœuds de transmission. Des études plus fouillées et associant l'épidémiologie moléculaire pourraient permettre de vérifier ces hypothèses et d'identifier plus précisément les zones où concentrer les efforts de contrôle afin d'enrayer les épidémies sur l'ensemble du Mozambique, voire dans les pays voisins.

Enfin, cette hétérogénéité à la fois spatiale et temporelle questionne radicalement la validité d'une méthodologie d'estimation du poids mondial du choléra basée sur l'extrapolation de seulement 3 études d'incidence du choléra et pourtant fréquemment citée [84]. De manière ironique, l'une de ces 3 études avait été conduite à Beira dans le cadre du suivi de l'efficacité de la campagne de vaccination [85] entre janvier et décembre 2004, année où le Mozambique fut frappé par la plus grosse épidémie de cette dernière décennie (20 080 cas rapportés [86] pour environ 25 millions d'habitants). Rien ne permet d'affirmer que l'incidence du choléra mesurée dans ce quartier vacciné ait été extrapolable au reste du pays. Encore moins peut-elle l'être pour les années suivantes et le reste du continent, même en prenant en compte les niveaux d'accès à l'eau et à l'assainissement comme le firent ces auteurs [87].

[84] Ali et al., « The global burden of cholera ».
[85] Lucas et al., « Effectiveness of mass oral cholera vaccination in Beira, Mozambique ».
[86] World Health Organization, « Cholera [every year since 1968] ».
[87] Ali et al., « The global burden of cholera ».

3 TRAVAUX SUR LE CHOLÉRA EN HAÏTI

3.1 Survivance du débat sur l'origine de l'épidémie en 2010

« Ce fut pour la première fois en 1832 que le gouvernement prit des mesures pour s'opposer à l'invasion en Haïti du choléra morbus qui avait déjà franchi l'Europe et pénétré aux Etats Unis. Il est à observer que cette maladie n'est jamais parvenue en Haïti, même quand elle s'est trouvée en même temps tout autour de notre île, à St-Thomas, à Porto-Rico, à la Jamaïque et à Cuba, au Vent comme sous le Vent. Cela tiendrait-il aux émanations de notre sol qui ne permettraient pas d'exister aux animalcules cholériques ou à un état particulier de notre atmosphère? »

Thomas Madiou,
Historien haïtien, 1847 [88]
Référence tirée de Jenson et al. [89]

Professor Colwell's « perfect storm » :
« You have this massive earthquake in January 2010 […] The geology of Haiti is limestone. With earthquake effects disrupting the rivers, the rivers become very alkaline […] Then Haiti had one of the hottest summers on record […] That was followed by a hurricane that skirted Haiti, causing heavy rain and flooding […] With all the river systems churned up with nutrients and warm water, and proper alkalinity, it would be ideal for the organism to become quite dominant. »

Pr. Rita Colwell
Interview to Shots, June 2012 [90]

3.1.1 En réponse à la controverse persistante autour de l'origine du choléra en Haïti

En octobre 2010, Haïti fut frappé pour la première fois de son histoire [91] par l'une des épidémies les plus violentes de la septième pandémie. Néanmoins, l'origine de cette épidémie fut indépendante du violent séisme ayant ravagé la zone métropolitaine 10 mois plus tôt. Des enquêtes de terrain menées par Renaud Piarroux et ses collaborateurs en novembre 2010 associées à l'analyse spatio-temporelle des premières semaines de l'épidémie [92], attribuèrent en effet clairement cette épidémie à la contamination massive

[88] Madiou, *Histoire d'Haïti*, 122.
[89] Jenson et Szabo, « Cholera in Haiti and Other Caribbean Regions, 19th Century ».
[90] Knox, « Scientists Find New Wrinkle In How Cholera Got To Haiti ».
[91] Jenson et Szabo, « Cholera in Haiti and Other Caribbean Regions, 19th Century ».
[92] Piarroux et al., « Understanding the cholera epidemic, Haiti ».

d'un affluent du fleuve Artibonite par les déjections d'un camp de la MINUSTAH (MIssion des Nations Unies pour la STAbilisation en Haïti) situé à Mirebalais dans le département du Centre. Un contingent de casques bleus venait d'y arriver en provenance du Népal, pays alors frappé par une importante épidémie de choléra [93]. Ces suspicions furent ensuite confirmées en juillet 2011 par une équipe américano-suédoise ayant comparé le génome total d'isolats cliniques haïtiens et ceux de divers pays dont le Népal [94]. Les souches haïtiennes isolées en octobre 2010 se montrèrent quasiment identiques – à 1 ou 2 paires de bases près – à des souches népalaises isolées au mois d'août de la même année.

Malgré ces évidences, l'équipe du Professeur Colwell publia en juillet 2012 dans le PNAS une étude affirmant notamment que 21% des cas de choléra en novembre 2010 étaient dus à des *Vibrio cholerae* non-O1/non-O139 polyclonaux et non toxinogènes, dont certains présentaient une proximité génétique avec des souches isolées dans l'environnement haïtien elle-même considérées comme des réservoirs d'îlots génomiques et de pathogénicité transmis horizontalement. Le reste des cas étaient dus à des *V. cholerae* O1 proches de souches sud-asiatiques et africaines [95]. En jetant ainsi un doute sur l'origine importée du choléra en Haïti et en tentant de remettre le paradigme environnemental au cœur des débats autour de la plus grosse épidémie de ces dernières décennies, cet article entraîna la publication par le PNAS de deux lettres de réponse, dont l'une cosignée par Renaud Piarroux et deux médecins du Ministère de la Santé Haïtien notamment [96]. Ces deux lettres pointaient du doigt de nombreuses imprécisions, biais de raisonnement, omissions, voire fautes de ce travail : données cliniques non mentionnées ; résultats contradictoires avec ceux du Laboratoire National de Santé Publique d'Haïti ; comparaisons génétiques mal interprétées ; non prise en compte surprenante des isolats népalais ; utilisation sans référence d'une carte publiée par OCHA et détournée de manière ambiguë de sa signification réelle… De fait, l'article avait été publié dans le PNAS selon la procédure de « contributed submission », qui ne prévoit pas de peer-reviewing indépendant… En juillet 2013, un consortium de chercheurs américains et haïtiens publièrent un article comparant le génome total de 108 souches

[93] Frerichs et al., « Nepalese origin of cholera epidemic in Haiti »; Orata, Keim, et Boucher, « The 2010 Cholera Outbreak in Haiti ».
[94] Hendriksen et al., « Population genetics of Vibrio cholerae from Nepal in 2010 ».
[95] Hasan et al., « Genomic diversity of 2010 Haitian cholera outbreak strains ».
[96] Frerichs et al., « Source attribution of 2010 cholera epidemic in Haiti »; Mekalanos et al., « Non-O1 Vibrio cholerae unlinked to cholera in Haiti ».

cliniques de *V. cholerae* dont 59 isolées en Haïti de 2010 à 2012, et 24 isolées au Népal en août 2010 [97]. Ces analyses montrèrent sans ambiguïté que l'épidémie haïtienne était causée par un clone de *V. cholerae* ayant divergé de souches népalaises entre juillet et octobre 2010, et ne présentant aucune acquisition de gène par transfert horizontal.

En juillet 2013 également, l'équipe du Professeur Colwell publia dans l'American Journal of Tropical Medicine and Hygiene une nouvelle étude relative aux facteurs environnementaux favorisants les épidémies de choléra, qui analysait des données indiennes de 1875 à 1900 et celles des premières semaines de l'épidémies en Haïti [98]. Sans nier directement la possibilité d'une importation du choléra en Haïti, les résultats et la discussion de cet article suggèrent que son apparition du choléra en octobre 2010 en Haïti serait d'origine environnementale et déclenchée par la conjonction d'un été particulièrement chaud susceptible d'avoir favorisé la multiplication de *V. cholerae* environnementaux préexistants, d'un pic de pluviométrie dans les semaines précédentes responsable d'inondations, de la destruction par le séisme de janvier 2010 d'infrastructures d'eau et assainissement déjà fragiles, et des conditions de vies difficiles dans les camps de sinistrés. À nouveau, une lecture attentive de ce travail révéla de nombreuses imprécisions conceptuelles et statistiques, erreurs, biais de raisonnements, voire suspicions que nous avons résumés dans une lettre à l'éditeur publiée en décembre de la même année [99] et qui est reproduite ici.

3.1.2 Lettre 1 : « Environmental factors influencing epidemic cholera »

A lire ci-après.

[97] Katz et al., « Evolutionary Dynamics of Vibrio Cholerae O1 Following a Single-Source Introduction to Haiti ».
[98] Jutla et al., « Environmental Factors Influencing Epidemic Cholera ».
[99] Gaudart et al., « Environmental Factors Influencing Epidemic Cholera ».

Am. J. Trop. Med. Hyg., 89(6), 2013, pp. 1228–1230
doi:10.4269/ajtmh.13-0499a

Letter to the Editor

Environmental Factors Influencing Epidemic Cholera

Dear Sir:

We have concerns with the recent publication by Jutla and colleagues, which aims to describe the environmental factors influencing epidemic cholera.[1] Regarding cholera in Haiti, the authors challenged the findings of many studies showing that the epidemic likely originated from the importation of toxigenic *Vibrio cholerae* by Nepalese peacekeepers in October 2010.[2–5] Instead, they attempted to show that environmental conditions conducive to rapid growth and transmission of *V. cholerae* played a substantial role in epidemic onset. Their hypothesis is based on the claim that increased temperatures and rainfall during the months preceding the epidemic favored the proliferation of *V. cholerae* in the Haitian waters and its subsequent transmission to the local population[1]; we believe their claims are based on misinterpretations of our published data and statistical correlations that fail to establish causality.

Our field investigation of the Haitian cholera epidemic has clearly indicated that outbreaks started in Meye, near Mirebalais, before subsequently spreading downstream, following the Artibonite River.[2] Using a Spearman's rank statistical test, Jutla and colleagues stated that the correlation between cholera cases in Mirebalais and the Lower Artibonite was "very high," thereby inferring that the epidemic started simultaneously in the two locations.[1] According to our report, each of the Lower Artibonite communes displayed a markedly higher correlation with the other Lower Artibonite communes than with Mirebalais.[2] Therefore, we did not report a strong correlation between Mirebalais and the Lower Artibonite communes, as claimed by Jutla and colleagues.[1] Nevertheless, correlation analyses are irrelevant to question the chronological progression of the epidemic. Indeed, our field investigation revealed that no suspected cases of cholera or severe diarrhea were reported in the Lower Artibonite before October 19, although the epidemic began on October 14 near Mirebalais.[2] Furthermore, an UN-appointed panel of scientists has further confirmed our findings.[3]

The objective of the Jutla report was to "understand the relationship between hydroclimatological processes and cholera." They claim that a climatic anomaly (400 mm rainfall in September versus < 200 mm average monthly rainfall for year 2010, see Figure 7B) may have played a role in the proliferation of the bacterium present in the environment and subsequently provoked the cholera epidemic.[1] As we were unaware of this climatic anomaly, we have repeated an extraction of the TRMM 3B46RT data in an attempt to replicate their findings. However, using the same data source, we could not highlight any climatic anomaly during September 2010, neither in the entire territory of Haiti nor when focusing on the Artibonite Basin. Figures 1 and 2 display that before the initial outbreak, rainfall levels were in the average range both in Haiti and the Artibonite Basin. In particular, we did not identify the 400-mm rainfall peak shown in Figure 7B. Note that our data correlates with the more detailed Figure 8 of the Jutla and colleagues report,[1] which fails to indicate excessive rainfall during the 30-day period preceding epidemic onset.

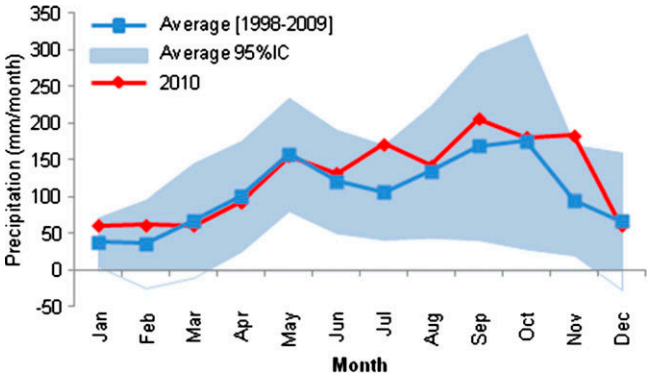


FIGURE 1. Monthly rainfall in Haiti in 2010 (red) and the national historical rainfall average of 1998 to 2009 (dark blue) (average 95%IC, light blue). Rainfall data was obtained from the NASA and JAXA Tropical Rainfall Measuring Mission (TRMM 3B46RT) (http://pmm.nasa.gov/node/158).

Indeed, by totaling the rainfall peaks shown in Figure 8 from September 15 to October 14, we obtained 130 mm total precipitation. These data represent a stark contradiction to the "anomalously high rainfall" during September and October claimed in Jutla's article. As the authors did not identify the exact data source, we could not assess their suggested correlation between elevated air temperatures and cholera. Nevertheless, we do not understand why temperatures "above the long-term climatological average by one standard deviation" are considered "significantly high" by Jutla and colleagues.

Finally, studies comparing the genomes of the Nepalese and Haitian *V. cholerae* isolates collected in 2010 have been ignored. It is important to note that just before embarking for Haiti, the Nepalese soldiers were exposed to a cholera epidemic in Nepal.[2,4] A study by Hendriksen and others has shown that the Haitian *V. cholerae* isolates were almost indistinguishable from strains collected in Nepal, with only one or two base-pair differences throughout the entire genome.[5] Additional studies have further supported these findings, which have never been revoked.[3] Whole-genome analysis of a 154-strain panel of *V. cholerae* isolates collected throughout the globe could not find any other strain as similar to the Haitian epidemic strains as the strains collected in Nepal in 2010.[6] Moreover, a recent molecular clock analysis published by Katz and others has estimated the most recent Nepalese and Haitian *V. cholerae* strain common ancestor date at September 28, 2010 (95% credibility interval: July 23 to October 17, 2010).[7] Therefore, the molecular clock results are incompatible with a prolonged presence of the epidemic strain in the Haitian environment. Disregarding these studies, Jutla and colleagues have only indicated that the epidemic isolates resembled those from South Asia and Africa, thereby suggesting that the strain responsible for the Haitian epidemic was already globally widespread before the epidemic.

Other misinterpretations were noted in this article, including but not limited to the 6% "rate of cholera" in Madagascar in 2000, the alleged link between cholera and the refugee camps

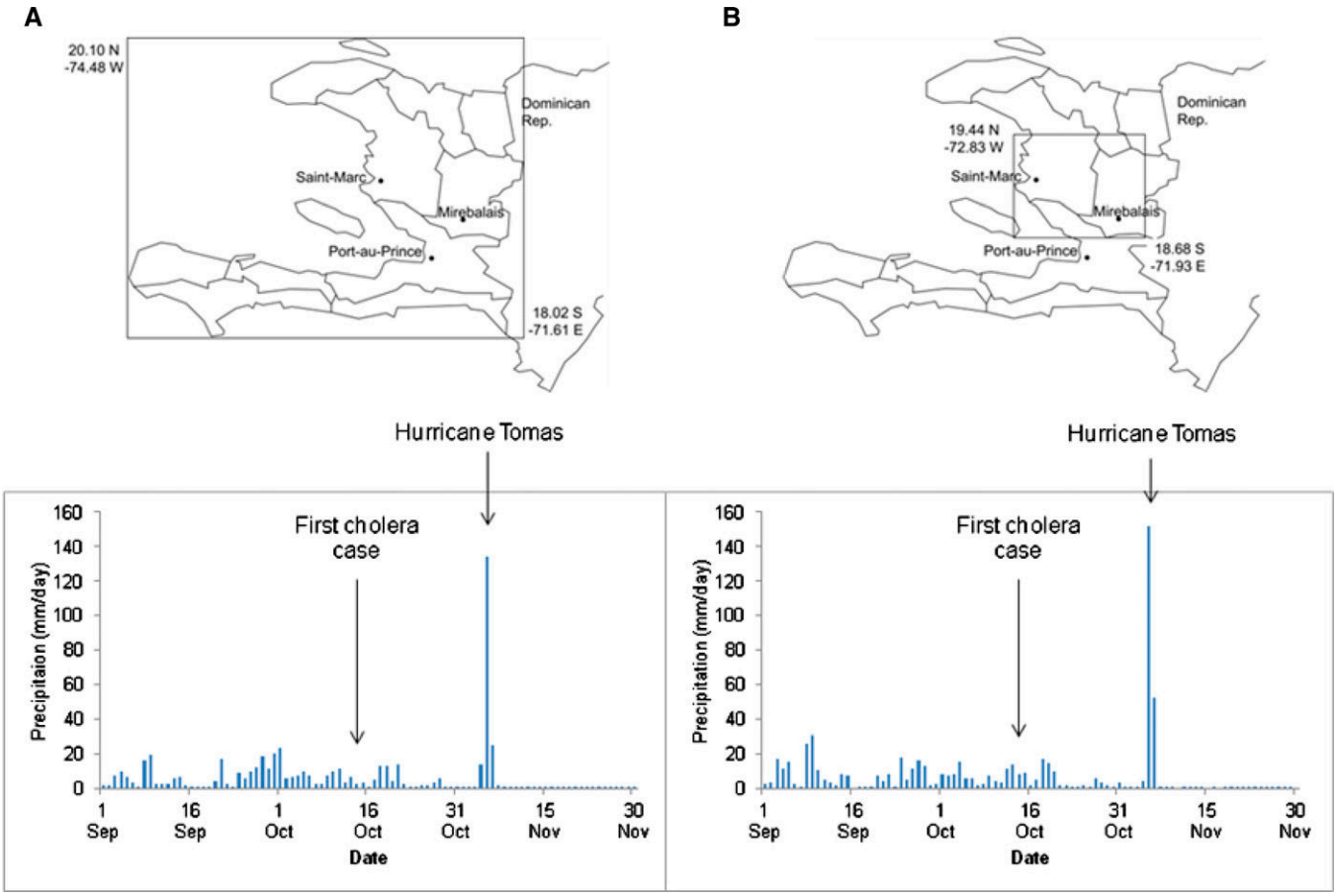


FIGURE 2. Daily rainfall for the entire territory of Haiti (A) and the Artibonite Basin (B). The zones applied to assess rainfall at each scale are indicated in the maps (square box), and the first cholera case and Hurricane Tomas are indicated on the precipitation histograms. Rainfall data was obtained from the NASA and JAXA Tropical Rainfall Measuring Mission (TRMM 3B46RT) (http://pmm.nasa.gov/node/158).

established in Haiti after the 2010 earthquake, and the claimed role that non-O1/O139 *V. cholerae* strains played in the Haitian epidemic, although they do not produce cholera toxin. Indeed, as stated by Mekalanos and others "non-toxigenic non-O1 *V. cholerae* can be diarrheagenic" but "neither causes cholera."[8] Overall, most conclusions are based on statistical correlations that are not suitable to show a causal relationship between hydroclimatological factors and cholera emergence. Moreover, the main results at the foundation of their conclusions could not be reproduced, although established evidence, including the results of field investigations and genomic comparisons of Nepalese and Haitian strains, was inadequately considered.

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3.2 Dynamique du choléra pendant la première année de l'épidémie en Haïti

3.2.1 Quels ont été les déterminants du choléra en Haïti au cours de la première année d'épidémie ?

Afin d'adapter au mieux les moyens mis en œuvre pour le contrôle de cette épidémie sans précédent, différentes prédictions épidémiques furent conduites dans les mois suivants son apparition en octobre 2010 [100]. Confrontées à l'épreuve des faits, parfois même avant leur publication, ces premières modélisations mathématiques démontrèrent toutes leur inaptitude à comprendre la dynamique de cette épidémie de choléra. En collaboration avec la Direction d'Épidémiologie de Laboratoire et de Recherche (DELR) du Ministère de la Santé Publique et de la Population d'Haïti (MSPP), nous nous mîmes donc au travail pour décrire et analyser l'évolution spatio-temporelle de cette épidémie au cours de sa première année. Ma mission consista à participer sous la direction de Jean Gaudart à la description temporelle et spatiale du choléra et à l'identification des clusters de transmission au cours des différentes phases épidémiques. Cette étude fit l'objet d'un article publié en avril 2013 dans la revue PLoS Neglected Tropical Diseases [101].

3.2.2 Article 1 : « Spatio-temporal dynamics of cholera during the first year of the epidemic in Haiti. »

A lire ci-après.

[100] Andrews et Basu, « Transmission dynamics and control of cholera in Haiti »; Tuite et al., « Cholera epidemic in Haiti, 2010 »; Chao, Halloran, et Longini, « Vaccination Strategies for Epidemic Cholera in Haiti with Implications for the Developing World »; Bertuzzo et al., « Prediction of the Spatial Evolution and Effects of Control Measures for the Unfolding Haiti Cholera Outbreak ».
[101] Gaudart et al., « Spatio-Temporal Dynamics of Cholera during the First Year of the Epidemic in Haiti ».

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PLOS | NEGLECTED TROPICAL DISEASES

Spatio-Temporal Dynamics of Cholera during the First Year of the Epidemic in Haiti

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Abstract

Background: In October 2010, cholera importation in Haiti triggered an epidemic that rapidly proved to be the world's largest epidemic of the seventh cholera pandemic. To establish effective control and elimination policies, strategies rely on the analysis of cholera dynamics. In this report, we describe the spatio-temporal dynamics of cholera and the associated environmental factors.

Methodology/Principal findings: Cholera-associated morbidity and mortality data were prospectively collected at the commune level according to the World Health Organization standard definition. Attack and mortality rates were estimated and mapped to assess epidemic clusters and trends. The relationships between environmental factors were assessed at the commune level using multivariate analysis. The global attack and mortality rates were 488.9 cases/10,000 inhabitants and 6.24 deaths/10,000 inhabitants, respectively. Attack rates displayed a significantly high level of spatial heterogeneity (varying from 64.7 to 3070.9 per 10,000 inhabitants), thereby suggesting disparate outbreak processes. The epidemic course exhibited two principal outbreaks. The first outbreak (October 16, 2010–January 30, 2011) displayed a centrifugal spread of a damping wave that suddenly emerged from Mirebalais. The second outbreak began at the end of May 2011, concomitant with the onset of the rainy season, and displayed a highly fragmented epidemic pattern. Environmental factors (river and rice fields: $p<0.003$) played a role in disease dynamics exclusively during the early phases of the epidemic.

Conclusion: Our findings demonstrate that the epidemic is still evolving, with a changing transmission pattern as time passes. Such an evolution could have hardly been anticipated, especially in a country struck by cholera for the first time. These results argue for the need for control measures involving intense efforts in rapid and exhaustive case tracking.

Citation: Gaudart J, Rebaudet S, Barrais R, Boncy J, Faucher B, et al. (2013) Spatio-Temporal Dynamics of Cholera during the First Year of the Epidemic in Haiti. PLoS Negl Trop Dis 7(4): e2145. doi:10.1371/journal.pntd.0002145

Editor: Hélène Carabin, University of Oklahoma Health Sciences Center, United States of America

Received August 16, 2012; **Accepted** February 15, 2013; **Published** April 4, 2013

Funding: This study was supported by the French Embassy in Port-au-Prince. Dr. Jean Gaudart was also supported by the ADEREM association for biological and medical research development (Association pour le Développement des Recherches biologiques et Médicales, http://www.aderem.fr). The funders played no role in study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Cholera appeared in Haiti in October 2010, probably for the first time in the country's history [1]. Importation of the vibrio [2], [3] triggered an epidemic that rapidly proved to be the world's largest epidemic of the seventh cholera pandemic. In January 2012, a cholera elimination objective was adopted by Haitian and Dominican authorities, the World Health Organization (WHO), the United Nations International Children's Emergency Fund (UNICEF), and many of their partners [4]. However, to establish effective control and elimination policies, strategies rely on the analysis of the dynamics of cholera dissemination. To bolster control policies, various mathematical models have been established [5]–[][][8]. They have provided varying results, thereby demonstrating the importance of mathematical assumptions and parameter estimations [9], [10]. One model, issued in March 2011, has predicted 779,000 cases and 11,000 deaths for November 2011 [5]. Another model has predicted that the principal peak of the epidemic would occur in April 2011 in several departments [6]. Other studies acknowledged that this peak occurred in December 2010 but predicted tens of thousands of cases for March and April 2011 [7], [8]. Among the various causes of inaccurate predictions, all reports have used observed cases at the departmental scale, which hardly exhibit outbreak dynamics. Andews *et al.* [5] have not explicitly modeled spatial diffusion, while other authors [7], [8] estimated parameters at the country level, assuming homogeneous dynamics between all locations [9]. In contrast, cholera epidemic curves provided during the year 2011 by the Haitian Ministry of Health and Population showed that the cholera evolution profiles greatly varied from that predicted by models, with a marked and unexpected reduction in cholera incidence during the first months of 2011 followed by a new outbreak in May. This observation reveal how crucial it is to generate a comprehensive description of cholera diffusion and monitor cases daily at a communal scale. In other areas affected by recurrent cholera epidemics, it has been

Author Summary

Cholera is the prototypical "waterborne" disease that can provoke deadly acute watery diarrhea epidemics in settings deprived of clean water and proper sanitation. In spite chronic deprivation, Haiti had been spared cholera for a century until the vibrio was imported in October 2010, which triggered the largest national epidemic ever recorded. To better understand the progression of the epidemic and adapt control measures, we describe and analyze the spatio-temporal dynamics and underlying factors associated with the first year of this cholera epidemic in Haiti. Attack rates reached highly heterogeneous levels between communes (from 64.7 to 3070.9 cases per 10,000 inhabitants), thereby suggesting disparate outbreak processes. While the first principal outbreak spread centrifugally like a damping wave that suddenly emerged from Mirebalais and Lower Artibonite, a second principal outbreak erupted at the end of May 2011, concomitant with the rainy season, and displayed a highly fragmented epidemic pattern. Environmental factors, such as rivers and rice fields, appeared to play a role in disease dynamics exclusively during the beginning of the epidemic. The dynamics of the cholera epidemic varied from place to place as time passed, following no clearly predictable scheme. Therefore, cholera control measures in Haiti should include rapid and exhaustive case tracking.

shown that studying the spatio-temporal dynamics of cholera outbreaks helped to define more effective control procedures [11]–[][15]. Currently, only one publication [16] describes data at this spatio-temporal scale; however, this report aimed only to understand the dynamics of the cholera epidemic during the initial weeks following the outbreak onset. Since this first phase of the epidemic, many other cases have been reported across Haiti with new peaks and possibly new patterns of transmission. Therefore, the objective of the present study was to describe the spatio-temporal dynamics of the first year of this cholera epidemic in Haiti, identify the principal factors explaining the heterogeneity, and assess the epidemic processes.

Methods

Cases and deaths

Cholera-associated morbidity and mortality data were prospectively and anonymously collected by the Departmental Health Directorates at the commune level. Departmental databases were sent to the Haitian Directorate of Health (*Laboratoire National de Santé Publique*, LNSP), where data were gathered and analyzed after quality control.

According to the WHO standard definition [17], a probable cholera case was defined as profuse acute watery diarrhea with severe dehydration. Bacteriological confirmation of cases was recurrently performed at the LNSP for samples collected throughout the entire country using standard methods [18]. The in-hospital case fatality rate (ICFR) was defined as the ratio of cumulative number of deaths reported at Cholera Treatment Centers (CTCs) to cumulative number of hospitalized cases (severe cases). The case fatality rate (CFR) was defined as the ratio of cumulative number of in-hospital deaths to cumulative number of cases (reported at any health structure). As some communes lacked proper health facilities, some cholera patients had to travel to health structures of the nearest commune. To avoid overestimating case numbers in such locations and underestimating case numbers in surrounding areas, the data derived from these neighboring communes were aggregated after interviewing local health actors and analyzing local reports.

In this study, we did not include personal medical data but included the number of incident cases anonymously reported at each health facility. This study was approved by the Haitian Ministry of Public Health and Population (*Ministère de la Santé Publique et de la Population*).

Methods

First, the mapping of global attack rates, mortality rates, ICFRs and CFRs observed between October 16, 2010 and October 15, 2011 was performed to assess the spatial distribution of the epidemic. Spatial autocorrelation was estimated using Moran's I statistic for areal data [19].

Second, temporal observations for the entire country were assessed to define epidemic phases and trends. Phases were specified using main slope changes in time series after mobile average (MA) smoothing (order two). The accuracy of this phase specification was then assessed by using sensitive analysis of the MA order, concordance with the wavelet analysis (see above), and the field expertise of the Haitian epidemiologists. For each epidemic phase, communal daily incidence rates (DIRs) were mapped, and spatial clustering was assessed using Kulldorff statistic [20]. To detect high-risk spatial clusters of cases, this algorithm moves a circular (or elliptic) scanning window over the study region, centered on each communal centroid with a radius ranging from 1% to 50% of the population at risk. This algorithm compares observed and expected case numbers inside and outside each window and estimates risk ratios based on the Poisson distribution. Using circular scanning windows, cluster significance (p-value) was calculated with a likelihood ratio test using the Monte Carlo approach with 999 random simulations under the null hypothesis of no clustering [19], [21]. Communal epidemic profiles of the different epidemic phases were compared and classified using hierarchical cluster analysis (HCA) based on Euclidean distance [22], and profile classes were then mapped. HCA is an unsupervised classification method that groups similar observations (the epidemiological curves for each commune) into classes depending on a similarity criterion (the daily case numbers recorded for each commune). Furthermore, to address the impact of population immunity, we assessed the impact of the accumulation of cases during the second outbreak. We compared the influence of cumulative incidences (aggregating phases 1 to 4) with the incidences observed during the second epidemic (phases 5 to 6) at the commune level using the Spearman correlation coefficient.

Third, to assess the environmental factors associated with outbreak spread, cases and rainfall time series at the country level were analyzed using wavelet spectrum analysis. By reducing the noise and capturing the local behavior of non-stationary time series [23], this approach detect underlying phenomena [24], [25], such as periodic variations, regime shifts or sudden perturbations and jumps. This method provides a multiscale analysis extracting the main evolution and trends of time series at different temporal scales and has been previously utilized to study cholera outbreaks [26], [27]. The relationship between cases and rainfall time series was assessed via cross-spectrum analysis [28]. Daily accumulated rainfall data were obtained from NASA Goddard Earth Sciences. These observations (TMPA-RT 3B42RT) were derived from the Tropical Rainfall Measuring Mission (see http://disc.sci.gsfc.nasa.gov/giovanni/overview/index.html for details). For each epidemic phase at the commune level, we also examined the relationship between cases and the following land cover surface factors: plains, mountains and hills, urban zones, rice fields, length of perennial rivers (10 km), area (km^2), and number of watersheds, which were

obtained from the MULTI-MENACES-HA team report [29]. These environmental factors were assessed via multivariate analysis using the Generalized Additive Model (GAM) derived from linear regression models [30], [31]. Standardized incidence ratios (SIRs) were estimated using log-transformed population density (as an offset variable) and were adjusted on the spatial distribution of communes modeled by thin plate splines following Wood's approach [30]. Because of the over-dispersion of cholera incidences, several models of the Negative Binomial and the Poisson families [32] were first graphically verified to meet the conditions of use and then compared using the Generalized Cross-Validation (GCV) score and the Un-Biased Risk Estimator (UBRE) score [30]. The stepwise selection of variables was performed using the GCV and UBRE scores. The explained deviance was also verified for model goodness-of-fit assessment. The SIRs and corresponding 95% confidence intervals (95CI) were estimated using the final selected model and tested.

Spatial cluster analyses were performed using SaTScan® v8.2.1 (Martin Kulldorff, Harvard Medical School, Boston, MA, USA and Information Management Services Inc, Silver Spring, MD, USA). Wavelet spectrum analyses were performed using Matlab® v7.1 (The Mathworks Inc., Natick, MA, USA). The other statistical analyses were performed using R® v2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria) with *mgcv* package (GAM modeling), the *DCluster* package (spatial analysis), and the *cluster* package (HCA). The p-values were compared with the probability threshold $\alpha = 0.05$. The maps were generated using Quantum-GIS® v1.7.3 (Open Source Geospatial Foundation Project, Beaverton, OR, USA).

Results

One year after October 16, 2010, 493,069 cases and 6,293 deaths associated with cholera had already been reported in Haiti. The global attack rate was 488.9 cases per 10,000 inhabitants, and the global mortality rate was 6.24/10,000 inhabitants. The global ICFR and CFR were 1.76% and 0.83%, respectively. During this first year, 852 of the 1,437 stool specimens collected in the ten departments of Haiti were positive for *Vibrio cholerae* O1 Biotype El Tor, serotype Ogawa. No switch to the Inaba serotype was observed until the second year of the epidemic.

The mapping of yearly attack rates (Figure 1a) showed that communes were disparately affected, as the rates ranged from 3,070.9 cases/10,000 inhabitants in Mirebalais (Department of Centre) to 64.7 cases/10,000 inhabitants in the western tip of the north peninsula (communes of Baie de Henne, Bombardopolis, Jean Rabel, and Mole St Nicolas). The Moran's I coefficient was particularly low ($I = 0.02$, $p = 0.5$), thereby indicating no significant spatial autocorrelation and confirming the highly fragmented pattern at this scale. The mapping of mortality (Figure 1b) displayed high yearly mortality rates in the western tip of the south peninsula with 58.5 and 45.1 deaths/10,000 inhabitants in Chambellan and Pestel, respectively. The low spatial autocorrelation together with the high degree of spatial heterogeneity of incident cases showed that outbreak dynamics in Haiti varied from location to location. This fragmented spatial pattern drew attention to the need for separate analyzes at each phase of the outbreak, both at the country and local levels.

At the country level (Figure 2), the epidemic course exhibited two principal outbreaks.

Studying the slope changes, the time series were divided into two periods separated by the main peak of the cholera epidemic (12/16/2011, 4,289 cases). The period preceding this peak was split into two parts separated by the nadir in cholera cases occurring on October 31, 2010 (1,053 cases), which was observed just before the violent increase in cholera cases reported in early November. The period succeeding the principal peak was split into four parts. During the first part (phase 3), the attack rate dramatically decreased (from 3,972 to 1,131 cases daily). Phase 3 ended on January 30, 2011 and was followed by a lull period characterized by a reciprocation of small increases and decreases (phase 4), with an average of 835 daily cases (standard deviation SD = 188 cases) until May 22, 2011. On May 22, the slope of the epidemic curve changed to a marked increase, thereby signalling the onset of a new epidemic wave and the beginning of phase 5. After a high tray above 2,000 cases per day (until 06/12/2011), the final recorded decrease characterized phase 6.

The first principal outbreak started during a period with very little rainfall (~2 mm/day during the last 15 days of October 2010). Outbreak onset lasted from mid- to late October (phase 1) and was associated with the introduction of *Vibrio cholerae* in Meille (commune of Mirebalais, Department of Centre) and the abrupt contamination of the Artibonite River [2],[16]. During this first phase, 23,587 cases were reported (DIR = 1.46 cases/10,000 inhabitants/day, 95CI[1.44–1.48]) (Figure 3). Spatial cluster analysis displayed only one significant high-risk cluster centered at the Artibonite Valley, with a significantly elevated relative risk (RR) of 42.72, 95CI[41.1–44.4] compared with the other regions of the country ($p = 0.001$), thereby confirming the link between cholera and proximity to the Artibonite River during the beginning of the epidemic.

During the second phase (November 1–December 15, 2010), cholera diffused out of the Artibonite Valley concomitant with Hurricane Tomas, and 119,347 cases were reported (DIR = 2.63 cases/10,000 inhabitants/day [2.62–2.65]). Among the five significant high-risk clusters, the largest cluster encompassed a large portion of the country including Port-au-Prince but spared the South Peninsula(RR = 3.49 [3.45–3.54], $p = 0.001$).

The hierarchical cluster analysis (HCA) of these first two phases of the epidemic profiles (Figure 4) identified the outbreak origin in Mirebalais (Class A), where cases occurred primarily during the first month (DIR = 19.81 cases/10,000 inhabitants/day [19.44–20.18]). The class B profile was primarily located in the low Artibonite valley, where the outbreak began a few days later (DIR = 6.22 cases/10,000 inhabitants/day [6.18–6.26]). In this area, most cases occurred at the beginning of the wave, and then the number of daily cases decreased. Classes C and D (DIRs = 3.41 cases/10,000 inhabitants/day [3.0–3.46] and 3.05 cases/10,000 inhabitants/day [3.01–3.09], respectively) displayed a smoother pattern after a delay of approximately one week. Finally, the communes of classes E and F (DIRs = 1.25 cases/10,000 inhabitants/day [1.24–1.27] and 0.83 cases/10,000 inhabitants/day [0.82–0.85], respectively) were the last affected areas. Overall, the mapping of epidemic profile classes exhibited a centrifugal spread from the Artibonite Valley: distant communes displayed delayed outbreak onsets, lower daily incidence rates, and delayed and smaller outbreak peaks.

With 104,784 reported cases (DIR = 2.26 cases/10,000 inhabitants/day [2.25–2.27]) from December 16, 2010 to January 30, 2011, phase 3 was characterized by a marked decrease that was observed in all communes but was more marked in urban communes. Six significant high-risk spatial clusters were identified; the main cluster was centered at the mountains of the Department of Centre with a RR of 2.36 [2.32–2.39] ($p = 0.001$). The subsequent forth phase was a lull period ending on May 22, 2011 with 93,474 reported cases (DIR = 0.83 cases/10,000 inhabitants/day [0.82–0.83]). During this lull phase, four significant clusters of elevated incidence rates persisted. The main

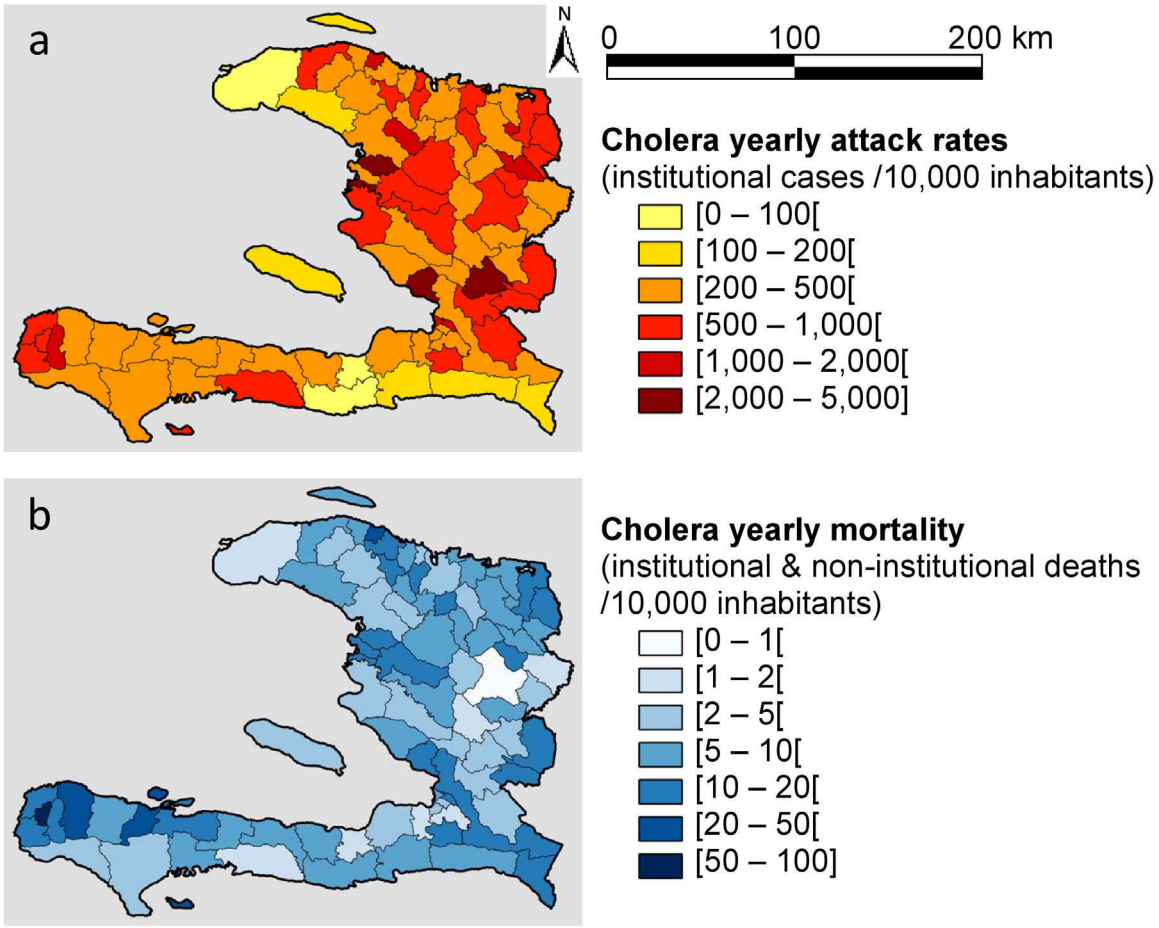

Figure 1. Mapping one year of cholera morbidity and mortality rates in Haiti. The colored scales represent yearly attack (a) and mortality (b) rates per 10,000 inhabitants in communes of Haiti (from October 16, 2010 to October 15, 2011).
doi:10.1371/journal.pntd.0002145.g001

cluster was again localized to the mountains of the Department of Centre with a RR of 3.27 [3.32–3.32] ($p = 0.001$). The remaining clusters displayed particularly local and brief outbreaks.

The second principal outbreak began at the end of May (phase 5), concomitant with the onset of the rainy season, which started late in 2011 and was associated with 35,356 cases (DIR = 1.67 cases/10,000 inhabitants/day [1.65–1.69]). This outbreak peaked on June 12. Five significant high incidence clusters were observed, the main cluster still remained localized to the mountains of the Department of Centre with a RR of 4.01 [3.92–4.1] ($p = 0.001$).

The subsequent decrease (phase 6) included 116,306 cases (DIR = 0.92 cases/10,000 inhabitants/day [0.86–0.98]). Five significant high incidence clusters were identified; the main cluster encompassed approximately five departments (North-East, North, Centre, Artibonite, and portion of the West department), with a RR of 2.81 [2.78–2.85] ($p = 0.001$). The remaining clusters were located at communes of the south peninsula with local outbreaks. The positive correlation (0.35, $p = 0.001$) between the two principal outbreaks suggests that population immunity did not play a major role in the cholera epidemic dynamics during the first year. The effect of immunity during this period may be concealed by spatial aggregation of the data at the communal level, population movement during the first weeks of the outbreak, and most notably environmental or social intra-communal determinants.

The patterns of these various phases were confirmed by spectral analysis of case time series (Figure 5a), which highlights the elevated velocity and intensity of the first phase in the Artibonite valley (phase 1) and the high (but less abrupt) intensity of phases 2 and 5. Spectral analysis of rainfall series (Figure 5b) highlighted the importance of rainfall during both Hurricane Tomas in November 2010 and the 2011 rainy season that began in May, which were the only two heavy rainfall periods associated with incident cases based on cross-spectrum analysis (Figure 5c).

Local environmental factors were assessed by quantifying their association with the spread of cholera at teach phase (Table 1). For phase 1, the results highlighted the role of the Artibonite River (Standardized Incidence Ratio for each 10 km portion of perennial rivers, SIR = 2.28 [1.86–2.79], $p<0.001$) and rice fields (SIR = 16.7 [3.0–93.7], $p = 0.002$). Conversely, urban zones (SIR = 0.034 [0.007–0.18], $p<0.001$) and mountainous zones (SIR = 0.113 [0.05–0.27], $p<0.001$) displayed a protective role. During phases 2, 4 and 6, no specific environmental factor was associated with outbreak spread. During phase 3, urban zones (SIR = 0.68 [0.47–0.97], $p = 0.03$) experienced a more rapid decrease in case numbers, thereby showing an apparent protective role. Other factors were no more significant during this phase. With the exception of phase 3, the spatial distribution of communes remained significant ($p<0.006$), thereby showing that environmental factors did not fully explain the spatial clustering of cases during each phase.

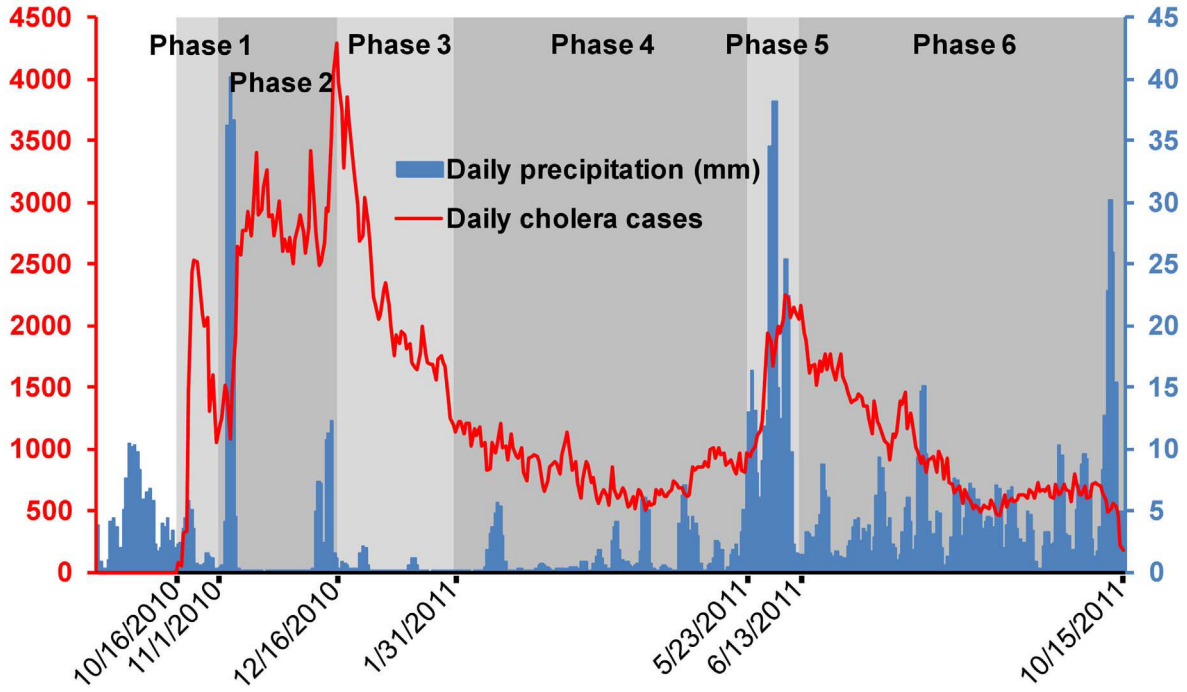

Figure 2. Temporal cholera dynamics. Daily cholera cases (red), daily rainfall (blue), and epidemic phases (grey) (September 15, 2010 to October 16, 2011) are presented. Accumulated rainfall data were obtained from the Daily Global and Regional Rainfall (TMPA-RT 3B42RT derived).
doi:10.1371/journal.pntd.0002145.g002

Discussion

With 493,069 cases after one year, the cholera epidemic in Haiti appear to be the largest ever recorded in a single country during the past 50 years. Although it began during the last trimester of 2010, the cases reported in Haiti accounted for more than 56% of the total cholera burden in 2010. Yearly attack rates were higher than 20% in several Haitian communes, such as Mirebalais (30.7%), L'Estere (29.2%), Grande Saline (22.1%), and Cabaret (26.6%). To make a comparison, the yearly attack rate during the 2008–2009 epidemic in Harare (Zimbabwe) was 1.29%, reaching a maximum in the Hopley suburb with 541 cholera cases per 5,994 inhabitants (9%) [33]. Before the Haitian epidemic, the largest cholera epidemic ever recorded during the seventh pandemic was the 1991 epidemic in Peru, which accounted for approximately 300,000 cumulative cases during the first year [34]. However, the yearly attack rate (approximately1.4%) of the Peruvian epidemic was approximately 3.5 times lower than that of the epidemic in Haiti (4.9%). Due to its exceptional amplitude, the cholera epidemic in Haiti led to a large number of fatalities. Because of the difficulties of identifying all cases and deaths in remote rural areas, it is likely that the recorded 6,293 deaths represent only a portion of the actual cholera death toll.

The analysis of epidemic profiles at different time phases reveals evidence of different spatio-temporal patterns. The first two phases of the epidemic (October 16–December 15, 2010) display a clear centrifugal expansion of cholera, with a damping wave centered at the location of the explosive outbreak onset in Mirebalais and the Artibonite Valley. Even if low rainfall had been recorded before early October, no heavy rain was associated with the outbreak onset (phase 1), and flooding cannot be incriminated. However, several environmental factors (rice fields, plains, rural zones, and rivers) were associated with a higher risk of contracting the disease during this early phase. These findings correlate with the results of previously published reports and studies that attribute the onset of the epidemic to massive contamination of the Artibonite River and downstream irrigation canals by an imported pathogenic strain of cholera [2], [16], [35].

Conversely, the particularly rapid diffusion of cholera out of the Artibonite Valley (November - mid-December 2010, phase 2) was not associated with any environmental factors but might be linked to other phenomena. Human-driven dissemination was favored by the massive contamination of the population living in the Artibonite Delta [16], the lack of immunity among Haitian population, and deficiencies in water, sanitation, and health care systems [35]. The explosive spread of the disease overwhelmed the humanitarian response and the initial attempts to broadcast awareness and hygiene messages. People who fled from the Artibonite Delta to neighboring communes [36] also played an aggravating role in cholera diffusion, thereby favoring the spread of cholera even in remote rural areas. The violent nature of this outbreak spread may also have been promoted by Hurricane Tomas, which reached Haiti on November 5 with rapid flooding in some areas already affected by cholera, such as Gonaives. Finally, riots in Port-au-Prince following the first round of presidential elections in early December 2010 may have also reinforced this explosive epidemic.

The relationship between rainfall and cholera spread in Haiti was attested by the association of phase 2 with Hurricane Tomas, the lull transmission period with the dry season (phases 3 and 4), and the second outbreak (phase 5) with the heavy rainfall during late May2011. This booster effect of rainfall on cholera outbreaks has been observed in many other countries [13], [14], [36]–[][38], where rainfall has caused latrine overflow or the washing up of waste with subsequent contamination of wells and surface waters. However, the relationship between rainfall and cholera likely involves other mechanisms, such as the seasonal modification of human water sources or human behavior such as rice culture

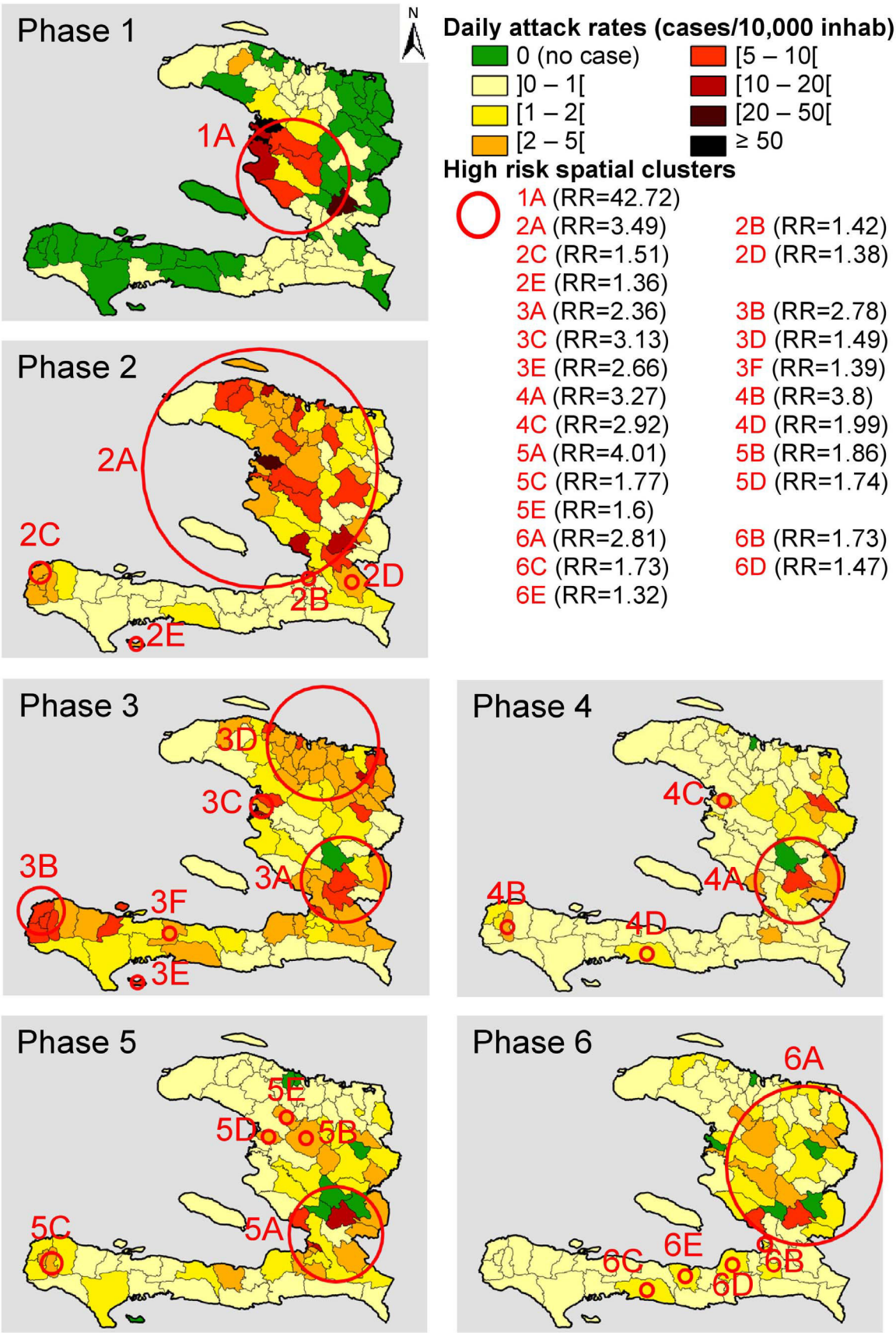

Figure 3. Daily incidence rates (DIRs) and high-risk spatial clusters for each epidemic phase.
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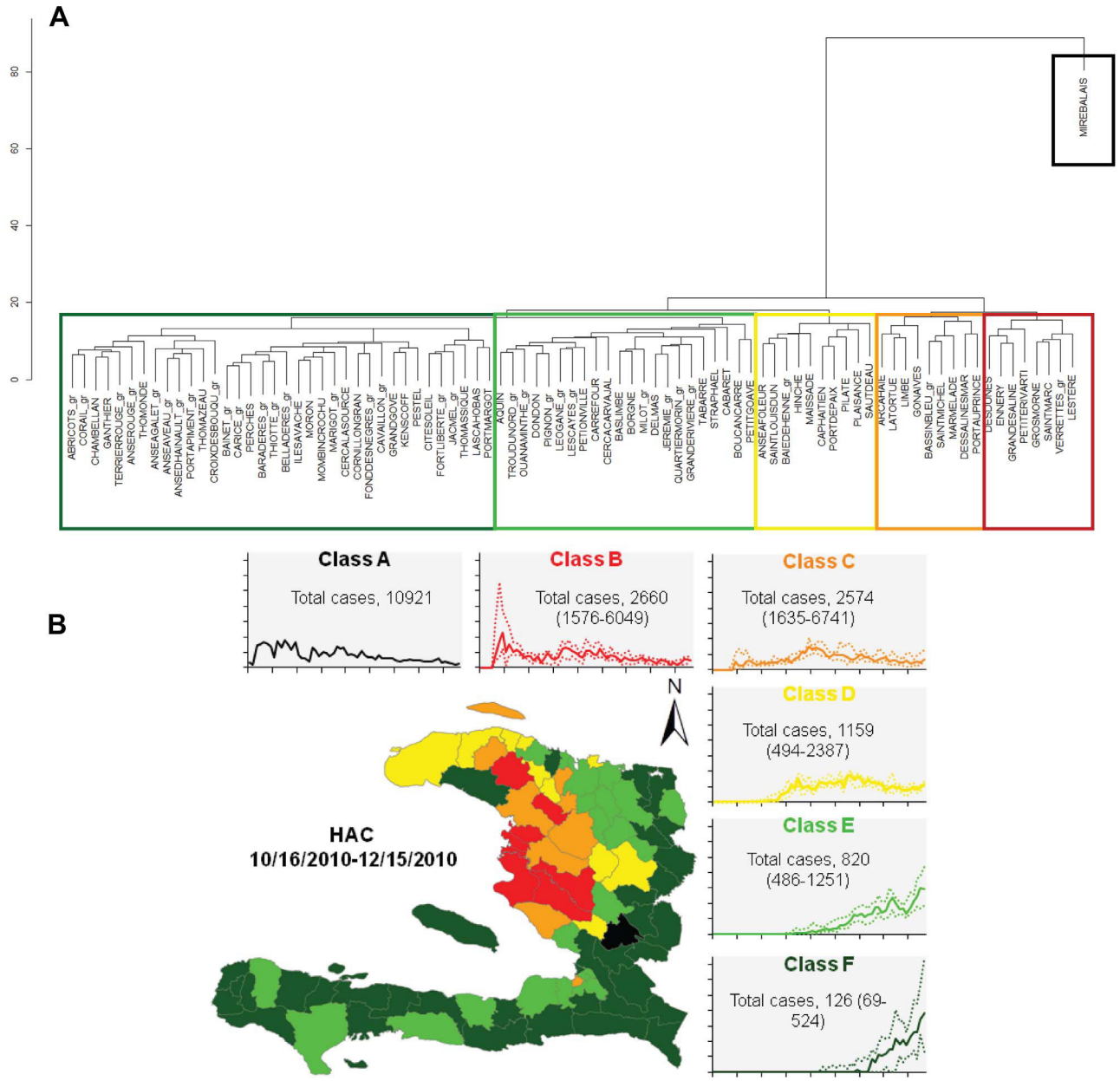

Figure 4. Epidemic profiles of the first outbreak phases (phases 1 and 2). a) Hierarchical cluster analysis (HCA) of communal epidemic profiles and b) Communal mapping of the epidemic profile classes. Median (25th–75th percentiles) communal cases observed during the period are provided for each class of profile. The graphs represent the median (solid line) and 25th–75th percentiles (dotted lines) of daily communal cases standardized by the total number of cases during the period.
doi:10.1371/journal.pntd.0002145.g004

activity. Overall, many phenomena affecting environment-to-human and human-to-human transmission may affect this relationship between rainfall and cholera outbreaks, which therefore should not be regarded in Haiti as in Bangladesh, where cholera onset has been associated with vibrio blooms in aquatic reservoirs [39], [40].

During phases 3, 4 and 5, cholera incidence was poorly associated with environmental factors. Cholera attack rates decreased more rapidly in the main towns than in rural areas during phase 3. Sequential identification of spatial clusters during the successive phases of the epidemic shows that mountainous rural areas located in the northern and eastern portions of the country likely functioned as a reservoir for cholera during the dry season until more favorable climatic factors triggered the second outbreak of late May 2011. During a field assessment in April 2011, we found that cholera persisted during the lull period in rural Haiti, circulating from one village to another, and provoking outbreaks linked with the transient local contamination of springs and streams. Due to the difficulty in reaching these mountainous remote areas, the fight against cholera was less efficacious than in towns and plains. Unlike observations made in Asia, where cholera outbreak patterns largely depend on human exposure to the aquatic reservoirs of *V. cholerae* [41], or eastern Democratic Republic of the Congo, where lakes play an important role in

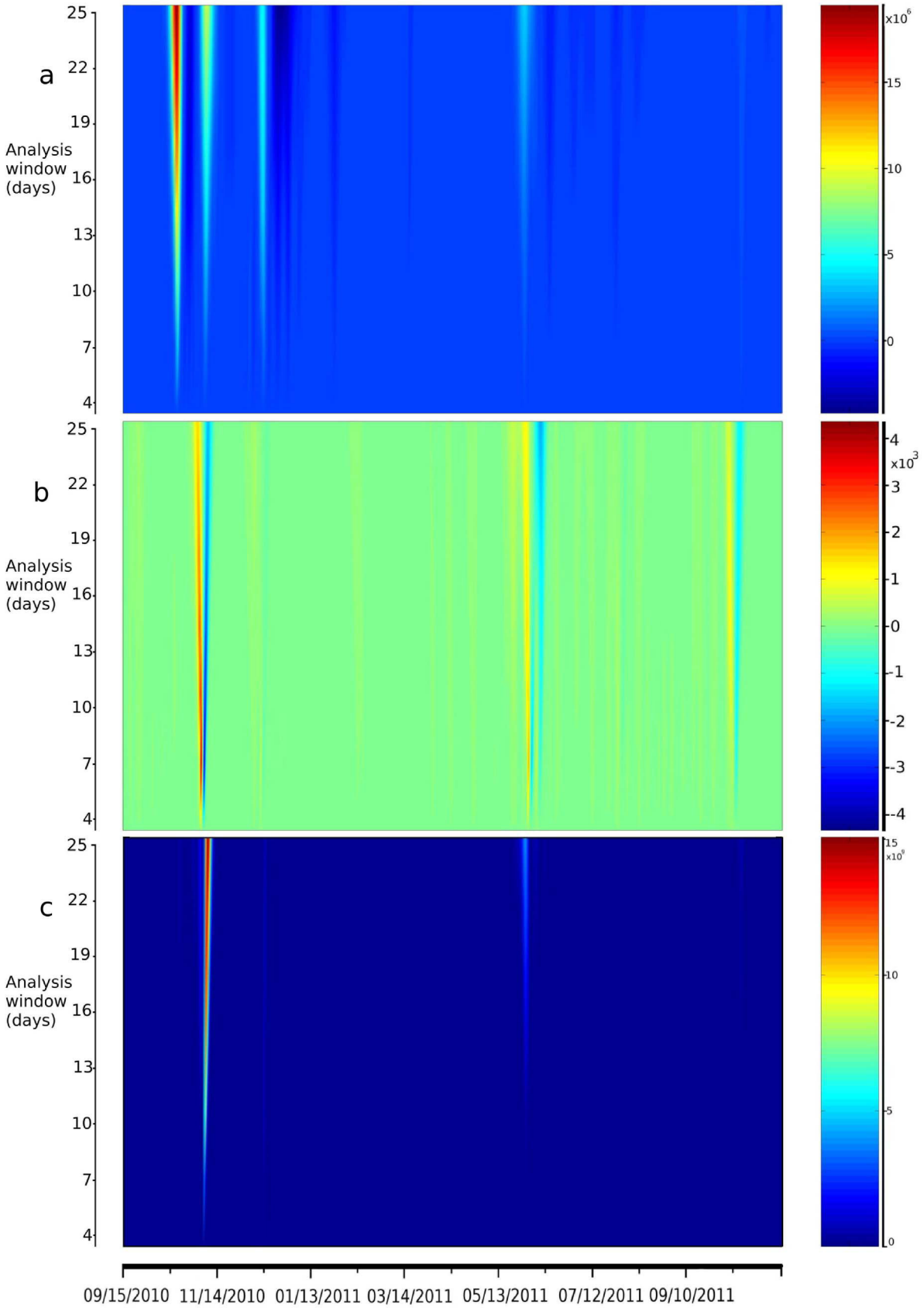

Figure 5. Spectral analysis of time series. Analysis of cases (a), rainfall (b), and cross-wavelet (c) between cases and rainfall are presented. The Y-axes represent length of the wavelet analysis window (from 3 to 26 days) and the color scales represent the spectral values for each length of the analysis window.
doi:10.1371/journal.pntd.0002145.g005

Table 1. Impact of local environmental factors during each epidemic phase.

	Phase 1: Oct 16, 2010 to Oct 31, 2010	Phase 2: Nov 1, 2010 to Dec 15, 2010	Phase 3: Dec 16, 2010 to Jan 30, 2011	Phase 4: Jan 31, 2011 to May 22, 2011	Phase 5: May 23, 2011 to Jun 12, 2011	Phase 6: Jun 13, 2011 to Oct 15, 2011
	SIR [95%CI] (p)					
Length of perennial rivers (10 km)	**2.28 [1.86–2.79] (<0.001)**†	-*	-*	-*	1.1 [1.0–1.21] (0.06)‡	-*
Number of watershed	-	-*	-*	-*	-*	-*
mountainous landscapes (vs. plains)	**0.113 [0.05–0.27] (<0.001)**†	-*	-*	-*	-*	-*
Urban zones	**0.034 [0.007–0.18] (<0.001)**†	-*	**0.68 [0.47–0.97] (0.03)**†	-*	-*	-*
Rice fields	**16.7 [3.0–93.7] (0.002)**†	-*	1.3 [0.95–1.8] (0.09)‡	-*	0.67 [0.43–1.06] (0.08)‡	-*
Spatial distribution of communes	**p<0.001**†	**p<0.001**†	-*	**p = 0.005**†	**p = 0.002**†	**p = 0.006**†

Standardized incidence ratios (p-values) were estimated using the multivariate regression model.
*Factor excluded using stepwise analysis.
†Significant factors (boldface).
‡Non-significant factors kept using stepwise analysis.
doi:10.1371/journal.pntd.0002145.t001

outbreaks [13], [15], our results do not suggest any environmental persistence of cholera. However, this has to be confirmed with environmental sample studies. Currently, cholera presence in the environment has been reported in two cross-sectional studies [42], [43], but no environmental spatio-temporal monitoring system has been developed. In contrast, rice fields tended to be protective during the second outbreak (phase 5). This may be partly due to population immunity acquired during the initial phases of the epidemics, particularly in the Artibonite Delta, which was heavily stricken during the first phase of the epidemic. The protection was likely also due to the action of nongovernmental organizations (NGOs) and local actors as well as the reinforcement of a population sensitization program that was implemented in the Artibonite plain [44].

Overall, our findings clearly show that the epidemic is still evolving. Such diversity in transmission patterns could hardly have been anticipated, especially in a country struck by cholera for the first time, which highlights the need for comprehensive studies such as the current investigation. Therefore, we believe it is too early to predict the future pattern of this epidemic, and especially to affirm that cholera will become endemic in Haiti. Notably, the presence of estuaries in an area hit by cholera does not necessarily mean that *V. cholerae* will perennially settle in the brackish waters and that seasonal outbreaks will recurrently occur in the future. Madagascar, another island with deficient sanitation, a susceptible hydro-geologic environment, a widespread rice culture, political tension, and a lack of resources, was hit by successive cholera waves from 1999 to 2001 [45]. Since this time, the country has not experienced new outbreaks. Like Madagascar, Haiti may benefit from its insular position far from usual endemic foci. The current spatio-temporal analysis shows that dynamics of the cholera epidemic varied from location to location as time passed, following no clearly predictable scheme. Excluding the first phase, no recurrent environmental factor was implicated, except rainfall involved in the exacerbation of the epidemic. After the first phases of the outbreak, the absence of constant spatial clusters and the changing pattern of cholera distribution in Haiti argue for the need for control measures that should include intense efforts in rapid and exhaustive case tracking.

Acknowledgments

We are grateful to the Haitian Ministry of Public Health and Population authorities, the medical teams in each Haitian department, the Cuban medical teams in Haiti for collecting the data, and the French Embassy in Port-au-Prince. We are also grateful to Sandra Moore, who helped in editing the manuscript.

Author Contributions

Conceived and designed the experiments: JG RB JB RM GT RP. Performed the experiments: JG RB BF RP. Analyzed the data: JG SR RB MP JB RP. Contributed reagents/materials/analysis tools: JG RB BF. Wrote the paper: JG SR RB MP BF RP.

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3.2.3 Une dynamique difficile à prédire et résultant de la contamination initiale du fleuve Artibonite

A partir du 16 octobre 2010, la transmission du choléra explosa initialement dans le périmètre limité de Mirebalais et de la Basse Vallée de l'Artibonite, une zone de rizières aux multiples canaux. La pluviométrie au cours de cette période demeura faible. A partir de ce foyer initial, le choléra se propagea très rapidement de manière centrifuge, sous la forme d'une onde de choc correspondant à la fuite des populations vers les départements voisins, et catalysée par les fortes précipitations de l'ouragan Thomas. Une accalmie relative fut ensuite observée début 2011 à la faveur de la saison sèche. Cette régression de la transmission n'avait pas été anticipée par les modèles mathématiques n'ayant pas pris en compte la contamination initiale du fleuve Artibonite. Au cours de cette saison sèche, la transmission du choléra apparut se rétracter dans des régions rurales et montagneuses difficiles d'accès. Une deuxième vague épidémique fut alors subie par la population haïtienne, concomitamment au retour de la saison des pluies fin mai 2011. La distribution spatiale de cette deuxième vague fut beaucoup plus fragmentée et non déterminée par les variables environnementales selon nos analyses.

Ces résultats ne suggéraient donc pas l'enracinement du choléra dans l'environnement haïtien. Ils démontraient par contre les difficultés à anticiper l'évolution d'une épidémie sans précédent, et notamment son risque d'endémisation.

3.3 Réservoirs environnementaux du choléra en Haïti

« L'élimination du choléra sur l'ile Hispaniola se traduit par l'interruption de la transmission. Cependant la bactérie étant dans l'environnement, des cas sporadiques seront toujours diagnostiqués. »

Plan d'élimination du choléra en Haïti 2013-2022 [102]

« It is still a transmission from person to person, or from temporary contamination of water points »

Peter de Clercq,
Représentant spécial adjoint pour le
Secrétariat Général des Nations Unies en Haïti,
Mai 2014 [103]

3.3.1 Le choléra s'est-il installé dans l'environnement haïtien depuis son importation ?

Après avoir participé à démontrer l'origine importée de l'épidémie de choléra en Haïti puis avancé dans la compréhension de sa première année d'évolution, il était dans la logique de l'équipe de chercher à savoir si la souche épidémique de *V. cholerae* O1 toxinogène ne s'était pas installée dans l'environnement aquatique haïtien, afin de mieux appréhender les perspectives d'élimination à court terme et d'adapter les stratégies de lutte.

Renaud Piarroux sollicita donc une équipe de l'ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) spécialisée dans la recherche de vibrios dans l'environnement aquatique et les produits de pêche, ainsi qu'un financement de l'Ambassade de France en Haïti, afin de conduire une étude de microbiologie environnementale en collaboration avec le Laboratoire National de Santé Publique d'Haïti. Réalisé en juillet 2012, ce travail ne retrouva pas de *V. cholerae* O1 toxinogène dans les sites échantillonnés. Afin d'en valoriser les résultats, j'ai été amené à participer activement à leur ré-analyse et à la rédaction d'un article publié dans la revue en ligne PLoS Current Outbreaks [104], la dernière née du groupe PLoS garantissant un peer-reviewing rapide pour des sujets d'actualité.

[102] République d'Haïti, Ministère de la Santé Publique et de la Population, et Direction Nationale de l'Eau Potable et de l'Assainissement, *Plan d'Élimination du Choléra en Haïti. 2013-2022*.
[103] « As dry season ends in Haiti, significant gains seen in fight against cholera, UN official says ».
[104] Baron et al., « No evidence of significant levels of toxigenic V. cholerae O1 in the Haitian aquatic environment during the 2012 rainy season ».

3.3.2 Article 3 : « No Evidence of Significant Levels of Toxigenic *V. cholerae* O1 in the Haitian Aquatic Environment During the 2012 Rainy Season »

L'article publié par PLoS Current Outbreaks peut être consulté à l'adresse suivante : http://currents.plos.org/outbreaks/article/no-evidence-of-significant-levels-of-toxigenic-v-cholerae-o1-in-the-haitian-aquatic-environment-during-the-2012-rainy-season/ (accédé le 1/10/2014)

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PLOS | CURRENTS OUTBREAKS

No evidence of significant levels of toxigenic *V. cholerae* O1 in the Haitian aquatic environment during the 2012 rainy season

September 13, 2013 · Research

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doi:10.1371/currents.outbreaks.7735b392bdcb749baf5812d2096d331e.

ABSTRACT

Background: On October 21, 2010, Haiti was struck by a cholera epidemic for the first time in over a century. Epidemiological and molecular genetic data have clearly demonstrated that the bacterium was imported. Nevertheless, the persistence of the epidemic for more than two years, the high incidence rates in some coastal areas and the seasonal exacerbations of the epidemic during the rainy seasons have prompted us to examine the levels of toxigenic *Vibrio cholerae* in the Haitian aquatic environment.
Methods: In July 2012, during the warm and rainy season, 36 aquatic stations were sampled to search for toxigenic *V. cholerae*. These stations included fresh, brackish and saline surface waters as well as wastewater; the sampling sites were located in both rural and urban areas (around Port-au-Prince and Gonaïves) located in the West and the Artibonite departments. *V. cholerae* bacteria were detected in enrichment cultures of 3 to 6 serial volumes of each water sample (sample volumes included: 1 L, 100 mL, 10 mL, 1 mL, 0.1 mL, 0.01 mL and 0.001 mL). Detection methods included both culture on selective agar (for strain isolation) and PCR targeting the genes *ompW (V. cholerae* species), *O1-rfb* and *O139-rfb* (O1 and O139 *V. cholerae* serogroups, respectively), and the cholera toxin gene *ctxA*, which is present exclusively in cholera strains.

Results: A total of 411 *V. cholerae* isolates from 29 stations were obtained via selective culture; however, only one of these isolates displayed a late positive reaction with polyvalent anti-O1 serum. Positive *V. cholerae* PCR results were obtained from each of the 32 tested stations (a total of 77 enrichments out of 107 yielded a positive result); only one sample yielded a positive *V. cholerae* O1 PCR result. The cholera toxin gene *ctxA* was never detected via PCR with either primer pair, which includes samples derived from the two stations yielding positive O1 culture or positive O1 PCR results. Therefore, we could not demonstrate the presence of toxigenic *V. cholerae* O1 among the 36 stations sampled. This suggests that all water samples analyzed contained less than 10 toxigenic *V. cholerae* O1 bacteria per liter, a level 1000-fold below the infective dose necessary to provoke cholera.
Conclusions: Currently, there is no evidence of a significant level of contamination of the aquatic environment in Haiti by the imported toxigenic *V. cholerae* O1 strain. The reemergence of cholera outbreaks in Haiti during rainy seasons is therefore more likely due to persisting outbreaks insufficiently tackled during the dry periods rather than the commonly suspected aquatic reservoir of toxigenic bacteria.

INTRODUCTION

On October 21, 2010, Haiti was struck by cholera for the first time in over a century [1]. The Haitian epidemic represents the largest national cholera epidemic of the seventh pandemic with 604,634 cases and 7,436 deaths reported from October 2010 to October 2012 [2].

Epidemiological data has demonstrated an exact spatiotemporal correlation between the first reported cholera cases in Meille, a small village 2 km south of Mirebalais, and the arrival of UN Nepalese peacekeepers in Haiti [3]. According to Frerichs *et al.* (2012), the Nepalese soldiers were exposed to a cholera epidemic in Nepal in late September just before embarking for Haiti, where they were primarily stationed in a camp near Mirebalais, situated on the banks of the Meille River [4]. The initial cases were biologically confirmed as *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor [5]. Genetic analysis has demonstrated that the Haitian cholera isolates were almost identical to isolates collected in Nepal a few weeks prior, which displayed only one- or two-base pair differences throughout the entire genome, thereby strongly suggesting that the Haitian cholera strains were very recently imported from Nepal [6].
During the first two years of the epidemic, cholera was disseminated throughout almost every region of Haiti, including the most remote rural areas. Meanwhile, outbreaks seemed to be aggravated by major climatic events, such as Hurricane Tomas in November 2010 and the hot and rainy seasons of 2011 and 2012. Moreover, it appears that cholera particularly affected certain coastal areas, such as the Artibonite Delta following the floods provoked by Hurricane Tomas and the low altitude wards of Port-au-Prince during the 2011 rainy season. In contrast, a major reduction in the number of

cases was observed during the dry seasons [2,7], when cholera transmission retracts in a few rural locations and urban quarters [8]. The persistence of cholera in the country for more than two years associated with a seasonal exacerbation of the epidemic during the rainy seasons, especially in coastal areas, has prompted us to examine the level of toxigenic *V. cholerae* contamination in the Haitian aquatic environment.

Indeed, in the Bay of Bengal, it has been shown that the environment can play a role in the durable establishment of cholera. Studies in the 1980s and 1990s have demonstrated that *V. cholerae* species can grow in various aquatic ecosystems, such as fresh waters, brackish waters and estuaries [9]. In such environments, *V. cholerae* species associates with phytoplankton and zooplankton in a pH- and salinity-dependent manner [9]. Increases in water temperature and subsequent plankton blooms have been shown to correlate with the fluctuation in cholera cases in the Bay of Bengal [10,11].

To investigate whether the aquatic environment presents a major risk of cholera transmission to local populations, we conducted a microbial assessment of toxigenic *V. cholerae* O1 levels on a panel of water samples isolated from several areas in the West and Artibonite departments. These areas were selected because they were heavily affected by cholera during either the floods that followed hurricane Tomas in 2010 (Artibonite Department) or the 2011 rainy season (Port-au-Prince and Gonaïves areas). Most of the sample sites were coastal areas considered to be favorable environments for *V. cholerae* growth; although, other inland sites (Cul-de-Sac Plain) suitable for *V. cholerae* proliferation were also examined.

MATERIALS AND METHODS

Study period

To search for toxigenic *V. cholerae* present in the Haitian environment, the study was performed during the warmest period of the rainy season (Figure 1). Aquatic samples were collected between July 3 and July 10, 2012, a period characterized by high surface water temperatures. During this period, approximately 168 suspected cholera cases were reported per day in the departments of West (including the Port-au-Prince metropolitan area) and Artibonite (Figure 1).

Sampling sites

Thirty-six stations were sampled from four distinct areas (Figure 2, Table 1). The sampling sites were selected based on field observations, focusing on the nature of the water bodies (surface water or wastewater), excluding well water and domestic tanks. The urban sampling areas included Port-au-Prince (seven stations) in the West department, and Pont-Sondé (one station), L'Estère (one station) and Gonaïves (four stations) in the department of Artibonite. Lakes and ponds unconnected to coastal waters were also tested, as their salinity levels are compatible with *V. cholerae* proliferation. These sites were located at lakes (Trou Caïman and Etang Saumâtre in the Cul-de-Sac plain, three stations) and a large pond located southwest of Saint Marc (Etang Bois-Neuf, one station). Rural areas of the Artibonite Plain were represented by 17

stations at both the river and diversion canals. Among these 17 stations, nine were frequently accessed for toilet or laundry activity. The two remaining stations were salt marshes located near Gonaïves.

Sampling collection and processing

Grab water samples were collected 20 cm below the surface with sterilized narrow-mouth plastic bottles. Sample collection was performed by boat at Etang Saumâtre Lake, Etang Bois-Neuf Pond and the Artibonite estuary. The other samples were collected from the shore with a telescopic pole. Water samples were transported in a cooler (containing frozen packs at the bottom for minimum contact with the bottles) to the Haitian National Laboratory of Public Health (LNSP), and the analysis was performed within 6 to 24 hours of collection. A mildly cool temperature during transport and lag time in the laboratory prior to analysis served to stabilize the *Vibrio* population as much as possible, as the sampling methodology intended to assess bacterial abundance in the water.

Surface water temperature and pH levels were measured at the sampling sites using a field pH meter (Hanna HI-98127, Grosseron, Nantes, France); conductivity was assessed at the laboratory with a field conductometer (Hanna HI-99301, Grosseron, Nantes, France). Fecal contamination was determined using Petrifilm™ Select E. coli (Département Microbiologie Laboratoires 3M Santé, Cergy, France), which was incubated overnight at 42°C.

Enrichment and selective cultures

The analyzed serial volumes for each water sample were selected based on the type of water and the expected abundance of *V. cholerae*. At each station, 3 to 6 serial volumes (selected from the following range: 1 L, 100 mL, 10 mL, 1 mL, 0.1 mL, 0.01 mL and 0.001 mL; sample volumes of 100 mL and 10 mL were always included) were analyzed, with an exception at 6 stations in the estuary of the Artibonite, where only 1 sample volume of 1 L was assessed. Sample volumes of 10^{-2} mL were analyzed from 12 stations, and volumes of 10^{-3} mL were assessed from 6 stations.

Sample volumes of 1 L and 100 mL were filtered (Diaphragm pump N035.3 AN.18 KNF Neuberger, Village-Neuf, France) successively with glass microfiber filters GF/D (grade D, 2.7 µm; Whatman, Maidstone, UK), glass microfiber filters GF/C (grade C, 1.2 µm; Whatman, Maidstone, UK) and 0.45 µm cellulose ester membranes (Millipore, Watford, UK). The filters were sequentially used for the filtration of water samples. The various filter sizes guaranteed the isolation of both fixed-form and free-living bacteria. The filters were placed in 250 mL of sterile Alkaline Saline Peptone Water (ASPW; composition for 1 liter: 10 g peptone, 20 g NaCl and 5 g yeast extract; post-autoclave pH: 8.6 ± 0.2). Sample volumes of 10 mL were incorporated in 100 mL of ASPW, and sample volumes of 1 mL to 0.001 mL were incorporated in 10 mL of ASPW.

The enrichment cultures were incubated from 16 to 24 hours at 41 ± 1°C [12] and subsequently cultured on selective TCBS (Thiosulfate Citrate Bile Sucrose) agar (Difco, provided by Bio-Rad, Marne la Coquette, France) to isolate *V. cholerae* colonies.

The screening procedure was based on phenotypic traits. Up to 20 sucrose-fermenting colonies were transferred with sterile toothpicks onto nutrient agar without NaCl (NA_0 – Difco, provided by Bio-Rad, Marne la Coquette, France) to test for growth at 37°C and then submitted for an oxidase test (Bactident oxidase strips, Merck, Darmstadt, Germany). All sucrose-fermenting isolates that were able to grow on NA_0 agar and tested oxidase-positive were considered to be presumptive isolates of *V. cholerae* [13].

Isolate serotyping

Presumptive *V. cholerae* isolates were examined to determine whether they were members of the O1 serogroup via slide agglutination using a polyclonal antibody specific for the O1 surface antigen (Bio-Rad, Marne la Coquette, France). A saline solution was used as a control to identify self-agglutinating isolates.

Molecular identification

At the LNSP laboratory, DNA extraction of the enrichment cultures was performed automatically after adding 10 µL proteinase K to 200 µL of each enrichment broth using Boom technology (TANBead viral auto kit, Taïwan) with the robot Medipro super pure system-32 (Taïwan). All 107 DNA extracts obtained from 32 sampling sites (stations 6 to 37), both pure and 10-fold dilutions, were assessed via two multiplex PCR [14,15]. The detection of *V. cholerae* species was performed via PCR targeting a gene encoding an outer membrane protein (*ompW*) [14] (Table 2). Two different cholera toxin gene (*ctxA*)-specific PCR assays were used to detect the cholera toxin [14,15] (Table 2). The gene coding for the O1 and O139 surface antigens (*rfb*) was assessed via PCR using O1- and O139-specific primers [15] (Table 2). The PCR assays were conducted using a G-Storm thermal cycler (Gene Technologies Ltd, Braintree, UK) with the cycling conditions described in Table 2.

RESULTS

Surface water characteristics and fecal contamination

The disparity in pH levels between the different stations was low (Table 1), with a mean pH of 7.4 (standard deviation: 0.4, minimum: 6.5 and maximum: 8.4). The Etang Bois-Neuf site displayed the highest water pH levels (8.4), which is a pH level known to be favorable for *V. cholerae* growth. The *in situ* water temperatures ranged between 27.7°C (Etang Saumâtre) and 38.8°C (Mariani) (mean: 33.3°C; standard deviation: 3.3°C). Salinity levels varied greatly between the sampling zones. Therefore, the sampling sites were categorized into 3 groups based on this characteristic: (1) 20 freshwater stations: salinity levels inferior to 0.5‰; (2) 11 brackish water stations (including 3 oligohaline wastewaters): salinity levels between 0.5‰ and 16‰; and (3) 5 saline water stations: salinity levels between 16‰ and 40‰.

The levels of fecal contamination varied greatly between samples (Table 1). Accordingly, the risk of contracting intestinal infections was low in the rice field and salt marshes (less than 100 Colony Forming Units (CFU) per 100 mL), high at the Artibonite plain stations located a pronounced distance from housing (101 to 1000 CFU per 100 mL), and very high near housing settlements (more than 1000 CFU per 100 mL). In wastewaters, fecal contamination levels ranged from 10,000 to 100,000 CFU per 100 mL.

Absence of toxigenic *Vibrio cholerae* O1

From a total of 141 enrichment cultures derived from the water samples collected at the 36 sampling sites, 411 presumed isolates of *V. cholerae* were isolated. The distribution of the isolates by sampling site is provided in Table 3. Nine sampling sites failed to yield any *V. cholerae* isolates. Five of these sites (sites 20, 21, 22 and 23 at Gonaïves and site 35 at Port-au-Prince) were saline waters. The other 4 stations (sites 8, 11, 12 and 13), which were fresh waters, were located at the mouth of Artibonite estuary.

The O1-agglutination test was performed on 390 presumed *V. cholerae* isolates, of which 56 strains were positive for auto-agglutination. Only a single isolate, which was isolated from sampling site 27 (a large canal south of L'Estère), displayed a late positive reaction with polyvalent anti-O1 serum, without auto-agglutination.

The *V. cholerae* PCR assays were found to be more sensitive than the culture assay, as positive results were obtained from all of the 32 tested stations (a total of 77 enrichments out of 107 yielded a positive *V. cholerae*-specific PCR on *ompW* gene). However, only 1 sample derived from station 31 in the rice field near Desdunes yielded a positive result for *V. cholerae* O1-specific PCR (*rfb* gene). The *ctxA*-specific PCR was performed on all water enrichments, including from station 27 (where an isolate displayed a late positive reaction with polyvalent anti-O1 serum) and from station 31 (positive O1 PCR). However, the cholera toxin was never detected with either pair of *ctxA*-specific primers, even from 100 mL or 1 mL water samples. Therefore, despite the high number of *V. cholerae* isolates obtained, we could not demonstrate the presence of toxigenic *V. cholerae* among all the samples collected.

Abundance of *Vibrio cholerae* in surface waters and wastewaters

Table 3 provides the smallest sample volume displaying the presence of *V. cholerae*, or in case of non-detection of *V. cholerae*, the largest volume analyzed for each station. The results of *V. cholerae* detection via culture or PCR are consistent with an abundance ranging from 1 to 10^6 bacteria per liter depending on the type of water.

DISCUSSION

The aim of this study was to ascertain whether toxigenic *V. cholerae* resides in Haitian aquatic ecosystems in sufficient concentrations to present a significant risk to the local population. Our purpose was therefore not to prove the absence of toxigenic *V. cholerae* in the environment, a goal that would require other methods and sampling strategies.

Aquatic samples characterized by a wide range of salinity levels were collected during a warm period, at both the time point and locations one would expect to find a high abundance of *V. cholerae* in the environment. Sampling sites were located in Haitian areas profoundly affected by cholera, and most of locales presented medium to high fecal contamination levels, thereby presenting aquatic conditions appropriate for the study of contamination by the epidemic *V. cholerae* clone.

To enhance culturable *V. cholerae* detection, environmental water samples were enriched. Sample enrichment facilitates the detection of *V. cholerae* (including toxigenic *V. cholerae*) regardless of the bacterial form (i.e., free-living bacteria or bacteria attached to phytoplankton, zooplankton and copepods). Various dilutions of the samples were enriched to maximize the chances of isolating and identifying *V. cholerae* clones, and every enrichment culture was analyzed.

Moreover, as it has been reported that cholera bacterium are also found in a viable but non-culturable state in the environment [16,17], we also performed several PCR assays on each enrichment culture to detect toxigenic *V. cholerae* bacteria that may remain non-culturable. The application of both bacterial culture and PCR techniques on enrichment samples has been proposed by other groups [18,19]. This two-sided approach has proven to be effective as every station presented positive results by either one method or the other with respect to the presence of *V. cholerae*. However, the two approaches may yield conflicting results for technical reasons; a gene associated with a specific phenotype may be detected via PCR, while it may remain unexpressed or absent in isolated colonies. The inverse situation is also possible, as we analyzed diverse aliquots. It is probably for these reasons that we observed two discrepancies between selective culture and PCR regarding the detection of *V. cholerae* O1 with samples 27 and 31. Finally, to improve our chances of detecting toxigenic *V. cholerae*, we used two distinct PCR assays targeting the *ctxA* gene and systematically tested both pure and 10-fold dilutions of the DNA extracts.

Nevertheless, despite all the precautions that were taken, we found no evidence of the presence of toxigenic *V. cholerae* O1 in any of the samples collected. The absence of the *ctxA* gene not only highlights the absence of *V. cholerae* CT + bacterium, but it also suggests the absence of phages carrying the gene in water samples. These findings seem to contrast with those described by Hill *et al.* (2011) who isolated two toxigenic *V. cholerae* O1 strains from two 30-L water samples among 14 samples [19]. However, these 2 studies were not carried out in the same context. Hill *et al.* searched for toxigenic *V. cholerae* during the first epidemic wave in October-November 2010, when attack rates of cholera exceeded 2,000 new cases per day, with an epicenter around the Artibonite coast [3,7]. At that time, many more infected individuals were likely to contaminate the environment with toxigenic *V. cholerae* via open-air defecation compared with the July 2012 period.

In contrast with the absence of toxigenic *V. cholerae* O1, numerous non-toxigenic non-O1 *V. cholerae* isolates were isolated in our study, even in very small sample volumes. Our results indicate that non-toxigenic *V. cholerae* are well established in freshwater and brackish Haitian aquatic environments. This is not surprising, as it has been demonstrated that the *V. cholerae* species can be isolated from many aquatic ecosystems throughout the world, including cholera-free areas, whether in freshwater [20,21], brackish water [22–24] seawater [25–27] or even wastewater [28].

Our study shows that in July 2012 the bacterial levels of the imported toxigenic clone were far below the levels required for direct transmission to local human populations, despite the massive biomass disseminated in 2010-2011 by more than half a million cholera patients in a country where open-air defecation [29] and the washing of clothes in rivers are widely practiced. The true level of exposure required to contract cholera is difficult to precisely assess. In a study performed in rural Bangladesh, Spira *et al*. (1980) have shown that people infected during the course of the study were unlikely to have ingested more than 10^5 viable organisms per day [30], whereas a study by Cash *et al.* (1974) has established 10^4 as the minimum inoculum required to provoke diarrhea in healthy volunteers with neutralized gastric acidity [31]. In our study, the lack of toxigenic *V. cholerae* O1 detection using PCR assays on enrichment cultures was well established for 31 stations with 100 mL or 1L sample volumes. This strongly suggests that all water samples analyzed contained less than 10 toxigenic *V. cholerae* bacteria per liter, a level 1000-fold below the infective dose necessary to provoke diarrhea. Notably, as well water and domestic tanks were excluded from our sampling design, our findings do not preclude the possibility of higher levels of toxigenic *V. cholerae* O1 in peri-domestic water bodies following recent contamination by infected individuals.

Non-toxigenic *V. cholerae*, such as those identified in the Haitian aquatic environment in this study, may provoke gastroenteritis or sporadic cholera-like diarrhea in humans; however, these strains have never been implicated in large-scale cholera epidemics [32]. Only *V. cholerae* serogroup O1, both 'classical' and 'El Tor' biotypes, and the derivative serogroup O139 are known to cause cholera epidemics [32]. The relationship between all cholera isolates implicated in the seventh pandemic has recently been elucidated by Mutreja *et al.* (2011) in a study based on whole-genome sequencing of 154 *V. cholerae* strains collected from all over the world [33]. By analyzing high-resolution markers (genome-wide single nucleotide polymorphisms), they showed that all strains isolated from various outbreaks during the seventh pandemic have a single common ancestor that emerged during the 1950s. Over time, the clones diversified. Most strains disappeared within a few years, while the remaining strains gave way to new pandemic waves spread by human activity. As we demonstrate that the imported epidemic *V. cholerae* strain has failed to settle in high levels in the aquatic environment of Haiti, our results are in total accordance with the Mutreja *et al.* findings. If the epidemic strains disseminated by humans could gain a foothold in the environment for an extended duration and eventually proliferate to levels compatible with epidemic reactivation via environment-to-human contamination, phylogenetic assessment of the 154 *V. cholerae* isolates analyzed by Mutreja et al. would not have revealed the diversification and extinction phases that characterized the distinct pandemic waves since the emergence of the seventh pandemic [33].

According to Faruque and Mekalanos, the precursors of the pandemic clones probably displayed traits that are lacking in environmentally adapted *V. cholerae*, regardless of the serogroup [32]. As specified by these authors, the evolution of environmental strains into typical pathogenic strains would require more widespread gene transfer events than that shown to occur with known phages [32]. Inversely, the transmission of a

toxigenic *V. cholerae* O1 strain could be dependent on the amplification-via-disease lifestyle, and the inability of the bacteria to re-establish in the environment might be due to the requirement of human host-dependent replication and transmission. Importantly, a recent study has found that the Haitian *V. cholerae* strain has failed to acquire any genes via horizontal gene transfer from the population of non-toxigenic *V. cholerae* bacteria residing in the local aquatic environment, thereby suggesting that environmental strains have probably played no role in the evolution of the outbreak strain [34].
In fact, our findings suggest that despite its massive dissemination, the toxigenic strain imported into Haiti has failed to colonize the environment at a level required for transmission to human. This may also explain the lack of gene exchange found between epidemic and environmental strains in Haiti [34].

In conclusion, these findings provide hope that cholera could be eliminated from Hispaniola with the recovery of the last patient. Such an objective seems all the more realistic as the elimination of epidemic-causing *V. cholerae* strains has already been observed in Latin American countries such as Peru and Mexico [35]. Mexican coasts present many aquatic environments conducive to *V. cholerae* proliferation, and many rural populations still suffer from limited access to potable water and suitable health care [29]. As over 43,000 cases were reported in Mexico with a higher incidence in coastal states from 1991 to 1996, Mexico was predicted to become a cholera-endemic region [36]. However, this pessimistic prediction failed to materialize, and the annual cholera incidence throughout the entire country dwindled down to 5 cases by the year 2000 [37] and 1 case the following year [38]. Strikingly, the disease has not been observed in this supposedly endemic country since 2001, and has obviously been extinguished in Mexico. Based on the current observations, the same outcome also seems plausible for Haiti, where cholera outbreaks during the rainy season appear to reemerge from persistent transmission foci insufficiently tackled during the dry season [8].

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ACKNOWLEDGMENTS

We are grateful to Ito Journel, who performed the DNA extractions at the National Laboratory of Public Health, in Haiti.

This study was funded by a grant from the French Embassy in Haiti supporting a trans-national cooperation between the French Agency for Food Environmental and Occupational Health Safety (ANSES) and the National Laboratory of Public Health.

Authors report no conflict of interest.

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FIGURES AND TABLES

Figure 1 – Evolution of the daily suspected cholera cases in the departments of West (including Port-au-Prince conurbation) and Artibonite, daily accumulated rainfall in the area and the daily mean temperature in Port-au-Prince in 2012. Time point of the sampling period (July 3 to 10 2012).

Accumulated rainfall data were obtained from satellite estimates (TMPA-RT 3B42RT derived) averaged on the position 18.25N-19.75N / 74.25W-71.75W and available at http://disc2.nascom.nasa.gov/Giovanni/tovas/realtime.3B42RT_daily.2.shtml (accessed April 29, 2013).

Mean daily temperatures observed at Port-au-Prince airport were obtained from the following:
http://gis.ncdc.noaa.gov/map/viewer/#app=cdo&cfg=cdo&theme=temp&layers=1&node=gis (accessed April 29, 2013).

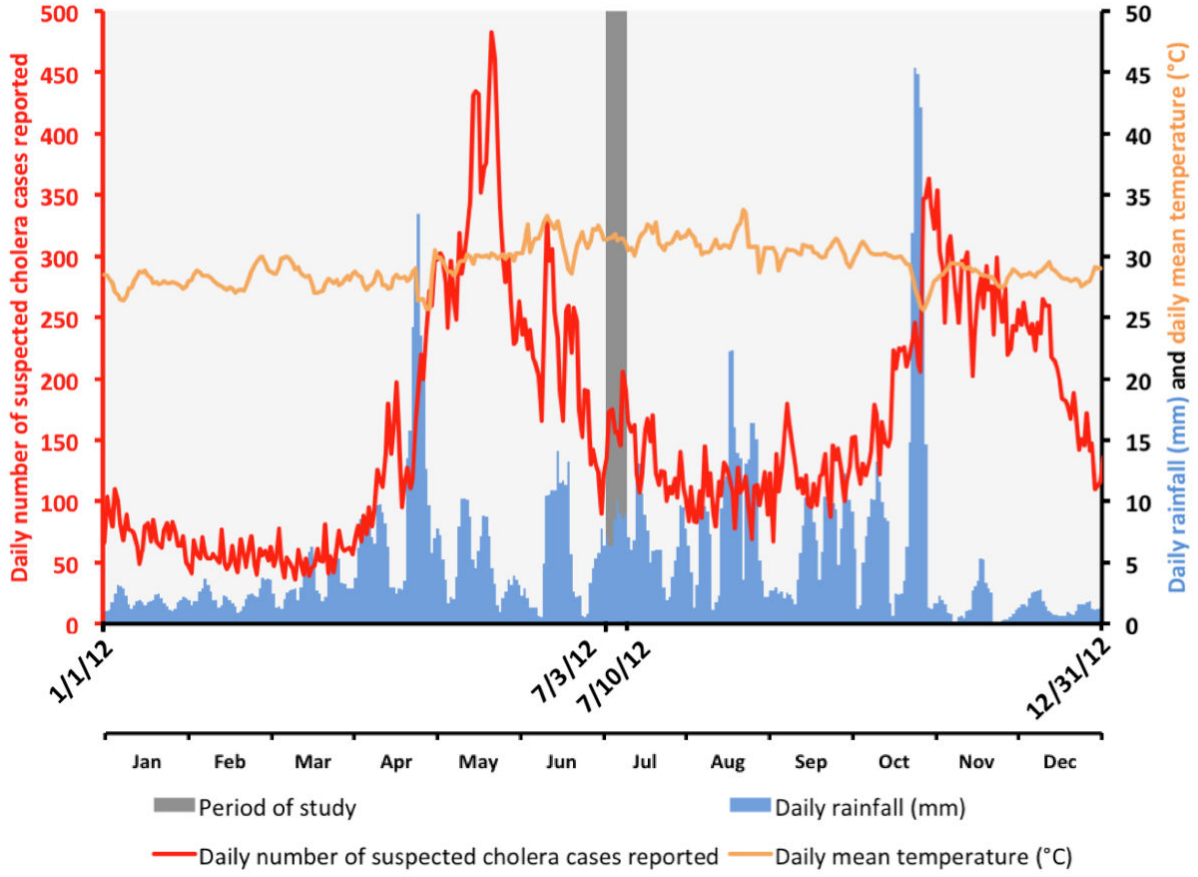

Figure 2 – Localization of the sampling stations in the West and Artibonite departments.
(See Table 1 for the corresponding characteristics)

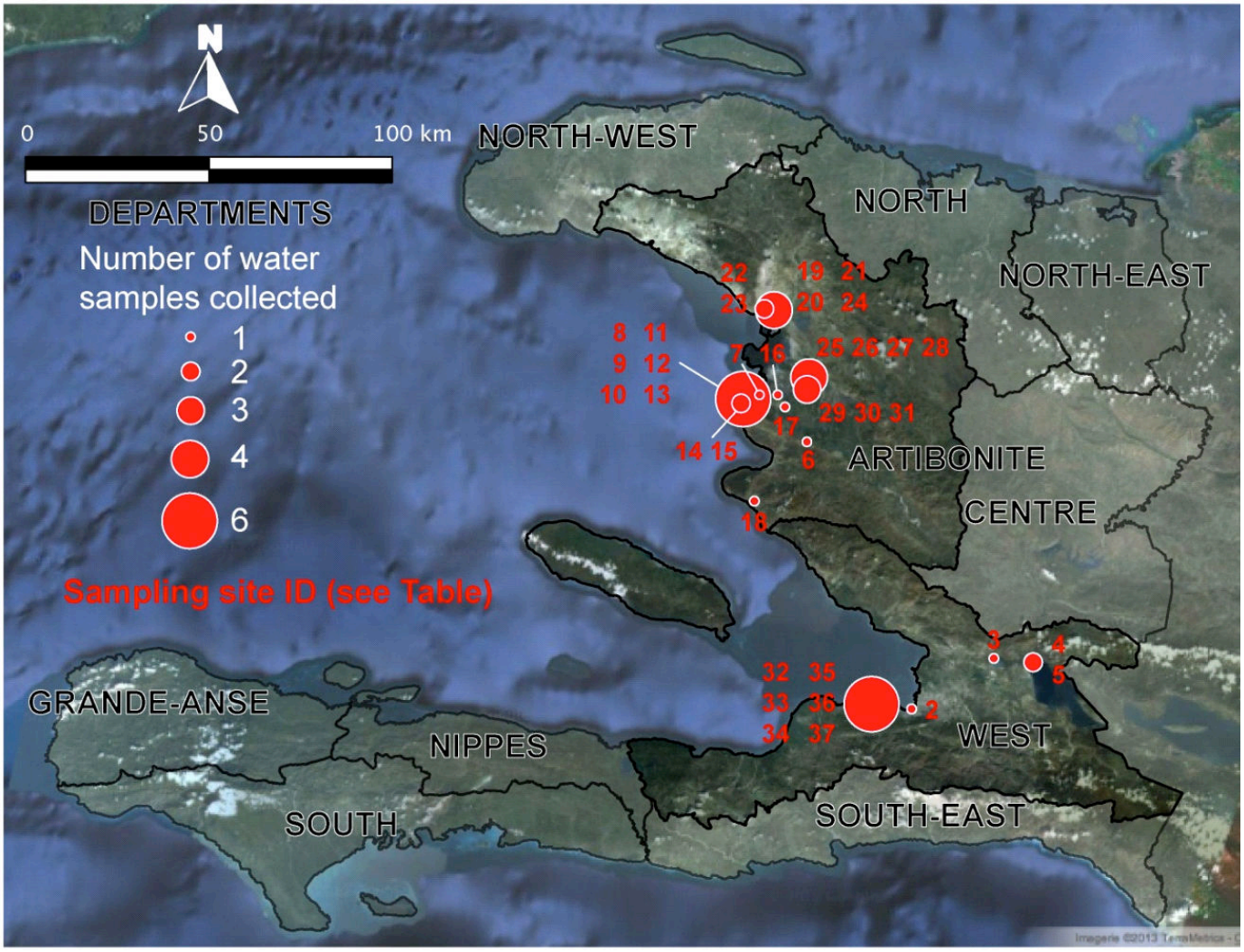

Table 1 – Characteristics of the sampling stations

(see Figure 2 for corresponding localization)

Sampling stations				Water characteristics			
Department	Commune	ID	Location	Salinity (‰) [1]	Temperature (°C)	pH	Fecal contamination (*E. coli* /100 mL)
West (Metropolitan area)	Port-au-Prince	2	Martissant (street wastewater)	ND	ND	ND	ND
	Carrefour	32	Mariani (wastewater in the river)	0.21	38.5	6.9	106,000
		33	Mariani (river shore)	0.20	38.5	7.0	35,000
		34	Mariani (river shore)	0.21	38.5	7.1	46,000
		35	Mariani (macrophyte lagoon)	25.02	38.5	7.5	12,400
		36	Mariani (microphyte lagoon)	10.72	38.5	6.7	50,000
		37	Mariani (river output into the sea)	0.31	38.8	6.5	1,000
West	Thomazeau	3	Trou Caïman lake	1.27	29.8	7.9	ND
		4	Etang Saumâtre lake (shore)	4.90	28.6	7.8	ND
		5	Etang Saumâtre lake (far from the shore)	5.62	27.7	7.5	ND
Artibonite	Gonaïves	19	Small canal of wastewater	2.09	ND	ND	91,000
		20	Small canal output into the sea	28.49	33.9	7.4	13,700
		21	Seashore near the small canal output	28.49	32.7	7.5	1,100
		22	Salt marsh: canal	32.17	36.1	7.7	<100
		23	Salt marsh: basin	29.54	33.9	8.0	<100
		24	Large canal of waste water	1.40	33.8	7.5	36,000
	Saint Marc	18	Etang Bois-Neuf	12.41	32.0	8.4	2,000
		6	Pont-Sondé (river Artibonite)	0.14	28.8	7.3	4,800
	Grande Saline	17	Main canal 1	0.15	32.5	7.5	2,800
		16	Main canal 2	0.15	32.4	7.4	3,600
		7	Drouin - main canal 3 (point-of-use)	0.15	30.2	7.3	2,100
		9	Artibonite river estuary 1	0.14	30.2	7.4	2,100
		10	Artibonite river estuary 2	0.15	30.5	7.3	3,100
		8	Artibonite river estuary 3 (pontoon)	0.15	31.1	7.3	2,900
		13	Artibonite river estuary 4	0.14	ND	7.4	2,000
		11	Artibonite river estuary 5	0.20	ND	ND	1,200
		12	Artibonite river estuary 6	4.87	30.0	7.2	800
		14	Basin 1	0.75	36.1	7.1	1,000
		15	Basin 2	0.27	34.8	7.9	<100
	L'Estère	25	L'Estère (river)	0.15	32.5	7.6	300
		26	L'Estère (small canal)	0.15	31.9	7.4	400
		27	L'Estère (large canal)	0.14	32.2	7.4	300
		28	L'Estère (roadside)	0.37	31.9	6.5	4,700
	Desdunes	29	Route de Desdunes (small canal)	0.26	ND	ND	200
		30	Route de Desdunes (large canal)	0.52	34.1	7.0	300
		31	Route de Desdunes (rice field)	0.30	33.5	8.0	<100

[1] fresh water, salinity <0.5‰; brackish water, 0.5-16‰; saline water, ≥16‰

ND, no data

Table 2 – Primer sequences and multiplex PCR assay conditions

Primer ID	Sequence	Amplicon size (bp)	Target	PCR conditions[1]	Reference
O139F2 (forward)	5'-AGCCTCTTTATTACGGGTGG-3'	449	*rfb* gene: O139-specific region	1	Hoshino *et al.*, 1998 [15]
O139R2 (reverse)	5'-GTCAAACCCGATCGTAAAGG-3'				
O1F2-1 (forward)	5'-GTTTCACTGAACAGATGGG-3'	308	*rfb* gene: O1-specific region	1	Hoshino *et al.*, 1998 [15]
O1R2-2 (reverse)	5'-GGTCATCTGTAAGTACAAC-3'				
VCT1 (forward)	5'-ACAGAGTGAG TACTTTGACC-3'	192	*ctxA* gene: A subunit of the cholera toxin	1	Hoshino *et al.*, 1998 [15]
VCT2 (reverse)	5'-ATACCATCCATATATTTGGGAG-3'				
ompW (forward)	5'-CACCAAGAAGGTGACTTTATTGTG-3'	588	*ompW* gene: Outer Membrane Protein	2	Nandi *et al.*, 2000 [14]
ompW (reverse)	5'-GAACTTATAACCACCCGCG-3'				
ctxA (forward)	5'-CTCAGACGGGATTTGTTAGGCACG-3'	301	*ctxA* gene: A subunit of the cholera toxin	2	Nandi *et al.*, 2000 [14]
ctxA (reverse)	5'-TCTATCTCTGTAGCCCCTATTACG-3'				

[1] PCR conditions 1: initial denaturation (5 min at 94°C); 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 55°C) and extension (1 min at 72°C); final extension (7 min at 72°C).
PCR conditions 2: initial denaturation (5 min at 94°C); 30 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 64°C) and extension (30 sec at 72°C); no final extension.

Table 3 – Results of *Vibrio cholerae* cultures, identifications and PCR assays

	Culture and identification		Specific PCRs			
Station ID	**Culturable *V. cholerae* [1] (limit volume [2])**	**No. O1 / No. tested *V. cholerae* strains**	***V. cholerae*: *ompW gene* (limit volume [2])**	***V. cholerae* O1: *rfb* gene**	***V. cholerae* O139: *rfb* gene**	**cholera toxin: *ctxA* gene**
2	pos (0,1 mL)	0/1	ND	ND	ND	ND
32	pos (100 mL)	0/1	pos (10 mL)	neg	neg	neg
33	pos (0,1 mL)	0/17	pos (1 mL)	neg	neg	neg
34	pos (0,1 mL)	0/11	pos (0,001 mL)	neg	neg	neg
35	neg (100 mL)	NI	pos (0,1 mL)	neg	neg	neg
36	pos (100 mL)	NT	pos (0,01 mL)	neg	neg	neg
37	pos (10 mL)	0/8	pos (10 mL)	neg	neg	neg
3	pos (0,1 mL)	0/40	ND	ND	ND	ND
4	pos (10 mL)	0/25	ND	ND	ND	ND
5	pos (10 mL)	0/8	ND	ND	ND	ND
19	pos (0,01 mL)	0/18	pos (0,01 mL)	neg	neg	neg
20	neg (100 mL)	NI	pos (0,01 mL)	neg	neg	neg
21	neg (100 mL)	NI	pos (0,01 mL)	neg	neg	neg
22	neg (100 mL)	NI	pos (10 mL)	neg	neg	neg
23	neg (100 mL)	NI	pos (100 mL)	neg	neg	neg
24	pos (0,01 mL)	0/15	pos (0,01 mL)	neg	neg	neg
18	pos (0,1 mL)	0/34	pos (0,1 mL)	neg	neg	neg
6	pos (0,1 mL)	0/30	pos (1 mL)	neg	neg	neg
17	pos (100 mL)	0/16	pos (100 mL)	neg	neg	neg
16	pos (0,1 mL)	0/27	pos (1000 mL)	neg	neg	neg
7	pos (100 mL)	0/12	pos (100 mL)	neg	neg	neg
9	pos (1000 mL)	0/2	pos (1000 mL)	neg	neg	neg
10	pos (1000 mL)	0/8	pos (1000 mL)	neg	neg	neg
8	neg (1000 mL)	NI	pos (1000 mL)	neg	neg	neg
13	neg (1000 mL)	NI	pos (1000 mL)	neg	neg	neg
11	neg (1000 mL)	NI	pos (1000 mL)	neg	neg	neg
12	neg (1000 mL)	NI	pos (1000 mL)	neg	neg	neg
14	pos (1 mL)	0/24	pos (1 mL)	neg	neg	neg
15	pos (0,1 mL)	0/33	pos (1 mL)	neg	neg	neg
25	pos (10 mL)	0/6	pos (10 mL)	neg	neg	neg
26	pos (10 mL)	0/3	pos (10 mL)	neg	neg	neg
27	pos (10 mL)	**1[*]/9**	pos (10 mL)	neg	neg	neg
28	pos (1 mL)	0/1	pos (1 mL)	neg	neg	neg
29	pos (0,01 mL)	0/11	pos (1 mL)	neg	neg	neg
30	pos (1 mL)	0/22	pos (1 mL)	neg	neg	neg
31	pos (10 mL)	0/8	pos (10 mL)	**pos**	neg	neg
Total	27 pos	**1[*]/390**	32 pos	**1**	**0**	**0**

[1] characteristic appearance on TCBS (sucrose-fermentation), translucent colony growth on 0% NaCl nutrient agar (Difco), and positive oxidase reaction

[2] Limit volume is the smallest with positive culture or the biggest with negative culture

[*] Late agglutination

NI: no isolate ; NT: no isolate tested ; ND: no DNA extraction

3.3.3 Une absence de réservoir environnemental significatif… contredite par une nouvelle étude aux conclusions discutables… et discutées

Malgré un échantillonnage conséquent et une méthodologie ayant permis d'isoler plusieurs centaines de souches de *V. cholerae*, il ne fut pas possible de détecter la présence de *V. cholerae* O1 toxinogène dans les 29 eaux de surface testées. On ne peut évidemment écarter l'hypothèse d'une présence de ces souches épidémiques à des concentrations inférieures au seuil de détection. Néanmoins cette étude, réalisée pendant la saison chaude et pluvieuse et quelques semaines seulement après un fort pic de transmission, incitait à penser que le choléra ne s'était pas installé de manière *significative* dans l'environnement haïtien. En l'absence de réservoir environnemental pérenne et significatif, il devenait alors réaliste d'envisager la possibilité d'éliminer le choléra à court terme en Haïti… à condition de bloquer la transmission entre les malades, conformément aux thèses présentées par l'OMS dans les années 1950 [105].

Sur le terrain en Haïti, ces résultats furent perçus avec un certain soulagement par les autorités sanitaires. Leur accueil par les experts de l'OMS et certains autres acteurs fervents défenseurs du paradigme environnemental fut cependant beaucoup plus mitigé.

En mars 2014, une équipe de l'Université de Floride ayant installé un laboratoire de microbiologie environnementale sur la commune de Gressier, situé à quelques dizaines de kilomètres au sud-ouest de Port-au-Prince, publia dans Emerging Infectious Diseases les résultats d'un important monitoring bactériologique de 4 rivières de la zone [106]. Quatorze sites furent échantillonnés chaque mois entre avril 2012 et mars 2013. Les auteurs isolèrent *V. cholerae* O1 dans 7 des 179 échantillons d'eau et des 144 échantillons d'animaux et de plantes aquatiques. Le gène de la toxine cholérique fut retrouvé chez 3 des 7 *V. cholerae* O1. En conclusion de leur article, ces auteurs suggérèrent que cette découverte pouvait être le reflet d'un réservoir environnemental établi susceptible de compliquer l'élimination du choléra en Haïti. Lors de la présentation de leurs résultats en février 2013 au Forum scientifique de la Direction d'Épidémiologie de Laboratoire et de Recherche, ils se firent cependant beaucoup plus

[105] Pollitzer, « Cholera studies- 11. Prevention and control ».
[106] Alam et al., « Monitoring Water Sources for Environmental Reservoirs of Toxigenic Vibrio Cholerae O1, Haiti ».

affirmatifs. De même que l'article consacré à ces travaux sur le site internet de l'Université de Floride [107].

Jugeant ces résultats intéressants mais leur interprétation largement excessive compte-tenu de la persistance de cas suspects de choléra pouvant avoir transitoirement contaminé ces rivières, nous récupérâmes, en collaboration avec MSF-Suisse qui tenait alors le seul centre de traitement de la zone, les adresses de tous les malades pris en charge sur cette période d'une année. Sans surprise, de nombreux cas suspects étaient signalés dans les environs de tous les sites retrouvés positifs. En outre, l'étude de la pluviométrie centrée sur la zone montrait toujours des précipitations persistantes.

Une lettre de réponse fut donc adressée à Emerging Infectious Diseases qui, acceptée depuis peu, devrait être publiée en janvier 2015 [108].

3.3.4 Lettre 2 : « Uncertain cholera environmental reservoir in Haiti »

A lire ci-après dans sa version acceptée pour publication par Emerging Infectious Diseases. Son édition est en cours.

[107] University of Florida, Department of Environmental & Global Health, et College of Public Health and Health Professions, « Dr. Afsar Ali and UF research team demonstrate established environmental reservoirs of toxigenic V. cholerae in Haiti ».
[108] Rebaudet, Stanislas et Piarroux, Renaud, « Uncertain cholera environmental reservoir in Haiti ».

EMERGING INFECTIOUS DISEASES®

In Response to "Monitoring Water Sources for Environmental Reservoirs of Toxigenic Vibrio cholerae O1, Haiti"

Journal:	*Emerging Infectious Diseases*
Manuscript ID:	Draft
Manuscript Type:	Letter
Date Submitted by the Author:	n/a
Complete List of Authors:	Rebaudet, Stanislas; Aix-Marseille Université, UMD 3 Piarroux, Renaud; Aix-Marseille Université, UMD 3
Keywords:	

Cover Page

Title:

In Response to "Monitoring Water Sources for Environmental Reservoirs of Toxigenic Vibrio cholerae O1, Haiti"

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Financial support and disclosure:

This work was co-financed by Assistance Publique – Hôpitaux de Marseille (APHM) and the Haiti Office of the United Nations Children's Fund (UNICEF-Haiti).

Both authors declare no conflict of interest related to this article.

Text:

In Response: In the March issue of *Emerging Infectious Diseases*, Alam *et al.* have reported a survey of Haitian water sources aiming to isolate *Vibrio cholerae* (1). Each month, from April 2012 through March 2013, they sampled 15 sites located at 3 rivers and 1 estuary in West Department. Seven *Vibrio cholerae* O1 isolates, including 3 *ctx*-positive toxigenic strains, were isolated out of 179 water samples and 144 aquatic animals and plants. Unfortunately, the results for all 7 *V. cholerae* O1 isolates were aggregated, and no details were provided concerning the exact time point and location corresponding to the 3 *ctx*-positive strains. The authors posed the question "*has V. cholerae O1 become established in environmental reservoirs in Haiti*?", subsequently warning that "*as long as the causative microorganism is present in the environment, eradication of the disease will not be possible*".

However, after challenging their results with more accurate epidemiological data, we found that the presence of these 3 *ctx*-positive toxigenic strains could more likely be due to recent fecal contamination of the sampled rivers (Figure 1). Indeed, many cholera cases were reported in the corresponding communal sections (i.e. the smallest Haitian administrative unit, corresponding to 25 km^2 on average) when the 7 *V. cholerae* O1 isolates were collected. In this context of an ongoing cholera epidemic associated with persisting rainfall (Figure 1), generalized open-air defecation inevitably leads to contamination of water sources. It is therefore impossible to determine whether positive rivers constitute perennial cholera reservoirs or rather they act only as transient vectors of the pathogen. The current dramatic decrease in cholera transmission may however provide a good opportunity to address this issue (2). We thus encourage Alam *et al.* to continue the search for *ctx*-positive toxigenic *V. cholerae* O1 strains in surface waters especially during cholera-free periods in the neighborhood.

Acknowledgments:

We are grateful to the Haitian Directorate of Epidemiology Laboratory and Research (*Direction d'Épidémiologie de Laboratoire et de Recherche* – DELR) and the Swiss section of Doctors without Borders (*Médecins Sans Frontières – Suisse*) for providing cholera case data from Leogane cholera treatment unit. We are indebted to Sandra Moore for her fine editing on this manuscript.

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Figure Legend:

Figure 1. Weekly cholera incidence in communal sections where water sampling spots where found positive for *V. cholerae* O1 ; accumulated precipitation in the studied area by week during April 2012-March 2013 ; and number of environmental sites from which *V. cholerae* O1 were isolated, by month.

Incidence calculated from cases hospitalized in Leogane cholera treatment center and living near the four sites found positive for *V. cholerae* O1 by Alam *et al.*: 2nd communal section of Leogane for *Lassale* site; 2nd section of Gressier for *Gressier* and *Gressier Beach* sites; and 3rd section of Gressier for *Jeffra* site; satellite-measured rainfall extracted from the NASA website (http://disc2.nascom.nasa.gov/Giovanni/tovas/realtime.3B42RT_daily.2.shtml, 18.5°-18.6°N, 72.6°-72.5°W).

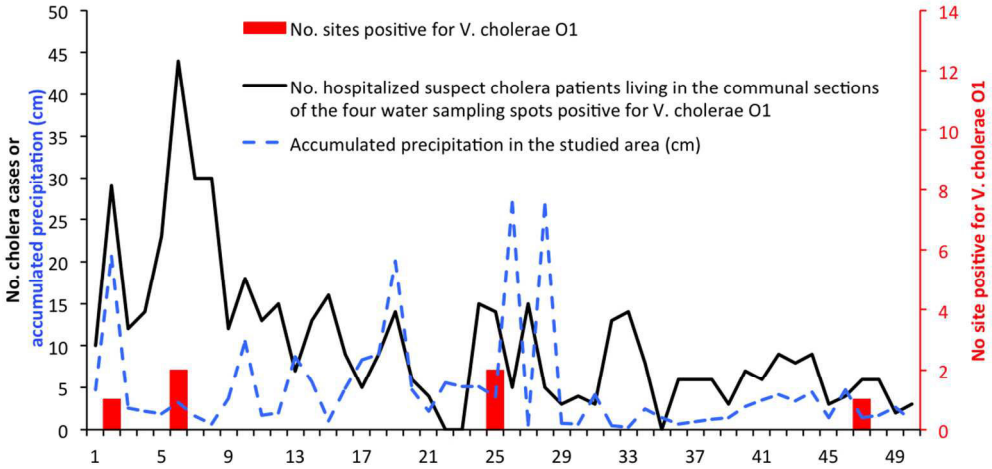

Figure 1. Weekly cholera incidence in communal sections where water sampling spots where found positive for V. cholerae O1 ; accumulated precipitation in the studied area by week during April 2012-March 2013 ; and number of environmental sites from which V. cholerae O1 were isolated, by month.
Incidence calculated from cases hospitalized in Leogane cholera treatment center and living near the four sites found positive for V. cholerae O1 by Alam et al.: 2nd communal section of Leogane for Lassale site; 2nd section of Gressier for Gressier and Gressier Beach sites; and 3rd section of Gressier for Jeffra site; satellite-measured rainfall extracted from the NASA website (http://disc2.nascom.nasa.gov/Giovanni/tovas/realtime.3B42RT_daily.2.shtml, 18.5°-18.6°N, 72.6°-72.5°W).
212x102mm (150 x 150 DPI)

3.4 Situation du choléra et des moyens de lutte en saison sèche

3.4.1 Où le choléra persiste-t-il en saison sèche et cette persistance est-elle due à une lutte inefficace ?

Début 2013, il était établi que le choléra avait indiscutablement été importé en Haïti, qu'il ne s'y était pas installé dans des réservoirs environnementaux significatifs, et qu'il présentait une composante saisonnière marquée. Depuis Marseille et en lien avec les autorités sanitaires haïtiennes nous suivions l'évolution de l'épidémie à distance. Nous étions alors étonnés de constater la persistance de l'incidence à un niveau aussi élevé en saison sèche, période pourtant favorable pour interrompre la transmission du choléra, et ce malgré des moyens de lutte sans précédent. Nous émettions alors l'hypothèse que cette persistance en saison sèche était due à une lutte inefficace sur le terrain. Afin de la tester à l'épreuve des faits, deux missions successives furent donc organisées avec le soutien financier de la Cellule de Crise du Ministère des Affaires Étrangères, via l'Ambassade de France en Haïti.

La première mission fut conduite en février-mars 2013 avec comme objectif principal d'identifier, caractériser et comprendre les foyers de persistance de la transmission du choléra pendant la saison sèche. Une investigation de terrain fut menée conjointement avec des épidémiologistes de la Direction d'Épidémiologie de Laboratoire et de Recherche (DELR). Ses conclusions furent notamment présentées au Ministère de la Santé et de la Population (MSPP) ainsi qu'au HCT (Humanitarian Country Team) qui regroupe les chefs des différentes agences onusiennes et des principales ONG présentes dans le pays. Elles donnèrent lieu à la rédaction d'un rapport [109] puis, très rapidement afin de remobiliser les acteurs, d'un article paru dans la revue PLoS Current Outbreaks début juin 2013 [110].

[109] Rebaudet et Piarroux, *Persistance du choléra en saison sèche en Haïti*.
[110] Rebaudet et al., « The dry season in Haiti: a window of opportunity to eliminate cholera ».

3.4.2 Article 2 : « The dry season in Haiti: a window of opportunity to eliminate cholera. »

L'article publié par PLoS Current Outbreaks peut être consulté à l'adresse suivante : http://currents.plos.org/outbreaks/article/the-dry-season-in-haiti-a-window-of-opportunity-to-eliminate-cholera/ (accédé le 2/10/2014)

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PLOS | CURRENTS OUTBREAKS

The dry season in Haiti: a window of opportunity to eliminate cholera

June 10, 2013 · Research

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ABSTRACT

Background: Since the beginning of the cholera epidemic in Haiti, attack rates have varied drastically with alternating peak and lull phases, which were partly associated with the fluctuating dry, rainy and cyclonic seasons. According to a study conducted in 2012, the toxigenic V. cholerae O1 strain responsible for the outbreak did not settle at a significant level in the Haitian aquatic environment. Therefore, we hypothesize that some areas of lingering cholera transmission during the dry season could play an important role in the re-emergence of outbreaks during the rainy season. Our objective was therefore to describe the dynamics of cholera and assess the fight against the disease during the dry season.Methods: A field study was conducted from February 19 to March 29, 2013. After identifying the affected communes by analyzing the national cholera database, we visited corresponding health facilities to identify patient origins. We then conducted a field assessment of these foci to confirm the presence of cholera, assess factors associated with transmission and examine the activities implemented to control the epidemic since the beginning of the current dry season.

Methods: A field study was conducted from February 19 to March 29, 2013. After identifying the affected communes by analyzing the national cholera database, we visited corresponding health facilities to identify patient origins. We then conducted a field assessment of these foci to confirm the presence of cholera, assess factors associated with transmission and examine the activities implemented to control the epidemic since the beginning of the current dry season.

Results: We found that the great majority of Haitian communes (109/140) presented no sign of cholera transmission in February and March 2013. Suspected cases were concentrated in a small number of urban and rural areas, almost all of which were located in the northern half of the country and often in inland locales. In these areas, community health activities appeared insufficient and were often inappropriately targeted. Out of 49 analyzed foci, only 10 had benefited from at least one intervention involving the distribution of water treatment products together with an awareness campaign since December 2012.

Conclusion: Cholera continues to affect Haiti as observed in early 2013; however, activities implemented to interrupt cholera transmission appear insufficient and poorly suited. This

deficiency in the fight against cholera, especially at a period when transmission is weak, may explain the persistence of cholera even in the absence of significant aquatic reservoirs in Haiti.

INTRODUCTION

In October 2010, the importation of a toxigenic *Vibrio cholerae* strain in Haiti [1–3] led to a massive epidemic that rapidly spread throughout all 140 Haitian communes [4,5]. By the end of March 2013, the Public Health and Population Ministry (MSPP) had reported 652,730 cases, corresponding to an attack rate of 6.4%. With 8,060 reported deaths, the global case fatality rate was 1.2%. This historic epidemic, which occurred in a small but densely populated country (10.2 million inhabitants in 27,750 km^2), accounted for over half of cholera cases and over one-third of cholera-associated deaths reported globally between 2010 and 2011 [6]. The disease also spread throughout the rest of Hispaniola island, and by late 2012, the Dominican Republic had recorded 29,433 cases and 422 deaths associated with cholera [7].

Since the beginning of the epidemic, cholera incidence in Haiti has been characterized by alternating peak and lull phases, which were partly associated with the fluctuating dry, rainy and cyclonic seasons [4] (Figure 1). Haiti experienced the first marked decrease in cholera transmission during the dry season in early 2012 (67 cases/day reported in March). At that time, cholera had almost completely disappeared from the Southern Peninsula as well as the North-East and North-West departments according to the Haitian National Cholera Surveillance System [8]. In contrast, residual transmission was recorded in a few dozen communes located in the North (DSN), Artibonite (DSA) and Centre (DSC) departments as well as the conurbation of Port-au-Prince (West department). During the rainy season of April-May 2012, the reappearance of the epidemic was first reported in these geographic areas, before spreading to the areas where transmission seemed to have stopped during the dry season. By the end of 2012, 903 additional cholera-associated deaths had been reported.

However, the evolution of cholera epidemics is always difficult to predict, and there is no undisputable study demonstrating that cholera will settle in Haiti for decades to come. Despite poor access to improved drinking water sources (69% of the population in 2010) and very poor use of improved sanitation facilities (17% of the population in 2010) [9], the country and the rest of the island of Hispaniola have been spared from cholera for at least a century and probably since the beginning of recorded history [10]. Moreover, a Franco-Haitian study conducted in 2012 (Baron S *et al.*, in process) has shown that the O1 strain responsible for the outbreak was overwhelmed by local non-O1 *V. cholerae* strains in a large set of Haitian aquatic environments. None of the rare O1 strains isolated in this study proved to be toxigenic. Thus, there is currently no evidence of a perennial and significant environmental reservoir for toxigenic *V. cholerae* in Haiti.

Therefore, we hypothesize that some areas of lingering cholera transmission during the dry season play an important role in the persistence of epidemics from one rainy season to the next and that the struggle against cholera in Haiti has failed to take advantage of the dry season opportunity. In this study, we aimed to describe both the dynamics of cholera during the dry season and the fight against the disease by targeting the remaining foci.

METHODS

At the request of the MSPP, a field study was conducted from February 19 to March 29, 2013 to both establish an inventory of the remaining cholera transmission foci during the dry season and assess the prevention actions carried out by Haitian and international organizations. The four components of the survey consisted of the following aspects: (1) identify affected communes by analyzing the information available in the national database; (2) identify transmission foci based on the basic data recorded by cholera treatment health structures; (3) conduct a field study of the principal foci to confirm the presence of cholera, assess factors associated with transmission and examine the actions taken to control the epidemic since the beginning of the current dry season (in collaboration with representatives of the National Department of Drinking Water and Sanitation (DINEPA)); and (4) perform interventions at the household level involving public awareness campaigns and the distribution of chlorine tablets.

Cholera-associated morbidity and mortality data at the commune level were provided by the Haitian Directorate of Epidemiology Laboratory and Research (DELR), which gathers, validates and analyzes anonymous data that are prospectively collected in the field by epidemiological surveillance officers. According to the WHO standard definition [11], a probable cholera case is defined as a patient aged 5 years or older who develops acute watery diarrhea, with or without vomiting, located in an area where there is a cholera epidemic. In Haiti, all acute watery diarrhea cases are reported as suspected cholera, but the surveillance system separately records cases <5 and ≥5 years-old. Bacteriological confirmation of cases is routinely performed at the National Laboratory of Public Health (LNSP) using standard methods [12].

The identification of concentrations of active cholera transmission was carried out by analyzing February and March 2013 cholera national databases. We assessed any commune presenting a bacteriological-confirmed cholera case OR a suspected cholera death (associated with concomitant reported cholera cases) OR more than 1 suspected cholera case per day (excluding patients less than 5 years of age to enhance the case definition specificity). We then visited the health facilities of as many at-risk communes as possible to identify the exact origin of cholera patients by interviewing the medical staff and reviewing the case registers of the last 1 to 3 months.

Field investigations were subsequently performed in identified suspected cholera foci to search for possible factors linked to cholera transmission. People and local health actors were also interviewed regarding the actions undertaken to stop the spread of cholera since the beginning of the dry season in December 2012. In Port-au-Prince, data on patient origin was provided by the staff of Médecins sans Frontières – Holland, who also described the actions performed to limit cholera transmission. Due to the time limitation, not all identified at-risk communes and transmission foci were investigated. However, medical staff declaring the suspected cholera cases and/or local epidemiologists were interviewed by phone.

Maps of cholera morbidity, mortality and prevention interventions were generated using Quantum-GIS® v1.8.0 (Open Source Geospatial Foundation Project, Beaverton, OR, USA).

RESULTS

From December 1, 2012 to March 31, 2013, 21,695 suspected cholera cases (≥5 years of age) and 238 related deaths were reported by the national surveillance system. However, most cases occurred in December and January (16,700 cases and 208 deaths) and a strong decrease in cholera incidence was noted in February and March with only 4,995 recorded cases and 30 deaths. In parallel, the percentage of positive samples cultured at the LNSP dropped from 68% in December and January to 41% in February and March (Table 1).

As shown in Figure 2, cholera morbidity and mortality distribution also exhibited a high degree of spatial heterogeneity, particularly at the end of the study period, when cholera seemed to persist almost exclusively in the 3 departments of DSN, DSC and DSA. Notably, only a few communes were significantly affected, even at these sites. In the South Peninsula and Port-au-Prince, the few sporadic cases were associated with a very low fatality rate and were probably non-cholera-associated diarrhea.

Twenty-four provincial communes with likely active cholera transmission were identified (Table 2, Figure 3). All but 8 communes were localized in the Artibonite (DSA), Centre (DSC) and North (DSN) departments. These 24 communes accounted for 69% of the total cases (≥5 years of age) recorded throughout the country, with a monthly attack rate of 6.0 cases/10,000 inhabitants. A total of 26 deaths was reported in these communes, and the cholera case fatality rate was 0.8%.

A few dozen confirmed cases were reported in Port-au-Prince conurbation with only one suspected cholera-associated death (Table 1 and Table 2). However, transmission remained sporadic in the capital, thereby yielding a low attack rate (1.4 cases/month/10,000 inhabitants). Moreover, the low case fatality rate (0.1%) demonstrates that most of these cases of acute watery diarrhea were probably not due to cholera infection.

In the 109 remaining communes, the attack rate (0.8 cases/month/10,000 inhabitants) was even lower than that observed in Port-au-Prince. Only 3 deaths were recorded (1 in Camp Perrin, 1 in Grande-Rivière-du-Nord and 1 in Bainet), and cholera was not biologically confirmed for any of these cases. Therefore, in the absence of other suspected cholera cases at the same period, we considered it unlikely these cases were associated with a local transmission of cholera.

Investigations were carried out in 12 of the 24 communes with suspected active cholera transmission. These communes were located in DSN (Cap-Haïtien, Quartier-Morin, Port-Margot and Borgne), DSC (Hinche, Mirebalais and Cerca-la-Source) and DSA (Saint-Marc, Gonaïves, Saint-Michel-de-l'Attalaye, Ennery and Gros Morne). Based on the field investigations, we identified 49 areas where clusters of cases had recently been reported. Taken together, these 49 areas accounted for 56% of cases reported in these 12 communes during the analysis period.

In DSN, the principal cholera residual focus was located in the city of Cap-Haïtien. Some cases were biologically confirmed by the LNSP. Note that patients from Cap-Haïtien were also treated in the nearby commune of Quartier Morin, which remained almost free of cholera. In Cap-Haïtien, cases were concentrated in several neighborhoods without proper access to clean drinking water. The water supply network had been out of order for 25 years throughout most of the city, and the population used water treated via reverse osmosis, which does not have the anti-bacterial properties of chlorinated water. The field assessment highlighted the presence of numerous boreholes and traditional shallow wells widely contaminated with fecal matter due to their proximity to unprotected latrines. The collection

of feces in plastic bags thrown onto the roof was said to be a common practice. Markets with no access to clean water and very poor food preservation practices were also likely sites of contamination.

In DSC, the commune of Cerca-la-Source reported the highest attack rates (126.0 and 83.4 cases/month/10,000 inhabitants in February and March, respectively). Only one suspected cholera-related death was reported (Figure 2). The field assessment showed that patients treated at the Cholera Treatment Center (CTC) were primarily children. As all samples delivered to LNSP for bacteriological confirmation were negative for V. cholerae and 90% (38/42) of the cholera rapid tests performed during the first half of March were negative, we noted a low level of cholera in this commune.

In DSA, the most active remaining foci were located in the town of Saint-Marc and several of its neighborhoods. Even though a private company sold chlorinated water, most of the population still relies on manually operated boreholes without home water treatment. In certain fishermen quarters, the general practice of defecating along the beach renders the contamination of open storage buckets of water highly likely. Cholera outbreaks occurred in several rural localities near Saint-Marc, where people rely exclusively on unprotected natural water sources. In addition to Saint-Marc, some cases were regularly reported in Gonaïves, where the municipal water network had been severely damaged by the hurricanes, as well as Saint-Michel-de-l'Attalaye. In the latter commune, most recent cases originated from the urban and peri-urban areas. Two water networks supplied this town with water and standposts. However, water was not treated for several weeks because of a chlorine shortage. In-house chlorination practices were almost never observed in those quarters, which were also largely deprived of latrines.

In all areas affected by cholera, community health activities appeared insufficient and inappropriately targeted since the beginning of the dry season 2012-2013 (Figure 4). Twenty-three of the 49 identified foci had not been previously investigated since December 2012. Thirteen had been investigated, especially in Gonaïves and Saint-Michel-de-l'Attalaye, but no intervention aimed to prevent transmission in the community had been initiated by MSPP, DINEPA or international partners. Only 13 foci among those visited had thus benefited from at least one intervention since early December 2012. At 3 of these 13 foci, prevention activities had been limited to awareness campaigns, which was sometimes due to the absence of available chlorination products (such as that found in Borgne, DSC). At least one distribution of water treatment products +/- soaps associated with an awareness campaign had nevertheless been organized in the 10 remaining foci, often with the assistance of NGOs such as Action-Contre-la-Faim (e.g., Saint-Michel-de-l'Attalaye in December) or Partners in Health (e.g., the Goyaviers section of Saint-Marc in December and January). A few distributions had been organized by the MSPP (e.g., Ennery in December and the rural Grand-Boucan portion of Mirebalais in January), while only one distribution event was launched by DINEPA in Borgne in December. Unfortunately, the majority of these distributions failed to target the principal transmission foci of the corresponding communes, which were primarily located in towns or the close outskirts, and instead exclusively focused on rural communities. Yet, some of these well-performed rural prevention events, such as that carried out in Gros Morne (DSA), proved effective with the subsequent local disappearance of cholera.

In Port-au-Prince conurbation, the low incidence and death rate associated with cholera may be due to the community actions implemented by NGOs. In particular, during the 2013 dry

season, Médecins sans Frontières – Holland staff continued identifying the areas associated with clusters of cases, where they organized awareness campaigns, water treatment product distribution and free bucket chlorination stations.

DISCUSSION

Thirty months after cholera onset, the disease is still present in Haiti. The disease attack rate has varied considerably since the beginning of the epidemic, with peaks during the rainy season and relative lulls during the dry season in 2011 and 2012 [5]. Our results show that the lull was even more pronounced in February and March 2013. In particular, of the 140 Haitian communes, 109 showed no sign of cholera transmission during early 2013 for more than two months. Indeed, in these 109 communes, there has been no confirmed case, significant group of cases, or death in the context of grouped diarrheic patients. Even if small cholera outbreaks may have remained unnoticed by the surveillance system in resource-deprived areas, it is unlikely, in the current context of Haiti, that an outbreak with subsequent cholera deaths would go completely unnoticed for two consecutive months. Only sporadic suspected cholera patients were reported, with an average of less than 5 cases per commune per month, which represents less than 1 case per 10,000 inhabitants per month. It is thus likely that, in these 109 communes, almost all of isolated and unconfirmed cases were associated with afflictions other than cholera. Note that, even if these cases of diarrhea were not due to cholera, the background noise of 1 monthly case of acute watery diarrhea per 10,000 inhabitants would yield 1,000 cases per month or 12,000 cases per year for the entire country of Haiti, which could portray the false impression of persistent endemic cholera. It is therefore of upmost importance to perform a microbiological confirmation of cases as attack rates decrease.

In February and March 2013, the majority of suspected cases were concentrated in a small number of urban and rural foci, almost all of which were located in the northern half and often in inland locales. Haitian estuaries did not seem to be particularly affected. Even in these residual foci, an overestimation of cases is possible. A pooled case fatality rate of only 0.8% in the 24 communes with potential cholera transmission appears indeed implausible, considering persisting difficulties at numerous CTCs [13]. A marked proportion of these cases of acute watery diarrhea, especially in children under 5 years of age, were probably not due to cholera. For instance, in the commune of Cerca-la-Source (DSC), biological tests performed on various inpatient samples were negative.

Nevertheless, cholera did not completely disappear, as active foci with laboratory-confirmed cases remained, such as that observed in the urban community of Cap-Haïtien. At this site, the vast majority of reported cases (87%) corresponded to patients over 5 years of age, which represents a percentage consistent with an ongoing cholera outbreak. Finally, the metropolitan area of Port-au-Prince displayed a markedly lower attack rate (1.4 cases per 10,000 inhabitants per month) than the 24 municipalities that were likely affected. Moreover, the extremely low lethality rate (0.1%) indicates that the majority of cases were probably

associated with other diarrheal diseases. However, even in Port-au-Prince, a few laboratory-confirmed cases were reported at least until the beginning of March.

Currently, no evidence of persistence of toxigenic V. cholerae in the Haitian environment at a significant level has been reported. In fact, an environmental study performed during the warm and rainy season 2012 failed to detect toxigenic V. cholerae via both culture and polymerase chain reaction analyses, even in estuaries (S Baron et al., in publication process). This does not allow to completely exclude the presence of a few toxigenic V. cholerae bacteria in the aquatic environment; however, considering that a minimum inoculum is required to provoke cholera [14], it is unlikely that undetectable concentrations of culturable or viable-but-non-culturable toxigenic V. cholerae in Haitian surface waters may greatly influence the dynamic of the current epidemic. Conversely, the remaining cases and small outbreaks that were still ongoing at the end of the dry season will thus likely play a leading role in the re-emergence of cholera during the rainy season. The fight against the infectious agent must therefore target this persisting human reservoir. As incidence of cholera decreases during the dry season, it is all the more important to enhance the fight against cholera transmission. Unfortunately, our field investigations show that low attack rates were often interpreted as evidence of "residual disease". The few reported cases and deaths were considered as "acceptable", and the investigation of small outbreaks and the hunt for the last remaining cases were therefore neglected. Such an attitude does not appear relevant if, as stated in the governmental strategic plan to fight cholera, the aim of the struggle is to eliminate cholera [15]. In 2012, the lull of the dry season had been followed by new epidemic waves responsible for 903 additional deaths [8]. Such a number of deaths is too high to not consider cholera as a priority. This is all the more important now that international funding is currently lacking, which has resulted in a deterioration in the quality of care at treatment centers as recently noted in an MSF press release [13] and a halt in community prevention activities.
To enhance the effectiveness of the fight during the dry season, interventions should be targeted on active foci, which must be detected as early as possible and immediately investigated. Suspected cases should be confirmed via microbiological testing, as the risk of inaccurate cholera diagnosis is important especially when the number of reported cases diminishes. Actions should primarily focus on access to clean water via the establishment or rapid repair of distribution networks when possible and the free distribution of treatment products in the other cases. These actions are all the more effective when competent technicians apply practical solutions adapted to the local context in populations still plagued by recent cholera cases.

Vaccination should only be a supplementary element in the strategy to eliminate the disease. Indeed, a meta-analysis conducted in 2011 by the Cochrane collaboration showed that the effectiveness of current vaccines was only 52% in the first year and 62% during the second year [16]. This overall rate of protection is lower in children less than 5 years of age (38% versus 66% for 5 years and older). As long-term effectiveness of the vaccine has not been demonstrated, WHO recommends re-vaccination after two years [17]. Given the lack of demonstrated efficacy in young children, vaccination should therefore be reserved for individuals over 5 years of age. Vaccination programs should always be accompanied with public awareness campaigns and actions to improve access to safe water and sanitation. In the

current context of vaccine shortages, massive untargeted vaccination throughout the country remains unrealistic. Instead, vaccination sites should be determined in real time based on epidemiological observations and microbiological confirmation. Inadequately targeted campaigns would have a high cost and yield poor results in impeding cholera transmission. Finally, there is a need to ensure that the financial and manpower resources required for vaccination campaigns do not impair those necessary to conduct preventive actions based on the promotion of enhancing hygienic practices and supplying safe water.

CONCLUSION

As observed at the beginning of 2013, it is evident that cholera continues to affect Haiti. Epidemiological data and studies on environmental strains have shown that toxigenic *V. cholerae* O1 persists only in the human reservoir, without a significant presence in the aquatic environment. As seen in 2011 and 2012, there is a great risk that cholera will re-emerge during the upcoming rainy season. The current situation should neither be seen as an acceptable background of cases nor justify a cutback in preventive actions. On the contrary, during this period of low incidence, control activities targeting residual foci are more likely to be effective. Unfortunately, community health activities appear insufficient and poorly suited. The present analysis, mildly severe but objectively documented, should be received as an incentive to maximize efforts to prevent new outbreaks during the rainy season and ultimately eliminate cholera from Haiti and the island of Hispaniola. Not long ago, other Latin America countries were able to achieve this radical goal [18]. Cholera must remain a health emergency and not a development issue. Cholera will not become endemic in the country if the disease is not endemic in the minds of the people.

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ACKNOWLEDGEMENTS

We are grateful to everybody who took part in the field investigations, especially: Mentor Ali-Ber LUCIEN, Fred-Shero CENADIN, Carol ANTOINE, Paul Menahel JASMIN, Nadège JACQUES (MSPP); Raoul TOUSSAINT, Jean Petit MARSEILLE, Clervil HENRISSAINT (DINEPA); and Olivier THONET (Unicef-Haiti).

We are also indebted to the French Embassy in Haiti and Assistance Publique-Hôpitaux de Marseille (APHM), who co-funded this work.

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FIGURES AND TABLES

Figure 1 – Evolution of the daily suspected cholera cases and rainfall between September 2010 and March 2013.

Accumulated rainfall data were obtained from satellite estimates (TMPA-RT 3B42RT derived), averaged on the position 18.25N-19.75N / 74.25W-71.75W, and available at: http://disc2.nascom.nasa.gov/Giovanni/tovas/realtime.3B42RT_daily.2.shtml.

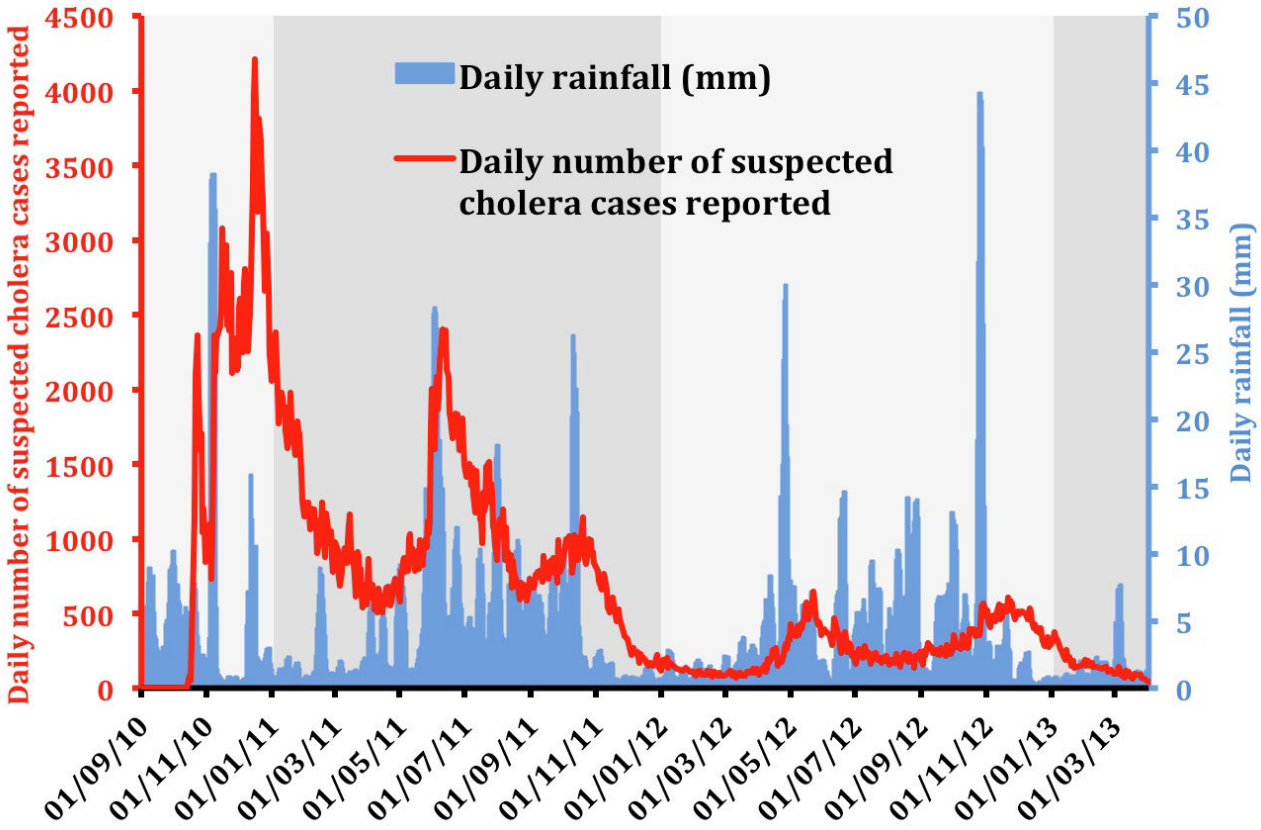


Table 1 - Bacteriological confirmation of cholera at the LNSP. Evolution of the monthly number of positive and negative samples (% of total) from December 2012 to March 2013

	December 2012		January 2013		February 2013		March 2013		Entire study period		
Department	**pos**	**neg**	**pos**	**neg**	**pos**	**neg**	**pos**	**neg**	**pos**	**neg**	**Total**
West	85	25	50	38	38	59	32	31	205	153	358
	(77%)	(23%)	(57%)	(43%)	(39%)	(61%)	(51%)	(49%)	(57%)	(43%)	
Artibonite	44	19	31	13	23	18	7	20	105	70	175
	(70%)	(30%)	(70%)	(30%)	(56%)	(44%)	(26%)	(74%)	(60%)	(40%)	
South-East	25	13	12	3	6	10	4	7	47	33	80
	(66%)	(34%)	(80%)	(20%)	(38%)	(63%)	(36%)	(64%)	(59%)	(41%)	
Centre	15	5	0	8	0	0	0	15	15	28	43
	(75%)	(25%)	(0%)	(100%)	(NA)	(NA)	(0%)	(100%)	(35%)	(65%)	
North	2	0	0	2	0	4	6	1	8	7	15
	(100%)	(0%)	(0%)	(100%)	(0%)	(100%)	(86%)	(14%)	(53%)	(47%)	
Total	171	62	93	64	67	91	49	74	380	291	671
	(73%)	(27%)	(59%)	(41%)	(42%)	(58%)	(40%)	(60%)	(57%)	(43%)	

LNSP, National Laboratory of Public Health, Port-au-Prince
pos, positive culture ; neg, negative culture

Figure 2 – Monthly cholera attack rates and number of cholera-associated deaths in patients ≥5 years of age during the dry season 2012-2013

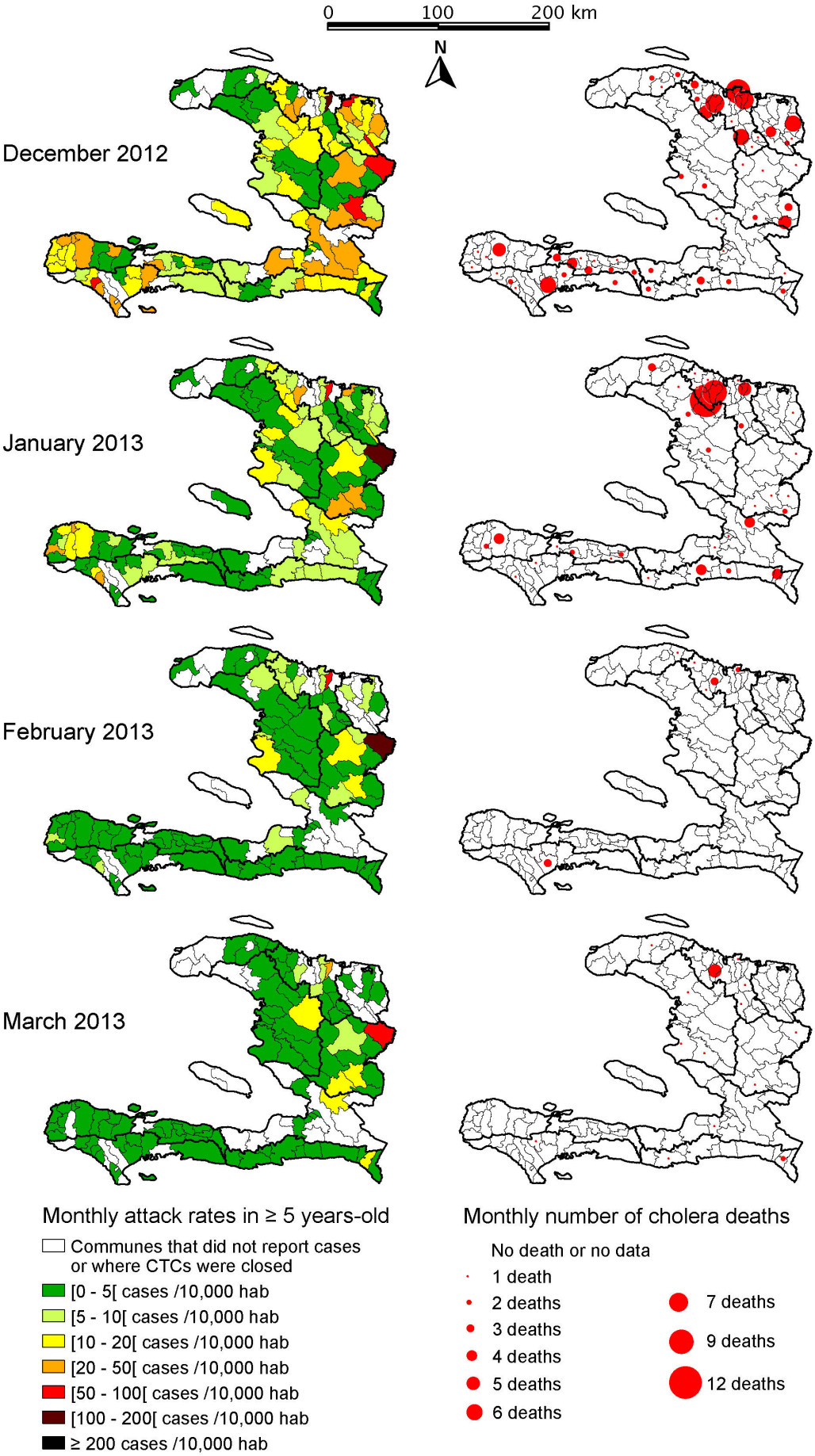


Table 2 – Communes with likely active cholera transmission in February and March 2013
(See Figure 3 for localizations)

			<5[3]	≥5[3]	≥5[3]	≥5[3]		
			No. of cases[4]	No. of cases[4]	Attack rate[5]	Cholera deaths	Bacteriological confirmation	Date of last confirmation
Artibonite	Gonaïves	340,155	70	198	5.8	1		13/12/12
Artibonite	Gros Morne	148,627	13	141	9.5	0	Yes	14/02/13
Artibonite	Saint-Marc	254,543	60	515	20.2	1	Yes	05/03/13
Artibonite	Saint-Michel	143,679	81	198	13.8	0		
Artibonite	Verrettes	138,242	44	110	8.0	1		
Centre	Cerca-La-Source	5,397	97	113	209.4	1		
Centre	Hinche	115,381	160	223	19.3	0		
Centre	Lascahobas	43,790	60	147	33.6	0		07/12/12
Centre	Mirebalais	93,319	98	251	26.9	1		21/12/13
North	Borgne	63,885	32	55	8.6	1		
North	Cap-Haïtien	261,952	43	359	13.7	3	Yes	22/03/13
North	Limbe	81,431	28	146	17.9	8		
North	Pilate	51,597	7	66	12.8	0		
North	Plaisance	66,426	13	50	7.5	1		
North	Quartier Morin	26,117	87	247	94.6	0	Yes	11/03/13
North	Saint Raphael	51,316	7	44	8.6	1		
North-West	Port de Paix	194,719	13	65	3.3	1		
North-West	Saint Louis du Nord	111,070	11	60	5.4	1		
West	Cabaret	65,148	95	65	10.0	0		
West	Leogane	190,746	0	147	7.7	0	Yes	22/02/13
West	Thomazeau	50,558	5	74	14.6	0		
South	Les Cayes	144,813	3	56	3.9	3		
South-East	Jacmel	178,756	2	40	2.2	0	Yes	25/02/13
South-East	Thiotte	33,341	5	54	16.2	2		
Total of the 24 selected provincial communes		2,855,008	1,034	3,424	12.0	26		
Port-au-Prince conurbation (7 communes)[1]		2,599,952	231	738	2.8	1	Yes	01/03/13
Rest of Haiti (109 communes)		4,913,113	301	833	1.7	3	No	
Total Haiti (140 communes)		10,368,073	1,566	4,995	4.8	30		

[1] Port-au-Prince Metropole = communes of Port-au-Prince, Carrefour, Delmas, Petionville, Cite-Soleil, Tabarre and Kenskoff

[2] calculated from 2003 Census and provided by Haitian Ministry of the Interior and Territorial Collectivities

[3] <5 years of age and ≥5 years of age

[4] cases suspected by acute watery diarrhea

[5] attack rates presented in cases /10,000 inhabitants

Figure 3 – Communes likely affected by active cholera transmission in February and March 2013 (See Table 2 for characteristics)
Several communes, such as Jacmel, Leogane and Plaisance, were only affected in February. Thiotte was exclusively affected in March.

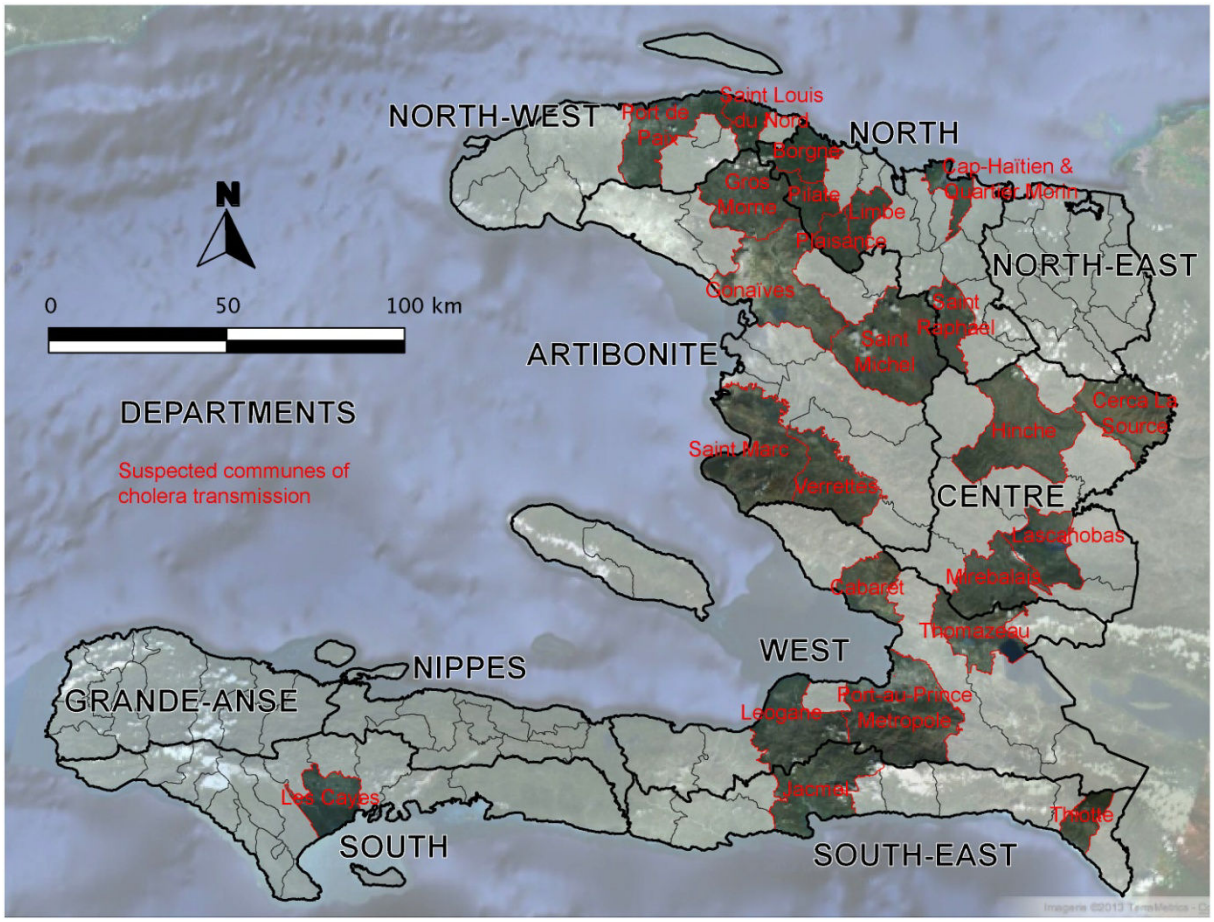
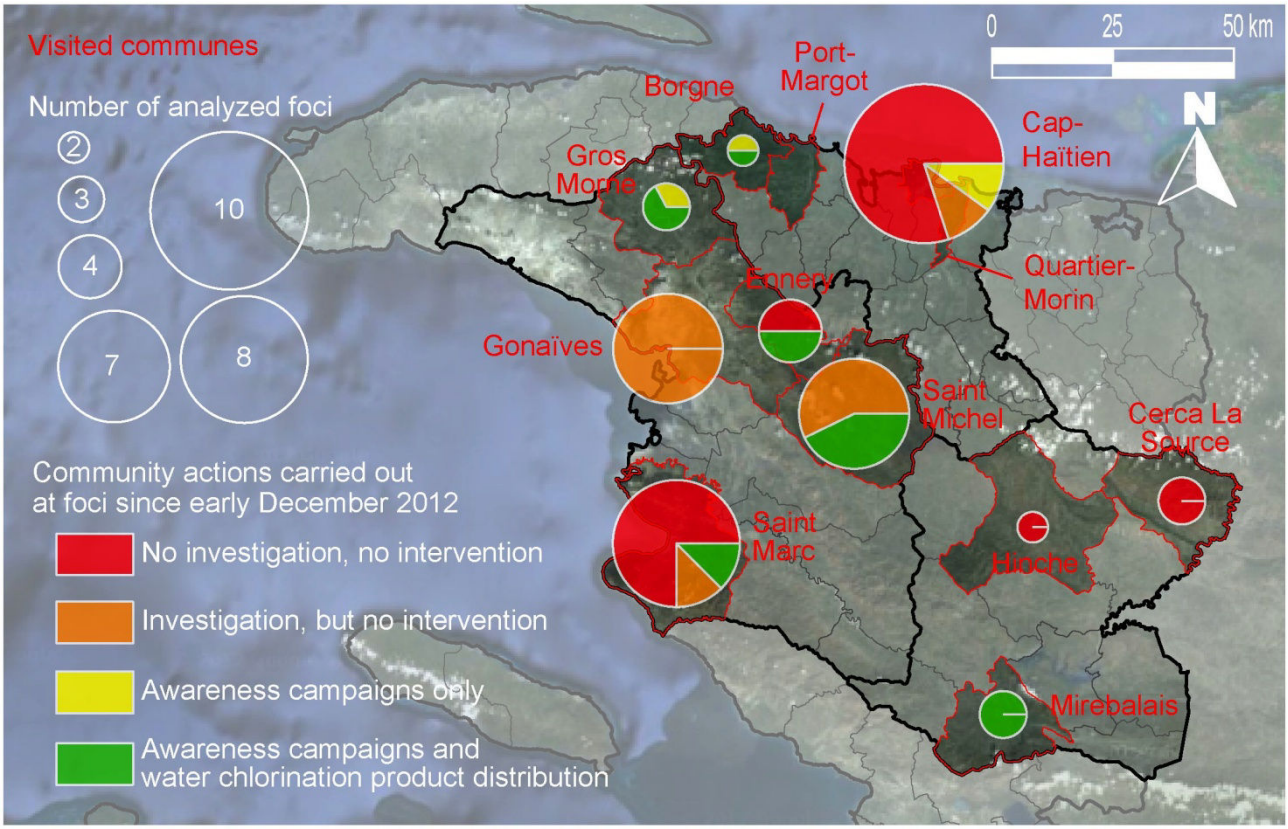


Figure 4 – Residual transmission foci identified in the 12 visited communes and community actions carried out since early December 2012



3.4.3 Une opportunité d'élimination non saisie en saison sèche

Au cours de la saison sèche 2012-2013, la transmission du choléra s'était donc rétractée dans un petit nombre de communes situées dans la moitié nord du pays et souvent dans des zones non côtières. De fait, la grande majorité de ces foyers résiduels n'avaient pas reçu de réponse adaptée depuis plusieurs mois.

Sur le terrain, cette opportunité manquée d'étouffer la transmission avant le retour de la saison des pluies était la conséquence de plusieurs phénomènes. De nombreux acteurs apparaissaient victimes d'un biais de perception consistant à être rassuré par la spectaculaire régression de l'incidence du choléra depuis 2010-2011, alors que l'épidémie haïtienne était pourtant toujours, début 2013, la plus importante du monde. En conséquence, les bailleurs avaient drastiquement réduit leur budget et la plupart des ONGs avaient quitté le pays à la fin de l'année 2012. Les partenaires toujours présents considéraient les cas résiduels en saison sèche comme un « bruit de fond » acceptable et se préparaient à retourner à l'assaut à la saison des pluies suivante. Par ailleurs, l'idée d'un choléra endémique et enraciné dans l'environnement haïtien était devenue largement partagée jusque dans le Plan d'élimination du choléra en Haïti 2013-2022 [111]. Dans ces conditions, le choléra n'apparaissait plus comme une urgence mais comme une problématique de développement.

La présentation de ces résultats ne fut pas toujours bien accueillie. Néanmoins, elle fut à l'origine d'un regain d'intérêt d'agences comme L'UNICEF ou ECHO en panne de stratégie de contrôle d'une épidémie qui avait fini par épuiser les forces en présence.

[111] République d'Haïti, Ministère de la Santé Publique et de la Population, et Direction Nationale de l'Eau Potable et de l'Assainissement, *Plan d'Élimination du Choléra en Haïti. 2013-2022*.

3.5 Stratégie de réponse ciblée en Haïti

« It's really an integrated approach within the UN and between the UN and other actors to support the national authorities. »

Peter de Clercq,
Représentant spécial adjoint pour le
Secrétariat Général des Nations Unies en Haïti,
Mai 2014 [112]

3.5.1 Mise en place d'une stratégie de lutte ciblée suivant les recommandations de l'APHM

La mission du mois de juin 2013 fut principalement consacrée à aider les services de l'UNICEF-Haïti à mettre sur pieds une stratégie d'appui au volet court terme du plan gouvernemental d'élimination du choléra en Haïti [113], intégrant les recommandations de l'APHM [114].

Cette stratégie fut lancée en juillet 2013 et se poursuit toujours. Elle comporte 4 objectifs : (1) renforcer la surveillance du choléra et notamment la production d'alertes de qualité ; (2) améliorer la coordination de la lutte contre le choléra à l'échelle nationale, départementale et locale ; (3) appuyer la réponse aux flambées, à travers la sensibilisation et la distribution ciblées de kits choléra comportant savon et produits de traitement de l'eau à domicile, ainsi que la réparation rapide des points d'eau défectueux ; et (4) renforcer les activités de prévention dans les zones les plus à risque, notamment par la réhabilitation de systèmes d'adduction d'eau. Pour ce faire, un appui financier direct est apporté au Ministère de la Santé Publique et de la Population (MSPP) et à la DIrection Nationale de l'Eau Potable et de l'Assainissement (DINEPA), et une organisation WASH partenaire est contractée pour chacun des 10 départements afin d'appuyer les autorités dans la mise en œuvre de ces activités de lutte ciblée.

3.5.2 Contractualisation de l'APHM pour guider la stratégie par l'épidémiologie

En parallèle, l'UNICEF engagea l'APHM dans un partenariat de 1 an afin que nous guidassions l'implémentation de la stratégie « par la production régulière d'informations épidémiologiques ». Ce partenariat fut prolongé pour une seconde année en juillet 2014.

[112] « As dry season ends in Haiti, significant gains seen in fight against cholera, UN official says ».
[113] UNICEF Haïti, *Stratégie pour appuyer le Plan national du Gouvernement Haïtien pour l'élimination du choléra. Juin 2013 - Décembre 2015*.
[114] Rebaudet et Piarroux, *Persistance du choléra en saison sèche en Haïti*.

Sur place les activités de suivi épidémiologique ont jusqu'à présent été menées avec l'aide de 4 médecins: Pierre Gazin, chercheur à l'IRD ; Aruna Abedi, qui travaille pour le ministère de la santé de RDC ; Robert Barrais, Chef de la surveillance à la DELR actuellement en congé d'études pour un master de santé publique ; et Lindsay Osei, jeune pédiatre à l'APHM.

Concrètement, la mission de l'équipe de l'APHM a jusqu'ici consisté à : (1) participer à mettre en place un système d'alerte permettant à la DELR d'identifier les communes devant être ciblées par les actions de réponse et de diffuser ces informations ; (2) participer aux investigations de terrain sur les foyers identifiés ; (3) participer aux réorientations de la stratégie UNICEF ; (4) recueillir des informations sur la situation épidémiologique et les activités de réponse ; (5) produire régulièrement des rapports synthétisant la situation épidémiologique, l'état de la lutte et les perspectives [115] ; (6) effectuer le plaidoyer nécessaire à l'adaptation et à la mise en œuvre de la stratégie ; et (7) participer activement à la coordination des différentes activités de la stratégie.

Afin de dresser un bilan de cette première année de la stratégie de réponses ciblées, un article est également en cours de rédaction dont une version provisoire vous est présentée ici.

3.5.3 Article 8 : « On the laborious way to cholera elimination in Haiti» (*en cours d'écriture*)

[115] Rebaudet et Piarroux, *Volet épidémiologique de l'appui de l'UNICEF au Plan National d'Elimination du Choléra en Haïti : Rapport intermédiaire*; Rebaudet et Piarroux, *Note sur l'évolution du choléra en Haïti, prospective pour 2014*; Rebaudet et Piarroux, *Choléra en Haïti : situation fin janvier 2014 et propositions pour avancer vers l'élimination*; Rebaudet et Piarroux, *Élimination du choléra en Haïti. Situation au 31 mai 2014*; Rebaudet et Piarroux, *Rapport de mission et point de situation sur le choléra en Haïti. Septembre 2014.*

On the laborious way to cholera elimination in Haiti

Introduction

Cholera hit Haiti on October 2010 [1], and soon occasioned the largest epidemic of the past decades at a nation level, with 706,291 suspect cases and 8,584 suspect deaths recorded on 27 August 2014 by the Haitian Ministry of Public Health and Population (MSPP* (meaning of acronyms is mentioned in Table 1]) [2]. Through following years, incidence progressively receded, alternating lull periods and outbreaks following seasonal rainfalls [3]. It was rapidly stated that cholera would inevitably become endemic on the island of Hispaniola. Nevertheless the Haitian government and its partners (UNICEF*, CDC* and PAHO*) launched an ambitious 10-years elimination plan [4] in February 2013, which called for thorough improvements of Haitian poor water and sanitation infrastructures. Meanwile oral cholera vaccination campaigns were advocated to limit the spread of cholera in the population [5].

Environmental studies have been implemented looking for the settlement of the *V. cholerae* epidemic strain in coastal and river environments. Until now no significant environmental reservoir of this strain could be highlighted, despite sporadic isolations of toxigenic *Vibrio cholerae* O1 [6,7]. Besides, field investigations showed that transmission during the dry season 2012-2013 retracted in various inland rural and peri-urban residual foci, which were mostly located in the northern half of the country. Surprisingly, very few prevention activities were conducted in these still active transmission areas during the dry season [8]. Therefore, we hypothesized that a strategy based on rapid detection of cholera outbreaks followed by early targeted preventing interventions would likely accelerate cholera elimination in Haiti, especially during the dry season [8].

Led by UNICEF, a two-years alert and response strategy was therefore implemented from June 2013, to support implementation of the short-term phase of the ten-years elimination plan by MSPP and DINEPA*. It aimed to detect and turn off every active cholera foci through targeted community awareness campaigns and interventions securing drinking water. We present here the first results of this approach, both in terms of implemented activities and cholera epidemic evolution.

Methods

Cholera alert and response strategy in Haiti

UNICEF's current "Strategy to support the national plan of the Haitian government to eliminate cholera" was launched in July 2013, aiming to reinforce four main components of cholera elimination activities: (1) coordination at national and departmental levels; (2) cholera surveillance; (3) WASH (WAter, Sanitation and Hygiene) response to cholera alerts through targeted awareness campaigns, improvement of drinking water quality and light water adduction system reparations; and (4) prevention in most vulnerable areas with mass awareness campaigns and rehabilitation of WASH infrastructures. Direct financial and human support was offered to MSPP* and DINEPA*, and at least one implementing WASH NGO was contracted for each of the ten Haitian departments (Table 1). At the same time, sibling health organizations were contracted by ECHO and PAHO to complement targeted response to cholera alerts. In certain areas, Directorate for Civil Protection and International Red Cross and Red Crescent Movement also took part in outbreak mitigation activities. From March 2013, capacities of MSPP have been progressively reinforced by the implementation of 10 departmental rapid response mobile teams. Financed by UNICEF and the World Bank, these EMIRA* performed rapid interventions in affected communities for household decontamination, awareness campaigns, distribution of water treatment products, as well as searching and care of contacts and cases targeted on affected communities.

Cholera cases, deaths, confirmation cultures and alerts

From October 2010, daily cholera-associated morbidity and mortality data were prospectively recorded by cholera-treating institutions and anonymously transmitted to MSPP Directorate for Epidemiology (DELR*), for control, compilation and analyses. According to the WHO standard definition [9], a probable cholera case is defined as a patient aged 5 years or older who develops acute watery diarrhea, with or without vomiting, located in an area where there is a cholera epidemic. In Haiti, however, DELR also separately recorded cholera *suspect* cases <5 years-old. Bacteriological confirmation of cases was routinely performed at the National Laboratory of Public Health (LNSP*) using standard methods [10]. From July 2013, DELR implemented a prospective identification of cholera alerts at communal level in order to better guide the response interventions of field partners. Three levels of alert were defined – *red*, *orange* and *no* alert – according to criteria formally validated during a national cholera alert and response workshop with Haitian authorities and international partners in August 2013 (Table 2). DELR staff identified alerts using a semi-automated excel spreadsheet, mapped them using Philcarto software V5.6 (http://philcarto.free.fr/, accessed 17 September 2014), and communicated alert bulletins to all partners by email and public

epidemiological meetings, usually on a weekly basis. Cholera alerts were also identified from surveillance data by departmental health directorates, and shared with partners, which also gathered local information during their community interventions.

Response interventions to cholera outbreak alerts

Partners were required to respond to a cholera alert within 48 hours. They were asked to conduct an investigation in local cholera treatment institutions and communities, in order to confirm cholera transmission, identify triggering and aggravating factors (history of index case, population gathering, funerals, water and sanitation conditions…), and search contacts and community cases. Response activities performed by UNICEF partners were prospectively recorded in detail including date and location of intervention, type of activity, number of people targeted, and sent to UNICEF. Relevance and impact of interventions were prospectively monitored using the completeness of response interventions to identified alerts, the evolution of epidemiological data, follow-up community interventions, and investigations of external epidemiologists contracted by UNICEF.

Data management and statistical methods

The national epidemic curve of daily suspect cases and 15-days smoothed case fatality rates (CRF) were drawn on Excel 2011 for Mac (Microsoft), together with daily accumulated rainfall data obtained from satellite estimates (TMPA-RT 3B42RT derived), averaged on the position 18N-20N/74.5W-71.5W and available at: http://disc2.nascom.nasa.gov/Giovanni/tovas/realtime.3B42RT_daily.2.shtml (accessed 17 September 2014). Attack rates (AR) were computed using country and commune population estimates provided by the Haitian Institute of Statistics and Informatics (www.ihsi.ht/pdf/projection/DOC_POPTLE18_MENEST2012.pdf, accessed 19 September 2014)

A retrospective identification of cholera alerts was computed using cases and deaths criteria only, because DELR actually never used criteria based on stool culture, RDT, patients' origin and rumors to identify alerts (Table 2), as these data prove to be difficult to prospectively gather and analyze. Alerts were considered at commune and week levels and counted in commune-weeks starting in epidemiological week (EW) 27, 2013 up to EW31, 2014, which thus represented a total 7980 commune-weeks. New red alerts were defined as commune-weeks fulfilling red alerts criteria that did not meet such criteria during the previous week. Considering that identifying these "theoretical" red alerts was the main objective of the surveillance component of the UNICEF strategy, prospective DELR alert system was evaluated in terms of completeness (proportion of effectively identified red alerts) and promptness (delay of diffusion of red alerts message following the meeting of red alert criteria in a given commune at a given week, in weeks). Time distribution of

retrospective alerts was drawn at national and departmental levels. To evaluate the relevance of UNICEF partners' response to alerts, reported interventions were matched with red alerts according to commune and week. Completeness, promptness and relevance of interventions were evaluated. Response interventions were qualified as prompt when implemented within 2 weeks after alert onset or after the previous intervention if alert persisted.

Over the studied period, three phases were defined (2013 rainy season, 2013-2014 dry season and 2014 rainy season), using a sensitivity analysis comparing accumulated rainfalls with a Kruskal-Wallis test and a week time step. Epidemiological, alerts and interventions variables were summarized for each phase, and epidemiological variables were summarized for each alert level. Univariate comparisons between phases or alert levels were performed using Friedman tests taking into account communes and duration of observed periods.

To analyze factors influencing the appearance of red alerts since the end of the 2013-2014 dry season, a shared gamma frailty model was used to takes into account the recurrent structure of the data, as previously described [11]. For each commune and each week, this survival model tested the probability for a red alert to appear, and included several covariates: coastal location; rainfall accumulated in the commune during the previous and current weeks (obtained from the nearest satellite estimates from the commune centroid); occurrence of a red alert during the current or previous weeks in surrounding communes, i.e. order-2 contiguous communes (islands were also considered contiguous to communes connected by boat); occurrence of a mass gathering in the commune during the current or previous weeks (dates of carnival feasts and other festivals were gathered from Haitian Ministry of Tourism website (http://www.haititourisme.gouv.ht/fetes-champetres-patronales/, accessed 19 September 2014] and field investigations); and urban population in the commune ≥200,000 inhabitants (2012 estimates obtained from Haitian Institute of Statistics and Informatics (www.ihsi.ht/pdf/projection/DOC_POPTLE18_MENEST2012.pdf, accessed 19 September 2014]).

All statistical analyses were performed using RStudio version 0.98.994 for Mac (http://www.rstudio.com/, accessed 17 September 2014) with R version 3.1.1 for Mac (http://www.r-project.org/, accessed 17 September 2014). Maps were drawn using QGIS v2.4 (http://www.qgis.org/en/site/, accessed 17 September 2014).

Results

Time evolution of suspect cholera and cholera-related deaths

Since the effective launch of the UNICEF strategy to support governmental plan for cholera elimination in Haiti in July 2013, an unprecedented decrease of cholera transmission has been observed (Fig. 1). In 2012, MSPP recorded 101,354 suspect cases (global AR, 97.3 cases /10,000 inhabitants) including 18% of children <5 years of age, and 908 suspect deaths (global CFR, 0.9%). In 2013, 58,874 suspect cases (global AR, 56.5 cases /10,000 inhab.) including 17% of children <5, and 592 suspect deaths (global CRF, 1.0%) were notified. From January to August 2014, only 8625 suspect cases (provisional global AR, 8.3 cases /10,000 inhab.) including 23% of children <5, and 69 suspect deaths (global CFR, 0.8%) have so far been recorded. Mean rainfalls accumulated in Haiti over the same periods were 1465 mm, 1262 mm, and 779 mm, respectively. Initiation of the alert-response strategy during the 2013 rainy season was followed by a 35% reduction of cholera incidence between July and November 2013 as compared to the 2012 corresponding period, while accumulated rainfall showed a 14% decrease (Fig. 1). Cholera incidence peaked on mid-November, exactly at the end of the rainfall. It then exhibited a dramatic drop that deepened during the 2013-2014 dry season and still persists 5 months after the return of the 2014 rainy season (Fig. 1), with a 77% reduction of suspect cases recorded between December 2013 and August 2014 as compared to December 2012–August 2013. On the same period, national accumulated precipitations were reduced by 9% (Fig. 1).

Within our study period (July 2013 – July 2014), sensitivity analysis based on national accumulated precipitations defined a 2013 rainy season of 19 weeks, followed by a 2013-2014 dry season of 20 weeks and a 2014 rainy season of 18 weeks (Table 3). Taking into account the communes and these durations, numbers of suspect cases and deaths were significantly different between these periods, as well as the proportion of <5 years-old cases, which increased over time (Table 3).

Cholera alerts

From July 2013 to August 2014, DELR produced 59 alert bulletins, which covered 42 epidemiological weeks out of 57 (74%). Out of these 57 weeks and the 140 communes of Haiti, a total of 686 “red” commune-weeks were identified, grouped in 289 new red alerts. However, after identifying “theoretical” alerts from cholera surveillance database we determined 1094 “red” commune-weeks including 300 new red alerts, and 920 “orange” commune-weeks. Despite alert criteria restricted to ≥5 years-old cases and deaths, red alerts included 81% and 97% of the total suspect cases and deaths recorded on the period (Table 4). DELR identified and communicated 43% of these “theoretical” new red alerts within 1 week of onset; 11% were identified and communicated

with a 2 weeks delay; 8% with a 3 weeks delay; 23% of new red alerts were identified and communicated more than 3 weeks after their "theoretical" onset; finally, 16% were never identified and communicated by DELR to partners.

Time distribution of these "theoretical" alerts is illustrated on Figure 2A. It followed evolution of the epidemic curve (Fig. 1). During 2013 rainy season, the number of alerts peaked on early November 2013 (week 45), with 49 "red" communes and 23 "orange" communes". This occurred just before the onset of the 2013-2014 dry season. Number of alerts then decreased down to a nadir in March 2014, just before the return of the rains, with respectively 4 and 9 communes in orange and red alerts on week 11. Number of communes in alerts didn't increase significantly during the following 4 rainy months. Comparisons between 2013 rainy, 2013-2014 dry and 2014 rainy seasons exhibited significant differences between the weekly number of "red" communes as well as the mean duration of red alerts (Table 3). Besides, these fewer red alerts were less severe, as illustrated by a significant global decrease in the weekly number of suspect cases and deaths in "red" communes over time (Table 3).

Distribution of alerts exhibited a marked regional pattern (Fig. 2B). Most red alerts occurred in the central-north area of the country: North (DSN), Artibonite (DSA), Centre (DSC), and West (DSO) departments. Conversely, North-West (DSNO) and North-East (DSNE), as well as most of the South-Peninsula – Nippes (DSNi), South-East (DSSE), South (DSS) and, except from September to November 2013, Grand'Anse (DSGA) departments – were far less affected. In spite of the rainy season, these 6 latter departments didn't even exhibit more than 1 red commune-week during the last 4 months of the study.

Culture confirmation of alerts

From July 2013 to July 2014, 2,197 stool samples were received by LNSP for cholera confirmation culture (Table 3). Effort sampling was significantly less important during 2013-2014 dry season than during 2013 and 2014 rainy seasons. Sampling was also significantly more important during red alert periods (26%, 76/300) than during orange alert (9%) and no alert (8%) periods (Table 4). They came from 54 different communes, out of which 4 were the source of half samples (St Marc, Gonaïves, Delmas and Jacmel).

Culture positivity ratio was 50% over the global study period (95%IC, 48-52), and it significantly decreased along time, from 60 during 2013 rainy season down to 37% during 2014 rainy season (Table 3). It also was significantly higher during red alerts (58%) than during orange (46%) and no alert (27%) periods (Table 4). Although 64 out of the 79 red alert periods (81%) were confirmed by

at least 1 positive culture, all cultures remained negative for 15 red alert periods. On the contrary, all cultures remained negative for 23 out of the 67 sampled no alert periods (34%), whereas at least 1 culture was positive for 66% of them (Table 4).

Evolution of alerts between 2014 dry and rainy seasons

Number of communes in alert progressively decreased during the 2013-2014 dry season. On weeks 12 & 13, just before the return of rainfalls, a red alert persisted in only 8 communes (Fig. 3): Limbe and Plaisance in DSN; Gonaives and St Marc in DSA, Hinche and Mirebalais in DSC; and Delmas and Port-au-Prince in DSO. Four communes were important urban centers, and four of them were located inland. During the 2014 rainy season cholera alerts showed a mild spillover progression, which appeared mostly limited to the neighboring of previously persisting alerts (Fig. 3).

Using the shared gamma frailty model, search for risk factors associated with red alert appearance during 2014 rainy season (Table 4) confirmed the role of recent contiguity to another commune in red alert (OR, 2.90 [1.04 – 8.12]; *P*-value < 0.05). However, neither coastal location nor the amount recent rainfall appeared statistically associated with appearance of red alerts on this period.

Cholera response to alerts interventions

From July 2013 to July 2014, organizations contracted by UNICEF and DINEPA reported 1183 response-interventions (Table 3). Out of these interventions 42.3% were conducted during the 2013 rainy season, 34.7% during the 2013-2014 dry season and 23.0% during the 2014 rainy season. Besides, 38.5% of interventions took place in DSA, 19.9% in DSNi, 14.9% in DSNO, 11.6% in DSN, 5.5% in DSS and the remaining 9.6% in DSO, DSGA, DSC, DSNE and DSSE (data not shown). Only 50.9% of recorded interventions were targeted on currently “red” communes (Table 3 & Table 4), and this proportion decreased from 66.8% during 2013 rainy season down to 36.0% during the 2014 dry season (Table 3).

Conversely, out of the 1094 commune-weeks in red alert, 716 (65.4%) didn’t receive any response intervention during the current or following weeks. Intervention didn’t include awareness campaigns or distribution of cholera kits for 39 red commune-weeks (3.5%); intervention was limited to awareness campaigns for 30 red commune-weeks (2.7%); and 309 red commune-weeks (28.3%) received a distribution of cholera kits, including 237 (21.7%) receiving a prompt distribution within 2 weeks after onset of this red alert or after the previous distribution when red alert persisted. Time distribution of response interventions to red alert is illustrated on Figure 4. Interventions and responses including a prompt distribution progressively intensified during 2013 rainy season. But

about two-thirds of red alerts observed during cholera transmission peak didn't receive any intervention (Fig. 4). Besides, in spite of the subsequent drop of red alerts, 24.1% and 21.0% of red commune-weeks were targeted by a prompt distribution of water treatment products during 2013-2014 dry and 2014 rainy seasons, respectively (Table 3 and Fig. 4). After several consecutive weeks with no rapid, targeted and relevant response in March and April, activities subsequently improved. Finally, periods of of red alerts diminution since July 2013 always appeared contemporary with a rising of well-addressed responses (Fig. 4).

Discussion

As a prime component of this first nation-wide targeted-response strategy to eliminate cholera from Haiti, an alert system was successfully implemented in July 2013. These alerts could be identified, mapped and communicated on a regular basis by DELR. Compared with usual epidemiological indicators, they have become a sensitive, consistent and popular qualitative indicator used to spot the communes where partners are to focus cholera control interventions.

However, promptness of this prospective alert identification has suffered from important delays in notification of cholera surveillance data, and it has been nearly impossible to integrate culture and rapid tests results as initially planned. Used surveillance data don't include community cases, and community deaths have been very inconstantly reported [12]. Spatial information in the national surveillance database doesn't concern patients' place of origin but only institutions where they seek treatment, which is too broad and can be misleading. These difficulties have encouraged field partners to set complementary alert and surveillance systems at departmental and local levels, and to seek patients' addresses in cholera treatment institutions' register books. Besides, cholera alerts have exhibited a low specificity when compared to stool cultures, whose sampling remains too limited due to logistic constraints. This probably generated a background noise of fake alerts caused by non-choleric acute watery diarrhea cases heading to biggest or most popular cholera treatment centers [12].

This original nation-wide strategy allowed a marked increase of targeted cholera response interventions, which had so far remained uncoordinated, and had dramatically declined from late 2012 [8]. However, response efforts of UNICEF partners and DINEPA declined during 2013-2014 dry season and an important proportion of red alerts obviously remained unaddressed. Some of them were false alerts. Some interventions were implemented by other partners (like health NGOs and EMIRA) and could not be included in the present study. Response capacities of several NGO were also temporally weakened by administrative problems and the severe yet under-reported

Chikungunya epidemic which swept the country from late April [13]. Finally, like during the previous dry season [8], motivation was difficult to maintain in a context of fading transmission. Thankfully, reported coverage of alerts has improved in recent months.

Implementation of this alert-response program was followed by an unprecedented decrease of cholera transmission, which seemed unlikely according to the most recent and fitted model [14] and UN predictions [15,16]. Likewise, while Haiti 2013-2022 cholera elimination plan [17] targeted an annual attack rate of 0.5% in 2014, this objective was nearly achieved as soon as 2013 (0.57%), and 2014 cumulated attack rate reached a mere 0.08% after the first 8 months. Transmission was remarkably mitigated during 2013 rainy season, before it dramatically dropped during the following dry season and still maintained at low levels after 5 months of 2014 rainy season. Between March and July 2014, barely any cholera alerts was even identified in 6 out of 10 departments. Taking into account the decreasing proportion of the culture positivity ratio along the period, the actual reduction of cholera transmission likely was even more spectacular and has got closer to extinction.

This success may classically be attributed to more favorable environmental and climatic conditions. Indeed, global accumulated rainfall was lower in 2013 than in 2012, and on the first 8 months, lower in 2014 than in 2013 too. Nevertheless, reductions did not exceed 14%, and if no hurricane has hit Haiti since November 2012, precipitations remained steady enough in 2013 and 2014 to cause local floods. According to our regression model, red alerts onset since the beginning of 2014 rainy season didn't appear significantly influenced by the amount of recent local precipitations. Vast areas of the country have remained free from cholera despite the return of rainfall. Besides, cholera transmission reduction may have been a consequence of an increasing immunity within Haitian population. However, protective immunity induced by clinically apparent or not infections starts declining after a few months or years [14,18,19] and cumulated attack rates observed since 2012 are not likely to have conferred a significant additional immunity to Haitian population. Likewise, the 4 oral cholera vaccination campaigns implemented by MSPP and its partners in 2012 and 2013 reached less than 200,000 people in limited areas of Port-au-Prince (DSO), Saint-Marc (DSA), Cap-Haïtien (DSN) and in Cerca-Carvajal (DSC) [20–22], and they probably had a limited impact on the global dynamic of the epidemic.

Conversely and in spite of missing intervention reports, diminutions of red alerts always appeared contemporary with a rising of prompt and targeted responses, suggesting they played a key role. During 2013 and 2014 rainy seasons, these responses may have been sufficiently efficient to temper the usual booster effect of rainfall on cholera transmission [3,23]. Besides, red alert progressive reappearance since 2014 rainy season was significantly associated with the contiguity to recent or

current red communes, but neither with a coastal location nor with recent rainfall. This suggests that alerts didn't originate in putative aquatic environmental reservoirs, but rather through the human-related local spreading from persistent transmission areas where the response wasn't efficient enough. Conversely, areas whose alerts benefited from an efficient response can stay free from cholera for a long period, provided that they keep far enough from active transmission foci.

Thirteen months after its launch, this nationwide targeted alert-response strategy likely resulted in a spectacular and persistent drop in cholera transmission in Haiti. Despite important limitations, it allowed Haiti to take a major step towards cholera elimination. However, progresses have come to a halt since the beginning of 2014, and an important proportion of red alerts have remained unaddressed. These mixed results strongly encourage strengthening this cost-effective strategy for the months to come. Cholera elimination in Hispaniola could be at hand, provided a step-up of efforts in Haiti and a similar implementation in the neighboring Dominican Republic. It then will be important to maintain an armed surveillance of possible environmental reservoirs, residual cholera carriers and new importations. Moreover, massive efforts in water and sanitation infrastructures will remain critical to protect this highly vulnerable country from another similar catastrophe. If international community was responsible for importing cholera in Haiti, it should not become guilty to forget this as soon as the epidemic finally recedes.

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Tables and Figures

Table 1 – Organizations' acronyms* and zones of activity

Acronym	Name of organization	Zone of activity
Governmental entities		
DELR	MSPP Directorate for Epidemiology Laboratory and Research (*Direction d'Épidémiologie de Laboratoire et de Recherche*)	
DINEPA	National Directorate for Water and Sanitation (*Direction Nationale de l'Eau Potable et de l'Assainissement*)	
EMIRA	Departmental rapid response mobile teams (*Équipe Mobile d'Intervention RApide*)	
LNSP	National Laboratory of Public Health (*Laboratoire National de Santé Publique*)	
MSPP	Haitian Ministry for Public Health and Population (*Ministère de la Santé Publique et de la Population*)	
Foreign and international agencies and donors		
APHM	*Assistance Publique – Hôpitaux de Marseille*, Aix-Marseille University, France	
CDC	Centers for Disease Control and Prevention	
DFID	United Kingdom Department for International Development	
ECHO	European Commission's Humanitarian Aid and Civil Protection department	
OCHA	United Nations Office for the Coordination of Humanitarian Affairs	
PAHO	Pan American Health Organization	
UNICEF	United Nations Children's Fund	
WB	World Bank	
Implementing organizations contracted by UNICEF		
ACF	Action Against Hunger (*Action Contre la Faim*)	DSA and DSNO in 2013-2014
ACTED		DSS since 2013 and DSGA since 2014
Care		DSGA in 2013
CRF	French Red Cross, works with Haitian Red Cross	DSO in 2013-2014
FONDEFH		DSN and DSNE in 2013
Oxfam		DSN and DSNE in 2013-2014
Plan		DSSE in 2013
SI	Solidarités International	DSNi and camps in DSO in 2013-2014, and DSSE since 2014
ZL	*Zanmi Lasante*	DSC in 2013-2014

DSA, Artibonite department; DSC, Centre; DSGA, Grand'Anse; DSNi, Nippes; DSN, North; DSNE, North-East; DSNO, North-West; DSO, West; DSS, South; and DSSE, South-East department.

Table 2 – Criteria for cholera alerts at communal level according to DELR

Red alert	
	≥1 cholera-associated death ≥5 years of age during the past 7 days of record
or	≥10 cholera suspect cases ≥5 years of age during the past 7 days of record
or	≥5 cholera suspect cases ≥5 years of age coming from a similar locale during the past 7 days of record
or	≥50% of positive rapid diagnostic tests
or	≥1 stool culture positive for *Vibrio cholerae* O1 at LNSP
Orange alert	
	Twofold or more increase of suspect cases ≥5 years of age during the past 7 days compared to the previous 7 days
or	Red alert during the previous week
or	Outbreak rumor in the commune
No alert	
	No red or orange alert criteria for at least two weeks

DELR, Haiti Directorate for Epidemiology Laboratory and Research; LNSP, National Laboratory of Public Health

Figure 1 – Daily evolution, of the suspect cases of cholera, 15-days smoothed global case fatality rate and national rainfall between January 2012 and August 2014, with timeframe of main elimination programs.

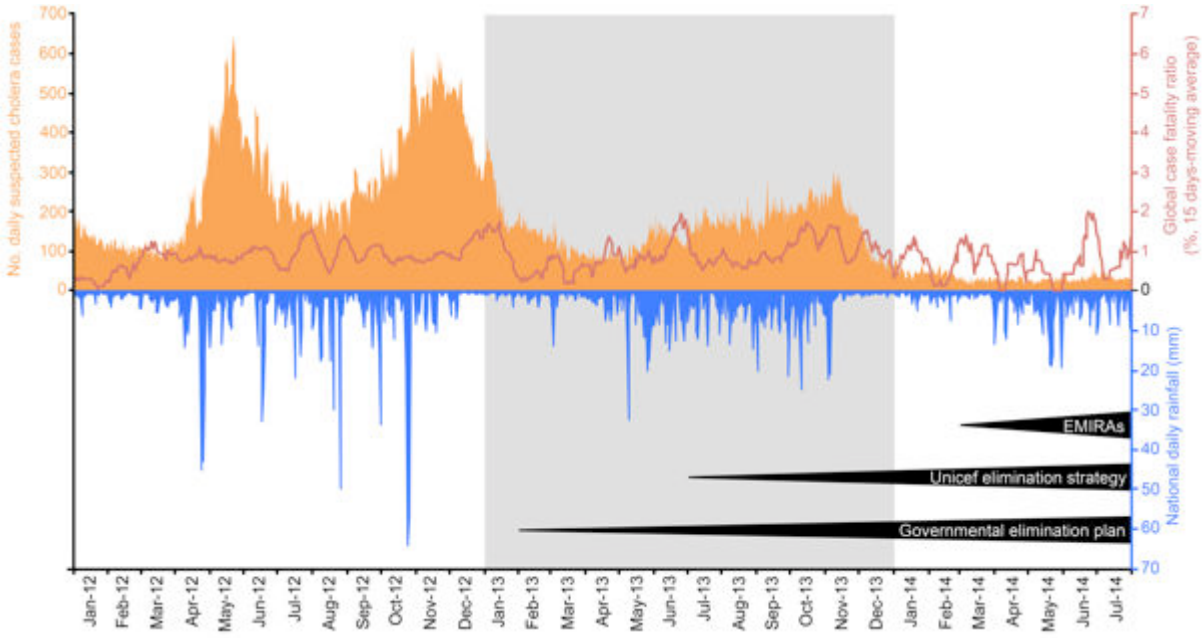


Table 3 - Synthesis of cholera cases, deaths, alerts and culture confirmations during the different periods from July 2013 to July 2014

	Whole period	Period 1	Period 2	Period 3
	2013W27-2014W31	2013 rainy season 2013W27-W45	2013-2014 dry season 2013W46-2014W13	2014 rainy season 2014W14-W31
Epidemic characteristics				
No. of weeks	57	19	20	18
No. of commune-weeks	7980	2660	2800	2520
Median weekly cumulated rainfall in Haiti, mm	23	34	5	26
[min-Max]	[3-88]	[8-68]	[3-15]	[12-88]
Cumulated no. of cholera suspect cases in Haiti	41256	26082	11191	3983
Percentage of cholera suspect cases <5 years-old	16%	15%	17%	21%
Cumulated no. of cholera associated deaths in Haiti	429	282	119	28
Cholera alerts				
No. of commune-weeks in red alert	1094	666	323	105
Median weekly no. of “red” communes	11	34	11	6
[min-Max]	[3-49]	[28-49]	[3-47]	[3-11]
Median duration of red alerts (in weeks)	3,7	5,1	1,8	1,7
[min-Max]	[1-34]	[1-34]	[1-14]	[1-5]
Median weekly no. of suspect cases in red alert communes	21	27	17	16
[min-Max]	[0-232]	[0-194]	[0-232]	[0-97]
Stool cultures for *V. cholerae* O1 at LNSP				
No. of received stool samples	2197	840	643	713
Median weekly no. of received samples	34	40	20.5	34.5
[min-Max]	[3-138}	[14-90]	[3-95]	[19-138]
Percentage of positive cultures	50%	60%	52%	37%
(95%IC)	(48-52)	(57-64)	(48-56)	(33-40)
Percentage of positive cultures during “red” commune-weeks	58.4%	62.0%	58.3%	49.8%
(IC95%)	(55.7-61.0)	(58.2-65.7)	(53.6-62.9)	(43.9-55.8)
Response interventions				
No. of response interventions	1183	500	411	272
(%)	(100%)	(42.3%)	(34.7%)	(23.0%)
No. of response interventions conducted in red alert communes	602	334	170	98
(% of interventions)	(50.9%)	(66.8%)	(41.4%)	(36.0%)
No. of commune-weeks in red alert with no intervention during the current or following weeks (% of red commune-weeks)	716 (65.4%)	445 (66.8%)	205 (63.5%)	66 (62.9%)
No. of commune-weeks in red alert with no awareness campaigns or distribution (% of red commune-weeks)	39 (3.5%)	19 (2.9%)	17 (5.2%)	3 (2.9%)
No. of commune-weeks in red alert with awareness campaigns without distribution (% of red commune-weeks)	30 (2.7%)	12 (1.8%)	8 (2.5%)	10 (9.5%)
No. of commune-weeks in red alert with distribution (% of red commune-weeks)	309 (28.3%)	190 (28.6%)	93 (28.8%)	28 (24.8%)
No. of commune-weeks in red alert with a prompt distribution (% of red commune-weeks)	237 (21.7%)	137 (20.6%)	78 (24.1%)	22 (21.0%)

Table 4 - Comparison of red, orange and no alert characteristics

	Red alerts	Orange alerts	No alerts
No. of alert periods	300	873	815
No. of commune-weeks	1094	920	5966
Cumulated no. of cholera suspect cases in Haiti	33079	3756	4242
Cumulated no. of cholera associated deaths in Haiti	420	7	7
No. of stool samples received at LNSP for cholera culture	1394	309	428
No. of alert periods with stool sample sent for culture	79	76	67
(%)	(26%)	(9%)	(8%)
Percentage of cultures positive for *V. cholerae* O1	58%	46%	27%
[95%-CI]	[56-61]	[40-52]	[23-31]
No. of alerts with ≥1 positive cultures	64	54	44
No. of alerts with negative cultures only	15	0	23
No. of response interventions conducted by UNICEF partners	334	170	98

Figure 2 – Weekly distribution of communal retrospective alerts from July 2013 to July 2014, (A) at national level with accumulated rainfall, and (B) at departmental level.
(DSA, Artibonite department; DSC, Centre; DSGA, Grand'Anse; DSNi, Nippes; DSN, North; DSNE, North-East; DSNO, North-West; DSO, West; DSS, South; and DSSE, South-East department.)

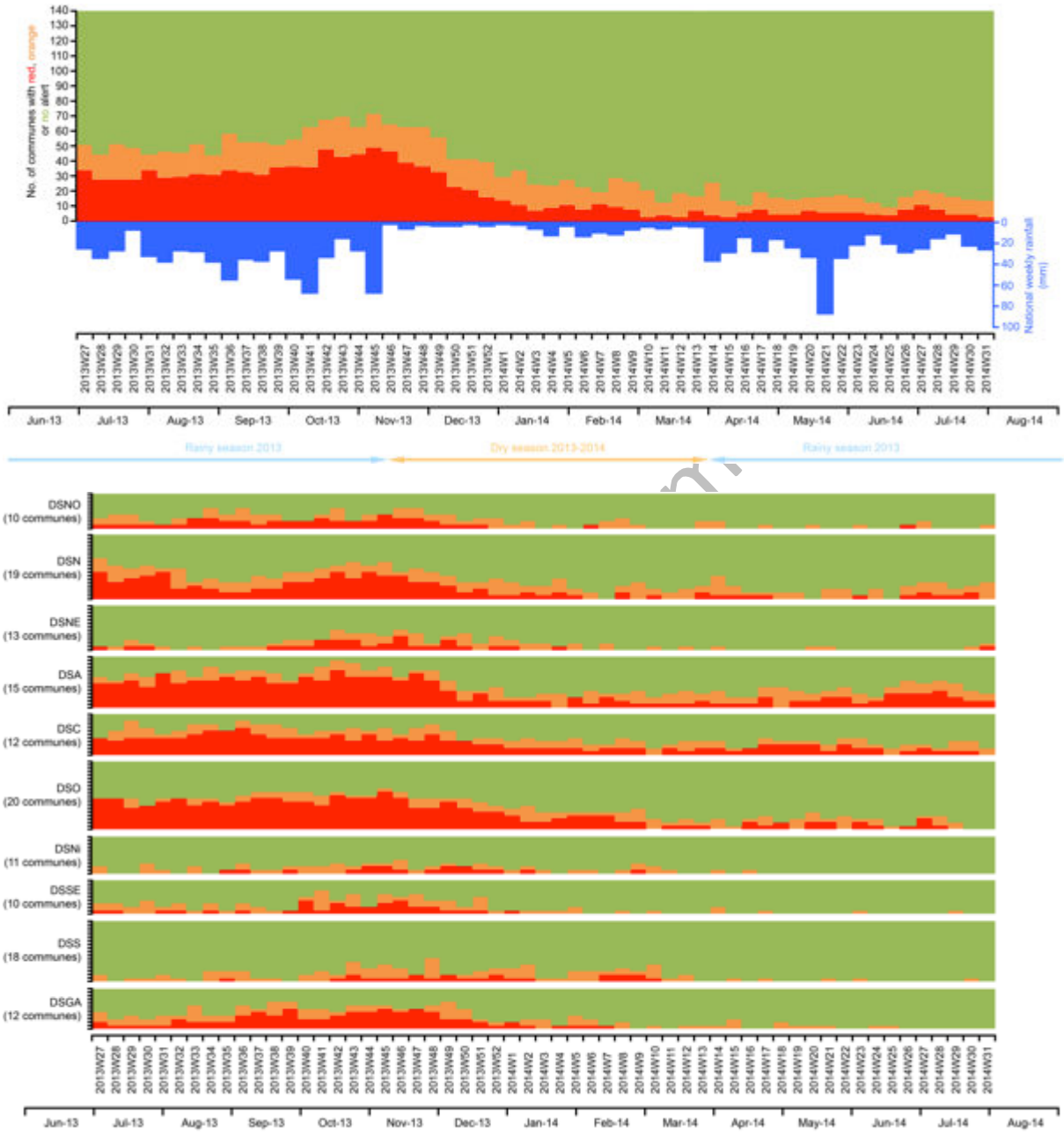


Figure 3 – Two-weeks step communal mapping of retrospective cholera alerts during the 2014 rainy season

(DSA, Artibonite department; DSC, Centre; DSGA, Grand'Anse; DSNi, Nippes; DSN, North; DSNE, North-East; DSNO, North-West; DSO, West; DSS, South; and DSSE, South-East department)

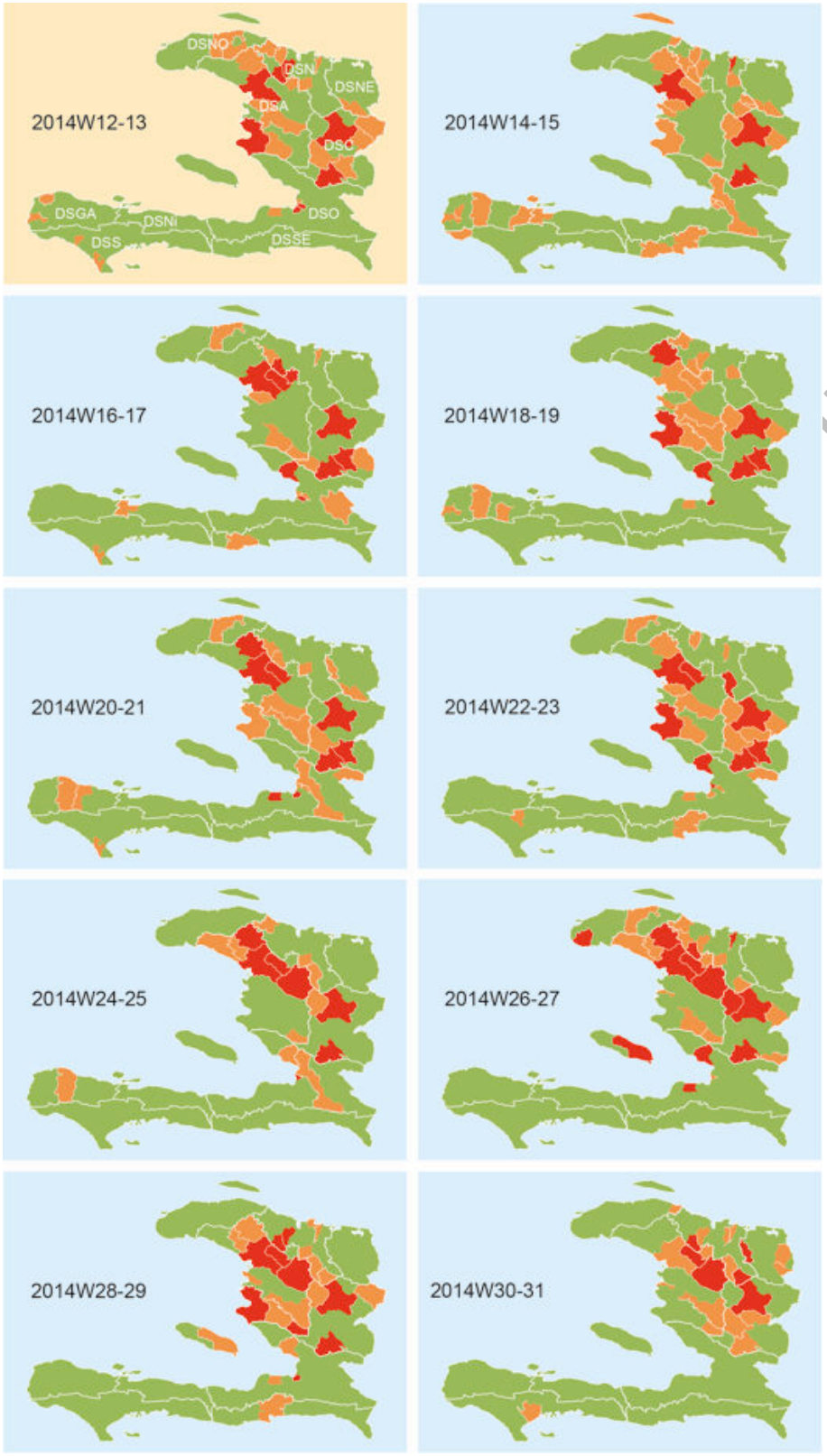
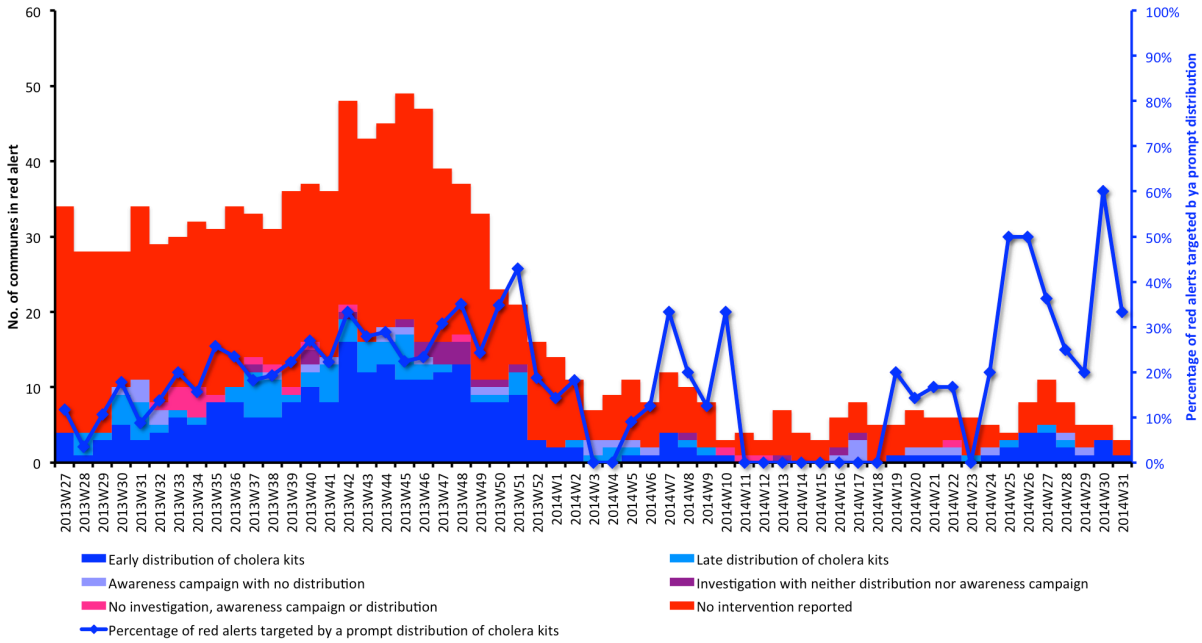


Table 4 - Risk factors associated with red alert apparition during 2014 rainy season

	Odd ratio	[95%CI]	*P*-value
Red alert in a rook 2 contiguous commune during the current or previous weeks	2.90	[1.04-8.12]	< 0.05
Mass gathering during the previous or current weeks	1.41	[0.44-4.49]	0.57
Coastal location	0.63	[0.20-1.93]	0.42
Urban population in the commune ≥200,000 inhabitants	5.12	[0.60-43.54]	0.13
Accumulated rainfall during the previous and current week in the department	1.01	[0.99-1.01]	0.11

coxph(Surv(Start, End, Episode)~Alert_contiguity+Mass_gathering+Coastal_location+Urban_50+Rainfall+frailty (ID, dist="gamma"),data=Apparition_dry)

Figure 4 – Time distribution of response interventions to red alerts, implemented and reported by UNICEF partners.



3.5.4 Des progrès initiaux spectaculaires suivis d'un échec à contrôler les derniers foyers

Entre juillet et novembre 2012, l'incidence du choléra avait fortement augmenté à la faveur de la saison des pluies. A l'inverse, les premiers mois suivant la mise en place de la Stratégie UNICEF en juillet 2013 ne furent marqués que par une augmentation très modérée de la transmission. Ensuite, celle-ci baissa de manière spectaculaire au cours des premières semaines de la saison sèche 2013-2014, jusqu'à atteindre un plancher sans précédent depuis 2010. Ainsi certaines semaines en mars-avril, on ne compta qu'une seule commune en alerte, généralement située dans les départements de l'Artibonite du Centre ou du Nord, et moins de 150 cas suspects âgés de 5 ans et plus. Le nombre réel était encore bien en deçà compte-tenu de la faible spécificité de la définition clinique des cas et de la diminution importante du taux de confirmation bactériologique des cas suspects.

Il est méthodologiquement difficile de prouver que cette amélioration de la situation épidémiologique fut la conséquence directe de la stratégie mise en place. D'autres facteurs comme la pluviométrie ont possiblement joué un rôle. Néanmoins, plusieurs observations plaident en faveur d'un impact réel du programme sur l'évolution de l'épidémie : (1) l'absence prolongée de transmission dans les vastes zones du pays débarrassées du choléra dès janvier ou février ; (2) la propagation de la transmission par contiguïté à partir des foyers non contrôlés ; (3) la mise en parallèle de l'évolution des alertes choléra avec les réponses apportées ; et bien sûr (4) les observations faites sur le terrain.

Malheureusement, malgré la diminution de l'incidence au cours de la saison sèche, la proportion d'alertes rouges recevant une réponse adaptée n'apparaît pas avoir significativement progressé par la suite. Cette inadéquation entre les activités de réponse et la situation épidémiologique explique probablement en grande partie la persistance des quelques foyers non stérilisés en fin de saison sèche, d'où la transmission s'est à nouveau propagée en tache d'huile avec le retour des pluies.

Ce bilan d'étape en demi-teinte illustre les difficultés à conduire un programme de ce type malgré les moyens mis en place. Les contraintes géographiques comme l'éparpillement de l'habitat et l'accès souvent compliqué dans les zones montagneuses expliquent en partie ces échecs. En outre, l'impact des campagnes de sensibilisation et la réelle utilisation des kits choléra par les populations touchées par le choléra se heurtent à des déterminants économiques et psycho-sociologiques souvent complexes à

décrypter et à anticiper. Par ailleurs, en dépit de termes de références identiques, les partenaires de l'UNICEF ont jusqu'ici fait preuve de niveaux d'engagement variables selon les départements, du fait de problèmes internes ou de difficultés relationnelles avec des autorités départementales plus ou moins coopératives.

Alors que le niveau de transmission était au plus bas durant les premiers mois de la saison des pluies, la situation s'avère plus compliquée depuis août 2014 avec une remontée sensible du nombre de nouveaux cas suspects, du taux de positivité des cultures et du nombre de décès. L'incidence demeure certes 5 fois plus basse qu'à la même période en 2012 et 2013, voire davantage si l'on prend en compte le taux de positivité des cultures. Mais nul ne saurait prédire dans quelles conditions les partenaires aborderont le retour de la prochaine saison sèche en novembre ou décembre 2014. En attendant, il convient de faire le maximum pour contenir voire éteindre les foyers actifs, afin d'aborder dans les meilleures conditions possibles la prochaine fenêtre d'opportunité. Il deviendra ainsi peut-être possible d'interrompre la transmission à l'échelle nationale dans les 6 mois à venir. Si tel est le cas, il conviendra alors d'être capable de détecter au plus vite les possibles reprises épidémiques à partir d'un foyer demeuré non détecté, d'un possible porteur chronique, ou d'un hypothétique réservoir environnemental.

4 DISCUSSION GÉNÉRALE

Ces différents travaux sur la dynamique de la transmission du choléra en Afrique et en Haïti mettent en évidence une importante hétérogénéité spatiale et temporelle, qui apparaît d'autant plus marquée que les échelles utilisées sont fines. D'importants facteurs environnementaux contribuent à déterminer cette hétérogénéité. Néanmoins leur impact sur la transmission du choléra semble moins lié à de possibles réservoirs environnementaux influencés par le climat, qu'à leur influence sur les comportements humains, notamment migratoires.

En effet, les résultats d'épidémiologie de terrain, d'épidémiologie géographique et d'épidémiologie moléculaire analysés et présentés ici suggèrent fortement un déplacement des épidémies de choléra via les mouvements de population. A l'instar des 3 vagues successives et partiellement superposées constituant la septième pandémie, l'endémicité du choléra dans de nombreux pays pourrait n'être en réalité que l'image lointaine et floue d'un phénomène de métastabilité constitué d'épidémies successives décrivant un double mouvement de migration, et d'expansion/rétraction.

L'endémicité peut être un concept utile pour catégoriser les pays selon leur niveau d'atteinte par le choléra. Néanmoins, il tend à biaiser la compréhension des épidémies en homogénéisant la distribution des cas dans l'espace et dans le temps. En détournant ainsi le regard des finesses de la dynamique de transmission du choléra, ce concept tend à servir de cheval de Troie au paradigme environnemental qui, bien que séduisant intellectuellement, n'a jusqu'à présent réussi à démontrer sa pertinence, ni en Afrique, ni en Haïti.

Faut-il le rappeler, l'objectif premier de ce travail n'est pas de remettre en cause la portée de ce paradigme *per se*, mais d'explorer, sans *a priori*, la dynamique des épidémies afin de proposer les stratégies de lutte les plus adaptées. L'exemple haïtien illustre pourtant à quel point les prétentions universelles de cette théorie peuvent être démenties par les réalités du terrain, et à quel point sa grande popularité a contribué à inhiber la lutte contre le choléra en négligeant la mise en place de stratégies ciblées dans les foyers de transmission.

A travers ces travaux, nous avons contribué à lever le voile sur ses incohérences. Sans conteste, le génotypage des souches de *V. cholerae* apportent une plus-value notable dans la compréhension des liens entre les épidémies, à condition de recourir à

un échantillonnage rigoureux associé à une description épidémique précise nécessitant enquêtes de terrain et analyses spatio-temporelles. La puissance de démonstration de ces nouveaux outils contribue déjà au déclin du paradigme environnemental, et pourrait même le renverser dans les années à venir. En démontrant la parenté phylogénétique de la quasi-totalité des épidémies analysées, ils ont d'ores et déjà replacé les mouvements de population au centre du débat sur l'origine des épidémies de choléra en Afrique et en Haïti. Je ne peux me prononcer sur le Golfe du Bengale, connaissant très mal la situation du choléra dans cette région du monde source des sept pandémies décrites depuis le début du 19e siècle.

A travers cette thèse, je me suis ainsi retrouvé plongé dans une controverse scientifique soulevant des questions d'ordre épistémologique, sociologique, voire éthique.

Le paradigme environnemental du choléra repose en effet sur un corpus de résultats essentiellement issus des côtes américaines et du Golfe du Bengale, dont la généralisation ne saurait aller de soi. Des constatations faites sur la grande diversité des *Vibrio cholerae* isolés dans l'environnement sont fréquemment extrapolées au sous-groupe clonal de *V. cholerae* O1 El Tor toxinogène responsable de la septième pandémie. Les nombreuses corrélations statistiques établies entre l'incidence du choléra et diverses variables climatiques ne sauraient garantir une relation de causalité, compte-tenu notamment du biais écologique inhérent aux données agrégées. Comme le faisait déjà Pollitzer dans les années 1950 [116], il demeure par exemple tout à fait possible d'interpréter l'impact de la pluie et de la température sur l'incidence du choléra sans recourir à des réservoirs environnementaux, mais en considérant simplement la contamination des points d'eau par les écoulements, la modification saisonnière des comportements, la concomitance de certains phénomènes, etc...

Pourtant l'existence ubiquitaire de réservoirs environnementaux du choléra apparaît aujourd'hui tellement établie, qu'on observe fréquemment un étonnant retournement de la charge de la preuve dans les discours qui y sont consacrées. A contrario du principe juridique de présomption d'innocence, 1% d'échantillons aquatiques positifs peut ainsi suffire à prouver l'existence d'un réservoir quelque soit sa

[116] Pollitzer, « Cholera studies- 10. Epidemiology ».

concentration ou le contexte local [117], alors que 100% d'échantillons négatifs ne suffisent plus à rassurer sur sa faible probabilité d'existence [118]. A ces biais de raisonnement s'ajoute également parfois la tendance de ces travaux à ne pas intégrer dans leurs analyses les éléments susceptibles d'infirmer l'hypothèse à prouver, comme par exemple les souches népalaises dans la recherche d'une parenté génétique au *V. cholerae* isolé en Haïti [119]. On y retrouve enfin des imprécisions voire des erreurs manifestes, qui posent la question d'un appauvrissement du raisonnement scientifique autour de l'origine des épidémies de choléra au cours des quatre dernières décennies. Les liens entre le choléra et l'environnement sont certainement plus subtils que l'idée que s'en faisaient Pollitzer et ses contemporains. Mais bien qu'entravés par les limites technologiques de l'époque, leurs raisonnements apparaissent souvent plus en phase avec la réalité du terrain que ce que l'on peut lire parfois aujourd'hui.

La popularité du paradigme environnemental et l'enfermement de certains de ses auteurs dans une interprétation univoque de l'épidémiologie du choléra s'expliquerait-elle au moins en partie par des facteurs sociologiques ? En ce début du 21e siècle, une grande partie de la recherche est financée par projet. Dans un contexte de grandes problématiques environnementales, on peut s'attendre à ce que des projets confortant l'impact du climat sur la transmission du choléra soient attractifs. Par ailleurs, on ne peut nier la part doctrinale et quasi-religieuse qui continue aujourd'hui de structurer le monde scientifique. Les rapports de certains reviewers en sont un signe fréquent. On touche ici l'une des limites du peer-reviewing qui rend difficile la publication de points de vue iconoclastes dans les journaux les plus diffusés. Ainsi, l'évaluation bibliométrique des équipes de recherche ne les incite-elle pas à prendre des risques. Enfin, on ne saurait négliger les raisons individuelles pouvant pousser certains chercheurs à s'entêter dans une voie sur laquelle ils ont bâti leur carrière.

On en arrive ici à la dimension éthique de cette controverse. Nous avons vu les doutes qu'il était permis d'émettre concernant certains résultats présentés dans deux publications sur l'origine présumée environnementale du choléra en Haïti [120]. L'une et l'autre avaient pourtant été publiées dans deux grandes revues à comité de lecture,

[117] University of Florida, Department of Environmental & Global Health, et College of Public Health and Health Professions, « Dr. Afsar Ali and UF research team demonstrate established environmental reservoirs of toxigenic V. cholerae in Haiti ».
[118] Baron et al., « No evidence of significant levels of toxigenic V. cholerae O1 in the Haitian aquatic environment during the 2012 rainy season ».
[119] Hasan et al., « Genomic diversity of 2010 Haitian cholera outbreak strains ».
[120] Ibid.; Jutla et al., « Environmental Factors Influencing Epidemic Cholera ».

Proceedings of the National Academy of Sciences of the United States of America (PNAS) et *The American journal of tropical medicine and hygiene*. Mais comme 6 autres articles publiés précédemment dans le PNAS [121] selon la procédure de *contributed submission* [122], rien ne permet de garantir que le peer-reviewing de l'article d'Hasan en 2012 [123] fut effectivement indépendant.

Suite à notre étude sur la saison sèche 2012-2013 en Haïti, une opportunité extraordinaire nous a été donnée par l'UNICEF-Haïti de participer activement à la mise en application d'une stratégie basée largement sur nos recommandations et consistant à répondre de manière ciblée aux flambées de choléra. Dans ce cadre, j'ai passé plus de la moitié de mon temps à travailler en Haïti aux côtés des autorités sanitaires et des épidémiologistes haïtiens, de l'équipe de coordination de l'UNICEF et des ONGs partenaires implémentant le projet sur le terrain, afin de produire de la connaissance et la transcrire en action.

Malgré de très nombreuses difficultés, les premiers mois du programme ont de toute évidence été un succès. A partir de mars 2014, la dégradation du niveau de réponse aux flambées de choléra et l'incapacité de réduire la proportion de celles restées sans réponse, ont contribué à une augmentation récente de la transmission dans laquelle j'ai ma part de responsabilité.

Obstacles administratifs et budgétaires, difficultés relationnelles entre certains partenaires, manque d'engagement voire obstruction de certains acteurs clefs, contraintes géographiques entravant l'efficacité de la réponse, difficultés à rester clairvoyant lorsque l'on est plongé dans l'action, épidémie de Chikungunya à partir d'avril 2014, sont autant de facteurs expliquant l'extrême complexité rencontrée dans cette mise en place d'une stratégie pourtant simple dans son énoncé. Ce constat quelque peu amer compte tenu de l'injustice de cette épidémie et de l'énergie personnelle engagée jusque là, génère un sentiment composite et fluctuant mêlant combativité et découragement, orgueil et humilité, voire culpabilité.

[121] Zo et al., « Genomic profiles of clinical and environmental isolates of Vibrio cholerae O1 in cholera-endemic areas of Bangladesh »; Colwell et al., « Reduction of cholera in Bangladeshi villages by simple filtration »; Faruque et al., « Emergence and evolution of Vibrio cholerae O139 »; Alam et al., « Viable but nonculturable Vibrio cholerae O1 in biofilms in the aquatic environment and their role in cholera transmission »; Constantin de Magny et al., « Environmental signatures associated with cholera epidemics »; Chun et al., « Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic Vibrio cholerae ».
[122] « Proceedings of the National Academy of Sciences of the United States of America. Information for Authors - Editorial Policies | Submission Guidelines ».
[123] Hasan et al., « Genomic diversity of 2010 Haitian cholera outbreak strains ».

Mais contre cette épidémie qui traîne en longueur, la lutte se poursuit plus que jamais. En ce début octobre 2014, la saison sèche approche et les prochains mois seront déterminants. Impossible de prévoir son issue, l'évolution de l'épidémie étant devenue trop dépendante de la survenue d'évènements imprévisibles et de la qualité des réponses mises en œuvre. Si la transmission du choléra venait à se poursuivre après la fin de la stratégie UNICEF en juin 2015, serait-on capable de maintenir une telle mobilisation financière et surtout humaine ? Quel serait l'impact d'un tel échec sur le débat autour des réservoirs environnementaux de choléra en Haïti et ailleurs, et sur les futurs programmes de lutte contre le choléra mis en place dans le reste du monde ? À l'inverse, un succès constituerait un exemple éclatant à tenter de reproduire dans d'autres contextes.

5 PERSPECTIVES

Indépendamment de ces difficultés et de ces inconnues concernant le processus d'élimination du choléra en Haïti, notre travail d'épidémiologiste consiste à produire des connaissances via une recherche aussi objective et honnête que possible. Notre mission est de poser des diagnostics à l'échelle d'une population, d'en tirer des recommandations thérapeutiques, voire d'y prendre part éventuellement. À ce titre, la compréhension des épidémies de choléra présente encore de nombreuses zones d'ombre, surtout en Afrique, et les travaux à mener restent nombreux. Notre équipe a peu à peu grandi ces trois dernières années. Sandra Moore, une biologiste américaine titulaire du master de santé publique de Marseille poursuit actuellement une thèse dans le laboratoire. Elle travaille plus spécifiquement sur le typage des souches africaines par microsatellite, et collabore étroitement avec le Sanger Institute de Cambridge pour leur séquençage total. Outre sa contribution aux résultats présentés dans cette thèse, ses travaux ont jusqu'à présent plus spécifiquement porté sur la République Démocratique du Congo, et un projet est en passe de débuter avec le Ghana, le Togo et le Bénin.

Néanmoins, si les diagnostics posés et les recommandations proposées peuvent parfois apparaître simples, la mise en application de ces dernières peut s'avérer extrêmement complexe. L'évolution de la situation du choléra en Haïti est là pour le rappeler. Avant d'être traduits en actions, les concepts et les messages doivent en effet traverser une série de filtres susceptibles de ralentir voire de biaiser leur compréhension. Car en étudiant les épidémies de choléra, on touche finalement à la complexité des comportements humains, qu'ils soient individuels ou collectifs. Le choléra est en effet la maladie d'une pauvreté en déséquilibre avec son environnement immédiat, et une maladie de la mondialisation des déplacements de population. Dans un cas comme dans l'autre, s'y joue une multitude de pratiques à risque difficiles à comprendre, à anticiper, éventuellement à influencer. A la lumière de notre expérience haïtienne, ces comportements apparaissent cependant moins faire obstacle au succès des stratégies de lutte que ceux de certains chercheurs et de certains décideurs. On ne saurait négliger l'impact des intérêts, des agendas politiques, de la manière dont l'ensemble des acteurs perçoivent les connaissances produites et les traduisent en volonté et moyens d'action.

Face à ce constat, on ne peut qu'appeler les sciences sociales à intégrer et élargir davantage l'étude pluridisciplinaire des épidémies de choléra. On ne peut aussi qu'être révolté par la perpétuation d'inégalités aussi criantes que l'épidémiologie peut, parfois, modestement contribuer à soulager.

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ANNEXES

Intitulés des doctorats AMU

Mentions et Spécialités des doctorats votés en CS le 16/10/2012

ED 62 – SCIENCES DE LA VIE ET DE LA SANTE

- Biologie
  - Biochimie structurale
  - Génomique et Bioinformatique
  - Biologie du développement
  - Immunologie
  - Génétique
  - Microbiologie
  - Biologie végétale
- Neurosciences
- Pathologie humaine
  - Oncologie
  - Maladies infectieuses
  - Génétique humaine
  - Conseil en Génétique
  - Pathologie vasculaire et nutrition
  - Ethique
  - Recherche clinique et Santé Publique

ED 67 – SCIENCES JURIDIQUES ET POLITIQUES

- Droit privé
- Droit public
- Histoire du droit
- Droit
- Science politique

ED 184 – MATHEMATIQUES ET INFORMATIQUE

- Mathématiques
- Informatique
- Automatique

ED 250 – SCIENCES CHIMIQUES DE MARSEILLE

- Sciences chimiques

ED 251 – SCIENCES DE L'ENVIRONNEMENT

- Anthropologie biologique

- Ecologie
- Géosciences de l'environnement
- Génie des procédés
- Océanographie
- Chimie de l'environnement

ED 352 – PHYSIQUE ET SCIENCES DE LA MATIERE

- Astrophysique et Cosmologie
- Biophysique
- Energie, Rayonnement et Plasma
- Instrumentation
- Optique, Photonique et Traitement d'Image
- Physique des Particules et Astroparticules
- Physique Théorique et Mathématique
- Matière Condensée et Nanosciences

ED 353 – SCIENCES POUR L'INGENIEUR : MECANIQUE, PHYSIQUE, MICRO ET NANOELECTRONIQUE

- Energétique
- Mécanique et Physique des Fluides
- Acoustique
- Mécanique des Solides
- Micro et Nanoélectronique
- Génie Civil et Architecture

ED 354 – LANGUES, LETTRES ET ARTS

- Etudes anglophones
- Etudes germaniques
- Etudes slaves
- Langue et littérature chinoises
- Langue et Littérature françaises
- Littérature générale et comparée
- Arts plastiques et sciences de l'Art
- Musicologie
- Etudes cinématographiques
- Arts du spectacle

ED 355 – ESPACES, CULTURES, SOCIETES

- Géographie
- Urbanisme et Aménagement du territoire
- Préhistoire
- Archéologie

- Histoire de l'Art
- Histoire
- Sciences de l'Antiquité
- Mondes arabe, musulman et sémitique
- Etudes romanes
- Sociologie
- Anthropologie
- Architecture

ED 356 – COGNITION, LANGAGE, EDUCATION

- Philosophie
- Psychologie
- Sciences du Langage
- Sciences de l'Information et de la Communication
- Sciences de l'Education

ED 372 – SCIENCES ECONOMIQUES ET DE GESTION

- Sciences de Gestion
- Sciences Economiques
- Sciences Economiques : AMSE

ED 463 – SCIENCES DU MOUVEMENT HUMAIN

- Sciences du Mouvement Humain
- Biomécanique
- Contrôle Perceptivo-Moteur et Apprentissage
- Physiologie de l'exercice
- Sciences de l'Homme et de la Société

RÉSUMÉ
Le choléra est une diarrhée hydrique sévère volontiers épidémique. D'origine bactérienne, elle est causée par des *Vibrio cholerae* O1 fabriquant la toxine cholérique. Ses déterminants environnementaux archétypaux ont donné naissance à un paradigme couramment cité. De nombreuses stratégies récentes de lutte contre cette maladie sont apparues peu efficientes en Afrique comme en Haïti. Elles peuvent cependant être améliorées par une meilleure compréhension de la dynamique des épidémies. Ce travail de thèse comporte donc une synthèse bibliographique des influences de l'environnement sur les épidémies de choléra en Afrique, qui montre les limites du paradigme environnemental du choléra sur ce continent. S'y joint une étude multidisciplinaire suggérant fortement que l'épidémie de choléra en Guinée en 2012 fut importée par voie humaine depuis la Sierra Leone voisine. Une description spatio-temporelle du choléra au Mozambique démontre ensuite l'hétérogénéité de sa transmission et amène à questionner le concept d'endémicité du choléra. Une seconde série de travaux est consacrée à l'épidémie de choléra en Haïti. Celle-ci présente également une répartition spatiale et temporelle très hétérogène. Son importante rétractation en saison sèche et son absence d'enracinement significatif dans l'environnement laissent espérer la possibilité d'une élimination rapide à condition d'apporter une réponse ciblée à tous les foyers épidémiques du pays. En dépit de débats persistants autour de l'existence de réservoirs environnementaux en Haïti, qu'ils aient précédé l'apparition de l'épidémie en octobre 2010 ou se soient constitué depuis, une stratégie d'élimination basée sur nos recommandations est actuellement menée par le Ministère de la Santé d'Haïti, l'UNICEF et leurs partenaires. Après des résultats spectaculaires en 2013 et au premier semestre 2014, les efforts semblent s'essouffler et la situation se détériore à nouveau. L'élimination effective du choléra dans saison sèche à venir demeure cependant réaliste si nous parvenons à convaincre et remobiliser les acteurs de terrain.

Mots clefs : choléra, *Vibrio cholerae*, épidémiologie, environnement, Afrique, Guinée, Mozambique, Haïti, élimination

ABSTRACT
Cholera is an epidemic acute watery diarrhea. It is caused by toxigenic bacteria *Vibrio cholerae* O1, and its environment determinants have been at the source of a popular paradigm. Many recent control strategies have shown little efficiency in Africa or in Haiti. They can however be improved by a better comprehension of the epidemics dynamic. This thesis work includes a bibliographic synthesis about environment influences on cholera in Africa, which highlights the limits of the cholera environmental paradigm on this continent. A multidisciplinary study is presented, which suggests that the 2012 cholera epidemic in Guinea was humanly imported from nearby Sierra Leone. A space-time description of cholera in Mozambique demonstrates heterogeneous transmission patterns and challenges the concept of cholera endemicity. A second part is dedicated to the current cholera epidemic in Haiti. Its transmission also exhibits marked spatio-temporal heterogeneity. Cholera important retraction during the dry season and its absence of significant establishment in the Haitian environment suggest it may be possible to rapidly eliminate cholera in the country, provided that every outbreak focus receives a targeted response. In spite of persisting debates about environmental reservoirs that may have preceded the onset of cholera in October 2010, or may have settled in the meanwhile, an elimination strategy based on our recommendations is currently implemented by Haitian Ministry of Health, UNICEF and their partners. After spectacular results in 2013 and during the first half of 2014, efforts seem to stall and the situation is slowly deteriorating again. Cholera elimination during the coming dry season remains realistic provided that we succeed in persuading and remobilizing the partners present on the field.

Key words: cholera, *Vibrio cholerae*, epidemiology, environment, Africa, Guinea, Mozambique, Haiti, elimination