

REVIEW ARTICLE

Wild birds as reservoirs for diverse and abundant gamma- and deltacoronaviruses

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One sentence summary: Although wild birds are hosts to numerous species of gammacoronaviruses and deltacoronaviruses, some of which infect domestic birds or are able to spill-over into mammals, we reveal the limitations to our current understanding of their diversity, ecology and evolution.

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ABSTRACT

Wild birds interconnect all parts of the globe through annual cycles of migration with little respect for country or continental borders. Although wild birds are reservoir hosts for a high diversity of gamma- and deltacoronaviruses, we have little understanding of the ecology or evolution of any of these viruses. In this review, we use genome sequence and ecological data to disentangle the evolution of coronaviruses in wild birds. Specifically, we explore host range at the levels of viral genus and species, and reveal the multi-host nature of many viral species, albeit with biases to certain types of avian host. We conclude that it is currently challenging to infer viral ecology due to major sampling and technical limitations, and suggest that improved assay performance across the breadth of gamma- and deltacoronaviruses, assay standardization, as well as better sequencing approaches, will improve both the repeatability and interpretation of results. Finally, we discuss cross-species virus transmission across both the wild bird – poultry interface as well as from birds to mammals. Clarifying the ecology and diversity in the wild bird reservoir has important ramifications for our ability to respond to the likely future emergence of coronaviruses in socioeconomically important animal species or human populations.

Keywords: avian coronavirus; *Coronaviridae*; coronavirus; deltacoronavirus; gammacoronavirus; infectious bronchitis virus; IBV; wild birds

INTRODUCTION: BIRDS AS ZONOTIC RESERVOIRS

The ongoing pandemic of SARS-CoV-2, and that of Ebola before it, brought a global focus on bats as a critical reservoirs for a variety of zoonotic diseases (Hayman *et al.* 2013; Mari Saez *et al.* 2015; Allocati *et al.* 2016; Openshaw *et al.* 2017; Brook *et al.*

2019). While the attention devoted to bats and other mammals is understandable, on a global scale birds are also an important host reservoir for a number of zoonotic diseases. Arguably, the most important of these are the avian influenza A viruses. These circulate in wild birds with no signs of disease (Olsen *et al.* 2006), but upon entering the poultry production system they may become highly pathogenic causing severe morbidity and

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mortality (Richard et al. 2017). Two subtypes of avian influenza A virus have been of recent pandemic concern: H5N1 (and recently its derivative H5N6) and H7N9. The former has caused a documented 861 human infections and 455 deaths (WHO 2020), while the latter is responsible for a reported 1567 human infections and 615 deaths (Naguib et al. 2019). In addition to avian influenza A virus, a number of other zoonotic diseases stem from the avian reservoir (Chan et al. 2015). While zoonotic transmission from wild birds to humans remains a rare event, birds play a role as reservoirs or vectors for zoonotic viruses including West Nile Virus (Murray, Mertens and Despres 2010), Crimean-Congo hemorrhagic fever virus (Lindeborg et al. 2012), tick-borne encephalitis virus (Waldenstrom et al. 2007), Louping-ill virus (Jeffries et al. 2014), Newcastle disease virus (Alexander 2000), Japanese encephalitis virus (Nemeth et al. 2012) and St. Louis encephalitis virus (Gruwell et al. 2000). In addition, birds harbor zoonotic disease-causing bacteria such as *Salmonella enterica* (Salmonellosis; Lawson et al. 2014), *Campylobacter jejuni* (Campylobacteriosis; EFSA Panel on Biological Hazards (BIOHAZ) 2010), *Mycobacterium avium* (Mycobacteriosis; Tell, Woods and Cromie 2001) and *Chlamydia psittaci* (Psittacosis; Eidson 2002), all of which regularly cause human infections.

Critically, wild birds interconnect all parts of the globe through annual cycles of migration (Bauer and Hoye 2014). Although many international borders closed due to COVID-19, numerous wild bird species continued to cross country and continental boundaries through their migrations. In addition to natural environments, birds can be found in our cities, using wastewater treatment plants, landfills and our drinking water reservoirs. Beyond wild birds, it is estimated that chickens raised for human consumption comprise three times the biomass of wild birds on the planet (Bar-On, Phillips and Milo 2018), creating an important amplifier of potentially zoonotic avian viruses, including avian influenza A virus (Wan 2012; Gao et al. 2013; Yoon et al. 2015). Despite our important relationship with birds, we have only a limited understanding of the diversity of avian viruses. In this review we explore the role of wild birds as hosts for coronaviruses, with a particular focus on their host range and the diversity of viruses they carry. We also discuss the limitations to the study of avian coronaviruses, make suggestions for the improved detection and characterization of these viruses and assess the risk for future cross species transmission.

A BRIEF HISTORY OF AVIAN CORONAVIRUSES

Coronaviruses cause numerous diseases in animals, including upper and lower respiratory diseases, gastroenteritis and central nervous infections. In humans, SARS-CoV-2 is currently causing a global pandemic of COVID-19. This virus, in addition to the recent zoonotic coronaviruses - SARS-CoV and MERS-CoV - are the result of cross-species transmission events (Lam et al. 2020; Lu et al. 2020), and all likely have their ultimate origins in bats (Li et al. 2005; Lau et al. 2013), before moving through intermediate hosts to humans (Song et al. 2005; Memish et al. 2014).

Despite the current focus on human and bat coronaviruses, the first coronavirus described was infectious bronchitis virus (IBV). Infectious bronchitis was first documented in 1931 in the USA (Schalk and Hawn 1931) in 2 day to 3 week old chicks with mortality in 40–90%. This disease soon spread across the USA. Confirmation that the disease was caused by a virus occurred in 1936 (Beach and Schalm 1935; Beaudette and Hudson 1937; Fabricant 1998). Coronaviruses were formally recognized as a viral family in 1968, which at the time included IBV, mouse hepatitis

virus and human strains of coronaviruses (B814, 229E) (Mallucci, McIntosh and Tyrrell 1968).

In birds, the Mass strain of IBV was the only one described until 1956 when a new strain was identified following failure to neutralize in a virus neutralization assay (Jungherr, Chomiak and Luginbuhl 1956). Since then, numerous IBV variants have been documented (Cavanagh 2007) and recently classified into six genotypes and 32 lineages (Valastro et al. 2016). Another coronavirus infecting poultry, Turkey coronavirus, was identified in 1971 as the causative agent of turkey enteritis, also called bluecomb disease (first described in 1951; Adams and Hofstad 1971). Today (2019 Update), the ICTV divides these viruses in two viral species: Avian coronavirus (AvCoV) and Avian coronavirus 9203, that together comprise all the genotypes of IBV and turkey coronavirus (although all species divisions are in reality arbitrary divisions of phylogenetic diversity). Despite decades of research, it was only recently that wild birds were screened for coronaviruses and, perhaps unsurprisingly, a large diversity of coronaviruses have now been described in wild birds without signs of disease. These viruses are all members of the gammacoronavirus and deltacoronavirus (Fig 1), of which there are now five and seven ratified species, respectively (de Groot et al. 2020).

THE ECOLOGY AND EPIDEMIOLOGY OF CORONAVIRUSES IN WILD BIRDS

Untangling the ecology of coronaviruses in wild birds is challenging due to non-conformity in screening and the characterization assays employed (see below). However, we may be able to infer aspects of the host range of these viruses from the available data. To date, coronaviruses have been detected in 15 avian orders comprising 30 families and across 108 species of wild birds (Table S1, Supporting Information). Avian groups from which these viruses are frequently detected include waterbirds (Anseriiformes, Charadriiformes, Pelecaniiformes, Suliformes and Spheniiformes), orders to which humans have close contact (Galliformes, Columbiformes and Passeriformes), in addition to the Gruiformes, Accipitriformes, Strigiformes, Falconiformes, Cathartiformes, Psittaciformes, Piciformes and Otidiformes (Table S1, Supporting Information). Avian orders that have been screened but in which coronaviruses have not yet been detected include the Caprimulgiformes, Procellariiformes, Coraciiformes, Apodiformes, Casuariiformes, Podicipediformes, Phaethontiformes and Cuculiformes.

Data collation reveals a substantial bias towards viral detection in waterfowl (ducks) as well as the identification of gammacoronaviruses rather than deltacoronaviruses. The former may be driven by studies that have screened samples originally collected for avian influenza A virus research: ducks and shorebirds are the main host reservoir for avian influenza A virus, resulting in a data bias towards the screening of Anseriiformes and Charadriiformes (e.g. Wille et al. 2015). Similarly, the relative frequency of gammacoronaviruses compared to deltacoronaviruses may reflect underlying technological biases, in which some studies use screening methods that may only be able to detect gammacoronaviruses as they were designed for AvCoV. More reliable conclusions may therefore be drawn from studies that employ screening methods that are able to detect both gammacoronaviruses and deltacoronaviruses (Chu et al. 2011; Hepojoki et al. 2017; Chamings et al. 2018; Paim et al. 2019; Table 1). These continue to reveal a high frequency of gammacoronaviruses in waterfowl, suggesting it is a true observation. Furthermore, we see detections of gammacoronaviruses

Table 1. Summary of host range of coronaviruses in wild birds¹.

Host taxonomy	Species	Number sampled	Number gamma-coronaviruses (percentage prevalence)	Number delta-coronaviruses (percentage prevalence)	Continent	Sample type	Screening method	Study
Anseriiformes, Anatidae, Anatinae	Mallard (<i>Anas platyrhynchos</i>)	227	16 (7%)	2 (1%)	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
	Mallard (<i>Anas platyrhynchos</i>)	129	28 (22%)	0	Europe	Cloacal swab, tracheal swab, oropharyngeal swab, or tissue	Murdrasoli et al. (2009)	Hepojoki et al. (2017)
	Pacific Black Duck (<i>Anas superciliosa</i>)	48	18 (38%)	1 (2.1%)	Australia	Combined oropharangeal and cloacal swab, or only cloacal swab	Callison et al. (2006), Chu et al. (2011), modified Chu et al. (2011)	Chamings et al. (2018)
	Northern Pintail (<i>Anas acuta</i>)	12	1 (8%)	0	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
	Northern Shoveler (<i>Anas clypeata</i>)	6	0	1 (16%)	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
	Northern Shoveller (<i>Anas clypeata</i>)	31	22 (71%)	2 (6.5%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
	American Wigeon (<i>Anas americana</i>)	1	0	1 (100%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
	American Wigeon (<i>Anas americana</i>)	18	1 (5.5%)	0	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
	Eurasian Wigeon (<i>Anas penelope</i>)	24	9 (38%)	1 (4%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
	Eurasian Teal (<i>Anas crecca</i>)	18	8 (44%)	1 (5.5%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
	Eurasian Teal (<i>Anas crecca</i>)	55	9 (16%)	0	Europe	Cloacal swab, tracheal swab, oropharyngeal swab, or tissue	Murdrasoli et al. (2009)	Hepojoki et al. (2017)
	Blue-winged Teal (<i>Anas discors</i>)	126	27 (21%)	6 (4.7%)	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
	American Green-winged Teal (<i>Anas carolinensis</i>)	110	27 (25%)	0	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
Grey Teal (<i>Anas gracilis</i>)	63	11 (17%)	0	Australia	Combined oropharangeal and cloacal swab, or only cloacal swab	Callison et al. (2006), Chu et al. (2011), modified Chu et al. (2011)	Chamings et al. (2018)	
Long-tailed Duck (<i>Ciangula hyemalis</i>)	8	4 (50%)	0	Europe	Cloacal swab, tracheal swab, oropharyngeal swab, or tissue	Murdrasoli et al. (2009)	Hepojoki et al. (2017)	
Anseriiformes, Anatidae, Aythiinae	Tufted Duck (<i>Aythya fuligula</i>)	1	1 (100%)	0	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
	Ring-necked Duck (<i>Aythya collaris</i>)	23	1 (4%)	0	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
Anseriiformes, Anatidae, Dendrocygninae	Lesser Whistling Duck (<i>Dendrocygna javanica</i>)	33	1 (3%)	0	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)

Table 1. Continued

Host taxonomy	Species	Number sampled	Number gamma-coronaviruses (percentage prevalence)	Number delta-coronaviruses (percentage prevalence)	Continent	Sample type	Screening method	Study
Anseriformes, Anatidae, Anserinae	Snow Goose (<i>Chen caerulescens</i>)	69	0	4 (5.8%)	North America	Cloacal swabs or fecal samples	Hu et al. (2018)	Paim et al. (2019)
	Whooper Swab (<i>Cygnus cygnus</i>)	78	1 (4%)	0	Europe	Cloacal swab, tracheal swab, oropharyngeal swab, or tissue	Murdrasoli et al. (2009)	Hepojoki et al. (2017)
Charadriiformes, Scolopacidae	Curlew Sandpiper (<i>Calidris ferruginea</i>)	34	3 (8.8%)	1 (2.9%)	Australia	Combined oropharangeal and cloacal swab, or only cloacal swab	Callison et al. (2006), Chu et al. (2011), modified Chu et al. (2011)	Chamings et al. (2018)
	Red-necked Stint (<i>Calidris ruficollis</i>)	534	9 (1.6%) ²	2 (0.3%) ²	Australia	Combined oropharangeal and cloacal swab, or only cloacal swab	Callison et al. (2006), Chu et al. (2011), modified Chu et al. (2011)	Chamings et al. (2018)
	Ruddy Turnstone (<i>Arenaria interpres</i>)	157	20 (12.7%) ²	5 (3.2%) ²	Australia	Combined oropharangeal and cloacal swab, or only cloacal swab	Callison et al. (2006), Chu et al. (2011), modified Chu et al. (2011)	Chamings et al. (2018)
Charadriiformes, Laridae	Herring Gull (<i>Larus argentatus</i>)	52	5 (10%)	0	Europe	Cloacal swab, tracheal swab, oropharyngeal swab, or tissue	Murdrasoli et al. (2009)	Hepojoki et al. (2017)
	Lesser Black-backed Gulls (<i>Larus fuscus</i>)	12	0	1 (8%)	Europe	Cloacal swab, tracheal swab, oropharyngeal swab, or tissue	Murdrasoli et al. (2009)	Hepojoki et al. (2017)
	Black-headed Gulls (<i>Chroicocephalus ridibundus</i>)	24	0	1 (4%)	Europe	Cloacal swab, tracheal swab, oropharyngeal swab, or tissue	Murdrasoli et al. (2009)	Hepojoki et al. (2017)
Pelecaniformes, Ardeidae	Grey Heron (<i>Ardea cinerea</i>)	10	0	4 (40%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
	Pond Heron (<i>Ardeola bacchus/speciosa</i>)	123	0	16 (13%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
	Pied Heron (<i>Ardea picata</i>)	7	0	5 (71%)	Australia	Combined oropharangeal and cloacal swab, or only cloacal swab	Callison et al. (2006), Chu et al. (2011), modified Chu et al. (2011)	Chamings et al. (2018)
Pelecaniformes, Threskiornithidae	Black-faced Spoonbill (<i>Platalea minor</i>)	3	0	1 (33%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)
Suliformes, Phalacrocoracidae	Great Cormorant (<i>Phalacrocorax carbo</i>)	24	0	13 (54%)	Asia	Cloacal swabs or fecal samples	Chu et al. (2011)	Chu et al. (2011)

¹Data are from four studies in which the assays used detect both gammacoronaviruses and deltacoronaviruses, and where the authors characterized the majority of the viruses identified. Only species that tested positive are presented. A full table of species from all studies is presented in Table S1 (Supporting Information).

²Chamings et al. characterized most, but not all, detections into gammacoronaviruses or deltacoronaviruses, so that the true prevalence is uncertain.

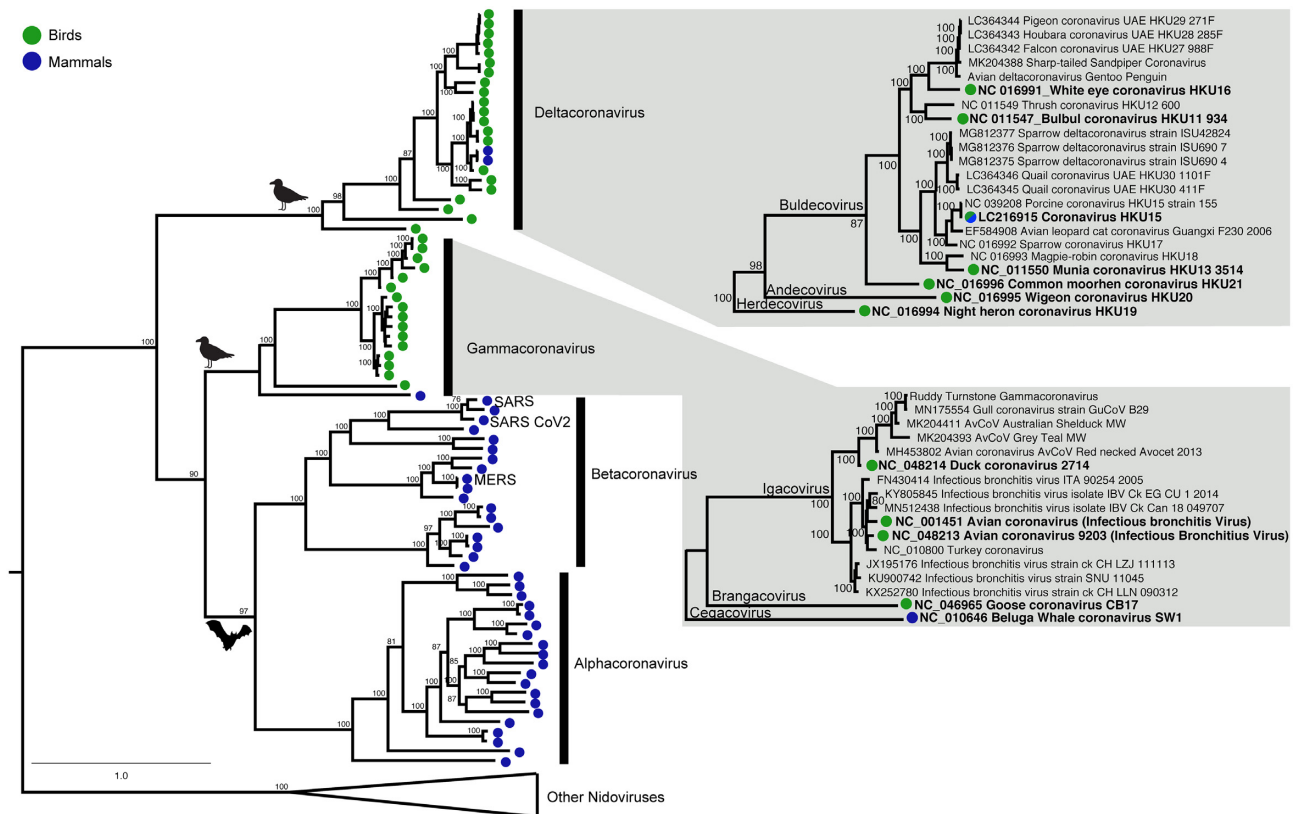


Figure 1. Maximum likelihood phylogeny of the ORF1ab protein that contains the RdRp of the *Coronaviridae*. Sequences shown include all those in RefSeq, in addition to other complete or near complete genomes from wild birds. Colored circles indicate whether viruses infect birds or mammals. As it has been predicted that bats are the source of all alpha and betacoronaviruses and birds the source of all gamma- and deltacoronaviruses, their silhouettes have been placed on the relevant nodes (Chan et al. 2013). Other members of the order *Nidovirales* are set as the outgroup. Bootstrap values > 70% are shown for key nodes. The scale bar indicates the number of amino acid substitutions per site. Amino acid sequences were aligned using the MAFFT E-INS-i algorithm and gaps were stripped using TrimAL, resulting in a 6747 amino acid alignment. The tree of ORF1ab amino acid sequences was estimated using IQ-TREE incorporating the best-fit model of amino acid substitution. The *Gammacoronavirus* and *Deltacoronavirus* genera have been expanded in grey boxes. Viral species ratified by the ICTV are indicated in bold and are adjacent to filled circles in the grey expansions. Subgenera ratified by the ICTV are indicated on the relevant nodes. Taxonomy reflects the ICTV 2019 update.

throughout the Charadriiformes (shorebirds, gulls and terns), with the occasional identification of deltacoronaviruses. Finally, there appears to be a bias towards the detection of deltacoronaviruses in herons and egrets; no gammacoronaviruses have been detected to date in these host species with the exception of AvCoV spill-over events (Rohaim et al. 2019; Table 1).

Understanding the ecology of wild bird coronaviruses at the level of viral species is complicated by severe limitations in the number of gene sequencing studies and the murkiness of species definitions of both gamma- and deltacoronaviruses. Despite this, it appears that most gamma- and deltacoronavirus species may infect multiple host species. Based on data from a short fragment of the RdRp (RNA-dependent RNA polymerase) of gammacoronaviruses (Fig 2), it appears that most sequences represent Duck coronavirus 2714 (DCoV) – a species recently ratified by the ICTV. DCoV appears to be common in both Anseriiformes and Charadriiformes. In the deltacoronaviruses, an unassigned species that we have tentatively referred to here as “novel wild bird deltacoronavirus” (described below), comprises sequences from a broad range of host species such as gulls, shorebirds, penguins, passerines and even bustards (Fig 3). Other deltacoronavirus species, such as Common Moorhen coronavirus, White-eye coronavirus

and Wigeon coronavirus have to date only been described in a single species, or within a single host order, such as *Munia* coronavirus and *Bulbul* coronavirus detected in Passerines (Fig 3).

Wild bird coronaviruses have been detected in all continents, with reports from Europe, Asia, Africa, North America, South America, Australia and even Antarctica. This suggests that these viruses are very common in wild birds, and are found whenever they are screened for. Although studies remain too limited to extrapolate any seasonal patterns, we previously demonstrated a high virus prevalence in a cohort of Mallards sampled across an autumn season (Wille et al. 2015, 2017), and low prevalence in other seasons (Wille et al. 2017). During the autumn, coronavirus prevalence was bimodal, reflecting a similar prevalence pattern of influenza A virus in these birds, such that there was a potential dependence of these viruses on the presence of influenza virus (Wille et al. 2015).

Although data is limited, the similarity in patterns in host range, geographic distribution and seasonality suggests that the ecology of wild bird coronaviruses, particularly DCoV for which we have the most data, may be similar to avian influenza A virus. However, it is clear that more large scale and standardized studies are needed to disentangle host ecology.

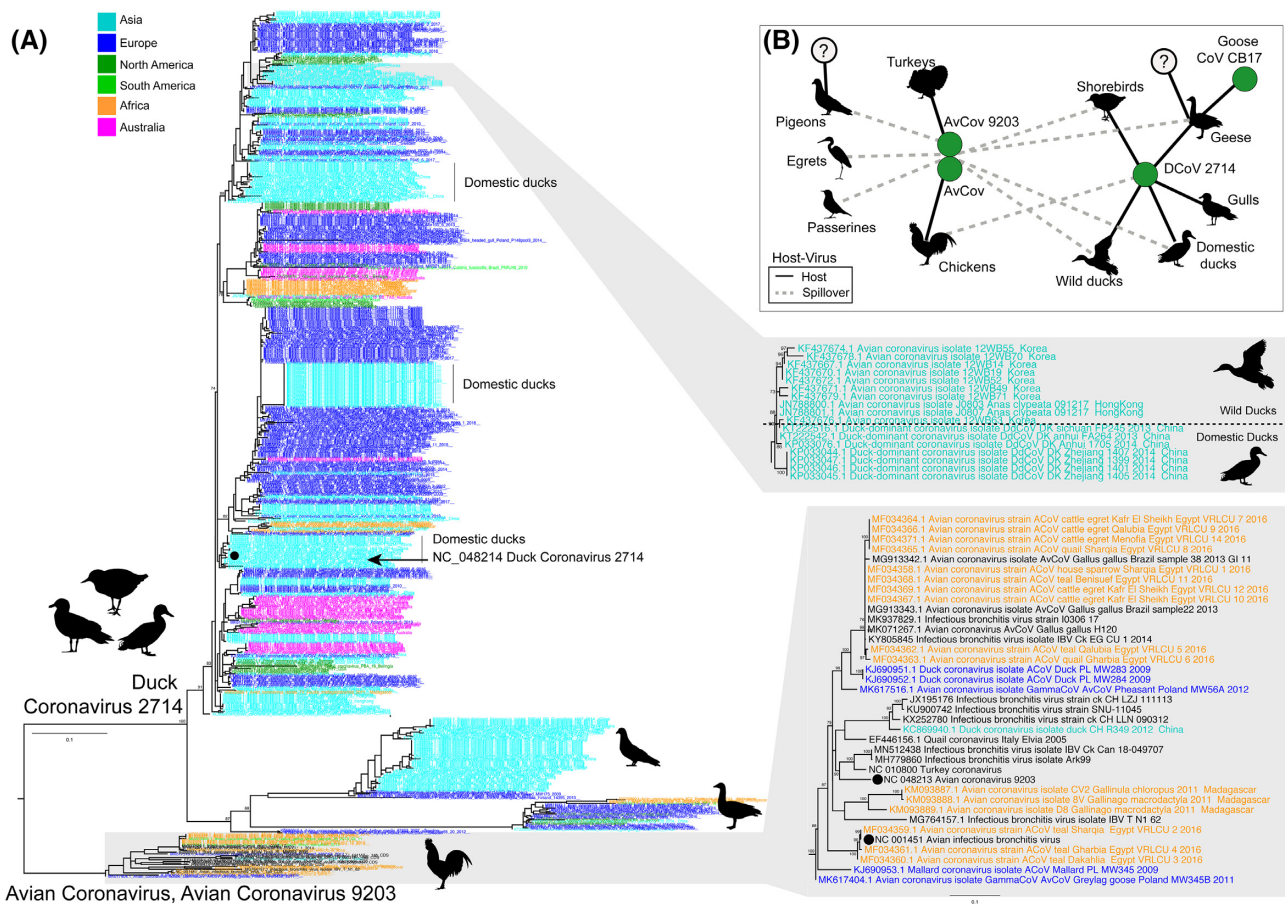


Figure 2. The clade comprising gamma-coronaviruses from wild birds is now a novel viral species. **(A)** Maximum likelihood tree of a 400 bp region of the RdRp of virus subgenus *Igacovirus* reveals a phylogenetic distinction between Duck coronavirus 2714 (DCoV), including those sequences from wild birds, and Avian Coronavirus plus Avian Coronavirus 9203 (AvCoV/AvCoV 9203). The phylogeny includes all sequences of DoV ($n = 557$), the clade comprising pigeon coronavirus (unratified; $n = 122$) and goose coronavirus (unratified; $n = 23$), in addition to all wild bird sequences and reference poultry sequences that comprise the AvCoV/AvCoV 9203 clade ($n = 38$). Sequences from RefSeq that represent each viral species are indicated with a filled circle. The clade containing viruses from pigeons and geese is indicated with a pictogram. Tips are colored by the geographic region of collection and those clades primarily comprising sequences from domestic ducks are shown. There is evidence of virus spill-over between domestic and wild ducks, such as the clade that has been expanded in grey. The phylogeny also highlights that some wild bird sequences fall into the same clade as AvCoV/AvCoV 9203, indicative of spill-over from poultry to wild birds. The scale bar represents the number of nucleotide substitutions per site. **(B)** Bipartite network demonstrating the currently described host range of gamma-coronaviruses in birds. Ratified viral species are denoted by a filled circle. Putative species are denoted by a clear circle with a "?". Hosts are indicated by a pictogram and connected by lines to the viruses from which they have been detected. Solid lines indicate an established host-virus relationship and dashed line indicate likely spill-over events. Silhouettes are distributed under a creative commons licence and downloaded from plyphopic.com.

TECHNICAL LIMITATIONS IN THE DETECTION AND CHARACTERIZATION OF WILD BIRD CORONAVIRUSES

A disparate choice in assays and sequencing-based characterization limits what we can say about the host species ecology of coronaviruses in wild birds. Across the 32 studies screening wild birds (PCR or qPCR), nine different molecular screening methods that have been used more than once (Adzhar *et al.* 1996; Stephensen, Casebolt and Gangopadhyay 1999; Cavanagh *et al.* 2001; Callison *et al.* 2006; Muradrasoli *et al.* 2009; Woo *et al.* 2009; Chu *et al.* 2011; Roh *et al.* 2014; Hu *et al.* 2018). This is problematic as different assays have different performance, limiting comparisons across studies. For example, Chamings *et al.* screened the same 918 samples using three different assays: a commonly used assay targeting the 5' UTR (Callison *et al.* 2006), a commonly used assay targeting the RdRp (Chu *et al.* 2011), and a modified version of that assay. They found that sensitivities for the UTR,

nested and modified PCRs were 46.8%, 70.9% and 77.3%, respectively, with a statistical difference in performance between the assay targeting the 5' UTR and that targeting the RdRp (Chamings *et al.* 2018). This is currently the only study directly comparing the sensitivity of multiple assays on the same sample set. Difficulty in drawing conclusions is exacerbated by the fact that these assays vary in the diversity of coronaviruses they detect. For example, some assays only detect AvCoV, some detect all gamma-coronaviruses and others detect both gamma- and delta-coronaviruses (Table 2).

Analysis of genome sequence data is crucial to describing novel species and understanding the diversity of coronaviruses within individual viral species. We are currently hampered by the combination of different choices in target gene as well as sequencing only 200–400 bp fragments of conserved genes, particularly the RdRp. Disparate choices in target genes limits inference as for many genes there are very few available wild bird sequences. For example, a study conducted in South America

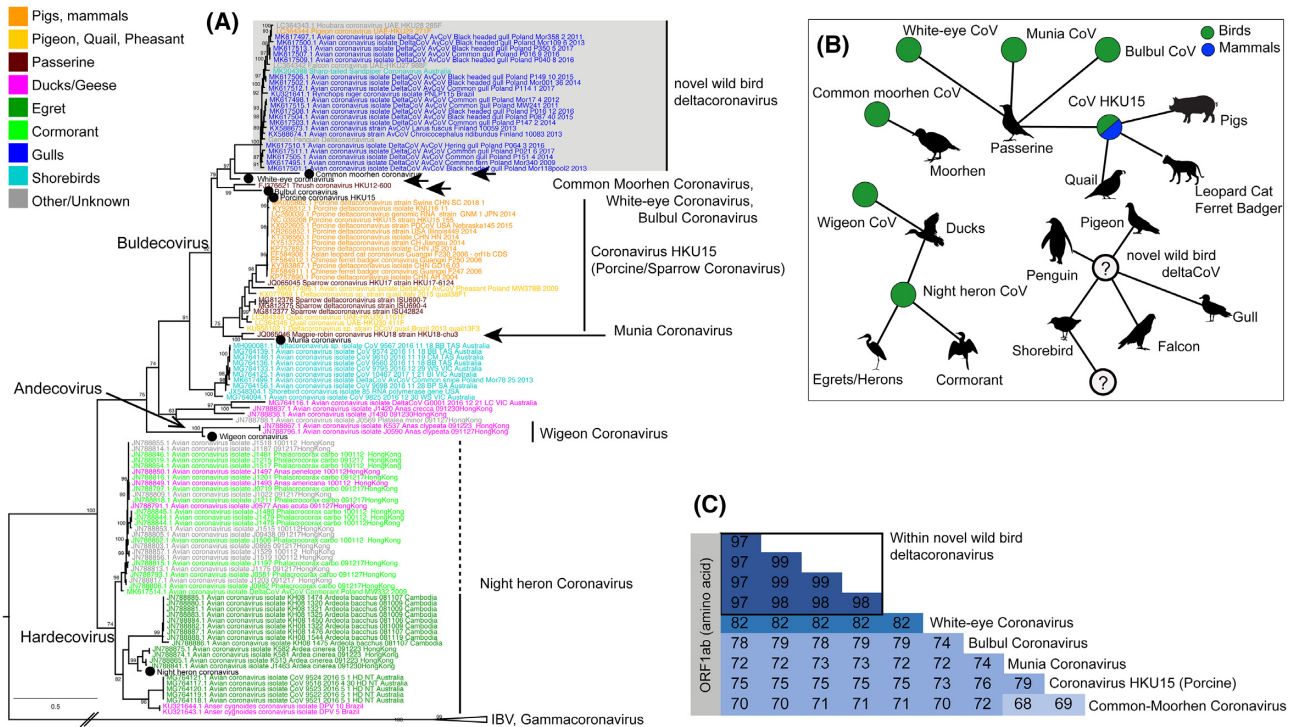


Figure 3. Extensive phylogenetic diversity of deltacoronaviruses and their complex host ecology. **(A)** Maximum likelihood tree of a 400 bp region of the RdRp comprising all available avian deltacoronavirus sequences in GenBank ($n = 110$), in addition to relevant sequences of porcine deltacoronavirus ($n = 11$) and other mammalian deltacoronavirus sequences ($n = 3$). Viral species ratified by the ICTV are denoted by a filled circle and clades including other sequences from the same species are indicated. Subgenera names are indicated on the relevant nodes. The clade comprising wild bird deltacoronavirus is in a grey box. Tips are colored by host species. The scale bar represents the number of nucleotide substitutions per site. AvCoV was used as the outgroup. Silhouettes are distributed under a creative commons licence and downloaded from phylopic.com. **(B)** Bipartite network demonstrating the currently described host range of deltacoronaviruses. Ratified viral species are denoted by a filled circle, colored by whether they infect avian or mammalian hosts. Putative species are denoted by a clear circle with a "?". Hosts are indicated by a pictogram and connected by lines to the viruses from which they have been detected. **(C)** Amino acid percentage identity of the ORF1ab of five members of the novel wild bird deltacoronavirus (LC364342, LC364343, LC364344, MK204388) and other ratified deltacoronavirus species

Table 2. PCR and qPCR assays currently used to screen wild birds for coronaviruses.

Study reference	Target gene	Amplicon Size (bp)	Detection of CoV	Wild bird studies
Adzhar et al. (1996)	S	466	AvCoV	Rohaim et al. (2017); Suryaman et al. (2019)
Callison et al. (2006)	5' UTR	143	AvCoV, potentially DCoV	Amery-Gale et al. (2018); Chamings et al. (2018); Domanska-Blicharz et al. (2014); Duraes-Carvalho et al. (2015)
Cavanagh et al. (2001)	3' UTR	214	AvCoV	Hughes et al. (2009)
Chu et al. (2011)	RdRp	400	Gammacoronavirus and deltacoronavirus	Barbosa et al. (2019); Chamings et al. (2018); Chu et al. (2011); de Sales Lima et al. (2015); Kim and Oem (2014)
Hu et al. (2018)	RdRp	668	Gammacoronavirus and deltacoronavirus	Hu et al. (2018); Paim et al. (2019)
Muradrasoli et al. (2009)	RdRp	179	Gammacoronavirus and deltacoronavirus	Hepojoki et al. (2017); Lebarbenchon et al. (2013); Muradrasoli et al. (2010, 2009); Rohaim et al. (2019); Wille et al. (2015, 2017, 2016)
Roh et al. (2014)	S1	Not provided	AvCoV	Rohaim et al. (2017)
Stephensen et al. (1999)	RdRp	250	AvCoV, potentially DCoV	Jonassen et al. (2005); Jordan et al. (2015); Verdugo et al. (2019)
Woo et al. (2009)	RdRp	440	Gammacoronavirus and deltacoronavirus	Lau et al. (2018); Woo et al. (2009, 2012)

sequenced the S gene of avian coronaviruses from 35 wild birds, although there are no other S genes from wild birds for comparison (Duraes-Carvalho et al. 2015). Similarly, a recent study from the USA sequenced the N gene, and comparisons of these data were limited to deltacoronaviruses for which there are full

genome sequences (Paim et al. 2019). By far the largest number of sequences available are of a short fragment of the RdRp. From these data, there appears to be no genetic structure based on host species or location in the gammacoronavirus (Wille et al. 2016). In studies of avian influenza A virus it is more informative

to sequence the HA gene (the external glycoprotein interacting with the host immune response) rather than the M (matrix) or PB1 (RdRp) genes, largely because the HA gene contains more genetic diversity. Studies of S gene sequences of coronaviruses may prove similarly informative, although this would require the acquisition of many more S gene sequences from wild bird viruses. While it is understandable that studies continue to sequence only gene fragments, we suggest that, at a minimum, sequencing both the RdRp and S genes is preferable.

As highlighted by the ongoing SARS-CoV-2 pandemic, full genome sequencing represents the gold standard for molecular analysis. In birds, the generation of full genome sequences is limited to a handful of screening studies (Woo et al. 2009, 2012; Chen et al. 2013; Lau et al. 2018), although full genome sequences have also been generated in metatranscriptomic studies (Wille et al. 2018, 2019, 2020; Fig. 1). Full genomes are important for understanding species distinctions because species proposals based on short sequence fragments will not be considered by the ICTV, so that the reliance on small genomic fragments such as the RdRp limits the number of ratified species in both the gamma- and deltacoronaviruses. This has resulted in murky viral species classifications, having downstream impacts on studies of viral ecology.

LACK OF TOOLS AND PROTOCOLS FOR VIROLOGICAL AND SEROLOGICAL ASSAYS

Serological tools allow the detection of virus antibodies and hence provide evidence of past infection. Currently, there are no tools for serological surveys of coronavirus species in wild birds and no established protocol for virus isolation. However, given almost a century of work on AvCoV (particularly IBV), we anticipate that, with validation, many tools may be repurposed.

A significant limiting factor in characterizing wild bird coronaviruses is the lack of primary viral isolates: all coronavirus species described in wild birds (except AvCoV) are only described from sequence data. Based on OIE protocols and available literature, IBV are isolated using a number of methods, including embryonated chicken eggs, as well as tissue or cell culture (De Wit and Britton 2018). Given the successful use of embryonated chicken eggs for isolation of other avian viruses (e.g. avian influenza viruses and avian paramyxoviruses/avulaviruses) this may be the most straightforward isolation technique to pursue. Cell culture methods are also routinely used, with detailed methods for tracheal organ culture provided by the OIE manual (De Wit and Britton 2018), and other cell lines such as chicken embryo kidney cells may also be useful (Ferreira et al. 2003). There is also the potential for screening of other cell lines commonly used for coronavirus research, such as Vero cells (Winter et al. 2006).

Currently available serological tools for IBV include ELISA, virus neutralization assays and haemagglutination inhibition assays (De Wit 2000; Villarreal 2010; De Wit and Britton 2018). These assays may prove useful for the detection of closely related viruses, particularly DCoV, although extensive validation is required. Development and validation of a commercially available ELISA test would be ideal and accessible for researchers to complement PCR-based surveillance. Virus neutralization assays are powerful, but highly specific and require isolates of all viral species. This assay is predominately used for strain differentiation within IBV (De Wit 2000; De Wit and Britton 2018). However, IBV, like most coronaviruses, is not naturally agglutinating, such that haemagglutination inhibition assays are

not ideal without neuraminidase treatment, in turn making the test difficult to standardize (De Wit 2000, De Wit and Britton 2018).

THE WILD BIRD CLADE OF AVCOV NOW CONSTITUTES A NOVEL SPECIES

With the most recent update of the ICTV (2019), there are now five ratified species in the genus *Gammacoronavirus*: (i) AvCoV that comprises some genotypes of IBV, (ii) AvCoV 9203 that similarly comprises some genotypes of IBV and turkey coronavirus, (iii) DCoV, (iv) Goose coronavirus CB17 and (v) Beluga Whale coronavirus (de Groot et al. 2011, 2020). For simplicity herein, we will refer to AvCoV and AvCoV 9203 as AvCoV. This update has addressed an important gap in the classification of wild bird coronaviruses: previously, viruses from wild birds fell into a clade that was phylogenetically distinct from AvCoV but were not assigned to a viral species. The first evidence for wild bird gammacoronaviruses came from studies of only a short fragment of the RdRp: these revealed a clear phylogenetic distinction between AvCoV and those viral sequences from wild birds (e.g. Muradrasoli et al. 2009; Chu et al. 2011). Since then, many more such short sequences have since been generated (Wille et al. 2016), revealing similar phylogenetic patterns (Fig. 2). In 2013, the first full genome of this potential novel coronavirus was generated from domestic ducks in China (Chen et al. 2013), with the authors suggesting that this may represent a new species. This was supported by work demonstrating that viruses found in domestic ducks are genetically different from those in chickens (i.e. AvCoV; Zhuang et al. 2015). The authors further suggested that these duck coronaviruses be named “duck-dominant coronavirus” due to their proliferation in domestic ducks. More complete or near complete gammacoronavirus genomes have now been generated in an array of wild bird species as part of metagenomic studies (Wille et al. 2018, 2019, 2020; Canuti et al. 2019) and these cluster with the sequences from wild birds, forming a clade that is distinct from AvCoV (Fig. 1). Although this viral species has recently been named Duck coronavirus 2714, reflecting that the first sequence was generated in ducks, this name does not depict the true diversity of host species infected by this virus. In addition to ducks, related viral sequences have been identified in a variety of Charadriiforme hosts, including shorebirds and gulls (Muradrasoli et al. 2010; de Sales Lima et al. 2015; Chamings et al. 2018; Fig 2B). Given the clear multi-host nature of this viral species, we expect that the host range of this viral species will expand further with additional screening.

Based on very short sequence fragments of the RdRp, there is tentative evidence for two additional species in this genera – goose coronavirus and pigeon coronavirus – although this is difficult to confirm in the absence of complete genome sequences (Zhuang et al. 2020). Combined, these data strongly suggest that the gammacoronaviruses may be more species diverse than currently appreciated.

EXTENSIVE VIRAL DIVERSITY OF THE DELTACORONAVIRUSES

The deltacoronaviruses currently contain seven ratified species spread across three subgenera, all of which have been detected in wild birds (de Groot et al. 2011, 2020; Fig 1). A phylogeny of the partial RdRp (Fig 3) reveals numerous sequences that cluster with the known species, such as Night Heron Coronavirus, but also clades that fall outside of ratified species. Without full

genome sequences it is again difficult to determine whether these viruses constitute new species. A large clade of sequences that fall outside of any ratified species, tentatively referred to here as novel wild bird deltacoronavirus, have been detected in a broad array of species (Fig 3) and are currently described by five complete genome sequences (Figs 1 and 3; Lau et al. 2018; Wille et al. 2019, 2020). The amino acid similarity of these five viruses is 97–99% (ORF1ab; Fig 3), strongly suggesting they represent a single virus species under ICTV guidelines. This is consistent with the findings in Lau et al. (2018), who also argue that the coronavirus found in falcons (*Falco* spp.), Houbara Bustard (*Chlamydotis undulata*) and pigeons comprise three members of a novel viral species. Despite generating full genome sequences, Lau et al. (2018) did not propose a name for this new viral species, although these sequences share less than 90% amino acid sequence similarity with other identified deltacoronavirus species, including the most closely related species, White-eye coronavirus (Fig 3). Based on phylogenies of the partial RdRp, we show here that this viral species is found in an array of Charadriiformes including gulls, skimmers and shorebirds and in many countries suggesting it may be widespread. Most surprising is the recent detection of this viral species in a penguin species of the Antarctic Peninsula: whether this represents a spill-over from gulls (*Larus dominicanus*) and skuas (*Stercorarius* spp.) inhabiting the region, or the detection in a potential host reservoir species is to be determined (Wille et al. 2020).

Overall, partial RdRp data indicate that the true diversity of deltacoronaviruses in wild birds may be far greater than currently described. With the sampling of avian species beyond Anseriiformes and Charadriiformes more novel deltacoronaviruses may be found in under sampled host taxa such as the Passeriformes.

WILD-BIRD – POULTRY INTERFACE AND THE SPILL-OVER OF AVIAN CORONAVIRUS INTO WILD BIRDS

As described above, gammacoronavirus sequences from wild birds largely comprise a distinct species from the poultry dominated AvCoV. By examining available sequence data (partial RdRp) in GenBank we can identify 557 gammacoronavirus sequences that comprise DCoV in addition to 23 representing an unrated viral species in geese, and 122 in an unrated species found in pigeons. Only 21 sequences from wild birds fall into the clade comprising AvCoV (Fig 2). Indeed, there is very little evidence for AvCoV in wild bird populations (e.g. Chu et al. 2011), and those that are reported likely reflect the spill-over of avian coronavirus from poultry. Two studies in Egypt screened wild birds in areas with endemic AvCoV in chicken flocks and found AvCoV in house crows (*Corvus splendens*), house sparrows (*Passer domesticus*) and ducks (Rohaim et al. 2017; Rohaim et al. 2019). House sparrows are known to frequent poultry barns and therefore are important spill-over hosts for notifiable avian viruses such as influenza A virus (Brown et al. 2009). House crows could fill the same niche, and might feed on the waste generated by the poultry industry if they are not found in barns. Not only did Rohaim et al. (2017) find poultry endemic strains in wild birds, they also found vaccine-derived strains, clearly demonstrating viral spill-over into wild birds. Pigeons fit a similar niche, and it is therefore unsurprising that AvCoV has been detected in these birds (Felippe et al. 2010; Martini et al. 2018).

Links with poultry production are important for the detection of AvCoV in wild birds and non-gallinaceous domestic birds.

For example, AvCoV has also been shown in *Eclectus* parrots (*Eclectus roratus*) from a breeder in West Java, Indonesia, and the authors suggested that the combination of backyard farming and low biosecurity may have contributed to this spill-over event (Suryaman et al. 2019). A study from Madagascar surprisingly detected AvCoV in two wild bird species: Common Moorhen (*Gallinula chloropus*) and Madagascan Snipe (*Gallinago macrrodactyla*). This is currently the only detection of AvCoV in wild birds with no obvious link to poultry production (de Sales Lima et al. 2015).

Wild ducks seem to be competent hosts for AvCoV without signs of disease, with the identification of endemic AvCoV strains in wild ducks in Egypt (Rohaim et al. 2017, 2019), Poland (Domanska-Blicharz et al. 2014) and the UK (Hughes et al. 2009), in addition to a detection of a vaccine derived strain in a duck in Egypt (Rohaim et al. 2017). Whether wild ducks transmit this virus is unclear. Due to the low proportion of AvCoV compared to DCoV detections in wild ducks, it is possible that these studies only detect direct spill-over events and that onward transmission is limited (Fig. 2).

There is very little evidence for DCoV infection in chickens. One exception is a study in Laos, where domestic ducks and chickens cohabit, in which DCoV was detected in one of the 22 sequenced coronaviruses from chickens (Pauly et al. 2019). As expected, they also found evidence of AvCoV in domestic ducks, as previously noted by others (Liu et al. 2005; Chen et al. 2013; Zhuang et al. 2015). Domestic ducks provide an interesting study system as the interface between domestic ducks and wild ducks is very porous, especially in countries where domestic ducks share habitat with wild ducks. As such, it is unsurprising that DCoV has been frequently detected in domestic ducks (Chen et al. 2013; Zhuang et al. 2015; Pauly et al. 2019), and that the first full genome of this virus species was from a domestic duck (Chen et al. 2013). Phylogenetic analysis reveals that the same lineages of DCoV are circulating in both domestic ducks and wild ducks. For example, viruses in a clade with > 99% RdRp sequence similarity were found in domestic ducks in China (2013, 2014), wild ducks in Hong Kong (2009) and wild ducks in Korea (2010–2012; Fig. 2). As with the ecology of avian influenza, wild ducks may play a role in the maintenance and evolution of DCoV in regions with high densities of domestic ducks, such as South-East Asia (Gilbert et al. 2006; Hill et al. 2015; Barman et al. 2017; Guinat et al. 2019; Kwon et al. 2020). Indeed, globally there are more domestic ducks raised for human consumption than wild Mallards (Gilbert, Xiao and Robinson 2017), and as such these birds may act as integral hosts for these viruses. Further work to understand the wild bird: domestic duck interface and the maintenance of DCoV in domestic ducks is clearly warranted.

CORONAVIRUSES CAUSING DISEASE IN WILD BIRDS

Until recently, the vast majority of viruses described in birds were those that caused mass mortality events in wild birds (e.g. Wellfleet Bay Virus; Ballard et al. 2017), resulted in production losses due to morbidity or mortality in economically relevant birds (e.g. Infectious Bronchitis Virus; Cavanagh and Naqi 1997, Newcastle Disease Virus; Alexander 2000, Avian Nephritis Virus; Koci and Schultz-Cherry 2002), or posed a zoonotic risk (e.g. Influenza A virus; Olsen et al. 2006). With the advent of metagenomic studies we are beginning to describe large numbers of viruses in wild birds that cause no overt signs of disease (Vibin et al. 2018; Wille et al. 2018, 2019, 2020; Canuti et al.

2019). This is creating a new narrative in which disease-causing viruses are the exception rather than the rule. Coronaviruses are no different. The first described avian coronavirus was IBV (in 1936) and is arguably still one of the most economically important viral respiratory diseases of chickens. Until around 2010, studies were largely focussed on disease-causing coronaviruses in chickens and turkeys (Cook, Jackwood and Jones 2012). This picture changed with the shift to molecular screening tools and PCR assays with broader detection power. Since then, thousands of wild birds comprising 108 species from all over the world have been screened for gamma and/or deltacoronaviruses (Table 1) and the vast majority that are positive for coronavirus do not have any signs of disease.

There is currently no evidence that DCoV causes disease in wild birds or domestic ducks, even when birds are co-infected with other avian viruses (Liu et al. 2005; Chu et al. 2011; Chen et al. 2013; Zhuang et al. 2015; Wille et al. 2016; Chamings et al. 2018). While AvCoV certainly causes disease in poultry, wild birds largely appear to lack disease signs when infected with these viruses (Domanska-Blicharz et al. 2014; Rohaim et al. 2017, 2019). A recent study found a gammacoronavirus, now called Goose coronavirus CB17, as the aetiological agent of a large mortality event of Canada Geese (*Branta canadensis*) and Snow Geese (*Chen caerulescens*) in the Arctic (Papineau et al. 2019). The virus was only identified in a single goose, and hence represents a rare occasion in which a gammacoronavirus causes disease in a wild bird. This novel virus is highly divergent (Papineau et al. 2019) and has been assigned its own subgenus (*Brangacovirus*) that falls as sister group to the subgenus *Igacovirus* containing AvCoV, AvCoV 9203 and DCoV (de Groot et al. 2020).

Multiple different deltacoronavirus species have been described in wild birds without any indication of disease. There have, however, been two instances of disease in domestic birds. First, a recent study from Domanska-Blicharz, Kuczkowski and Sajewicz-Krukowska (2019) described disease in farmed quail in Poland attributed to a Quail deltacoronavirus. This is in contrast to the study initially describing this virus in quails in the UAE wherein there was no sign of disease (Lau et al. 2018). Hence, deltacoronaviruses may cause disease when introduced into poultry. Second, a novel coronavirus was identified in a green-cheeked Amazon parrot (*Amazona viridigenalis*) suffering from psittacine proventricular dilatation disease (PDD) (Gough et al. 2006). At the time, deltacoronaviruses had not yet been described, but analysis of the short sequence generated (DQ233651) indicates that this virus is a deltacoronavirus with 92% similarity to *Munia* coronavirus. Current evidence suggests that PDD is caused by avian bornavirus (Staehele, Rinder and Kaspers 2010), so whether this bird died due to infection with *Munia* coronavirus or avian bornavirus is unclear.

There is one oddity. Puffinosis is a disease of Manx Shearwaters (*Puffinus puffinus*) in which young birds get blisters on their feet, conjunctivitis and problems with movement. Up to 70% of chicks that are diagnosed with puffinosis die (Miles and Stoker 1948). In an attempt to identify the aetiological agent of puffinosis, homogenates of lungs or blood from two affected shearwaters were inoculated into mice, and a coronavirus was subsequently described. However, virus isolation by eggs or cells was unsuccessful, this virus was not found in the control mice (Nuttall and Harrap 1982), and shearwaters inoculated with a suspension of this virus did not develop puffinosis. The authors therefore speculate that they may have isolated a virus similar to murine coronavirus, a betacoronavirus, that causes murine illness (Nuttall and Harrap 1982). Subsequent studies on puffinosis have not considered a coronavirus as the aetiological agent

(Nuttall, Perrins and Harrap 1982; Nuttall, Brooke and Perrins 1985; Kirkwood et al. 1995) and there is limited further work in the literature in attempting to ascertain whether this disease is caused by virus, including a coronavirus.

CROSS-SPECIES TRANSMISSION IN DELTACORONAVIRUSES AND THE EMERGENCE OF PORCINE CORONAVIRUS

Gammacoronaviruses and deltacoronaviruses are dominated by viruses found in birds, although the most divergent virus in the former group is beluga whale coronavirus from mammals. In the deltacoronaviruses, mammalian viruses are nested within the subgenus *Buldecovirus*. Porcine deltacoronavirus (PDCoV; Coronavirus HKU15) is a rapidly emerging swine virus, found globally. PDCoV was initially detected in 2009 in faecal samples from pigs in Asia, but its aetiological role was not identified until 2014 when it was associated with diarrhea in pigs in the United States (Woo et al. 2012; Zhang 2016). PDCoV is sister to a clade containing sparrow coronavirus and previous analysis showed that these two viruses likely constitute the same viral species, with > 96% amino acid identity in domains used for species demarcation (de Groot et al. 2011; Woo et al. 2012). This indicates that a cross-species transmission event from birds to mammals likely occurred relatively recently. Further, the S proteins of Bulbul coronavirus and *Munia* coronavirus exhibit greater sequence identity with the PDCoV S protein compared with that of sparrow coronavirus (70.2% and 71.2% versus 44.8%), suggesting that a recombination event has occurred in the evolutionary history of this virus (Woo et al. 2012). A study assessing PDCoV receptor specificity found that this virus uses host aminopeptidase N as an entry receptor, but strikingly was able to efficiently infect cells of an unusually broad species range, including human and chicken cells (Li et al. 2018). Further experimental infections have since demonstrated that chickens become infected and transmit the virus effectively to uninfected contact birds (Boley et al. 2020). Infection of chicken cells is perhaps unsurprising given the viral species Coronavirus HKU15, that includes PDCoV, also infects birds (e.g. Sparrow Coronavirus strain). That human cells may be easily infected is of concern as it suggests a limited barrier to zoonotic transmission, although without serology studies in piggery workers it is challenging to assess whether any zoonotic transmission has yet occurred. In addition to pigs, deltacoronaviruses have also been described in Asian leopard cats (*Prionailurus bengalensis*) and Chinese ferret badgers (*Melogale moschate*) in markets (Dong et al. 2007): these viruses comprise some of the first description of deltacoronaviruses. That these viruses have only ever been described in this one study in wet markets and/or exotic mammals is of considerable concern as it highlights the propensity for these viruses to spill over into mammals and vast under-sampling of mammalian deltacoronaviruses.

CONCLUSIONS

Members of the gamma- and deltacoronaviruses are common in wild birds, and we anticipate that more viral diversity will be observed with continued screening and sequencing, particularly through metagenomics. Species definitions of avian coronaviruses remain challenging due to limitations in the generation of full genomes of these viruses, preventing consideration by the ICTV. This, in turn, inhibits the true assessment of viral diversity in wild birds and creates limitations in understanding

cross-species transmission within wild birds, between wild birds and domestic birds and from wild birds to mammals.

Of particular importance is that we demonstrate the transmission of gammacoronaviruses across the wild bird:poultry interface, both between poultry and wild birds and domestic ducks and wild birds. This porous interface has led to control problems in viruses such as influenza A virus, and it would be of great concern, with potentially large socioeconomic ramifications, if another wild bird coronavirus entered the poultry reservoir. Cross-species transmission between the avian and mammalian reservoir appears to be limited in gammacoronaviruses, but there is evidence of more than one cross-species transmission event involving the deltacoronaviruses, with the detection of these viruses in wet markets and the emergence of porcine deltacoronaviruses. Indeed, we suggest that there is a systematic under-sampling of deltacoronaviruses in mammals in wet markets and in natural habitats. Cross-species transmission is a common feature of coronaviruses and it is therefore imperative that gamma- and deltacoronaviruses are included in future surveillance efforts. A basic understanding of the extent and pattern viral diversity in species that we currently monitor for zoonotic viruses, including birds, will be central to understanding the future emergence of viruses in both socioeconomically relevant animal species, as well as in humans.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSRE](#) online.

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