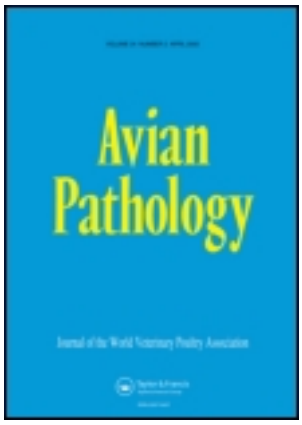

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Coronaviruses in poultry and other birds

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REVIEW

Coronaviruses in poultry and other birds

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The number of avian species in which coronaviruses have been detected has doubled in the past couple of years. While the coronaviruses in these species have all been in coronavirus Group 3, as for the better known coronaviruses of the domestic fowl (infectious bronchitis virus [IBV], in *Gallus gallus*), turkey (*Meleagris gallopavo*) and pheasant (*Phasianus colchicus*), there is experimental evidence to suggest that birds are not limited to infection with Group 3 coronaviruses.

In China coronaviruses have been isolated from peafowl (*Pavo*), guinea fowl (*Numida meleagris*; also isolated in Brazil), partridge (*Alectoris*) and also from a non-gallinaceous bird, the teal (*Anas*), all of which were being reared in the vicinity of domestic fowl. These viruses were closely related in genome organization and in gene sequences to IBV. Indeed, gene sequencing and experimental infection of chickens indicated that the peafowl isolate was the H120 IB vaccine strain, while the teal isolate was possibly a field strain of a nephropathogenic IBV. Thus the host range of IBV does extend beyond the chicken.

Most recently, Group 3 coronaviruses have been detected in greylag goose (*Anser anser*), mallard duck (*Anas platyrhynchos*) and pigeon (*Columbia livia*). It is clear from the partial genome sequencing of these viruses that they are not IBV, as they have two additional small genes near the 3′ end of the genome.

Twenty years ago a coronavirus was isolated after inoculation of mice with tissue from the coastal shearwater (*Puffinus puffinus*). While it is not certain whether the virus was actually from the shearwater or from the mice, recent experiments have shown that bovine coronavirus (a Group 2 coronavirus) can infect and also cause enteric disease in turkeys.

Experiments with some Group 1 coronaviruses (all from mammals, to date) have shown that they are not limited to replicating or causing disease in a single host. SARS-coronavirus has a wide host range. Clearly there is the potential for the emergence of new coronavirus diseases in domestic birds, from both avian and mammalian sources. Modest sequence conservation within gene 1 has enabled the design of oligonucleotide primers for use in diagnostic reverse transcriptase-polymerase chain reactions, which will be useful for the detection of new coronaviruses.

Viruses of Wild Birds and Minor Domestic Species

The outbreaks of highly pathogenic avian influenza viruses around the globe in the past few years have been devastating for the countries concerned (Capua & Marangon, 2003; Capua & Alexander, 2004), and have created fears with the public (Swayne & Beck, 2004). Attention has not only been on the epidemics in the major poultry species, the domestic fowl (Liu *et al*., 2003; de Wit *et al*., 2004; Ellis *et al*., 2004a, b; Lee *et al*., 2004a; Armin *et al*., 2004), but also in minor species, including turkeys (Capua *et al*., 2004), commercial ducks (Kwon *et al*., 2005), and waterfowl and other wild birds (Ellis *et al*., 2004a).

One of the other great scourges of poultry, Newcastle disease virus, has also been studied not only in the context of disease within domestic fowl (Meulemans *et al*., 2002; Nakamura *et al*., 2004), but also in turkeys (Wehmann *et al*., 2003), pigeons and doves (Aldous *et al*., 2003, 2004; Barbezange & Jestin, 2003, 2005; Terregino *et al*., 2003), geese (Wan *et al*., 2004), ducks (Aldous *et al*., 2003), and other species, including sea birds (Lee *et al*., 2004b) and parrots (Kommers *et al*., 2003).

Chicken anaemia virus is known to be the cause of the greatest economic loss in the poultry industry among the small DNA genome-containing viruses (Todd, 2000; Todd *et al*., 2003). Other members of the *Circoviridae* family, infecting species other than domestic fowl, have also been receiving attention; for example, beak and feather disease virus in psittacines (Hess *et al*., 2004; Johne *et al*., 2004; Raue *et al*., 2004), goose circovirus (Chen *et al*., 2003a; Ball *et al*., 2004; Smyth *et al*., 2005) and columbid circovirus in pigeons (Soike *et al*., 2001).

Geese have also been the subject of study with respect to parvovirus (Tatár-kis *et al*., 2004), goose haemorrhagic polyoma virus (Lacroux *et al*., 2004), reovirus infections (Palya *et al*., 2003) and West Nile virus (Banet-Noach *et al*., 2003) infections.

From the above it can be seen that there has been a lot of interest in virus infections of species other than the most numerous one (domestic fowl), notably in geese,

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pigeons, ducks and psittacines. These four types of bird also feature prominently in the very recent development of our understanding of coronavirus in avian species. Since the emergence of SARS-coronavirus in humans in 2002 there has been increased interest in coronaviruses in other species, including birds. Prior to that time, our knowledge of coronaviruses in birds was limited largely to three species: domestic fowl, turkeys and pheasants. The coronaviruses of these species (infectious bronchitis virus [IBV], turkey coronavirus [TCoV, also known as bluecomb disease virus] and turkey enteritis coronavirus) and pheasant coronavirus (PhCoV) are clearly very closely related in terms of gene sequences, and also antigenically. Other galliform birds (guinea fowl, partridge, peafowl) have been shown recently to be infected by coronaviruses that are very similar to IBV. Indeed, as discussed in the following, in some cases it probably was IBV that was recovered from these species. Perhaps the biggest recent step forward in the context of coronaviruses in birds has been the detection of coronaviruses in greylag goose (the ancestor of most domestic geese), mallard duck, pigeons and a parrot. These coronaviruses are clearly not simply isolates of IBV, but represent new coronavirus species.

In this review I shall be looking at these newly discovered viruses, putting them into context with respect to the well-known avian coronaviruses, and also with regard to what we know of the host range of coronaviruses that infect mammals.

Coronavirus is Not Necessarily Limited to Replicating in—or Causing Disease in—a Single Host

This was emphasized by the spread of SARS-coronavirus from mammals such as the palm civet cat (*Paguma larvata)* and racoon dog (*Nyctereutes procyonoides*) to humans in 2002, and the subsequent spread from China to other countries in the following year. The virus has also been shown to replicate in cynomolgus macaques (*Macaca fascicularis*), ferrets (*Mustela furo*), domestic cats (*Felis domesticus*) and mice (*Mus musculus*) (see references in Cavanagh, 2005)

Coronaviruses had often been described as being fastidious. This claim arose from the difficulty that virologists had experienced in finding types of cells in which to grow coronaviruses *in vitro*. However, some 20 years before the emergence of SARS-coronavirus in humans, experiments had already shown that coronaviruses were not limited to replication in a single host.

Porcine transmissible gastroenteritis virus (TGEV) is a Group 1 coronavirus that causes severe enteritis in young pigs. Over 20 years ago, Woods *et al*. had demonstrated that the Group 1 canine coronavirus (CCoV) and feline coronavirus (FCoV) can replicate in pigs (Woods *et al*., 1981, 1992). Moreover, inoculation of pigs with a virulent FCoV resulted in clinical signs typical of a virulent TGEV infection (Woods *et al*., 1981). Tissue-culture-adapted FCoV and CCoV did not give clinical signs. Thus, FCoV and CCoV are able to replicate in pigs, and some, but not all, strains are able to cause disease. The type II strains of these two viruses and TGEV have integral membrane (M) and nucleocapsid (N) proteins with very similar ($>90\%$) protein sequences (Gonzalez *et al*., 2003). Their surface spike (S) proteins, which are responsible for the attachment of the virus to cells, have $>80\%$ identity overall, including $>94\%$ identity from amino acid residues 275 to 1447. We know now that these three viruses can use the same cell surface molecule for attaching to cells *in vitro*. For example, feline amino peptidase N, which is a receptor for FCoV, also acts as a receptor for TGEV and for human coronavirus 229E, another Group 1 coronavirus (Kolb *et al*., 1997).

An isolate of a coronavirus apparently from a child experiencing diarrhoea had proteins with 99% identity with those of bovine enteric coronavirus (BCoV) (Zhang *et al*., 1994). Assuming that there had not been cross-contamination in the laboratory, then this indicates that bovines and humans can be infected with the same coronavirus.

The recently discovered canine respiratory coronavirus has 95% identity with the proteins of bovine coronavirus (Erles *et al*., 2003). Might each of these two virus species infect both dogs and cattle?

These observations make it clear that we should not assume that a given coronavirus species is limited to replication in—or to causing disease in—a single host.

Before discussing the coronaviruses that have been detected in avian species, it is appropriate to look at the *Coronavirus* genus as a whole, including structural, antigenic and genetic features.

Coronaviruses: the Three Groups

The known coronavirus species are presented in Table 1. They have been assigned to three groups. Initially this

Table 1. *Coronavirus species*[a]

Group 1
Subgroup 1a
Porcine transmissible gastroenteritis coronavirus (TGEV)
Canine enteric coronavirus (CECoV)
Feline coronavirus (FCoV)
Subgroup 1b
Porcine epidemic diarrhoea coronavirus (PEDV)
Human coronavirus 229E (HCoV) and other species (e.g. NL-63)
Bat coronavirus
Group 2
Subgroup 2a
Murine hepatitis coronavirus (MHV)
Human coronavirus OC43 (HCoV OC43) and other species
Bovine coronavirus (BCoV)
Canine respiratory coronavirus (CRCoV)
Porcine haemagglutinating encephalomyelitis coronavirus (HEV)
Puffinosis coronavirus (from a shearwater, *Puffinus puffinus*)
Subgroup 2b
SARS-coronavirus (SARS-CoV)[b]
Group 3
Infectious bronchitis coronavirus (IBV)
Turkey coronavirus (TCoV)
Pheasant coronavirus (PhCoV)
Goose coronavirus (GCoV)
Duck coronavirus (DCoV)
Pigeon coronavirus (PiCoV)

[a]Examination of the table shows that for a given species of animal there can be >1 species of coronavirus, and there can be coronavirus species from >1 group.

[b]The Coronavirus Study Group has recommended to the International Committee on the Taxonomy of Viruses that the SARS-coronavirus be included in Group 2.

was based on antigenic relationships (McKintosh *et al*., 1969; Bradburne, 1970; Pensaert *et al*., 1981), which were later largely supported by gene sequencing. Some 25 years ago it was suggested that TCoV was closely related to BCoV of Group 2 (Dea *et al*., 1990). We now know that this is not the case and that TCoV is unequivocally closely related to IBV, both in terms of gene and protein sequences (Breslin *et al*., 1999a,b; Cavanagh *et al*., 2001), and also antigenically (Guy *et al*., 1997; Breslin et al., 2000; Loa *et al*., 2000).

Structural Features of Coronaviruses

The composition of coronavirus particles is illustrated in Figure 1. The large surface S protein is responsible for the attachment of the virus to cells, and for the fusion of the virus envelope with the cell surface or endosomal membranes, thereby releasing the single-stranded, positive-sense RNA genome into the cytoplasm, where replication of the RNA takes place. The genome of IBV is a 27.6 kb single-stranded, positive-sense RNA. Studies with some coronaviruses have shown that the S protein is a determinant of host range and pathogenicity (de Haan *et al*., 2002; Haijema *et al*., 2004). In the case of IBV, the S protein is possibly a determinant but is not necessarily a determinant of pathogenicity. Thus when the S gene of the non-pathogenic Beaudette strain was replaced with that of the pathogenic M41 strain, the host range of Beaudette was altered *in vitro*: the virus no longer replicated in Vero cells (Casais *et al*., 2001, 2003). When this spike-swapped recombinant was inoculated (by nose and eye drop) into chickens, it was still non-pathogenic—although it did stimulate protective immunity against challenge by M41 (Hodgson *et al*., 2004).

In addition to the four structural proteins (S, E, M and N) that are possessed by all coronaviruses, Group 2 coronaviruses contain a fourth membrane-associated protein, the haemagglutinin-esterase protein (reviewed by Enjuanes *et al*., 2000; Cavanagh, 2005). The order of the structural protein genes within the genome is the same for all coronaviruses (Figure 2).

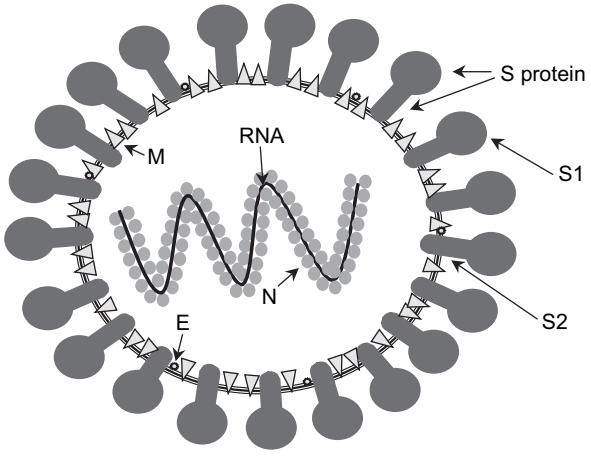

Figure 1. *IBV particles comprise a lipid envelope, derived from internal cell membranes, associated with four structural proteins and an RNA genome of 27.6 kb. The N protein surrounds the RNA. The other three proteins are in the envelope. The E protein is present in only trace amounts and is required for budding of the virus particle. At the surface is the large spike protein, S. The M protein is the most numerous of the envelope proteins, making contact with S, E and N.*

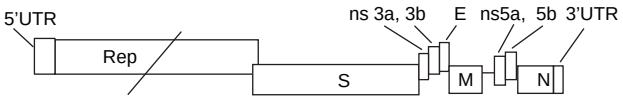

Figure 2. *Genome organization of IBV. Gene 1, which accounts for approximately two-thirds of the genome, encodes many proteins that are associated with RNA replication and transcription. All coronaviruses have the structural protein genes S, E, M and N (see Figure 1) in the same order. Where the genomes of coronaviruses differ is in respect of the number and location of genes that encode non-structural (ns) proteins that are situated among the structural protein genes. IBV has two such genes, 3 and 5, which encode four ns proteins, 3a and 3b, and 5a and 5b. The E protein is encoded by the third ORF of gene 3.*

Interspersed among the genes that encode the structural proteins are some relatively small genes that encode what are believed to be non-structural proteins (Figure 2). The number of such genes varies among coronaviruses, including among species within a given group (reviewed by Enjuanes *et al*., 2000; Cavanagh, 2005). In the case of IBV, TCoV and PhCoV there are two such genes, numbers 3 and 5 (Boursnell *et al*., 1987; Breslin *et al*., 1999a; Cavanagh *et al*., 2001, 2002). Each of these genes encodes two non-structural proteins: 3a and 3b, 5a and 5b (Figure 2). None of these four proteins are required for replication in cell culture, in tracheal organ culture or in chicken embryos; recombinant IBVs in which these genes have been inactivated or deleted still replicate to the same titres as the parent virus (Casais *et al*., 2005; Hodgson *et al*., 2005). The functions of the small non-structural proteins are not known for any coronavirus.

The virion proteins have $<40\%$ amino acid identity between species in different Groups. Group 1 is rather heterogeneous and has been split into two subgroups, with $<45\%$ structural protein amino acid identities between the subgroups (Gonzalez *et al*., 2003; Table 1). FCoV type II, canine coronavirus type II and TGEV of Subgroup 1a have M and N proteins that share $>90\%$ amino acid identity, and S proteins with $>80\%$ amino acid identity. To put that into context, this degree of amino acid identity is similar to that exhibited among many serotypes of IBV. Furthermore—and this is relevant to a topic (host range) to be discussed later—IBV, TCoV and PhCoV have a similar degree of protein relatedness to one another as is exhibited among TGEV, FCoV type II and CCoV type II.

Infectious Bronchitis

IBV was the first coronavirus to be isolated, in the 1930s. Given its importance to the economics of the poultry industry, which is related in part to its extensive antigenic variation (Meulemans *et al*., 2001; Farsang *et al*., 2002; Liu & Kong, 2004; Cavanagh *et al*., 2005; Gelb *et al*., 2005), it has been widely studied for more than half a century (reviewed by Cavanagh & Naqi, 2003). Although it causes extensive damage to the mucosae of the respiratory tract, its impact is magnified as a consequence of its enhancement of disease associated with co-infections by bacteria and mycoplasmas (Matthijs *et al*., 2003; Landman & Feberwee, 2004). Control is partially achieved by the ubiquitous use of live attenuated vaccines (Bijlenga *et al*., 2004), although they

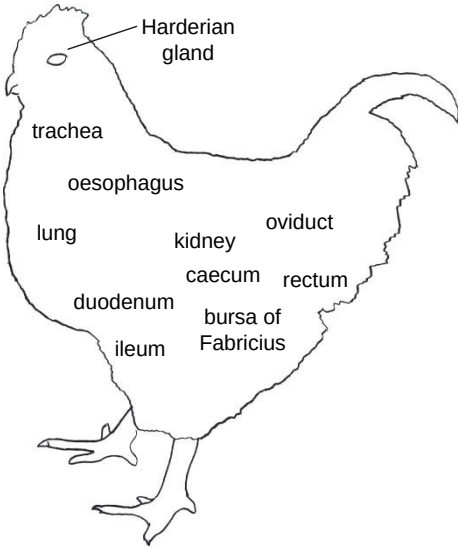

Figure 3. *IBV replicates at many epithelial surfaces. Pathology is most commonly observed in the respiratory tract. Some strains are highly nephropathogenic.*

can be associated with exacerbation of secondary bacterial infections (Matthijs *et al*., 2003).

Despite its name, IBV replicates at many epithelial surfaces (Figure 3; reviewed by Cavanagh, 2003). These include much of the alimentary canal, as well as kidney, gonads and bursa. Pathology is not usually associated with infection of the alimentary tract by IBV, although proventriculitis has been attributed to strains of IBV (Yu *et al*., 2001). It has long been known that some strains of the virus are highly nephropathogenic (Liu & Kong, 2004). It may be that some strains of IBV are associated with pathological manifestations that have not yet been recognized. The capacity of the virus to replicate in so many tissues may contribute to the sequence diversity of its proteins, especially that of the S protein. Strains of IBV differ in their virulence, and the genetic background of the host can influence the outcome of infection (see Bacon *et al*., 2004, and references cited therein). Thus, IBV is not a simple pathogen and its heterogeneity with respect to its protein sequences, broad tissue tropism and pathogenicity should be borne in mind in the context of the host range of the virus, which, as described later, may be wide.

Turkey Coronavirus

A coronavirus was shown 30 years ago to be involved with enteritis of turkeys in the USA where it has been most thoroughly studied. In young poults it can be associated with mortality, while in older birds it is debilitating, resulting in underperformance with regard to meat and egg production (Guy, 2000, 2003; Guy *et al*., 2000). The virus was confirmed as being in the UK in 2001 (Cavanagh *et al*., 2001) and has subsequently been demonstrated in Brazil and Italy. Field studies in my laboratory, involving virus detection by reverse transcriptase-polymerase chain reactions (RT-PCRs), and detection of antibodies in turkey sera by immunofluorescence with IBV-infected cells and an enzyme-linked immunosorbent assay (Chen *et al*., 2003b) indicate a prevalence of $>30\%$ (Culver *et al*., unpublished observations).

Relationship between IBV and TCoV

Research at the University of North Carolina in the mid-1990s showed that coronaviruses from turkeys were genetically (Breslin *et al*., 1999a,b) and antigenically (Guy *et al*., 1997, 2002; Breslin *et al*., 2000) closely related to IBV, confirmed elsewhere (Cavanagh *et al*., 2001; Ismail *et al*., 2001a) reviewed by Guy (2000). Indeed, antibodies binding to IBV antigens had been detected in turkey sera as early as 1987 by Weissman *et al*. (1987), using an IBV enzyme-linked immunosorbent assay. That overlooked report was perhaps the first to demonstrate the close relationship between TCoV and IBV.

An earlier suggestion from work in Canada, that TCoV was closely related to bovine coronavirus (Group 1), was wrong (reviewed by Guy, 2000). The sequences of the genes of the turkey coronaviruses in the US, and those subsequently detected in the UK (Cavanagh *et al*., 2001), had 85 to 90% identity with those of IBV, which is high given that strains of IBV commonly differ from each other by this amount. The exception was in the spike protein; recent research has revealed that there is only approximately 34% identity between the spike protein of the TCoV isolates and those of IBVs, a lower identity than the lowest yet seen between IBV strains (Lin *et al*., 2004). The spike proteins of IBV serotypes generally have between 75 and 85% identity, although some serotypes have as little as 60% identity.

Interestingly, the S proteins of the three TCoV isolates, although isolated many years apart, had 91% amino acid identity among each other—greater identity than is usually shown by serotypes of IBV. It is possible that TCoV will be less heterogeneous than IBV with respect to its spike protein; further study is required.

Pheasant Coronavirus

Electron microscopy and some antigenic analysis had indicated that pheasants were infected by a coronavirus (PhCoV), sometimes associated with respiratory disease and sometimes associated with kidney disease (Spackman & Cameron, 1983; Lister *et al*., 1985; Gough *et al*., 1996; Pennycott, 2000). Gene sequencing subsequently confirmed that it was coronavirus (Cavanagh *et al*., 2002). PhCoV has since been detected in many pheasants, collected from many pheasant ranges that were experiencing respiratory disease (Welchman *et al*., 2002). The degree of genetic relatedness to IBV is the same as that between IBV and TCoV except with regard to the S protein, in which PhCoV and IBV are more closely related to each other than to TCoV. The gene sequences of the dozen or so pheasant isolates that we sequenced differed by approximately 10% from those of IBVs that we worked with in the laboratory and differed similarly from all IBV sequences in the databanks (Cavanagh *et al*., 2002). There were differences in gene sequences amongst the pheasant coronaviruses, similar to that which exists among serotypes of IBV.

Host Range of IBV, TCoV and PhCoV

Is each of these avian coronaviruses able to infect other species? Experimental inoculation of TCoV into chickens resulted in replication in the alimentary tract, although asymptomatically (Ismail *et al*., 2003).

Inoculation of chickens with several PhCoVs resulted in production of antibodies, indicative of replication, but without disease (Gough *et al*., 1996). Thus these viruses have the capacity to replicate in other hosts but they might not cause disease. When the capacity of FCoV and CCoV to infect pigs was studied, the degree of pathology caused was strain dependent (Woods *et al*., 1981). Clearly only a few strains of TCoV and PhCoV have been examined in chickens, and then in good laboratory conditions, rather than in the field where other factors can exacerbate the consequences of infection by a virus. An open mind should be kept, therefore, with regard to the possibility that coronaviruses of one galliform species might be able to cause disease in another.

The relatedness at the genetic level and the capacity of IBV, TCoV and PhCoV to infect chickens, raises the question as to whether these three viruses should be considered as three distinct species or as one species, with different strains causing disease in one host species but not the other (Cavanagh, 2001). TCoV and IBV have been considered for many years to be distinct species. Given that TCoV is an enterotropic virus, certainly in terms of pathology, whereas IBV is a respirotropic virus in terms of pathology, and the great differences in the sequences of their S proteins, it seems reasonable to continue considering them as separate species. The lack of disease in chickens experimentally infected with three PhCoV isolates has likewise suggested that PhCoV be considered as a separate coronavirus species.

Coronaviruses in Other Galliform Birds

Chinese researchers have very recently sequenced the complete genomes of coronaviruses isolated from domestic peafowl (*Pavo cristatus*), partridge (*Alectoris* sp.), guinea fowl (*Numida meleagris*) and teal (*Anas* sp.). All these viruses were IBV-like with respect to their genome organization (which differs in various respects from the coronaviruses in Groups 1 and 2) and, indeed, with the sequences of the genes encoded by their genes.

The proteins of the peafowl virus had >99% identity with the widely used IBV H120 vaccine (Liu *et al*., 2005: databank accession number for the whole genome sequence, AY702085). This extremely high degree of identity makes it almost certain that it was the vaccine strain that had been recovered from the peafowl, which had chickens in the neighbourhood. When the isolate was inoculated into chickens, it caused no disease—which is what one would expect of the H120 vaccinal strain. No disease had been reported in the peafowl from which the coronavirus had been isolated. It would be helpful if sera from peafowl were to be analysed for antibodies cross-reactive with IBV (e.g. by virus neutralization test). This would be another way of demonstrating that peafowl had most probably been infected by the H120 infectious bronchitis vaccine.

Ito *et al*. (1991) isolated from 5-day-old and 10-day-old guinea fowl a coronavirus that was antigenically related to IBV. The guinea fowl had been suffering high mortality and low feed consumption, and enteritis was reported. When the isolate was inoculated experimentally (intranasally) into chickens and into guinea fowl, both species exhibited respiratory distress and aqueous faeces. Sera from nearby layer breeder chickens (which had been multiply vaccinated against IB) did neutralize the guinea fowl virus, indicating antigenic relatedness between the guinea fowl coronavirus and IBV. Whether the guinea fowl virus was a "genuine" guinea fowl coronavirus (i.e. a separate species) or an IBV that had spread from nearby chickens to the guinea fowl is not known. Nevertheless, the field observations and the laboratory infections indicate that the virus from the guinea fowl had a host range of more than one bird species.

From the above it would seem probable that other gallinaceous birds, at least, would be susceptible to coronaviruses that are genetically similar to IBV (i.e. Group 3 coronaviruses).

Coronaviruses in Non-galliform Birds

The isolate in China from a teal—also kept domestically, near chickens—had a spike protein that had ~90% identity with some known IBV strains, including a nephropathogenic one (Liu *et al*., 2005; databank accession number for the whole genome sequence, AY702975). When this isolate was inoculated into chickens, it caused disease, including kidney involvement. This, plus the very high relationship between the genes of the teal isolate and IBV, including the S gene, makes it probable that the teal isolate was actually an IBV strain that had spread to the teal from nearby chickens. Assuming that these findings did not result from cross-contamination in the laboratory, it would appear that IBVs can replicate not only in the chicken and other gallinaceous birds, but also in teal, a non-gallincaceous bird. Notwithstanding, it is possible that the virus was a "genuine" teal coronavirus that was able to infect and cause disease in chickens. No disease was reported in the teal at the time of isolation of the virus. As in the case of the peafowl virus, it would be helpful if serum from the teal were tested in a neutralization test with the IBV-like virus that had been recovered from the teal.

Transport of IBV by Avian Species other than Chickens

In summary, it would appear that IBV can replicate in other gallinaceous and in some non-gallinaceous birds, asymptomatically in at least some cases. Irrespective of whether IBV causes disease in species other than the chicken, these other birds would act as a vector of IBV. Given that birds fly, an IBV strain could be transported over long distances by other avian species.

Group 3 Coronaviruses in Greylag Goose, Mallard Duck and Pigeon

In Australia, in 1988, a coronavirus was isolated from tracheal/cloacal swabs, using chicken embryos, from racing pigeons that were exhibiting ruffled feathers, dyspnoea and excessive mucus at the beak (Barr *et al*., 1988). The virus caused changes in the embryos typical of IBV, and it was neutralized by serum against an Australian serotype of IBV. When the virus was inoculated (intranasal, intraocular and oral routes) into pigeons and chickens, the chickens but not the pigeons developed respiratory disease. It is possible that the clinical signs observed in the racing pigeons from which the virus was obtained were not caused by the coronavirus, or that the coronavirus had been able to cause

disease in the pigeons because their resistance had been lowered by intercurrent disease. Notwithstanding, it would appear that an IBV replicated in pigeons.

Recent research has revealed that pigeons are susceptible to a coronavirus, indeed to a Group 3 coronavirus. Jonassen *et al*. (2005) used a pan-coronavirus RT-PCR (able to detect coronaviruses of all coronavirus groups; see later) to look for the presence of coronaviruses in gut contents. They detected coronaviruses in faecal samples or cloacal swabs of 40/163 graylag geese, 2/100 pigeons and 1/5 mallard ducks. Further RT-PCRs for the 3′ end of the genome revealed that these viruses were Group 3 coronaviruses. On the basis of nucleotide sequencing, the viruses in these three species were clearly different from each other, and were clearly different from Group 3 coronaviruses of chickens, turkeys and pheasants. For example, there was a large insert in the hypervariable part of the 3′ untranslated region of the goose viruses when compared with IBV. Moreover, the goose virus had two additional open reading frames (ORFs) downstream of the nucleocapsid protein gene, while the pigeon virus had one of these ORFs (Figure 4). (The sequence of that part of the genome was not available for the mallard duck virus). Upstream of these two ORFs and of the N protein gene was a putative transcription-associated sequence (TTTAACAA), very similar to the transcription-associated sequence (CTTAACAA) of the other Group 3 coronaviruses. Consequently, one would expect that the goose and pigeon viruses would generate two and one, respectively, additional mRNAs, and the corresponding proteins.

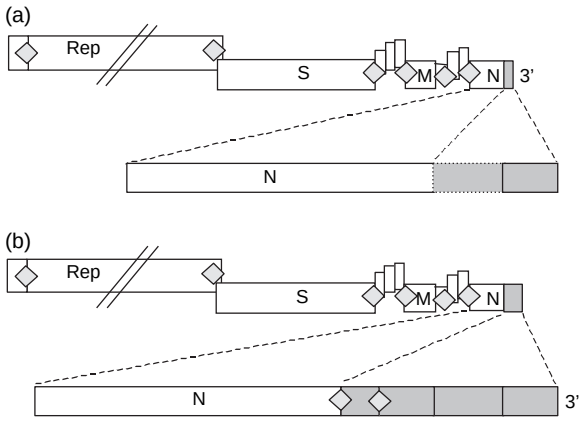

Figure 4. *Additional genes in the genomes of goose coronavirus and pigeon coronavirus. At the beginning of each gene is a transcription-associated sequence (TAS; illustrated as a diamond) that causes subgenomic mRNAs to be made, which are subsequently translated to give the IBV proteins. 4a: At the 3′ end of the IBV genome is a region without a TAS, hence no mRNA is made. This is called the 3′ UTR, comprising two parts. The extreme 3′ part has highly conserved sequence among Group 3 coronaviruses. The part (rectangle with dotted lines) between this conserved region and the N gene is very variable, and is absent from some strains (e.g. M41). 4b: Jonassen et al. (2005) have shown that the region of the greylag goose coronavirus genome downstream of the N gene is much larger than that of IBV, TCoV and PhCoV. Moreover, the additional sequence contains two TASs. Most probably this would result in the production of two additional mRNAs, and two additional proteins. The pigeon coronavirus had one, not two, of these additional TASs.*

Attempts to isolate live coronavirus from the geese, ducks and pigeons, using embryonated domestic fowl eggs inoculated by the allantoic cavity route, were unsuccessful. Consequently it was not possible to perform host range studies in chickens, which would have been very interesting.

The investigation of Jonassen *et al*. has revealed greater heterogeneity among Group 3 coronaviruses than had previously been observed in the viruses isolated from galliform birds. It would be particularly interesting if other parts of the genome were to be sequenced.

Are Poultry Susceptible to Non-Group 3 Coronaviruses?

"Yes" is the simple answer to that question. When bovine coronavirus (Group 2) was experimentally inoculated into turkeys, it replicated, causing enteritis (Ismail *et al*., 2001b). Therefore we have to keep an open mind to the possibility that turkeys might be infected, potentially with economic consequences, with coronavirus transmitted from domestic cattle—or from wild bovines. Perhaps poultry are susceptible to other non-Group 3 coronaviruses, including some that have not yet been discovered. It is possible that avian species might be susceptible to a Group 1 coronavirus and, indeed, coronaviruses that might not be in any of the three currently recognized groups.

Often overlooked is the discovery over 20 years ago of a coronavirus (puffinosis coronavirus) from the Manx shearwater (*Puffinus puffinus*), a bird that visits the shores of Britain in summer (Nuttall & Harrap, 1982). The virus is a Group 2 coronavirus. As the virus was isolated by passage of shearwater material in the brains of mice, it is possible that the virus is actually a murine coronavirus (a Group 2 coronavirus) that was present in the mice before inoculation. Notwithstanding, it is possible that birds are naturally infected by coronaviruses that are not Group 3 viruses.

Detection of Coronaviruses in Birds

Although many IBV isolates, plus several TCoV and PhCoV isolates, have been sequenced, mostly the sequence has been of the spike protein gene. As this is the most variable gene among these viruses, it is not the most suitable for the selection of sequences with which to make oligonucleotide primers likely to work in RT-PCRs designed to detect new Group 3 coronaviruses. The next most sequenced regions of the genome are those of the N gene and the adjacent 3′ UTR. Andreasen *et al*. (1991) designed a RT-PCR involving a forward primer within the M protein gene and a reverse primer near the beginning of the N protein gene. That primer pair has not only been used with IBV, but also to detect TCoV (Breslin *et al*., 1999a, 2000). A RT-PCR within the N gene has been developed that detects both TCoV and IBV (Sellers *et al*., 2004; Spackman *et al*., 2005).

The final 300 or so nucleotides of the 3′ UTR are among the most conserved parts of the IBV genome, being essential for replication (Dalton *et al*., 2001). Adzhar *et al*. (1996) designed four oligonucleotides in this region. The primer set UTR1$^-$/UTR2$^+$ gave positive results with RNA from all the 41 IBV isolates tested. The set UTR3$^-$/UTR4$^+$ worked well with all 25 IBV isolates tested. As additional sequence data became available for the 3′ UTR of TCoV (Breslin *et al*., 1999b)

and novel strains of IBV (Sapats *et al*., 1996), these oligonucleotides were modified. The modified primer combination UTR11^{-}/UTR41^{+} has proven to be particularly effective as it produces a very sensitive RT-PCR (Culver & Cavanagh, unpublished results) and was used to detect TCoV in gut contents and PhCoV on oropharyngeal swabs (Cavanagh *et al*., 2001, 2002). The 3′ UTRs of the coronaviruses detected in greylag goose, mallard duck and pigeons are very similar to those of IBV, TCoV and PhCoV, confirming the highly conserved nature of this region of the 3′ UTR (Jonassen *et al*., 2005). Comparison of the sequences of primers UTR11^{-} and UTR41^{+} with the 3′ UTR sequences of these three viruses reveals a perfect match in respect of UTR11^{-} (situated at the extreme 3′ end of the genome) and a small degree of heterogeneity within URT41^{+}. The utility of the latter would probably be improved by extending it in the 3′ direction. Situated between the positions corresponding to these two oligonucleotides is the highly conserved s2m sequence, which could be the basis for the design of another oligonucleotide primer that would be likely to work with RNA from many Group3 avian coronaviruses. It should be kept in mind that this s2m sequence is not unique to Group 3 coronaviruses—it is present in the SARS-coronavirus—and has been found in astroviruses and in one picornavirus species (Jonassen *et al*., 1998).

There are few parts of the genomes of coronaviruses that are sufficiently conserved to enable the design of oligonucleotides that could be used as primers in RT-PCRs with the aim of detecting new coronaviruses, irrespective of the group to which they belonged. One moderately conserved region is the RNA-dependent RNA polymerase domain of the gene 1b ORF. Stephensen *et al*. (1999) were the first to demonstrate the application of several oligonucleotides in this region for the detection of coronaviruses in Groups 1, 2 and 3 (Figure 5). Even then, not all combinations worked with all of the coronaviruses, and degeneracy had to be introduced into the primer sequences to maximize their utility. We have used the Stephensen *et al*. (1999) primers to successfully detect TCoV in many faecal samples (Culver & Cavanagh, unpublished results), and to confirm as being a coronavirus a virus isolated from a green-cheeked Amazon parrot (*Amazon viridigenalis Cassin*; Gough *et al*., 2005).

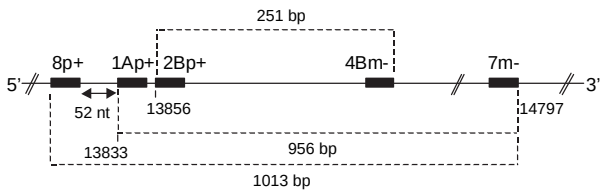

Figure 5. *Relative positions on the genome of IBV Beaudette of the pan-coronavirus oligonucleotide primers designed by Stephensen* et al. *(1999). The dashed lines (----) and associated numbers indicate the size of the PCR product (bp, base pairs) generated with pairs of primers. nt, nucleotides. The five-figure numbers in the smallest font size are the nucleotide positions from the 5′ end of the genome of IBV Beaudette. Stephensen et al. reported that primer pair 1Ap^{+}/7m^{-} did not work with RNa from turkey coronavirus or porcine haemagglutinating encephalomyelitis virus, but that these two viruses did give a PCR product with primer pair 8p^{+}/7m^{-}.*

The strategy used by Jonassen *et al*. (2005) to detect coronaviruses in greylag geese, pigeons and mallard ducks was to use two of the Stephensen *et al*. (1999) degenerate primers, 4Bm and 2Bp (Figure 5). As more sequences became available, modified versions of these primers were made, better suited to the sequences exhibited by the coronaviruses from these particular bird species. Jonassen *et al*. also used a primer based on the s2m sequence, as a positive sense primer in conjunction with oligo(dT), as the reverse primer in a PCR to generate sequence data from the 3′ end of the goose, duck and pigeon viruses.

Final Comments

Although there have been great advances during the past 2 years, we have surely only scratched the surface with regard to the discovery of coronaviruses in birds. We should keep in mind that the host range of coronaviruses is greater than was once thought, as has been shown with studies of Group 1 coronaviruses (Woods *et al*., 1981; Woods &Wesley, 1992). The experience of the SARS-coronavirus is only the latest example of this. The keeping of chickens 'free range' is common in some parts of world, and is increasing in some of those countries that have long had intensive poultry rearing. The extensive systems mean that poultry are exposed more to pathogens of other birds and mammals, and vice versa, including an increase in the chance that poultry will be exposed to coronaviruses of other species.

We should also keep an open mind about the diseases caused by the known avian coronaviruses. To date we consider TCoV to be only enterotropic. Perhaps there are some respiratory forms out there, as there are with variants of TGEV (i.e. porcine respiratory coronavirus) and BCoV. PhCoV is associated with respiratory disease in pheasants and also with nephritis, while IBV replicates at many epithelial surfaces in chickens and may be associated with more diseases than we are yet aware.

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REVIEWNon-English Abstracts

Coronaviruses in poultry and other birds

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Coronavirus chez les volailles et autres oiseaux

Le nombre d'espèces aviaires chez lesquelles les coronavirus ont été détectés a doublé ces vingt dernières années. Si, les coronavirus isolés chez ces espèces appartiennent tous au groupe 3 comme les coronavirus les plus connus de la poule domestique, (*virus de la bronchite infectieuse,* IBV, chez *Gallus gallus)*, de la dinde (*Meleagris gallopavo)* et du faisan (*Phasianus colchicus*), il y a des preuves expérimentales suggérant que les oiseaux ne doivent pas être infectés uniquement avec des coronavirus du groupe 3.

En Chine, des coronavirus ont été isolés de pans (*Pavo*), de pintade (*Numida meleagris,* également isolé au Brésil), de perdrix (*Alectoris*) et aussi d'un oiseau, n'appartenant pas à la famille des gallinacés, la sarcelle (*Anas*), mais tous ces animaux ont été élevés au voisinage de poules domestiques. L'organisation du génome de tous ces coronavirus est très proche de celle de l'IBV, particulèrement la séquence génétique. En ce qui concerne l'isolat du pan, le séquençage du gène et l'infection expérimentale des poulets ont montré qu'il s'agissait de la souche H120 du vaccin de l'IB, alors que l'isolat de la sarcelle correspondrait peut-être à une souche terrain du virus néphropathogène de l'IB. Ainsi la diversité des hôtes du virus de l'IB dépasse le poulet.

Plus recemment des coronavirus du groupe 3 ont été détectés chez des oies cendrées (*Anser anser*), des canards colvert (*Anas platyrhynchos*) et des pigeons (*Columbia livia*). Il est clair qu'au vu du séquençage d'une partie du génome, ces virus ne sont pas des IBV du fait qu'ils ont deux petits gènes additionnels près de l'extrémité 3' du génome.

Il y a vingt ans, un coronavirus a été isolé après inoculation de tissu provenant d'un puffin des anglais (*Puffinus puffinus*) à une souris. Toutefois, il 'est pas sûr si ce virus provient vraiment du puffin où s'il provient de la souris, des expériences récentes ont montré que le coronavirus bovin (coronavirus du groupe 2) peut infecter mais peut aussi entraîner une maladie entérique chez la dinde.

Des expériences avec quelques coronavirus du groupe 1 (tous isolés de mammifères jusqu'à présent) ont montré qu'ils ne se limitaient à se répliquer ou à entraîner une maladie chez un seul hôte. Le coronavirus du SRAS a une grande variété d'hôtes. Clairement, il y a possibilité d'émergence de nouvelles maladies à coronavirus chez les oiseaux domestiques, à partir de sources mammifère et aviaire. La conservation d'une séquence de dimension modeste à l'intérieur du gène 1, a permis la sélection d'amorces oligonucléotidiques pour les réactions de transcription inverse et d'amplification en chaîne par polymérase (RT-PCRs) utilisées pour le diagnostic. Ceci sera très utile pour la détection de nouveaux coronavirus.

Coronaviren beim Geflügel und bei anderen Vögeln

Die Zahl der Vogelspezies, bei denen Coronaviren nachgewiesen worden sind, hat sich in den letzten Jahren verdoppelt. Während die Coronaviren bei diesen Spezies alle als Gruppe 3-Coronaviren klassifiziert wurden wie die besser bekannten Coronaviren beim Haushuhns (infektiöse Bronchitis Virus, IBV in *Gallus Gallus*), bei der Pute (*Meleagris gallopavo*) und dem Fasan (*Phasianus colchicus*), gibt es experimentell Anzeichen, die vermuten lassen, dass Coronavirusinfektionen beim Vogel nicht nur auf Viren der Gruppe 3 beschränkt sind.

In China sind Coronaviren aus Pfau (*Pavo*), Perlhuhn (*Numida meleagris*, auch in Brasilien), Rebhuhn (*Alectoris*) und auch von einem nicht-galliformen Vogel, der Krickente (*Anas*) isoliert worden, wobei alle diese Tiere in der Nachbarschaft von Hühnern gehalten wurden. Alle diese Coronaviren sind hinsichtlich der Genomorganisation und der Gensequenz eng mit dem IBV verwandt. Gensequenzierung und experimentelle Infektion von Hühnern ließ das Perlhuhnisolat als H120-IB-Impfstamm erkennen, während das Krickentenisolat wahrscheinlich ein Feldstamm eines nephropathogenen IBV war. So hat sich das Wirtsspektrum des IBV über das Huhn hinaus ausgedehnt.

Kürzlich wurden Gruppe 3-Coronaviren in einer Graugans (*Anser anser*), einer Stockente (*Anas platyrhynchos*) und einer Taube (*Columbia livia*) entdeckt. Aufgrund der partiellen Genomsequenzierung

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dieser Viren ist es klar, dass es sich nicht um IBV handelt, da sie zwei zusätzliche kleine Gene in der Nähe des 3'-Ends des Genoms besitzen.

Vor 20 Jahren wurde nach Inokulation von Organmaterial eines Sturmtaucher *(Puffinus puffinus)* in Mäuse ein Coronavirus isoliert. Während es nicht sicher ist, ob das Virus ursprünglich aus dem Sturmtaucher oder aus den Mäusen stammte, haben neuere Experimente gezeigt, dass bovines Coronavirus (ein Gruppe 2-Coronavirus) nicht nur Puten infizieren, sondern auch eine Darmerkrankung in ihnen hervorrufen kann.

Experimente mit einigen Gruppe 1-Coronaviren (alle vom Menschen, bis heute) ließen erkennen, dass sie nicht auf die Vermehrung und Verursachung einer Erkrankung in einem einzigen Wirt begrenzt sind. Das SARS-Coronavirus hat ein weites Wirtsspektrum. Offensichtlich gibt es sowohl aus aviären als auch aus Mammalierquellen ein Potential für die Entstehung von neuen Coronaviruserkrankungen bei domestizierten Vögeln. Durch die bescheidene Sequenzkonservierung im Gen 1 war es möglich, einen Oligonukleotid-Primer für die Anwendung in diagnostischen Reverse Transkriptase-Polymerasekettenreaktionen (RT-PCRs) zu erstellen, was für die Entdeckung neuer Coronaviren hilfreich sein wird.

Coronavirus en aves de producción y otras aves

El número de especies de aves en las cuales se ha detectado coronavirus se ha doblado en los últimos dos años. Mientras que los coronavirus en estas especies pertenecen al Grupo 3 de los coronavirus, como los ya bien conocidos coronavirus de los pollos y gallinas de producción (*Infectious bronchitis virus,* IBV, en *Gallus gallus*), pavo (*Meleagris gallopavo)* y faisán (*Phasianus colchicus*), hay evidencia experimental que sugiere que puede ser que las aves se infecten con otros coronavirus diferentes a los del Grupo 3.

En China, se han aislado coronavirus de pavo (*Pavo*), gallinas de Guinea (*Numida meleagris;* también aislada en Brasil), perdices (*Alectoris*) y también de aves no gallináceas, trullos (*Anas*), que se criaban cerca de aves domésticas. Todos los coronavirus están muy relacionados en cuanto a la organización genómica y, además, en la secuencia génica, al IBV. La secuencia génica y la infección experimental de pollos indicaron que el aislado de pavo fue la cepa vacunal H120 IB, mientras que el aislado de trullo fue posiblemente una cepa de campo de un IBV nefropatogénico. Por lo tanto, el rango de huéspedes de IBV se extiende más allá del pollo.

Los coronavirus del Grupo 3 estudiados más recientemente han sido detectados en oca vulgar (*Anser anser*), ánade real (*Anas platyrhynchos*) y palomas (*Columbia livia*). A partir de la secuenciación parcial del genoma de estos virus queda claro que estos virus no son IBV ya que tienen dos pequeños genes cerca de la región terminal 3' del genoma.

Hace veinte años, se aisló un coronavirus tras la inoculación en ratón de tejidos de pardela pichoneta (*Puffinus puffinus*). Mientras que no se sabe con certeza si el virus era de la pardela o del ratón, experimentos recientes han demostrado que el coronavirus bovino (un coronavirus del Grupo 2) es capaz, no únicamente de infectar pavos, si no también de causar un proceso digestivo en estos animales.

Los experimentos con algunos coronavirus del Grupo 1 (todos aislados de mamíferos, hasta el momento) han mostrado que se replican o causan enfermedad en un huésped único. El coronavirus del SARS presenta un amplio rango de huéspedes. Hay un claro potencial de emergencia de nuevas enfermedades causadas por coronavirus en aves domésticas, tanto a partir de aves como de mamíferos. La presencia de algunas secuencias conservadas en el gen 1 ha permitido el diseño de cebadores para su uso en la técnica de transcriptasa reversa- reacción en cadena de la polimerasa (RT-PCRs), que puede ser útil para la detección de nuevos coronavirus.