

Prevalence and genetic diversity of avian coronaviruses in Luxembourg, The Netherlands and Nigeria

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Abstract

Similar to avian influenza and Newcastle disease virus, avian coronaviruses (CoV) seem to use wild birds as reservoirs. The large variety of potential hosts raises the question of what impact avian CoV from wild birds may have on domestic poultry. Here we report surveillance data from three countries, namely Luxembourg, the Netherlands and Nigeria, showing a high genetic diversity of both gamma- and delta-coronaviruses, as well as a high diversity of avian hosts.

Introduction

Increasing evidence suggests that several wild bird species are asymptomatic carriers of a wide variety of avian CoV (Muradrasoli *et al.*, 2010; Wille *et al.*, 2015), but avian CoV prevalence, host range and infectious potential for poultry are currently unknown. In addition, CoV are prone to recombination and as-yet-unknown CoV strains might be the donors of new genes to infectious bronchitis virus (IBV), leading to the generation of new serotypes or new CoV species such as Turkey and Guinea fowl CoV (Brown *et al.*, 2016; Ducatez *et al.*, 2015). In this study, we aimed to detect and characterize the gamma- and delta-CoV strains in wild birds in Luxembourg, the Netherlands and Nigeria and thereby understand the links between viral strains from various species or regions.

Materials and methods

Cloacal and/or tracheal swabs and faeces from wild birds sampled during the surveillance for avian influenza in Luxembourg (2006-2010 and 2014-2015), the Netherlands (2007) and Nigeria (2007-2008 and 2011) were screened for the presence of gamma- and delta-CoV by a modified nested RT-PCR (Chu et al., 2011). Positive samples were sequenced using the same primers. Phylogenetic analyses including partial RNA-dependent RNA polymerase (RdRp) gene sequences available on GenBank and the sequences obtained in this study were performed with MEGA v6 (Tamura et al., 2013).

Results

In total, 5.3% (53/1000) of birds sampled in the framework of continuous surveillance between 2006 and 2010, tested positive for gamma- or delta-CoV in Luxembourg, compared to 7.5% (13/174) of birds sampled during winter 2014-2015. In the Netherlands and Nigeria, 4.4% (34/776) and 1% (16/1576) of birds tested positive, respectively.

Ninety-three partial RdRp sequences were obtained and phylogenetic analyses based on a 366 bp fragment revealed great genetic diversity. Gamma- and delta-CoV were identified in the three countries. In total, 19, 24 and 7 gamma-CoV and 26, 10 and 7 delta-CoV strains were found in Luxembourg, the Netherlands and Nigeria, respectively. Most of the gamma-CoV strains from wild birds grouped within three clusters (A, B and C) distinct from infectious bronchitis viral strains, and interspersed with other strains found in Europe, Asia, Africa or the USA, resulting in no clear geographic or species-specific cluster. In Anseriformes the majority of strains clustered within the gamma-CoV cluster A which includes the largest number of strains, but also within gamma-CoV cluster B and to a lesser extent within delta-CoV. The strains found in pigeons also formed a separate cluster C including mainly strains from pigeons from China.

A greater genetic and host diversity (Anseriformes, Pelaeconiformes, Gruiformes, Charadriiformes, Passeriformes) was observed in delta-CoV compared to gamma-CoV. However, most sequences clustering within delta-CoV grouped together with sequences obtained from related bird species. For instance, two strains from great cormorants found in Luxembourg grouped with sequences from great cormorants from Hong Kong. A strain from a Sudan golden sparrow from Nigeria was most closely related to sequences from tree sparrows from Luxembourg.

In addition, inter-species transmission involving poultry was also observed. In Luxembourg, an IBV-like strain was found in a common blackbird. A chicken was harbouring a gamma-CoV strain belonging to the cluster C including mainly pigeon strains, and a delta-CoV strain from a tree sparrow cluster was found in a peafowl.

Discussion

Our data show differences in gamma- and delta- CoV prevalence according to country which is most likely to be due to species sampling differences. However, seasonality and the age of the birds could also play a role in the frequency of virus detection, since the influx of birds at migratory sites and the presence of juveniles could influence virus transmission (Wille *et al.*, 2015). Similarly to avian influenza virus, gamma-CoV seem to be easily exchanged between migratory birds, especially ducks, as shown by the similarity of strains shared by various species. Shared environments such as feeding and resting places, might facilitate inter-species CoV transmission. On the other hand, the surveillance of gamma- and delta-CoV is generally biased by the cohorts used. Indeed, Passeriformes or Columbiformes also host specific delta or gamma-CoV strains. Our results suggest that despite their diversity, delta-CoV tend to be more restricted to certain bird species, compared to gamma-CoV, although exceptions were noted. Three cases of IBV-like strains in wild birds or other gamma- and delta-CoV in poultry were also observed, showing that bridge species with potentially more frequent contacts with poultry could participate in increasing the interspecies transmission of CoV to and from poultry.

In the future, more efforts towards sequencing a larger fragment of the RdRp gene to improve phylogenetic inference, especially of gamma-CoV, is desirable. This would enable a more precise understanding of the species and geographic distribution of viral strains. In addition, sequencing of the spike gene will be needed in order to investigate the relationship between avian CoV strains from wild and domestic birds.

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