Online Comment #60187

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in response to:Mathilde Richard et al. Limited airborne transmission of H7N9 influenza A virus between ferrets. *Nature* **501**, 560–563 (26 September 2013) doi:10.1038/nature12476

What was the purpose of the second ferret experiment in this paper (ferret pairs F5-F8, referred to hereafter as Passage 2)? From reading the paper, we inferred that the intention was to compare the transmissibility of the original human isolate, A/Anhui/1/2013, assessed in ferret pairs F1-F4 (hereafter Passage 1), to that of virus isolated from ferret F1, by measuring transmission in a second group of 4 ferret pairs, Passage 2. We inferred this intention because:

- the sample size (2 groups of 4 ferret pairs) was justified by citing a paper (ref. 29) that suggests using 4 vs. 4 ferret pairs to compare transmissibility of viral isolates
- the paper states: "However, the rapid selection of substitutions in the HA and PB1 genes and the gain in clonality did not change transmission substantially enough to be detectable with the current group size of four ferrets."

Moreover, we could not think of (nor did the paper describe) another motivation for Passage 2.

Had the intention been to compare transmission of A/Anhui/1/2013 to that of the isolate from F1, the Passage 2 experiment would have been uninterpretable for two reasons:

1. The inoculum was 100x lower in Passage 2 than in Passage 1, rendering the experiments not directly comparable.
2. Given the results of Passage 1 (3/4 recipient ferrets infected), there was no possible result in Passage 2 that could have been statistically significantly different from Passage 1. The actual result was 1/4 ferrets infected (Fisher's exact p value 0.49). Had the results been 0 of 4, 2 of 4, or 4 of 4, the corresponding p values would have been 0.14, 1.0 and 1.0 respectively.

Following *Nature*'s recommendation, one of us (ML) raised these points in an email to the corresponding author before posting them. Prof. Fouchier replied in part: "The goal of both transmission experiments was to test the robustness of the ferret-to-ferret transmissibility of Anhui/1."

In light of the points above, we were surprised by this reply, but surely this must be taken as the authoritative statement of the intent of Passage 2. If so, the experimental design is faulty for a different reason. The isolate from recipient F1, which like A/Anhui/1/2013, was genetically heterogeneous, contained three genetic substitutions not detected in A/Anhui/1/2013, as well as polymorphisms at different frequencies from A/Anhui/1/2013. It is difficult to understand how two experiments, using inocula differing 100-fold in titer and using genetically distinct inocula, could provide reliable data on the transmissibility of a single virus isolate.

While the publication in *Nature* of flawed experiments is a surprise in any circumstance, it is especially unfortunate to undertake such experiments at all when they involve the sacrifice of 8 ferrets and entail a risk of a laboratory accident with a mammalian-transmissible, highly virulent pathogen.