Attenuated Vaccines Can Recombine to Form Virulent Field Viruses

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Live attenuated herpesvirus vaccines are widely used in human and veterinary medicine. The risk of vaccines recombining to generate virulent natural recombinants has been raised, but disease outbreaks caused by herpesvirus vaccine recombination have never been reported (*1*).

The herpesvirus infectious laryngotracheitis virus (ILTV or gallid herpesvirus 1) causes mild to severe respiratory disease in poultry worldwide. Economic losses result from mortality and decreased egg production. Attenuated ILTV vaccines are used widely (*2*). Concurrent with the introduction of a new vaccine, novel, genotypically distinct viruses (referred as class 8 and 9 viruses) were isolated from geographically distinct Australian regions starting in 2008 (*3*). These virulent strains have been associated with outbreaks causing mortality rates of up to 17.6% (*4*). In Australia, three ILTV vaccines are available. The closely related Australian-origin vaccines SA2 and A20 (Pfizer, Australia) are classified genotypically as class 1 viruses on the basis of patterns seen in polymerase chain reaction–restriction fragment length polymorphism (RFLP) analysis (*5*). The European-origin Serva vaccine strain (Intervet) was first registered in Australia in 2006 and has a class 7 genotype (*3*). The SA2 and A20 genomes are divergent from the Serva genome, having only 99.2% nucleotide sequence identity with it (*6*). The contemporaneous introduction of the Serva vaccine and emergence of new classes of virulent ILTV (classes 8 and 9), together with genetic relatedness of viruses causing recent isolates, led to the hypothesis that the class 8 and 9 viruses may represent subpopulations of viruses within the Serva vaccine or may have arisen after in vivo passages of the Serva vaccine (*3*).

We performed whole-genome sequencing and comparative sequence analysis of the class 8 and 9 viruses and the three vaccine strains. In genome alignments, much of the class 8 and 9 sequences were almost identical to Serva. Single-nucleotide polymorphisms (SNPs) were clustered in distinct genomic regions unique to each class. The sequence in these regions was identical (class 8) or almost identical (class 9) to the sequences of SA2 and A20 (Fig. 1A). These results are consistent with interspecies recombination between the co-circulating Australian-origin and European-origin vaccines giving rise to virulent class 8 and 9 field strains.

To confirm the likelihood of recombination, we compared the sequences of the class 8 and 9 viruses to those of Serva, SA2, and A20 by using Simplot. Two points of crossover were detected for each virus (Fig. 1, B and C). These corresponded to the recombination regions identified in genome alignments.

To determine whether other class 8 and 9 isolates had the same recombinant genomes as the two fully sequenced isolates, we performed in silico restriction fragment length polymorphism (RFLP) analysis with the genomes of the sequenced class 8 and 9 viruses. We also performed specific PCRs to amplify the recombination crossover points of additional class 8 and 9 isolates from different disease outbreaks. The in silico RFLP patterns were consistent with in vitro RFLP analyses of other class 8 and 9 viruses submitted to our diagnostic laboratory. The PCRs revealed that the same recombinant genomes were present in other isolates (figs. S1 and S2).

Lastly, the pathogenicity of class 8 and 9 viruses was studied in specific-pathogen-free chickens. Each recombinant had a distinct in vivo phenotype. After correcting for multiple hypothesis testing, both recombinants had significantly increased virulence or replication compared with their parent strains (fig. S3).

The rapid emergence of two virulent recombinants suggests that recombination between attenuated herpesvirus vaccines and resultant restoration of virulence may be rare but can bring about a fitness advantage, with severe consequences. The findings from this study raise concerns about the use of multiple distinct attenuated herpesvirus vaccines under conditions that favor recombination. These findings have implications for the use of herpesviruses, and possibly other DNA viruses, as attenuated vaccines or vaccine vectors.

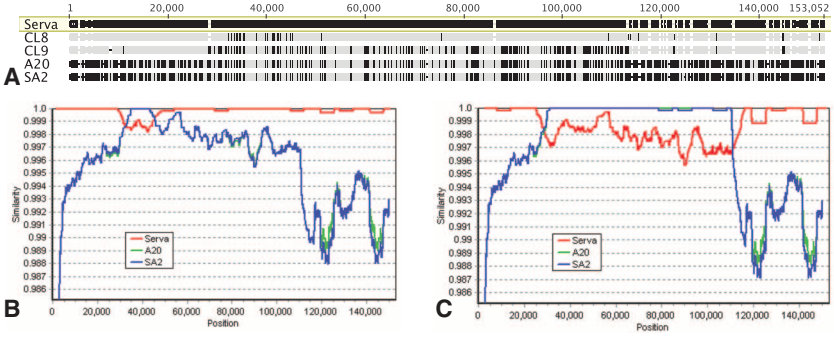

Fig. 1. Whole-genome alignments and similarity plots. (**A**) Alignment of the class 8 (CL8), class 9 (CL9), SA2, and A20 genome sequences with the Serva genome sequence. Vertical black lines indicate single-nucleotide differences from the Serva sequence. Dashes indicate single-base gaps. (**B** and **C**) Similarity plots of the sequences of the CL8 (B) and CL9 (C) genomes with those of Serva, A20, and SA2. The results indicate that the class 9 virus was derived from A20 and Serva. One parent of the class 8 virus could not be defined because the recombinant region was identical to both SA2 and A20. All recombinations were significant ($P < 10^{-3}$, maximum χ^2 test).

References and Notes

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Acknowledgments: The Rural Industries Research and Development Corporation, Australia, funded this study. This work is supported by NICTA, which is funded by the Australian government as represented by the Department of Broadband, Communications and the Digital Economy and the Australian Research Council through the ICT Centre of Excellence program. Sequence data for the class 8 and 9 viruses have been deposited in GenBank under accession nos. JN804826 and JN804827, respectively. The three reference sequences are Serva, GenBank accession no. HQ_630064; SA2, GenBank accession no. JN596962; A20, GenBank accession no. JN596963.

Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6091/188/DC1
Materials and Methods
Figs. S1 to S3
References (*7*–*12*)

28 November 2011; accepted 26 April 2012
10.1126/science.1217134

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