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## ACCEPTED MANUSCRIPT

## Viral interference between Low Pathogenic Avian Influenza H9N2 and avian infectious bronchitis viruses *in vitro* and *in ovo*

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#### Highlights

- AIV and IBV co-infection led to decreased growth of both viruses.
- During super-infection, the second virus decreased the growth of the first virus.
- The level of secreted IL-1beta varies, depending on the experimental conditions.

#### ABSTRACT

**Background:** Low pathogenic avian influenza (LPAI) H9N2 and infectious bronchitis virus (IBV) are important pathogens of poultry, causing important economic losses for the sector. Replication interference between these two viruses was described using cell cultures (CC) and embryonated chicken eggs (ECE). Chicken embryo lung (CEL) and ECE were simultaneously or sequentially infected with IBV vaccine strain (H120) and LPAIV-H9N2 (A/Ck/TUN/145/2012) to evaluate viral interactions *in vitro* and *in ovo*, respectively. Real-time RT-PCR was developed to specifically quantify both AIV and IBV genomes as well as viral gene copy numbers during mixed infections. The amount of IL-1 beta, in supernatants of co-infected cell cultures, was determined using an ELISA assay.

**Results:** Quantitative results of AIV and IBV co-infection showed that interferences between the two viruses yielded decreased viral growth. However, in the case of super-infection, the second virus, either AIV or IBV, induced a decrease in the growth of the first inoculated virus.

**Conclusion:** It appears that either AIV or IBV has a negative impact on the other virus growth when they are inoculated simultaneously or sequentially. The ELISA results showed that higher

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