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# Unbiased detection of infectious agents in respiratory syndromes of poultry using a metagenomic approach

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## Background

Respiratory viruses in poultry species are poorly investigated to date. In order to identify all the viral pathogens in duck respiratory tract, tracheal swabs were collected on ducks showing respiratory clinical signs and/or egg drop syndromes and were submitted to a metagenomic analysis.

## Objectives

Using a high-throughput sequencing approach, we aimed at describing the respiratory viral and bacterial flora of ducks showing respiratory clinical signs and/or egg drop syndromes

## Methods

Pools of ten swabs were put together to construct libraries. A total of 10 flocks of Peking ducks or Muscovy ducks were sampled. The preparation of specimens for deep sequencing was performed as previously described (Victoria & al., 2009; Phan & al., 2011; Shan & al., 2011). Briefly, clinical specimens were centrifuged, treated with DNase and RNase, then DNA and RNA were extracted and amplified by PCR using a random primer (Victoria & al., 2009). The PCR products were purified and loaded on a Miseq (Illumina) sequencer.

## Results

More than 2 million reads (of about 150 nucleotides) were initially generated. The assembled contigs and singlet sequences were compared to Genbank virus database using GAAS software. We identified a majority of sequences of (i) bacteriophages (*Siphoviridae*, *Myoviridae*, *Podoviridae*, *Microviridae*), and (ii) animal viruses (*Retroviridae*, *Reoviridae*, *Adenoviridae*, *Poxviridae*, *Picornaviridae*, *Orthomyxoviridae*, and *Paramyxoviridae*); as well as few sequences of plant and insect viruses. Altogether, these data resulted in the description of a duck respiratory virome.

In the same way, bacterial reads were identified by 16SRNA analysis: a majority of gram negative (*Enterobacteriaceae*) were identified.

## Conclusion

We have conducted a metagenomic analysis in Peking ducks or Muscovy ducks. Deep sequencing is a powerful approach to detect most infectious agents in a clinical case. It also allows for the detection of the opportunistic pathogen flora, unrelated to clinical signs. This study increases our understanding of the viral diversity in ducks and highlights the complexity of co-infections in poultry respiratory tract.