RESEARCH ARTICLE

Phylodynamic and Evolutionary analysis of Rotavirus G12 in Africa

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Keywords: Rotavirus, Virus Evolution, Africa.

Short Title: Molecular evolution of African Rotavirus G12

Significance of the study

Rotavirus genotype G12 has a recent history, and has spread rapidly causing outbreaks of acute gastroenteritis across Africa. To further understand the mechanisms behind its rapid spread, we investigated the Evolutionary dynamics, Phylogeny and population demography of the VP7 gene among African strains. Our study revealed that African G12 rotaviruses have diversified into 3 potential clades within their parental lineage III based on geographic boundaries. We also discovered that they have a constant demographic profile. Our findings reveal the potential for rapid genetic diversity of Rotavirus G12 and highlight the importance of molecular surveillance in Rotavirus control effort.

Abstract

***Objective*:** Rotavirus genotype G12 has spread globally and has caused outbreaks across Africa. It has become one of the most prevalent genotypes of rotavirus in Africa in recent years. To further understand the drivers for its genetic diversity and rapid spread we investigated the Bayesian phylogeny, viral evolution and population demography of Rotavirus genotype G12 in Africa. ***Methods*:** We downloaded full and partial VP7 gene sequences of Rotavirus genotype G12, from twelve African countries (n=96). We aligned the African G12 sequences with seven global reference G12 sequences. Phylogenetic analysis was carried out using MEGA Vs 6, Evolutionary analysis and Bayesian Phylogeny was carried out, using Markov Chain Monte Carlo (MCMC) implemented in BEAST. ***Results*:** Phylogenetic analysis revealed that all the African sequences fell into lineage III diversifying into two major clades. The evolutionary rate of the African rotavirus G12 sequences was estimated to be $1{,}678\times10^{-3}$, with 95% high posterior density interval (95%HPD) of 1.201×10^{-3} to 2.198×10^{-3} nucleotide substitutions/ site/ year. The time to most recent common ancestor $T_{MRC,}$ was 16.8, 95%HPD (15.0 -25.54). The MCMC tree topology clustered into three lineages (II, III, IV), with all the African trains falling into lineage III, and further diversified into three clusters within lineage III. The demographic history of the African G12 viruses was estimated using BSP model, and revealed a constant population dynamic. ***Conclusions*:** We have shown the potential for genetic diversification of Rotavirus genotype G12 in Africa. We recommend the adoption of Molecular surveillance across Africa to further control spread and diversification of Rotavirus.

Introduction

Group A rotavirus (RVA) has been established to be the main agent responsible for acute gastroenteritis (AGE) among children and infants worldwide. In 2016, it was reported to be responsible for about 128,000 deaths with over 2/3 of cases occurring in sub-Saharan Africa [Troegger et al, JAMA 2018]. There are two life attenuated rotavirus vaccines, Rotarix and Rotateq, which have been licensed for use in many countries after large phase 3 clinical trials were conducted in 2006 [Ruiz-Palacious 2006, Vesikari 2006]. In 2009 the world health organisation WHO, recommended the global use of the 2 live attenuated vaccines Rotarix and Rotateq. In Africa, several countries have included rotavirus vaccination in their EPI programs [Jonestellar et al, CID 2017,].

Rotavirus belongs to the virus family Reoviridae, it is a non-enveloped and has an icosahedral nueleocapsid structure, enclosing a double stranded (ds) RNA genome segmented into 11 compartments. The rotavirus RNA genome codes for six structural proteins, (VP1 to VP4, VP6 and VP7) and five/ six nonstructural proteins (NSP1 to NSP5/6) [Estes, 2013]. There are at least 10 distinct species/groups (A- I, J), differentiated by their VP6 antigenic properties [Banyai et al, 2017]. There are 32 G (VP7) genotypes and 47 P (VP4) genotypes identified through molecular epidemiologyhttps://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/7th-RCWG-meeting. update of the Rega Institute, KU Leuven, Belgium.

Molecular epidemiology has identified the widespread circulation of various genotypes of rotavirus in Africa, the globally prevalent genotypes G1P[8], G3P[4] have been reported in various African countries [Mwenda et al, 2018, Simwaka et al, 2018, Lartey et al, 2018, Moure et al, 2018, Motayo et al, 2018]. However these globally prevalent genotypes are being replaced by more recent genotypes such as G9P[8], G8P[4], G12P[6], G12P[8] [Japhet et al, 2013]. There have also been recent reports of widespread outbreaks in parts of West Africa such as Nigeria attributed to RVA G12 strains [Japhet et al, 2013, Inario et al, 2015, Japhet et al, 2017]. Report has also suggested the rapid evolution of RVA genotype G12 as a panacea for its global spread [Mattehijnssens et al, 2010]. The rapid emergence of this genotype in Africa has not been well understood, however mechanisms such as interspecies recombinations, RNA polymerase infidelity and accumulated point mutations have been attributed to rotavirus evolution [Ramman et al 2006, Matthijnsses et al, 2011, Ramman et al 2010, Estes 2013]. In other to answer some of the questions arising from the rapid spread of RVA genotype G12 in Africa, we investigated the Bayesian phylogeny and evolutionary dynamics of RVA genotype G12 strains in Africa.

Methods

Dataset

A total of 96, partial VP7 genotype G12 gene sequences spanning a period of 1998 to 2013 from twelve African countries (8 Nigerian, 11 Cameroonian, 4 Togolese, 12 Democratic republic of Congo, 3 Kenya, 12 Mozambique, 2 Ethiopia, 3 Malawi, 4 Ugandan, 17 Burkina Fasso, 1 Egypt, 1 Mauritius), were downloaded from Genebank with the help of the Rotavirus resource database. Along with these were seven reference VP7 rotavirus genotype G12 genome sequences from Argentina, Thailand, Korea, and Japan. The total number of sequences in the dataset was 103.

Phylogenetic analysis

Sequence data was edited and assembled using CLUSTALW software. Phylogenetic trees were constructed in Mega version 6.0 software using Maximum likelihood and Neighbor joining methods with p distance model and 1000 bootstrap replicates www.megasoftware.net.

Evolutionary rate, time scaled Phylogeny and Population dynamic analysis

Evolutionary and additional phylogenetic analysis was carried out on African Rotavirus genotype G12 strains, using a Bayesian evolutionary approach using Markov Chain Monte Carlo (MCMC) implemented in BEAST version 1.8 [Drummond et al, 2009]. For the MCMC run, a total of 103 rotavirus virus G12 sequences were aligned, consisting 96 African sequences (8 Nigerian, 11 Cameroonian, 4 Togolese, 12 Democratic republic of Congo, 3 Kenya, 12 Mozambique, 2 Ethiopia, 3 Malawi, 4 Ugandan, 17 Burkina Fasso, 1 Egypt, 1 Mauritius) also included are 2 reference sequences from Argentina, 2 from Japan one each from Thailand and Korea, and one Porcine G12 strain. A list of the sequences used for the analysis is contained in supplementary table 1. Several models with different priors were initially evaluated strict and relaxed molecular clock models, constant population size, exponential and two non-parametric models of population changes, Bayesian Skyride plot and Gausian Markov Random Field Skyride plot. Each selected model was run for an initial 30,000,000 states. Models were then compare using the Bayes factor with marginal likelihood estimated using the path sampling and stepping stone methods implemented in BEAST version 1.8 [Drummond et al, 2009]. Further analysis was then done using the relaxed clock with Bayesian Skyride plot coalescent prior. The MCMC run was set at 100,000,000 states with a 10% burn in. Results were visualised in Tracer version 1.8. (http://tree.bio.ed.ac.uk/software/tracer/). The effective sampling size (ESS) was calculated for each parameter, all ESS values were > 200 indicating sufficient sampling. Bayesian skyride analysis was carried out to visualise the epidemic evolutionary history using Tracer vs 1.8. (http://tree.bio.ed.ac.uk/software/tracer/). The maximum clade credibility tree was selected from the posterior tree distribution after 10% burn-in using TreeAnnotator vs 1.8. (http://beast.bio.ed.ac.uk/TreeAnnotator/) and a time scaled MCC tree was visualized in FigTree vs 1.4.

Results and Discussion

We report the evolutionary dynamics and phylogeny of rotavirus genotype G12 in Africa. Rotavirus genotype G12 has been reported globally, it was also reported that a single lineage of this genotype is responsible for its rapid global spread (Matthijnssens et al 2009, Matthijnssens et al 2010). We analysed, 96 partial rotavirus genotype G12 VP7 sequences from 13 African countries along with 7 global reference sequences. Phylogenetic analysis of the sequences revealed that all the African sequences fell into lineage III diversifying into two major clades, the West African clade highlighted in sky blue, and the East/South African clade highlighted in grey. Lineage 2 reference isolates are indicated in Red, while the lineage IV Porcine isolate is indicated in bright green (Figure 1). Our results show that African rotavirus G12 isolates have evolved into two sub lineages defined largely by geographical location. The reason for this genetic diversification was not investigated, although factors such as natural barriers to mass migration of human population such as the high mountain ranges of Central and East Africa, as well as the Sahara desert could serve as major factors. A similar observation of geographically bound genetic diversification was reported in a recent study of Lassa fever virus in Nigeria 2018 (Siddle et al, 2018), where genetic diversification was restricted by rivers which acted as barriers to the cross migration of rodent reservoirs of the virus.

Majority of RNA viruses have been reported to have an evolutionary rate of between 1.0×10^{-3} to 1.0×10^{-6} subs/site/year (Jenkins et al 2002). The evolutionary rate of the analysed African rotavirus G12 sequences was estimated to be $1{,}678\times10^{-3}$, with 95% high posterior density interval (95%HPD) of 1.201×10^{-3} to 2.198×10^{-3} nucleotide substitutions/ site/ year (subs/site/year). This is slightly lower than the evolutionary rate of 1.45×10^{-3}, and 1.89×10^{-3} of rotavirus genotypes G2, and G9 reported in a global study of rotavirus molecular evolution in 2010 (Matthijnsssis et al, 2010, Denis et al, 2014). The evolutionary rate observed in our study hovers around the global rate previously reported for rotavirus genotype G12 in 2010 (Matthijnssis et al, 2010), this shows that the African strains have a steady mutation rate but possess the potential to diversify rapidly through molecular evolution. This can also be due to repeated outbreaks in populated communities, giving opportunity for recombination and reassortment (Gomez et al, 2014, Japhet et al, 2018). The time to most recent common ancestor $T_{MRC,}$ was calculated to be 16.8 years, 95% HPD (15.0 – 25.54), dating back to around mid-1996. The time scaled MCMC tree topology clustered into three lineages (II, III, IV), with all the African trains falling into lineage III. The African G12 strains further diversified into three sub-clades within lineage III, the West African, East African and South African clusters as shown in figure 2. The porcine G12 isolate was the only lineage IV strain, while the reference strains from Argentina, Thailand and Korea fell into lineage II. From the MCMC tree the diversification of the 3 clusters within the African lineage III isolates occurred around the same time, between the year 2003 and 2004. The MCMC tree topology follows a similar trend with the neighbour phylogeny shown in Figure 1, further buttressing the earlier mentioned observation of geographically bound diversification of African rotavirus genotype G12 VP7 genes.

The demographic history of the African G12 viruses estimated through the BSP model, suggested that the genotype experienced a constant population dynamic (constant effective number of infections) after which began a gradual decrease in its population size around the year 2007. This is an indication of that the genotype appeared in Africa about the same time when advocacy for mass vaccination against rotavirus was adopted in many countries. Towards the end of the first decade in the new millennium some African countries have started already institutionalized rotavirus vaccination into their childhood immunization programs [Yen et al, 2011]

Conclusions

We have shown that Rotavirus genotype G12 has diversified based on geographical locations. There is also tendency for further diversification due to its evolutionary rate and fast spread. However the population dynamic of the genotype in Africa seems to be gradually declining. This shows the positive impact of Universal routine vaccination which has been implemented in some African countries, and is being advocated for in others. We recommend the adoption of Molecular surveillance across Africa to further control spread and diversification of Rotavirus.

Figure Legends

Figure 1. Phylogenetic tree of Rotavirus genotype G12 partial VP7 gene sequences. Tree was constructed using the Neighbour joining algorithm, with 1000 bootstrap replicates using MEGA version 6.0 software and visualised in Figtree. The West African cluster is highlighted in sky blue, the East/South African cluster highlighted in grey. Lineage 2 reference isolates are indicated in Red, while the lineage IV Porcine isolate is indicated in bright green. Scale bar is indicates number of substitutions per site.

Figure 2. Time scaled Bayesian MCMC tree of African Rotavirus virus VP7 genotype G12 sequences. Nigerian strains are indicated in Blue, Burkina Fasso strains are indicated in Sky blue, Cameroonian strains are indicated in purple, Democratic Republic of Congo are indicated in Green, Zambian strains are in Pink. South African strains are indicated in Brown, Mozambique are indicated in Ash, Kenyan strains are indicated in Bright green. The reference porcine strain is indicated in Orange. The coloured bare represent the identified clade within lineage III (Blue horizontal bar represents the West African clade, Ash coloured bar represents the East African clade, while the Pink bar represents South African clade.). The yellow shaded region represents reference sequences in lineage II, other lineage are indicated at the side of the sequences.

Figure 3. Bayesian skyplot reconstruction of African Rotavirus genotype G12 strains, showing the median exponential growth line, with the blue solid area representing the 95% HPD for the growth.

Funding statement

The authors did not receive and grants or funding for this study.

Conflicts of interest

The authors declare that they have no conflicts of interest

Supplementary materials description

Supplementary table list the names and ascension numbers of the sequences downloaded from Genbank utilised in the analysis of the data in this study.

References

Bányai K, Kemenesi G, Budinski I, Földes F, Zana B, Marton S, Varga-Kugler R, Oldal M, Kurucz K, Jakab F. Candidate new rotavirus species in Schreiber's bats, Serbia. Infect Genet Evol. 2017 Mar;48:19-26.

Dennis FE, Fujii Y, Haga K, et al. Identification of novel Ghanaian G8P[6] humanbovine reassortant rotavirus strain by next generation sequencing. PLoS One 2014; 9:e100699.

M. K. Estes and H. B. Greenberg, "Astroviridea," in *Fields Virology*, D. M. Knipe and P. M. Howley, Eds., vol. 6, Lippincot Williams andWilkins, 2013.

Gomez M.M., Resque H.U., Volotao E.M., Rose T.U et al. Distinct evolutionary origins of G12P[8] and G12P[9] group A rotavirusstrains circulating in Brazil. Infect. Genet. Evol. 2014. 28: 385-388.

G. Ianiro, R.Delogu, M. Baba et al., "Molecular characterization of group A rotavirus strains detected in children with diarrhea admitted to Nigerian hospitals in 2013," *Archives of Virology*, vol. 160, no. 6, pp. 1511–1517, 2015.

M. O. Japhet, O. Famurewa, M. Iturriza-Gomara et al., "Group A rotaviruses circulating prior to a national immunization programme in Nigeria: Clinical manifestations, high G12P[8] frequency, intra-genotypic divergence of VP4 and VP7," *Journal of Medical Virology*, vol. 90, no. 2, pp. 239–249, 2018.

M. O. Japhet, O. A. Adesina, O. Famurewa, L. Svensson, and J. Nordgren, "Molecular epidemiology of rotavirus and norovirus in Ile-Ife, Nigeria: high prevalence of G12P[8] rotavirus strains and detection of a rare norovirus genotype," *Journal of Medical Virology*, vol. 84, no. 9, pp. 1489–1496, 2012.

Jenkins GM, Rambaut A, Pybus OG, Holmes EC. 2002. Rates of molecular evolution in RNA viruses: a quantitative phylogenetic analysis. J Mol Evol. 54:156–165.

C. L. Jonesteller, E. Burnett, C. Yen, J. E. Tate, andU.D. Parashar, “Effectiveness of rotavirus vaccination: a systematic review of the first decade of global postlicensure data, 2006-2016,” *Clinical Infectious Diseases*, vol. 65, no. 5, pp. 840–850, 2017.

Lartey BL, Damanka S, Dennis FE, Enweronu-Laryea CC, Addo-Yobo E, Ansong D, Kwarteng-Owusu S, Sagoe KW, Mwenda JM, Diamenu SK, Narh C, Binka F, Parashar U, Lopman B, Armah GE. Rotavirus strain distribution in Ghana pre- and post- rotavirus vaccine introduction. Vaccine. 2018 Nov 12;36(47):7238-7242.

Matthijnssens J, Bilcke J, Ciarlet M, Martella V, Banyai K, Rahman M, Zeller M, Beutels P, Van Damme P, Van Ranst M. 2009. Rotavirus disease and vaccination: impact on genotype diversity. Future Microbiol. 4:1303–1316.

Matthijnssens J, De Grazia S, Piessens J, Heylen E, Zeller M, Giammanco GM, Bányai K, Buonavoglia C, Ciarlet M, Martella V, Van Ranst M. Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. Infect Genet Evol. 2011 Aug;11(6):1396-406.

Moure UAE, Banga-Mingo V, Gody JC, Mwenda JM, Fandema J, Waku-Kouomou D, Manengu C, Koyazegbe TD, Esona MD, Bowen MD, Gouandijka-Vasilache I. Emergence of G12 and G9 rotavirus genotypes in the Central African Republic, January 2014 to February 2016. BMC Res Notes. 2018 Jan 5;11(1):5.

Mwenda JM, Parashar UD, Cohen AL, Tate JE. Impact of rotavirus vaccines in Sub-Saharan African countries. Vaccine. 2018 Nov 12;36(47):7119-7123.

Rahman M, Matthijnssens J, Yang X, Delbeke T, Arijs I, Taniguchi K, Iturriza-Gomara M, Iftekharuddin N, Azim T, Van Ranst M. 2007. Evolutionary history and global spread of the emerging G12 human rotaviruses. J Virol. 81:2382–2390.

Rahman M, Matthijnssens J, Saiada F, Hassan Z, Heylen E, Azim T, Van Ranst M. Complete genomic analysis of a Bangladeshi G1P[8] rotavirus strain detected in 2003 reveals a close evolutionary relationship with contemporary human Wa-like strains. Infect Genet Evol. 2010 Aug;10(6):746-54.

Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, et al. (36 coauthors). 2006. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 354:11–22.

Siddle K., Eromom P., Barnes K.G., Mehta S. et al. Genomic analysis of Lassa fever during an increase in cases in Nigeria in 2018. N. Engl. J. Med.2018.379: 1745-1753.

Simwaka JC, Mpabalwani EM, Seheri M, Peenze I, Monze M, Matapo B, Parashar UD, Mufunda J, Mphahlele JM, Tate JE, Mwenda JM. Diversity of rotavirus strains circulating in children under five years of age who presented with acute gastroenteritis before and after

rotavirus vaccine introduction, University Teaching Hospital, Lusaka, Zambia, 2008-2015. Vaccine. 2018 Nov 12;36(47):7243-7247.

C. Troeger, I. A. Khalil, P. C. Rao et al., “Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years,” *JAMA Pediatrics*, 2018.

Vesikari T, Matson DO, Dennehy P, et al. (28 co-authors). 2006. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med. 354: 23–33.

Yen C., Tate J.C., Patel M.M., Cortese M.M. et al. Rotavirus vaccines: update on global impact and future priorities. Humm. Vaccin. 2011.7(12): 1282-90.

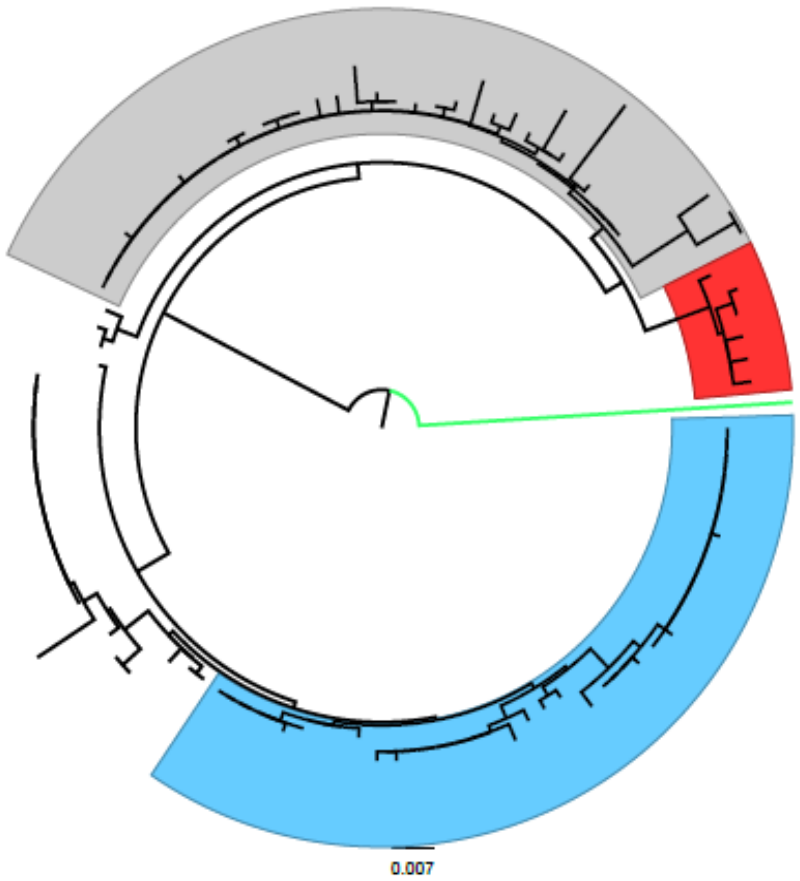

Figure 1

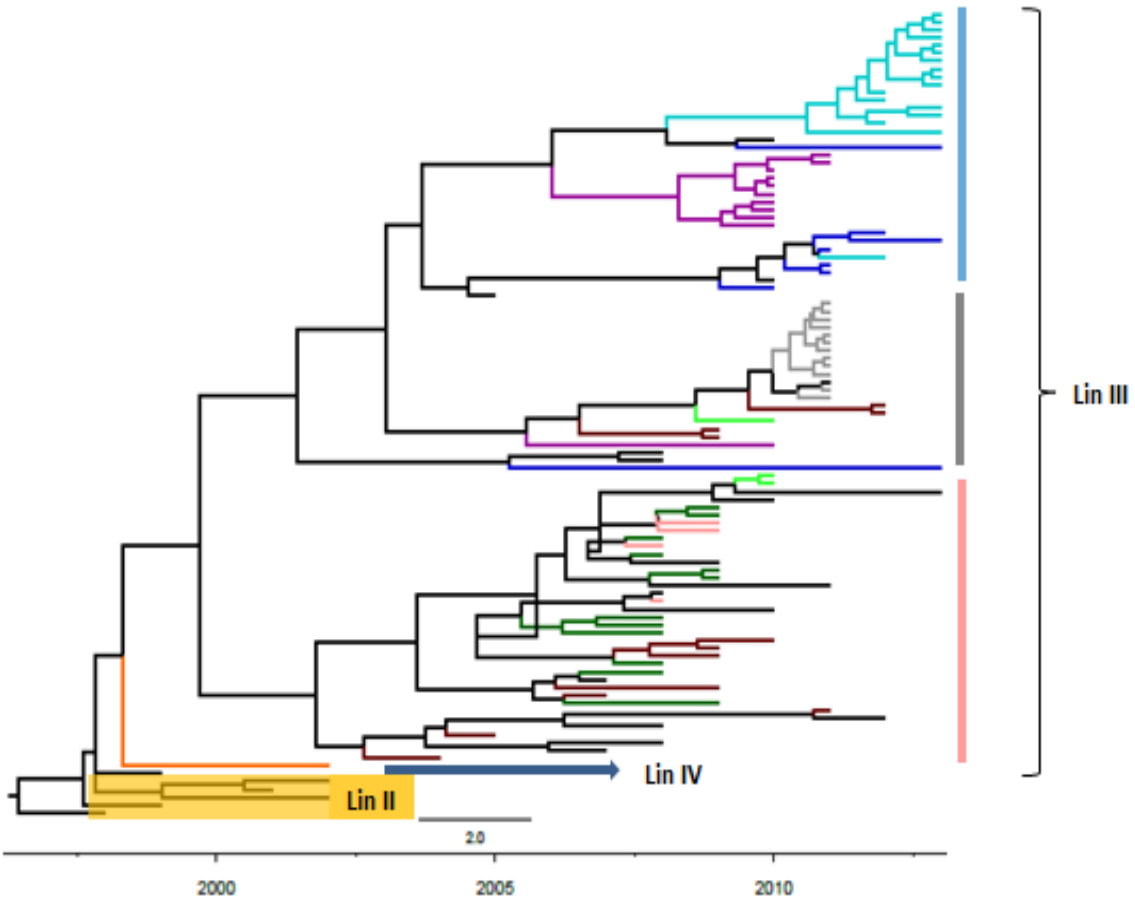

Figure 2

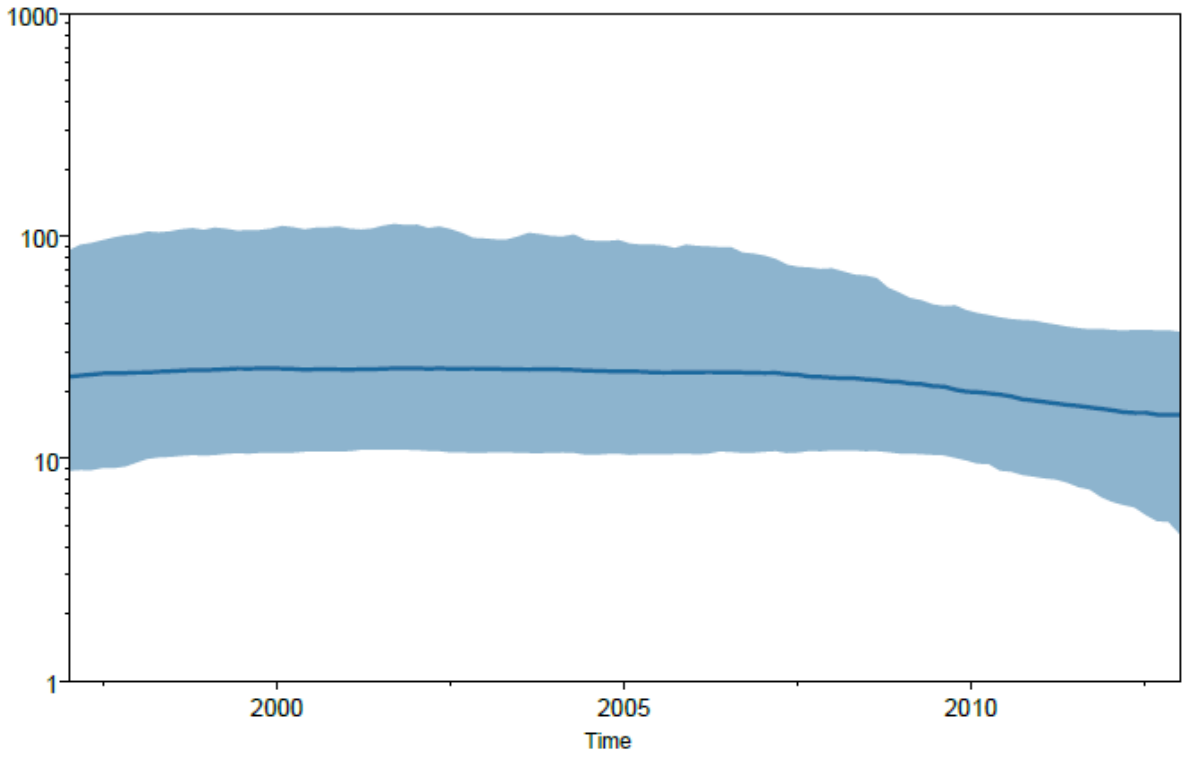

Figure 3