



COMMENTARY

A nomenclature for avian coronavirus isolates and the question of species status

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Currently, there is no agreed naming system for isolates of infectious bronchitis virus (IBV), whose host is the domestic fowl (*Gallus gallus domesticus*). A uniform, informative system for naming IBV isolates would be very helpful. Furthermore, the desirability of a single naming system has become more important with the recent discoveries that coronaviruses with genome organizations and gene sequences very similar to those of IBV have been isolated from turkeys (*Meleagris gallopavo*) and pheasants (*Phasianus colchicus*). To date, no genetic features have been found that are unique to turkey isolates and to pheasant isolates that would permit unequivocal differentiation from IBVs. Should the avian coronaviruses from turkeys, pheasants and other birds each be considered as distinct coronavirus species? Or should avian coronaviruses that have gene sequences similar to those of IBV be treated as host-range variants of IBV or, more objectively, as host-range variants of a species that might be called avian coronavirus (ACoV)? Clearly, the topic of avian coronavirus species differentiation requires debate. For the moment, a naming system for avian coronavirus isolates is overdue. Increasingly, papers will include data of coronaviruses isolated from more than one species of bird. It is desirable to have a nomenclature for avian coronaviruses that indicates the host species of origin. Furthermore, it would be helpful if the name of an isolate included the country/region of origin, an isolate number and the year of isolation. The names of avian paramyxovirus (APMV) and avian influenza virus (AIV) isolates have long since contained this information; I suggest that we adopt a similar convention for isolates of avian coronaviruses. For example, the D274 isolate of IBV could be named chicken/Netherlands/D274/78. Representatives of avian coronaviruses from turkey and pheasant would include turkey/United States(Nc)/NC95/95 and pheasant/UK/750/83. Two upper case letters would be used to denote country of isolation, whereas one upper and one lower case letter would be used to indicate state or province, e.g. Nc, North Carolina. The full-length names could be abbreviated, when desired, similar to the convention used for AIV isolates, e.g. chNL78, tyUS(Nc)95 and phUK83. If the serotype of an isolate has been clearly established, this might be included in the name at end, like the serotype designation of AIVs, e.g. chicken/China/NRZ/91 (Mass.) for the Chinese isolate of the Massachusetts serotype. This suggested naming system for isolates is essentially neutral with regard to whether viruses from different bird species should be considered as different coronavirus species or simply as variants of just one avian coronavirus species. In my opinion an informative nomenclature for avian coronavirus isolates is required now, to improve communication, and need not be delayed until a decision on the definition of coronavirus species has been made.

Recent Developments in our Understanding of Coronaviruses from Different Avian Species

For several decades, our knowledge of avian coronaviruses (ACoVs) was based on just two viruses: avian IBV, of domestic fowl, and turkey coronavirus (TCoV; turkey enteric coronavirus). The two viruses were considered as separate

coronavirus species. IBV was in coronavirus Group 3, by itself, whereas TCoV was in Group 2, together with such coronaviruses as murine hepatitis virus, bovine coronavirus (BCoV), haemorrhagic enteritis of pigs and human coronavirus OC43 (Cavanagh *et al.*, 1995). Antigenic analyses indicated that IBV and TCoV were not related (Verbeek & Tijssen, 1991; Verbeek *et al.*, 1991). Later, gene sequencing

further substantiated the view that IBV and TCoV were very different – this time in terms of genome organization and gene sequence. Moreover, TCoV was found to have gene sequences almost identical to those of BCoV (Dea *et al.*, 1990). Thus, there was no confusion when discussing the two viruses, they were separate virus species: one, TCoV, was like BCoV; the other, IBV, was genetically unique amongst coronaviruses. Two major developments have complicated this simple situation.

First, recent analyses of genes 4, 5 and 6 and the 3' untranslated region (UTR) of three coronavirus isolates from turkeys in the US have shown that they are very similar genetically not to BCoV, but to IBV (Guy *et al.*, 1997; Breslin *et al.*, 1999a,b; Stephensen *et al.*, 1999; reviewed by Guy, 2000). The same has been revealed by analysis of genes 3 to 6 and the 3' UTR of a coronavirus isolated in turkeys in Britain (Cavanagh *et al.*, 2001a). Second, many coronavirus isolates from pheasants in the UK are genetically similar to IBV in genes 2 to 6 and the 3' UTR (Cavanagh *et al.*, 2001b). The turkey and pheasant viruses not only have a genome organization like that of IBV, but their gene sequences have high identity with those of IBV. The turkey isolates differ from each other to about the same extent as do IBV isolates of different serotypes. Moreover, the genes of the turkey viruses have the same extent of identity with IBV genes as is seen when IBV serotypes are compared with each other. The same has been found for the pheasant viruses. The turkey and pheasant coronavirus genes that have been examined had no unique features that would immediately identify the viruses as coming from turkeys and pheasants, respectively. If the gene 3 sequences, for example, of three coronavirus isolates from each species (chickens, turkeys and pheasants) were stacked in a random order, one would not be able to identify which were from domestic fowl, turkeys and pheasants (Cavanagh *et al.*, 2001a,b).

Doubtless coronaviruses exist in many, maybe all, avian species; polymerase chain reactions using 'universal' oligonucleotides will be helpful in the hunt for them (Kwon *et al.*, 1993; Adzhar *et al.*, 1996; Gelb *et al.*, 1997; Keeler *et al.*, 1998; Capua *et al.*, 1999; Cavanagh *et al.*, 1999). We need a naming system to accommodate current and future discoveries. The immense economic importance of IBV has led to innumerable isolations of the virus but the 'names' assigned to them have been notable for their lack of information.

Definition of a Virus Species from the International Committee for the Taxonomy of Viruses

The International Committee for the Taxonomy of Viruses (ICTV) (Van Regenmortel *et al.*, 1997; Van Regenmortel, 2000) has endorsed the following definition of virus species:

A virus species is a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche.

A polythetic class consists of members that have a number of properties in common. A virus species will be defined by a number of properties, although it is not usually necessary for every member of a species to share all the common properties. It is considered inappropriate that a single characteristic be used to assign new isolates to a species.

A virus is a 'replicating lineage'. Genealogy and evolution should be reflected by taxonomic distinctions. The mutability of viruses, in particular RNA viruses, is one reason for not using a single characteristic to differentiate one virus species from another within a genus. Van Regenmortel *et al.* (1997, 2000) state that, while no particular value of genome sequence dissimilarity can be used to differentiate virus species, sequence data is nevertheless a good starting point.

The 'ecological niche' is not simply the host. Rather, 'the ecological niche is an attribute of a virus species related to its biotic habitat'. Biotic properties may include geographic localization, host range, vectors, pathogenesis and other host responses.

Van Regenmortel *et al.* go on to list a number of characteristics (diagnostic properties) that may be used to differentiate one species from another, within the same genus:

1. genome sequence relatedness
2. natural host range
3. cell and tissue tropism
4. pathogenicity and cytopathology
5. mode of transmission
6. physicochemical properties
7. antigenic properties.

Species Status for Coronaviruses from Different Species of Bird?

How should we consider the coronaviruses from the three types of bird that have been studied most, i.e. chickens, turkeys and pheasants? Should they be considered as different virus species, IBV, TCoV and pheasant coronavirus (PhCoV, perhaps)?

In his excellent essay, Guy (2000) has argued that it would be prudent to consider coronaviruses of domestic fowl and turkeys (that are similar genetically) as distinct virus species. Guy acknowledges that the viruses from the two species are genetically very similar, have some shared antigenic identity, and that the turkey virus will grow in chickens, although without causing disease. However, he believes that other properties outweigh these, supporting the notion of distinct turkey and chicken coronavirus species. Polyclonal serum against the chicken/Arkansas/Ark99/73 reacted with the turkey

virus but antibody against the latter did not bind to IBV in immunofluorescence analysis (Guy *et al.*, 1997). In addition, a broadly reactive monoclonal antibody against the IBV S2 protein did not bind to the turkey virus (Guy *et al.*, 1997). However, it has recently been shown that a commercially available enzyme-linked immunosorbent assay for IBV was able to detect antibodies in turkey sera from the field (Loa *et al.*, 2000), as was also shown in 1987 (Weisman *et al.*, 1987). That emphasizes the relatedness of the turkey viruses with IBV, supporting the sequencing data.

While IBVs can be readily propagated in the allantoic sac of fowl eggs, it has been necessary to grow turkey coronaviruses in the amniotic sac of turkey embryos. The turkey virus has been reported to replicate to titres about 10^4 -fold less in chick embryos than in turkey embryos (Adams & Hofstad, 1971). IBV isolates can be readily adapted to growth in some chicken cell cultures, whereas this has not been the case for turkey coronaviruses. Finally, the known coronaviruses of turkeys have been associated with enteritis (Barnes & Guy, 1997), whereas IBV is usually associated with respiratory disease in domestic fowl (Cavanagh & Naqi, 1997).

However, IBVs can grow in several gut tissues of the domestic fowl, as Guy acknowledges (Darbyshire *et al.*, 1975; Jones & Ambali, 1987; Dhinaker Raj & Jones, 1997), although without causing clinical disease. In addition, IBVs can grow in oviduct and kidney, resulting in decreased performance of the birds, and some IBVs are highly nephropathogenic, causing substantial mortality (Cumming, 1963; Albassam *et al.*, 1986; Meulemans *et al.*, 1987; Lambrechts *et al.*, 1993; Pensaert & Lambrechts, 1994). Coronaviruses in pheasants have been linked with kidney disease; the primary infection is probably of the respiratory tract (Lister *et al.*, 1985; Gough *et al.*, 1996; reviewed in Pennycott, 2000). Many pheasant coronaviruses that we have examined were associated with respiratory disease (Cavanagh *et al.*, 2001b). Notwithstanding, the turkey isolates studied to date do have a strict tropism for epithelia of gut tissues and bursa of Fabricius, unlike IBV.

Should avian coronavirus species be defined by host range? The domestic fowl is considered to be the only natural host for IBV, although experiments with other avian species have been few in number (Cavanagh & Naqi, 1997). Similarly, there have been few host range studies with coronaviruses from turkeys and pheasants. A turkey coronavirus and a pheasant coronavirus that are genetically related to IBV were able to replicate in chickens but without causing disease (Gough *et al.*, 1996; Guy, 2000). Since host range differences are not clear cut, one could use the presence or absence of clinical disease and the nature of it, e.g. enteric or respiratory, as factors in defining avian coronavirus species identity.

If a coronavirus genetically related to IBV, turkey and pheasant viruses were to be isolated from another species of bird, e.g. a pigeon, would that be considered to be a unique species, and so on with every avian species? It will not be possible to study the host-range of every avian coronavirus that is discovered, to establish relationships at that biological level. One could inoculate domestic fowl and turkey embryos to see if the biological behaviour of an isolate was like that of chicken or turkey isolate. Of course, one might obtain isolates that may not grow well in either type of embryo. Regarding coronavirus growth in the laboratory, pheasant coronaviruses have been readily grown in the allantoic cavity of domestic fowl eggs (Lister *et al.*, 1985; Gough *et al.*, 1996). Given also that the pheasant viruses are clearly IBV-like at the genetic level, including the protein sequence of the S1 spike glycoprotein (Cavanagh *et al.*, 2001b), should they be considered as being the species IBV, with the same name?

Whether to regard coronaviruses from turkeys, pheasants and other birds as virus species distinct from each other and from IBV or as variants of one avian coronavirus species is a difficult question to answer. Guy is right in saying that additional sequencing studies will be needed to fully address this taxonomic question. I would go further and say that we need to isolate ACoVs from more bird species and assess their biological behaviour in addition to genomic characteristics.

The Coronavirus Study Group of the ICTV, which has the authority to make taxonomic proposals to the ICTV, does not have strong criteria for defining the mammalian coronavirus species, so there is no precedent to follow within the *Coronaviridae* family. It would be interesting to know the views of 'aficionados' of avian and other coronaviruses.

Regarding the seven general characteristics of Van Regenmortel *et al.* (1997) for assigning an isolate to a species within a given genus, the foregoing notes indicate that numbers 1, 2, 3, 4 and 7 are appropriate for avian coronaviruses. Item 5, mode of transmission, might be included if one accepts that TCoV is spread largely by the faecal-oral route, whereas IBV is largely by aerosol. Vectors are unlikely to be involved. It might prove useful if someone were to re-visit the question of stability at low pH (a physicochemical property, item 6) of IBV and TCoV under standard conditions. In one study, the titre of some strains of IBV were reduced 1 to 2 \log_{10} at pH 3 at room temperature, while other isolates dropped 5 \log_{10} (Cowen & Hitchner, 1975). The turkey coronavirus studied by Deshmukh & Pomeroy (1974) was resistant to pH 3 for 30 min at 22°C, although whether that virus was IBV-like or bovine coronavirus-like (discussed later) is perhaps not clear.

A Naming System for Avian Coronaviruses is Needed Now

Unless and until that issue is resolved, we have the problem of how to name coronavirus isolates such that there is no confusion about their host of origin. The names of the very many isolates of IBV that have been reported over half a century often contain little information; a systematic naming system would be useful for IBV alone. The demonstration that coronaviruses in turkeys and pheasants can be genetically highly related to IBV increases, in my view, a need for a common naming system.

The gene sequences of turkey isolates have been compared with those of IBVs and the comparisons published (Breslin *et al.*, 1999a,b; Stephensen *et al.*, 1999). Sequence comparisons involving coronaviruses from several species of bird will doubtless increase in number. A naming system is required that makes it clear from which bird species an isolate was made. It would be helpful if the naming system gave additional information, e.g. location and year of isolation and, if known, serotype.

The Avian Paramyxovirus and Avian Influenza Virus Models

This type of information is contained within the names of APMVs and AIVs, which probably have the most useful names. For example, one Newcastle disease virus (NDV) isolate is APMV-1/chicken/Switzerland/Safnern/95, indicating that the isolate has the name Safnern (although a number might equally well have been assigned) and was isolated from a chicken in Switzerland in the year 1995 (Gohm *et al.*, 2000). There are, of course, a great many isolates of NDV, obtained from many species of bird in many countries over a large number of years. However, the APMV naming system accommodates them all very well. Similarly for AIVs; for example, A/chicken/Victoria/32972/85, isolated from a chicken in the state of Victoria, Australia, in 1985 and assigned the number 32972 (Heckert *et al.*, 1999).

APMV's have been divided into nine serotypes, PMV-1, PMV-2, etc. (Alexander, 1997). The differentiation into serotypes is based on haemagglutination-inhibition tests, haemagglutination being mediated by the haemagglutinin-neuraminidase surface glycoprotein. While some APMV serotypes comprise viruses from several bird species, e.g. APMV-1 has been isolated from many species, others are known only to contain viruses from one or a few species, e.g. APMV-9 isolated only from ducks.

AIVs have been divided into an even larger number of subtypes, 15, based on haemagglutination-inhibition tests, haemagglutination being caused by the haemagglutinin surface glycoprotein (Easterday *et al.*, 1997). There is further differentiation based on antigenic properties of the neuraminidase glycoprotein.

Finally, during this look at the AIV model, one must not overlook the 'A' at the front of the name of every AIV. This denotes that the viruses are type A viruses. The type A influenza viruses, which have common antigenic properties in relation to the nucleoprotein and matrix protein, include viruses from humans and many mammals in addition to birds (Easterday *et al.*, 1997). Humans are also infected by type B influenza viruses, which are differentiated from type A viruses by an antigenically distinct nucleoprotein and other antigenic differences. Type C influenza viruses are substantially different from types A and B, including having a single glycoprotein with haemagglutinin, esterase and fusion properties, whereas influenza A and B viruses have the haemagglutinin and fusion properties in one protein and neuraminidase in a distinct protein.

APMV and AIV Models Applied to ACoVs

It seems to me that we should have a naming system for avian coronavirus isolates that reflects the species of origin and also gives additional useful information. The core part of APMV and AIV names would serve very well for ACoVs, e.g. chicken/Korea/B4/86 (Song *et al.*, 1998), chicken/United States (De)/072/92 (Keeler *et al.*, 1998), turkey/United States (Nc)/NC95/95 (Guy *et al.*, 1997), pheasant/UK/438/94 (Gough *et al.*, 1996). Gelb and colleagues (Keeler *et al.*, 1998) have adopted a similar model, without a species indicator, for IBV isolates from the USA, e.g. DE/072/92 and CA/633/85 for isolates from the states of Delaware and California, respectively.

As already outlined, the naming systems for APMVs and AIVs give additional, very important information. The 'APMV-1', 'APMV-2', etc., tell us the serotype. The 'A' at the start of the names of AIVs reminds us that they are type A influenza viruses. Do we need a similar epithet for avian coronaviruses, e.g. ACoV? It would seem to be premature to choose such an epithet for *formal* usage. Such a name would be unnecessary if the various avian coronaviruses were considered to be distinct species and named after the host of origin, e.g. turkey coronavirus (TCoV), or named in some other way. However, even if the various ACoVs were to be assigned separate species status, the species might be named ACoV-1, ACoV-2, etc., or ACoV-A, ACoV-B, etc., or some such qualifying addition. (The Coronavirus Study Group of the ICTV recently proposed that 'Co' be used in coronavirus names, rather than simply 'C', to avoid confusion with other viruses, e.g. HCV (hepatitis C virus), the acronym for human coronavirus becoming 'HCoV' (Enjuanes *et al.*, 2000).

If the acronym 'ACoV' was considered to be appropriate for inclusion in a naming system for avian coronaviruses, would 'ACoV' be sufficient? Possibly not. It would be sufficient if all the avian

Table 1. Division of coronaviruses into three groups^a

Group 1
Porcine transmissible gastroenteritis virus
Feline coronavirus
Canine coronavirus
Porcine epidemic diarrhoea virus
Human coronavirus 229E
Group 2
Murine hepatitis virus
Bovine coronavirus
Porcine haemagglutinating encephalomyelitis virus
Human coronavirus OC43
Group 3
Avian infectious bronchitis virus
Turkey coronavirus ^b

^a Enjuanes *et al.* (2000).

^b Until the discovery of coronaviruses from turkeys that were genetically closely related to infectious bronchitis virus (IBV), turkey coronavirus had been placed in Group 2, because early studies had indicated that turkey coronavirus was very similar to bovine coronavirus. The name 'turkey coronavirus' has subsequently been used for the IBV-like viruses and the virus moved to Group 3 by the Coronavirus Study Group of the International Committee for the Taxonomy of Viruses (Enjuanes *et al.*, 2000.). Further discussion is required on the status of turkey coronavirus as a coronavirus species, and also on IBV-like coronaviruses from other avian species, e.g. pheasants.

coronaviruses were closely related, e.g. with a similar genome organization and broadly similar gene sequences. This might not be the case. As mentioned in the opening paragraphs of this essay, some coronaviruses isolated from turkeys are genetically similar to BCoV while others are related to IBV. The difference between BCoV and IBV at the genetic level is substantial. Sequencing revealed that the BCoV-like isolates had gene sequences 99% identical to those of bovine-origin BCoVs (Dea *et al.*, 1990). Another study has described the isolation of BCoV-like virus from scouring turkeys in the vicinity of cattle from which BCoV was isolated (Brown *et al.*, 1996). Most recently, it has been demonstrated experimentally that BCoV can cause enteritis in turkeys (Ismail *et al.*, 2001). Does this mean that we should consider the BCoV-like viruses isolated from turkeys as being BCoV, i.e. not as avian coronaviruses? It is not in the remit of this essay to attempt to answer that question; it is a matter for the Coronavirus Study Group of the ICTV.

It is very possible that two or even more types of genetically distinct coronaviruses may exist in the avian world. There are precedents for believing this. Some mammalian species are associated with more than one type of coronavirus. There are two known types of coronaviruses in humans, belonging to Groups 1 and 2, respectively (Table 1). Three types

of coronavirus, two in Group 1 and one in Group 2, are associated with diseases in swine (Table 1). It would not be surprising, therefore, if two – or more – types of coronavirus were present in avian species. However, this is a hypothetical point!

Finally, the convention used for AIVs whereby the serotype is given in parentheses at the end of the name, e.g. H7N5, might be selectively applied to avian coronavirus names. The serotyping of IBVs has been reviewed recently by De Wit (2000). Classically, virus neutralization tests have been used for this purpose, although the haemagglutination-inhibition test has also been used extensively to serotype IBVs as it is more amenable to routine, large-scale application. Dozens of serotypes have been defined; there may be a much greater number. Diagnostic laboratories can only use standard reference sera against a few serotypes on a routine basis. Notwithstanding, some serotypes have become very prominent, associated with economically importance disease on a large scale. Vaccines have been manufactured using a number of serotypes. Thus, there are practical reasons for including serotype in the name of an IBV isolate, where it is known.

A Nomenclature for Avian Coronavirus Isolates

As already stated, the core parts of the naming systems used for APMVs and AIVs would seem to be suitable for naming isolates of ACoVs. Using the system proposed would, for example, give a complete name such as chicken/Italy/2191/97 (624I) for an isolate reported by Capua *et al.* (1999), being an IBV isolate, number 2191, from chickens in Italy in 1997 and which was of the 624I serotype (Capua *et al.*, 1994). Another example is chicken/China/NRZ/91 (Mass.) to describe a Chinese isolate of the Massachusetts serotype (Wu *et al.*, 1998).

In papers where only IBV (chicken) isolates were under consideration, the name could be truncated to Italy/2191/97 (624I). The full name of isolates could be tedious if repeated frequently in a paper and be especially awkward when used in tables and figures. Therefore, one could adopt the abbreviations used for AIVs (e.g. as illustrated in Banks *et al.*, 2000), e.g. ckIT97 for chicken/Italy/2191/97 (624I). The unique isolate number could be included when a paper included several isolates from the same country, e.g. ckIT/2191/97. It has been suggested (D. Alexander, personal communication) that the double-letter country abbreviations used in electronic mail addresses could be used in the abbreviated forms of the names of viruses, where possible. A list of these abbreviations can be found at the web site: <http://www.crayne.com/country.html>.

In some countries, especially large ones, there is a desire to indicate the region, e.g. state or province, in which a virus was isolated. In that case, it is

suggested that the region be referred to in the way used for AIV isolates, i.e. an upper-case letter and a lower-case letter; for example, De, Mn, Pa for Delaware, Minnesota and Pennsylvania, respectively. This would avoid confusion with Germany, Mongolia and Panama, whose two-letter codes are DE, MN and PA, respectively.

Not everyone will be familiar with the abbreviations for states and provinces. Therefore, I would suggest that, when a region is indicated, the virus name should also include the country code, e.g. US(De)/072/92 for IBV isolate 072 in 1992 from Delaware, USA (Gelb *et al.*, 1997). The turkey/US(Nc)/NC95/95 isolate (Guy *et al.*, 1997) and pheasant/UK/438/94 (Gough *et al.*, 1996) could be abbreviated to tyNC95 and phUK96, respectively.

Conclusion

Whether avian coronaviruses comprise one or more species is a topic for debate, which, in the way of taxonomic issues, may continue for a long time. Indisputably, I believe, is an immediate need for a naming system for avian coronavirus isolates, irrespective of the species question. The nomenclature proposed, i.e. denoting host species/location/isolate number/year, is flexible. A name could easily and minimally be modified if it was decided that 'host species' was to be a major defining characteristic of an avian coronavirus species. For example, pheasant/UK/438/94 might simply become PhCoV/UK/438/94. Similarly, if some other epithet was used to denote a coronavirus species, most of the name could remain unchanged.

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