

Response to Reviewer 1 Comments

Dear Reviewer,

Please find below the response to your comments and suggestions

Point 1: There is no description about the number of samples testing positive for the virus in 502 poultry carcasses. The authors should add concrete figure. And there is no explanation about the principles for the sequencing samples selected from them. Is it randomly selected?

Response 1:

“Out of 502 tested carcasses, 177 out of 459 chickens or broilers, 4/8 ducks, 2/2 geese, 2/8 turkeys, and 16/22 common quails were positive for the virus. Tissue samples with positive results on AIV M-gene RT-qPCR showed a lower mean Ct value, with a mean Ct value of 19.78 (n = 58, standard deviation (SD) = 3.84), compared to individual oropharyngeal swabs from carcasses with positive results, with a mean Ct value of 23.80 (n = 201, SD = 4.88).”

“To increase the probability of obtaining sequences of good quality, RNA samples with a high (RNA) viral load have been selected. The viral RNA yield in the purified RNA samples was assessed based on the Ct values of the samples obtained with the AIV M-gene RT qPCR.”

“These samples consisted of pools of organs such as the lung, trachea, proventriculus, intestine, and spleen from animals based on the low Ct values of the samples obtained previously with the AIV M-gene RT-qPCR.”

Point 2: Line209-210. “The low number of nucleotide differences between the viruses...”, the authors should clearly illustrate the specific values of nucleotide identity of the eight gene segments between the viruses.

Response 2:

“The low number of nucleotide differences between the viruses collected from some of the outbreaks in commercial or backyard farms might be indicative of viral spread among them. In particular, viruses 3124-03, 3124-04, 3124-05 and 3124-07 collected from commercial farms cluster together, showing from 0 to 8 nucleotide differences along the entire genome, and separately from the backyard cases. On the other hand, some of the viruses collected from the backyard farms are closely related to each other, such as viruses 5162-15, 5162-19, 5162-07, 5162-04 (2 to 7 nucleotide differences) or viruses 5162-01, 5162-02, 5162-16 (1 to 5 nucleotide differences), indicating a potential virus spread among them.”