Evolutionary models: applications

Examples from Clinical Virology

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Case 1

Mystery illness causes paralysis in young Namibian adults
Panic is sweeping through suburbs north of Katutura (Windhoek) after 3 people died and 19 others were hospitalised with a disease that still has to be identified.

A press release from the Ministry of Health and Social Services last night indicated that the disease was not confined to the Khomas Region, and that cases of "undiagnosed paralysis" among adults had been reported in the Otjozondjupa and Hardap regions.

A media briefing is scheduled for this morning to reveal information related to the outbreak.

Well-placed hospital sources confirmed yesterday that 2 other people were fighting for their lives in the Intensive Care Unit (ICU) of the Windhoek Central Hospital after contracting the mystery disease.”
Another case of a ‘strange’ disease in Namibia

- 39 year old man from Aranos
- He complained of stomach pain, fever, weakness in his legs on 8 May 2006
- He was admitted in Windhoek with leg weakness and had to be intubated for respiratory failure (could not breathe adequately). He was ventilated and subsequently died after more than a week in intensive care.
- Prior history: He was admitted from his farm in Aranos on 25 April for a cholecystectomy on 27 April, done in a Windhoek private hospital.
- Stool specimen collected on the 15th of May was cultured for poliovirus – ‘wild type’ poliovirus was isolated
Could this be Polio?

- Namibia had been poliovirus free since 1995
- The only link between the ‘mystery illness’ in Katutura and the Aranos case was the man’s prior visit to Windhoek
- Poliovirus is usually a disease of children, but almost all cases in Katutura were young adult males
Experts are convinced it is not Polio

- Of the initial 34 cases with **acute flaccid paralysis** …
  - Age range between 5 and 76 years
  - 13 of the 18 cases with age indicated (72%) were aged between 20 and 35 yrs
  - Age: highly unusual for polio
  - Concentration around Windhoek
- Unusually high death rate: 17/136
- Experts said – this is not Poliovirus – the Aranos case is unrelated (they did not know of his short stay in Windhoek)
- Next page: Map of prior poliovirus circulation
As of 1 February 2006, Egypt and Niger were no longer considered endemic for wild poliovirus because neither country had indigenous transmission during the preceding 12 months. – Au 1er février 2006, l’Égypte et le Niger n’étaient plus considérés comme des pays où le poliovirus sauvage est endémique car aucune transmission indigène n’a été signalée au cours des 12 mois précédents.

The designations employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

Les désignations utilisées sur cette carte et la présentation des données qui y figurent n’impliquent, de la part de l’Organisation mondiale de la Santé, aucune prise de position quant au statut juridique de tel ou tel pays, territoire, ville ou zone, ou de ses autorités, ni quant au tracé de ses frontières.
The outbreak unfolds
Poliovirus detected

- Poliovirus-1 was isolated in 19 cases in Namibia
- All older than 14 years of age (14-59y)
- 79% were 15-19 years old
- 17/19 (89%) were male
- Explanation for this unusual outbreak
  - History of vaccination
  - Migration
  - Women care for children and may be indirectly vaccinated (children wearing nappies excrete vaccine virus)
Phylogenetics of Poliovirus

• Where did this virus come from?
• There are three polioviruses: PV-1,2 and 3
• An important structural gene, the VP1 gene (906 nucleotides) was sequenced
Findings from phylogenetic analysis

• All isolated viruses were PV-1 and were closely related
• Poliovirus molecular clock yields a 1% change per year
• Viruses had a 2.5% difference from parental Indian strains indicating that the virus had been in Africa for at least 2.5 years
• The Namibia viruses matched closely with a strain isolated in Angola
• The viruses belonged to the South Asia Strain Polio virus 1 group (SOAS) – originating from India
Another poliovirus tree: SOAS introduced into Australia

Role of phylogenetics in outbreaks

• How will phylogenetic trees compare?
  – A viral strain that has recently been imported into a country
  – A viral strain that has been circulating for a long period
• How can one trace the source of the outbreak?
• Why is this important for outbreak control?
References


• World Health Organisation. Weekly Epidemiological Record. 2006, 81, 425–432

• Gert van Zyl. The Namibian polio outbreak: re-introduction into fertile soil. SA Fam Pract 2006:48(7)
Case-2

Largest hospital-related HIV outbreak ever recorded:

*Foreign health care workers, working in Libya accused of deliberately infecting their patients*
Foreign doctors accused of deliberately infecting children in Al-Fateh Hospital (Benghazi, Libya) in 1998

Largest hospital acquired outbreak of HIV in history
The story

- World Organization, Libya investigates hospital acquired outbreak
- > 400 children infected with HIV
- Specimens sent for analysis in September 1998
- 6 Foreign HCWs: 5 Bulgarian Nurses and 1 Palestinian Doctor accused of deliberately infecting the children (these HCWs all arrived from March 1998 to August 1998)
CONCISE COMMUNICATION

Nosocomial Outbreak of Multiple Bloodborne Viral Infections

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In resource-limited countries, nosocomial transmission of bloodborne pathogens is a major public health concern. After a major outbreak of human immunodeficiency virus (HIV) infection in ∼400 children in 1998 in Libya, we tested HIV, hepatitis C virus (HCV), and hepatitis B virus (HBV) markers in 148 children and collected epidemiological data in a subgroup of 37 children and 46 parents. HIV infection was detected in all children but one, with HCV or HBV coinfection in 47% and 33%, respectively. Vertical transmission was ruled out by analysis of parents’ serology. The children visited the same hospital 1–6 times; at each visit, invasive procedures with potential blood transmission of virus were performed. HIV and HCV genotypic analyses identified a HIV monophyletic group, whereas 4 clusters of HCV sequences were identified. To our knowledge, this is the largest documented outbreak of nosocomial HIV transmission.
Report in JID

- No epidemiological data except that the children ‘were infected during 1998’ were provided by WHO in Lybia
Two of the world’s foremost HIV experts Vittorio Colizzi and Luc Montagnier investigated the outbreak: “Colizzi and Montagnier were able to obtain blood samples and medical records from the children, examine the hospital, and interview its staff.

It soon became apparent, says Colizzi, that “this is a classic nosocomial infection” in which tainted blood is accidentally passed between patients through poor hygiene practices, such as the reuse of disposable syringes and catheters, insufficient sterilization of instruments…”

Court overrules scientific evidence

• Libyan court disregarded the ‘Western’ evidence and only considered the ‘evidence’ from Libyan scientists that supported the claims of deliberate infection

• All 6 health care workers were sentenced to death (for second time) on 19 Dec 2006 for deliberately infecting children

• The Libyan scientists’ arguments were based on a poor understanding of phylogenetics and a premise of discounting that the poor infection control could account for such a large outbreak

-Libyan Journal of Medicine (O. Bagasra 2007)
Advanced phylogenetics to the rescue

de Oliveira, Tulio, et al. Molecular Epidemiology: HIV-1 and HCV sequences from Libyan outbreak. Nature 444.7121
Methods

• HIV-1 $gag$ gene sequences from 44 children
• 61 Hepatitis C Virus $E1E2$ gene sequences
• Phylogenies were estimated and assessed using algorithmic, Bayesian and maximum-likelihood methods
Figure 1 | HIV-1 and HCV sequences from 1998 Al-Fateh Hospital (AFH) outbreak. a–c, Estimated maximum-likelihood phylogenies for HIV-1 CRF02_AG (a), HCV genotype 4 (b) and HCV genotype 1 (c). Source of sequences used for analysis: AFH, red; Egypt, green; Cameroon, blue. Black circles mark the common ancestor of HCV subtype 4a and 1a; numbers above AFH lineages give clade support values using bootstrap and bayesian methods, respectively. Scale bar units are nucleotide substitutions per site. For visual clarity, AFH clusters are represented by triangles and some non-informative reference strains are excluded.
Results

- HIV-1 sequences: Monophyletic cluster within CRF02_AG (a circulating recombinant form of HIV)
- The cluster is closest to West African reference strains
- HCV sequences: Three monophyletic clusters - related to Egyptian 4a strains
- Evolution was analysed using an established Bayesian Markov chain Monte Carlo approach
- Irrespective of model used the most recent common ancestor predated March 1998 when the accused HCW’s arrived at the Al-Fateh Hospital
- It suggested that the Al-Fateh Hospital had a long-standing *infection control* problem
Figure 2 | Estimated dates of the most recent common ancestor for each cluster. Results obtained by using different evolutionary models. Vertical lines show the 95% highest posterior density intervals. Red line shows time of arrival of the foreign staff in March 1998. For further details, see supplementary information. ‘Const’, constant size; ‘Expo’, exponential growth.
Phylogenetics in Forensics

• Importance of established methodology
• Timing of transmission events
• Model bias could be excluded by concordance of different models
• The importance of clusters in providing evidence of common source outbreaks!
The aftermath of the Libyan case

• Accused were sentenced to death for the second time in 19-Dec-2006
• Extradited to Bulgaria, where they were later released
• Libya complained about the release
• In February 2011 the resigned minister Mustafa Abdel-Jalil alleged that “Gaddafi and his government were entirely responsible for the infection of the children with HIV”
• The reason for the extremely high rate of transmission has not been resolved in literature but is not inconceivable (HIV transmission is highly variable and dependant on viral load and nature of exposure)
Sofia airport: Bulgarian president Georgi Parvanov pardons the medical team convicted of infecting Libyan children with HIV.

References

• Omar Bagasra, Mohammed Alsayari, Rebecca Bullard-Dillard and Mohamed A Daw. The Libyan HIV Outbreak: How do we find the truth? Libyan J Med 2007
• de Oliveira, Tulio, et al. "Molecular Epidemiology: HIV-1 and HCV sequences from Libyan outbreak." Nature 444.7121
Case 3

Influenza: Phylogenetics, evolution and selection pressure
ssRNA in 8 segments

- highly mutable
  (lack of proofreading by RNA polymerase)

Picture by Prof Wolfgang Preiser
Annual influenza

- Three influenza strains are causing annual epidemics
- Influenza uses haemagglutinin (HA) to bind to and infect cells in the patient’s airway, whereas neuraminidase (NA) plays a role in release from the ‘mother cells’ to infect a new generation of cells.
- When the host (human) forms antibodies against HA and NA proteins these could prevent the virus from infecting cells
- Immunity towards influenza is based on these ‘neutralising’ antibodies
- After each seasonal epidemic many people who became infected have neutralising antibodies and are therefore not susceptible to the circulating strain(s)
Immune pressure

• There is immune system selection pressure on the HA and NA genes
• Mutations in HA or NA could either be ‘detrimental’ – limit the fitness of the virus or be ‘beneficial’ by resulting in escape from antibody pressure
• Some variants that are both fit and have the ability to escape the immune pressure have a survival advantage
• There is therefore selection for and enrichment for these immune escape variants
• During summer there are only few cases of influenza infection (transmission rates are lower than in winter)
• Therefore only a few variants survive the combined immune pressure and unfavourable conditions during summer (or are re-introduced from other countries during the following winter season)
• Influenza viruses therefore evolve along various bottle-necks and expand again during a favourable winter season
Figure: Immune escape of influenza viruses

Evolutionary bottle-neck

Immune pressure AND unfavourable conditions

- Original viral population
- Immune escape variants after selection

Time
Influenza Phylogenetic Tree

Bush RM, Bender CA, Subbarao K, Cox NJ, Fitch WM. Predicting the evolution of human influenza A.
Influenza exposed to frequent bottlenecks

- Influenza phylogenetics are used to decide on the selection of each year’s vaccine strains (in order to give the best immune protection)
- How does the influenza tree compare to the HIV-1 CRF02_AG tree in case 2?
- What kinds of pressures are infectious agents exposed to?
  - Host immunity
  - Functional and structural constraints
  - Anti-infective drug pressure
- Where on the tree do you find the ‘old’ versus the current circulating strains?
References

Thank You

Questions?