## HPAI H5N1 virus from suboptimally vaccinated chickens was not transmitted to unvaccinated pen mates and not detected in environmental dust

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Suboptimal vaccination is considered a risk for the control of Highly pathogenic Avian Influenza (HPAI), because it may result in birds protected against clinical disease that are still able to transmit the virus to birds in the same flock, or even to birds in other flocks (e.g. by dust). In this study we examined virus transmission among suboptimally vaccinated chickens and determined the amount of virus in dust originating from these birds. Groups of 10 chickens were formed and per group 5 chickens were vaccinated with either 1/3, 2/3, or none of the recommended dose. Two weeks post vaccination the vaccinated birds were challenged with HPAI H5N1 virus, and thereafter placed with the 5 unvaccinated birds. Tracheal and cloacal swabs were collected daily to monitor virus transmission, and the amount of virus in dust was determined. None of the vaccinated birds showed clinical signs post inoculation, whereas all birds in the non-vaccinated groups did and died. Vaccinated birds only shed virus via trachea, as cloacal swabs remained negative. The amount of virus detected in tracheal swabs was  $10^{4.5}$ ,  $10^{3.7}$  and  $10^{6.4}$  EID<sub>50</sub> for the 1/3, 2/3 and non-vaccinated group, respectively. None of the contact birds in the vaccinated groups became infected, whereas all contacts to the non-vaccinated birds did. All dust samples collected in the vaccinated groups appeared negative in RT-qPCR; dust samples in the non-vaccinated group showed on average  $10^{2.8}$  EID<sub>50</sub> per m<sup>2</sup>. This study showed that birds vaccinated with only 1/3 dose could be infected, but were unable to infect unvaccinated cage mates. Moreover, no virus was detected in dust collected around the cages. Possibly, the absence of cloacal shedding reduced deposition of viral particles via faeces and therefore reduced the amount of virus into the environment and transmission of virus to cage mates.