Cleverly designed vaccine blocks H5 avian influenza in models

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Until now most experimental vaccines against the highly lethal H5N1 avian influenza virus have lacked effectiveness. But a new vaccine has proven highly effective against the virus when tested in both mice and ferrets. It is also effective against the H9 subtype of avian influenza. The research is published online ahead of print in the *Journal of Virology*.

The strength of the new vaccine is that it uses attenuated, rather than "killed" virus. (Killed viruses are broken apart with chemicals or heat, and they are used because they are safer than attenuated viruses.) Killed virus vaccines against avian influenza are injected into the bloodstream, whereas this vaccine is given via nasal spray, thus mimicking the natural infection process, stimulating a stronger immune response.

The danger of current attenuated virus vaccines is that they might exchange dangerous genetic material with garden variety influenza viruses of the sort that strike annually, potentially rendering a lethal but very hard to transmit influenza virus, such as H5, easily transmissible among humans. To mitigate those dangers, the study authors, led by Daniel Perez of the University of Maryland, came up with an ingenious design. Influenza viruses carry their genetic material in eight "segments," explains coauthor and University of Maryland colleague Troy Sutton. When viruses reassort, they exchange segments. But each segment is unique, all eight are needed, and the viruses are unfit if they contain more than eight segments.

The vaccine is based on an attenuated version of the H9 virus, with an

H5 gene added into one of the H9 virus' segments, to confer immunity to the H5 virus. Segment 8, which is composed of the so-called NS1 and NS2 genes, was split apart, and the NS2 gene was moved into segment 2, adjacent to the polymerase gene, which copies the virus' genetic material during replication. Placing NS2 next to the polymerase gene slowed its function, interfering with the virus' replication. That makes the vaccine safer.

The next step was to engineer the H5 gene into the vaccine. It was inserted into segment 8, where the NS2 gene had been.

Another aspect of the new vaccine's design makes it safer still, by rendering successful reassortment less likely. Both NS1 and NS2 are needed for viral replication. Since the two genes are now separated into different segments, any reassortment will have to include both segments, instead of just segment 8, in order for a reassortant virus to be viable. This greatly reduced the probability of successful reassortment.

The World Health Organization (WHO) recognizes avian influenza subtypes H5, H7, and H9 as potential pandemic viruses, because they all have in rare instances infected humans, and because they circulate in wild birds. Single reassortants could be sufficient to breach the species barrier, and since they do not circulate among us, we lack any immunity. Moreover, H5 is unusually lethal, having killed roughly half of those few it is confirmed to have infected.

More information: L. Pena, T. Sutton, A. Chockalingam, S. Kumar, M. Angel, H. Shao, H. Chen, W. Li, and D.R. Perez, 2013. Influenza viruses with rearranged genomes as live-attenuated vaccines. *J. Virol.* Online ahead of print, 28 February 2013, doi:10.1128/JVI.02490-12