LETTER

REPLY TO RAMEY ET AL.:

Let time be the arbiter

Scott Krauss[a], David E. Stallknecht[b], Richard D. Slemons[c], Andrew S. Bowman[c], Rebecca L. Poulson[b], Jacqueline M. Nolting[c], James P. Knowles[a], and Robert G. Webster[a,1]

We thank Ramey et al. (1) for their opinion that the article "The enigma of the apparent disappearance of Eurasian highly pathogenic H5 clade 2.3.4.4 influenza A viruses in North American waterfowl" (2) provides insufficient evidence to support the hypothesis that wild aquatic birds do not serve as the reservoir of highly pathogenic (HP) influenza A viruses. However, most of the concerns raised by Ramey et al. (1) are already discussed in our report (2) or resulted from a misinterpretation of our work. With regard to the disappearance of these viruses from North America, Krauss et al. (2) state that "we cannot conclude that the HP H5Nx viruses were eradicated from wild aquatic birds" and the title purposely includes the word "apparent" to further reflect this uncertainty. Ramey et al. (1) correctly point out that many of the postoutbreak samples were not from areas in the Pacific flyway where these viruses were initially detected in North American wild birds during November–December 2014 and January 2015; however, HP H5N2 influenza A virus infections became widespread in Midwestern poultry flocks along the Mississippi flyway, where most of our post-outbreak surveillance samples originated. The virus was also isolated from wild birds in the Mississippi flyway. If these viruses were established in a North American wild bird reservoir, it is expected that they could have been detected in any part of the country.

There is no doubt that wild aquatic birds play a role in the spread of HP influenza A viruses (3–9); this is acknowledged and clearly stated in our report (2). With regard to emergence of HP influenza A viruses, there is no historic or current evidence that these viruses—H5 or H7—have emerged directly from wild bird populations. Ramey et al. (1) refer to the documented reassortment of the Eurasian clade 2.3.4.4 H5N8 virus with North American viruses as evidence of emergence, and these reassortment events are already acknowledged in our report (2). Although these reassortment events do result in a HP influenza A virus with a novel genotype, they do not support the emergence of a HP phenotype from wild birds because the H5 gene responsible for the HP phenotype originated from poultry reservoirs in Asia (10).

We acknowledge that additional surveillance is needed to fully resolve whether wild aquatic birds perpetuate (i.e., provide a reservoir of) HP influenza A viruses. The identification by real-time PCR of HP H5N2 in an Alaskan wild mallard duck in August 2016 does not provide evidence that a wild bird reservoir exists for this virus, and such an occurrence is consistent with the multiyear detection of HP H5N1 virus observed in Europe during 2005–2007. The "proof of the pudding" will be whether these HP H5Nx viruses continue to be detected in wild birds and commercial poultry in North America, or whether they disappear. Regardless of outcome, and considering the increasing number of both low-pathogenic and high-pathogenic influenza A viruses that are currently maintained in domestic birds globally, it is essential that we understand the mechanisms that either enhance or prevent the establishment of these viruses in wild bird populations.

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2 Krauss S, et al. (2016) The enigma of the apparent disappearance of Eurasian highly pathogenic H5 clade 2.3.4.4 influenza A viruses in North American waterfowl. *Proc Natl Acad Sci USA* 113(32):9033–9038.

3 US Department of Agriculture (2016) Wild Bird Positive Highly Pathogenic Avian Influenza Cases in the United States: December 2014 to June 2015 and July 2015 to June 2016. Available at https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian-influenza-disease. Accessed September 9, 2016.

4 Ramey AM, et al. (2016) Evidence for common ancestry among viruses isolated from wild birds in Beringia and highly pathogenic intercontinental reassortant H5N1 and H5N2 influenza A viruses. *Infect Genet Evol* 40:176–185.

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The authors declare no conflict of interest.

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E6554 | www.pnas.org/cgi/doi/10.1073/pnas.1614678113 Krauss et al.