Molecular epidemiology of rabies in KwaZulu Natal, South Africa

Peter Coetzee

University of Pretoria
Molecular epidemiology

- **Molecular epidemiology**: Scientific field of study in which molecular markers (unique patterns of nucleotide or amino acid substitutions) are used to track the diversity, and routes of dissemination of a pathogen within and between susceptible host populations.

- **Aim**: Identifying factors which influence the incidence, occurrence and spread of a disease
Molecular epidemiology - Basic principles

- **Mutations** – random errors caused during genome replication.
- **Viral mutation** up till a million times higher than mutations in Eukaryotes
- **Reason:** RNA-dependant RNA polymerases lacks proofreading function.
- **Viral population** - quasispecies.
- **Genetic drift, neutral mutation** - evolution of geographic variants.
Molecular epidemiology - Basic principles

- Genetic typing techniques
- Molecular sequence data
  - Quantitative measurement of relatedness between viral variants
  - Construction of phylogenetic trees which allow for the visualization of evolutionary relationships between taxa (phylogenetic characterization).
Molecular epidemiology – Methodology

- Essential to select correct region of genome for characterization
- Rates of mutation vary from gene to gene – selective pressures can either constrain or promote evolution
- Ideal target: region with maximum genetic information flanked by two regions of low variation - primer sites
Molecular epidemiology – Methodology

- RT-PCR, automated fluorescent nucleotide sequencing, multiple alignment, phylogenetic analysis.
- Distance based methods - NJ
- Sequence based methods – MP, ML
- Publications: NJ – simplest, fastest, most commonly used, genetic distances indicated on tree
Molecular epidemiology – Methodology

- Multiple trees with varying topology may be reconstructed from a given dataset – essential to test robustness of reconstructed tree topologies.

- Bootstrap tests:
  1. Multiple random re-sampling of nucleotides from multiple sequence alignment.
  2. New tree constructed from every pseudo-replicate dataset.
  3. Number of times a specific grouping is reconstructed from total number of datasets – statistical support for clustering of isolates in a specific monophyletic group

- 80-100% - strong support for grouping of isolates
- Below 70% - generally considered non-significant
KwaZulu Natal

- South Africa’s most populous province.
- 54 independently governed magisterial districts.
- Borders Swaziland and Mozambique in the north, Eastern Cape in the south.
- Bound inland by Lesotho, the Free state province and southeastern Mpumalanga
- Agricultural, industrial and recreational hubs of province occur primarily along the coast – connected by N2 highway.
- Hubs typically surrounded by informal settlements
Rabies in KwaZulu Natal – Historical perspectives

- Dog rabies introduced from Angola in 1947
- Spread throughout African subcontinent in susceptible domestic and wild canid populations.
- Two epidemic initiated in northern KwaZulu Natal:
  1. 1964 – 1968: brought under control through vaccination and control of dog movement.
  2. 1976 – 2006: initiated by refugees fleeing political unrest in Mozambique, remains intractable
Rabies in KwaZulu Natal – Epidemiological perspectives

- Epidemic in KwaZulu Natal - majority of human and animal cases in South Africa per annum.
- Majority of human cases – children.
- Domestic dog - principal disseminator and transmission host.
- Apparently no involvement of wildlife in the epidemiology of the disease.
- Highest number of cases reported from coastal regions - Durban, Richards Bay and Port Shepstone (highest population density).
- Fewer cases reported from internal regions.
Rabies in KwaZulu Natal – epidemiological perspectives

- Reasons for the persistence of the second epidemic.
  1. Inability to control dog movement.
  2. Inadequate vaccination coverage.
  3. AIDS – feral dogs.
- Hotspot vaccination and euthanasia campaigns (2003) - effective in reducing number of rabies cases in target regions
Problem statement

- Little known about the molecular epidemiology of canid-borne rabies in KwaZulu Natal.
- Project aimed to contribute to the currently available knowledge base by using phylogenetic methods to characterize viral isolates from the province.
Materials and methods

- Number of cases (2003): 409 animal, 11 human
- Rabies cases not evenly distributed – localized in densely populated coastal regions.
- Representative sampling – attempted to sequence at least 50% of submitted isolates from each magisterial district from which rabies was reported from during the study year
- Pseudogene and glycoprotein cytoplasmic domain targeted – highly variable, suitable for distinguishing between closely related variants
- 123 animal and 5 human isolates included in final dataset.
Analysis strategy

- Two phylogenetic trees constructed in order to address the different aspects of the epidemic in KwaZulu Natal.
  - **First tree** – KZN, Zimbabwe, South Africa
  - Regional perspective
  - **Second tree** – KZN isolates only
  - Clarification of the relationships which exist specifically between isolates from the different magisterial districts of the province.
Phylogenetic analysis of canine viral isolates from southern Africa – Origin of epidemic and involvement of wildlife
Phylogenetic analysis of viral isolates from KwaZulu Natal – identification of regional variants

- Viral isolates from KwaZulu Natal divisible into two viral groups (Subfamily A and B).
  1. Subfamily A – core of current epidemic among dogs
  2. Subfamily B – putative dog-jackal cycles

- Grouping of viral isolates into clusters (clades) based on geographic region of isolation.
  - Subfamily A (cluster 1A-8A), Subfamily B (cluster 1B-2B)
  - Phylogenetic characterization of regional topotypes – future surveillance
Phylogenetic analysis of viral isolates from KwaZulu Natal – Investigation of the origins of current epidemic

- Subfamily B
- Group A
- Remnant cycle (1960-1964)
- Group B
- Group C
- Freestate
- Lesotho
Reconstruction of human case histories from the province (2002-2003)

- Five human cases from KwaZulu Natal analyzed
- Cases among children, terminal patients - complicates reconstruction of human case histories - contact routes, control measures
- Database of regional variants – useful for comparative studies.
- Human isolates without exception clustered with variants in regions these exposures presumably occurred in.
What’s new?

- Characterization of geographic variants
  - sequence database for future comparisons
- Clear definition of the emergence of epidemic in KwaZulu Natal.
- Possible involvement of wildlife
Co-authors: Prof L. H Nel (UP), Mrs. W. Markotter (UP), Dr. J. Randles (Allerton Regional Veterinary Laboratory)

Acknowledgement: Dr. Claude Sabetha (OP,), Dr. Felicity Burt (NICD), Nobantu, Jackie, Nicolette, Liz (UP)
Thank you !!!

Stop Rabies!