



Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid



Short communication

Evolutionary relationships among human-isolated and wildlife-isolated West Nile viruses

John M. Drake*

Odum School of Ecology, University of Georgia, Athens, GA 30602-2202, USA

ARTICLE INFO

Article history:

Received 10 March 2009
Received in revised form 23 July 2009
Accepted 27 July 2009
Available online xxx

Keywords:

West Nile virus
Evolution
Assortative mixing

ABSTRACT

The evolutionary relationships among pathogen lineages in multi-host systems are often the only observable signature of unobserved ecological and epidemiological processes. The evolution of viruses infecting humans, particularly, is of interest because of the public health importance of understanding the relationship of virus exposure to disease risk. Here I report results of two analyses of the evolutionary relationships among West Nile viruses in North America. These analyses suggest that (1) assortative mixing occurs between virus groups and human vs. non-human hosts and (2) human-derived isolates are related to each other. The ecological processes generating these viruses and the epidemiological consequences of West Nile virus host preference are unknown.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The evolutionary ecology of multi-host pathogens is an active area of research and a foundation for understanding host shift and infectious disease emergence (Woolhouse et al., 2001). West Nile virus (WNV), a mosquito-borne flavivirus in the Japanese encephalitis antigenic complex, provides a key example of how a pathogen with multiple competent hosts can modify ecological communities at multiple spatial scales and how this ecological interaction exposes the human population to disease risk (LaDeau et al., 2007). The expansion of WNV to the Western hemisphere in the late 1990s and simultaneous emergence of a pathogenic lineage, followed by subsequent genetic evolution, has provided a unique opportunity to study how an emerging multi-host pathogen evolves. It is well known that there is modest variation among circulating West Nile viruses (Beasley et al., 2003; Davis et al., 2005; Grinev et al., 2008). It is unknown, however, if strains differentially infect humans and if evolutionary forces acting on the virus in wildlife have additional consequences for human health. A clear picture of these cross-species interactions is necessary, however, if we are to understand how the ongoing epizootic of West Nile viruses in North America wildlife exposes the human population to disease risk.

A recent study by Grinev et al. (2008) provides new insight into the relationship between viruses infecting humans and viruses infecting wildlife. Grinev et al. (2008) obtained complete genome

sequences and structural gene sequences of WNV from 8 and 22 human isolates, respectively, that had been obtained from infected blood donors. A phylogenetic tree of the gene *env*, which codes for the envelope glycoprotein, showed little variation in the WN02 lineage, consistent with previous findings. They then constructed a tree from their 8 complete human-isolated genomes and 39 additional complete genomes sequences including 14 isolates from humans, 19 isolates from birds, 5 isolates from mosquitoes, and 1 isolate from a horse. This tree provided strong evidence for the existence of two outgroups within the more recently derived WN02 lineage. In summary, Grinev et al. (2008) interpreted these findings to provide little evidence for immune selection. They did not answer, however, the question of whether or not viruses and hosts mix assortatively, *i.e.*, whether or not WNVs infecting humans are a non-random sample of viruses circulating in wildlife. Here I take two lines of reasoning to address this idea.

First, inspection of the evolutionary relationships among the 30 completely sequenced WN02 genomes (Fig. 3 in Grinev et al., 2008) suggests that human cases (60%) may more likely belong to the recently diverged Outgroup 2 than to Outgroup 1 and basal lineages. Cross-classifying these genomes, we have the contingency table shown in Table 1.

To test the hypothesized relationship, I performed one-tailed tests of the null hypothesis $H_0: D(\text{Host}|\text{Group}) = D(\text{Host})$, using the framework of General Independence Tests developed by Hothorn et al. (2006) implemented in the R package coin (Hothorn et al., 2008). Significant departure from independence was detected using both asymptotic ($p = 0.0192$) and exact ($p = 0.0415$) conditional null distributions. For comparison, the more familiar two-sided Pearson's χ^2 -test using Monte Carlo simulations to obtain the

* Tel.: +1 706 583 5539; fax: +1 706 542 4819.
E-mail address: jdrake@uga.edu.

Table 1

Contingency table cross-classifying inferred clades in the WN02 lineage with host taxonomy.

Group	Host	
	Human	Bird/mosquito
Outgroup 2	13	4
Outgroup 1 and basal lineages	5	8

conditional null distribution was marginally non-significant ($p = 0.0608$). Together, these tests provide evidence that evolutionary groups and host type are differentially associated with each other.

A second line of reasoning looks at the relatedness of human and non-human isolates. Assigning each fully sequenced genome a nearest evolutionary neighbor (ties broken with a random number), one finds that 14 out of 18 (77.8%) human isolates had a human-isolated nearest neighbor. From the binomial distribution function with parameter $p = 0.6$ (for the 60% of human isolated genomes overall), we have the chance of more than 14 of 18 human-isolated genomes also having a human-isolated nearest neighbor to be $p = 0.033$. This suggests that, in general, viruses isolated from humans are more closely related to other viruses isolated from humans than to viruses isolated from wildlife.

Taken together, these analyses suggest, (1) lineages and hosts mix assortatively and (2) isolates infecting human hosts are evolutionarily related. Whether assortativity results from differential infectivity or differential replication, it is probable that it reflects host selection on a collection of quasispecies that are simultaneously inoculated. The statistical significance of these findings despite the small sample size of the analysis is noteworthy, and implies that the association is probably quite strong. However, the small sample size also requires that these findings be qualified. Because isolates were collected non-randomly (e.g., 8 out of 18 fully sequenced human-isolated genomes are from one study), it is possible that the over-

representation of human isolates in Outgroup 1 is spurious. The most obvious artifact would be if the 18 human-isolated viruses were of more recent derivation simply due to sampling. A Spearman rank-order correlation between isolate year and host does not support such an explanation ($r = -0.09$, $p = 0.646$). At present, alternative hypotheses such as spatial segregation cannot be ruled out. An additional possibility worth investigating is assortativity of viruses infecting enzootic vectors and bridge vectors, which are considered to be important for causing human infections. We tentatively conclude, therefore, that human-isolated West Nile viruses are a biased set of those circulating, but recommend further empirical confirmation as additional data become available. The epidemiological consequences of this evolution remain to be explored.

Acknowledgements

This work was supported by National Science Foundation grants EF-0824507 and EF-0723601.

References

- Beasley, D.W.C., Davis, C.T., Guzman, H., Vanlandingham, D.L., Travassos da Rosa, A.P.A., Parsons, R.E., et al., 2003. Limited evolution of West Nile virus has occurred during its southwesterly spread in the United States. *Virology* 309, 190–195.
- Davis, C.T., Ebel, G.D., Lanciotti, R.S., Brault, A.C., Guzman, H., Siirin, M., et al., 2005. Phylogenetic analysis of North American West Nile virus isolates, 2001–2004: evidence for the emergence of a dominant genotype. *Virology* 342, 252–265.
- Grinev, A., Daniel, S., Stramer, S., Rossman, S., Caglioti, S., Rios, M., 2008. Genetic variability of West Nile Virus in US Blood Donors, 2002–2005. *Emerging Infectious Diseases* 14, 436–444.
- Hothorn, T., Hornik, K., Van de Wiel, M.A., Zeileis, A., 2006. A lego system for conditional inference. *The American Statistician* 60, 163–257.
- Hothorn, T., Hornik, K., Van de Wiel, M.A., Zeileis, A., 2008. Implementing a class of permutation tests: the coin package. *Journal of Statistical Software* 28 (8), 1–23.
- LaDeau, S.L., Kilpatrick, A.M., Marra, P.P., 2007. West Nile virus emergence and large-scale declines of North American bird populations. *Nature* 447, 710–713.
- Woolhouse, M.E.J., Taylor, L.H., Haydon, D.T., 2001. Population biology of multi-host pathogens. *Science* 292, 1109–1112.