



Review

Molecular evolution and emergence of avian gammacoronaviruses

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ARTICLE INFO

Article history:

Received 4 March 2012

Received in revised form 8 May 2012

Accepted 9 May 2012

Available online 17 May 2012

Keywords:

Coronavirus

Nidovirus

Evolution

Emergence

Virus diversity

ABSTRACT

Coronaviruses, which are single stranded, positive sense RNA viruses, are responsible for a wide variety of existing and emerging diseases in humans and other animals. The gammacoronaviruses primarily infect avian hosts. Within this genus of coronaviruses, the avian coronavirus infectious bronchitis virus (IBV) causes a highly infectious upper-respiratory tract disease in commercial poultry. IBV shows rapid evolution in chickens, frequently producing new antigenic types, which adds to the multiple serotypes of the virus that do not cross protect. Rapid evolution in IBV is facilitated by strong selection, large population sizes and high genetic diversity within hosts, and transmission bottlenecks between hosts. Genetic diversity within a host arises primarily by mutation, which includes substitutions, insertions and deletions. Mutations are caused both by the high error rate, and limited proof reading capability, of the viral RNA-dependent RNA-polymerase, and by recombination. Recombination also generates new haplotype diversity by recombining existing variants. Rapid evolution of avian coronavirus IBV makes this virus extremely difficult to diagnose and control, but also makes it an excellent model system to study viral genetic diversity and the mechanisms behind the emergence of coronaviruses in their natural host.

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1. Introduction

The majority of emerging infectious diseases are caused by RNA viruses. High rates of mutation, short generation times and large population sizes drive their rapid evolution. A wide variety of animals, including humans, are affected and examples include severe

acute respiratory syndrome coronavirus (SARS-CoV), influenza virus, human immunodeficiency virus, poliovirus, hepatitis A, B, and C virus, yellow fever virus, dengue virus, West Nile virus, and many others (Lai and Holmes, 2001). Understanding viral evolution is important because it can lead to outbreaks of known diseases (e.g. the 2009 human pandemic H1N1 influenza virus), host shifts and the emergence of new diseases (e.g. hantavirus), as well as escape from immune response and antiviral drugs (e.g. human immunodeficiency virus).

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Because of their high mutation rates, RNA viruses exist as a genetically diverse population of related virus particles within a host. Genetic diversity, defined as a measure of all the genotypes (genetic variation), as well as the frequency of those genotypes in the population, is created by mutations, including substitutions, deletions, and insertions, and recombination events that occur when the viral genome is replicated. Viral evolution is the result of a combination of high mutational input and the forces that act on the genetic diversity: selection both within and between hosts, which will increase the frequency of adaptive mutations and decrease the frequency of deleterious ones, and genetic drift during severe transmission bottlenecks, which will stochastically and indiscriminately affect the frequency of all types of mutation (Elena and Sanjuan, 2007; Manrubia and Lazaro, 2006). The processes involved in the emergence and spread of RNA viruses can often be measured in real time, making them perfect candidates for the study of the evolution of infectious diseases (Holmes, 2009).

2. Coronaviruses

Coronaviruses are positive sense single stranded RNA viruses. Their viral genome is approximately 27–31 Kb in length, which is the largest of the positive sense single stranded RNA viruses. Recently, the Coronavirus Study Group of the International Committee for Taxonomy of Viruses reorganized the family *Coronaviridae* (<http://talk.ictvonline.org/media/p/1230.aspx>). The *Coronaviridae*, in the order *Nidovirales*, are now divided into two subfamilies *Coronavirinae* and *Torovirinae*. The *Coronavirinae* are further subdivided into genus *Alphacoronavirus*, *Betacoronavirus* and *Gammacoronavirus* based on genetic analysis of the conserved domains in the replicase protein pp1ab and on the structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N). The alpha and betacoronaviruses infect and cause disease in mammals whereas the gammacoronaviruses are largely avian viruses.

The most infamous coronavirus is SARS-CoV, which is currently placed in the betacoronavirus group. However, SARS-CoV and

SARS-CoV-like viruses have a different gene order and sequence divergence distinct from many of the viruses in that group. In only a few months the virus rapidly spread to over 24 countries, infecting 8098 people and killing 774 (www.who.int/csr/sars/en/). Extensive testing and quarantine of infected individuals contained the outbreak. Although there are currently no known cases of SARS, surveillance continues and rapid recognition and response plans have been put into place. The SARS-CoV emerged from a reservoir of coronaviruses in bats that transmitted to an intermediate host then to humans (Li et al., 2005). Thankfully it has not reemerged in humans.

Gammacoronaviruses frequently emerge in avian species. One example is turkey coronavirus (TCoV), which emerged from a recombination event that replaced the spike gene of avian coronavirus infectious bronchitis virus (IBV), a virus of chickens, with a spike from an as yet unknown source. Another example is the repeated emergence of new genetic and antigenic types of IBV in chickens (Fig. 1), which leads to multiple serotypes of the virus that do not result in immunological cross protection.

3. Infectious bronchitis

Infectious bronchitis is a highly contagious upper-respiratory tract disease of chickens and a severe economic burden on the poultry industry worldwide (Cavanagh and Gelb, 2008). The causative agent, IBV, has also been found in peafowl (*Galliformes*) and IBV-like viruses have been isolated from teal (*Anas crecca*), geese (*Anserinae*), pigeons (*Columbiformes*), and ducks (*Anseriformes*) (Cavanagh, 2007). Clinical signs of the disease in chickens are watery eyes, mucus in the nares and trachea, gasping, coughing, and tracheal rales. The disease can also cause a decrease in egg production and egg quality, and some strains of the virus can cause an interstitial nephritis. When secondary pathogens like avian mycoplasmas, *Escherichia coli*, *Ornithobacterium rhinotracheale*, and/or *Bordetella avium* complicate the disease, pneumonia, airsacculitis and peritonitis can occur. Morbidity is almost always 100%

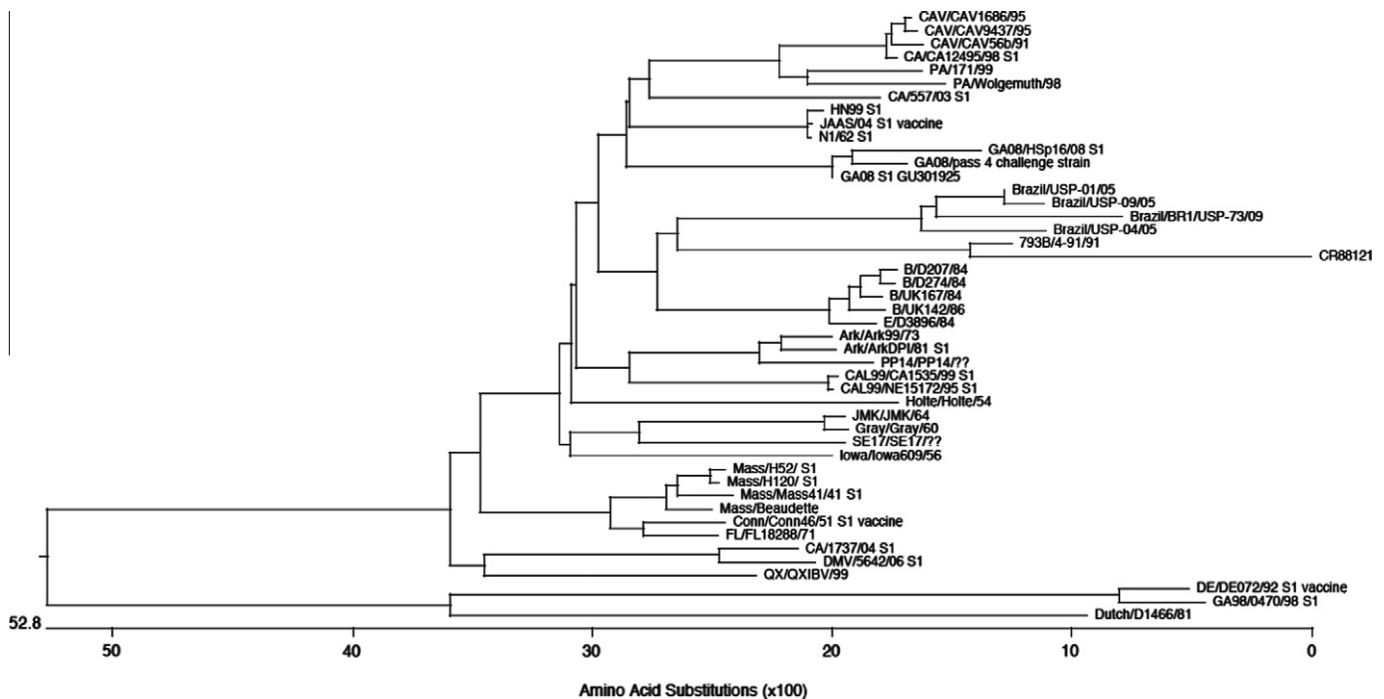


Fig. 1. Phylogenetic tree of different strains of avian coronavirus IBV showing S1 glycoprotein amino acid sequence relatedness. The sequences were aligned using Clustal W and the Neighbor-Joining method was used to reconstruct the phylogeny (DNASTAR, Inc., v.8.0.2, Madison, WI).

whereas mortality can be quite variable depending on the age of the birds, strain of the virus and secondary pathogens involved in the disease and can range from 14% to 82% (Cavanagh and Gelb, 2008). The virus replicates in epithelial cells causing lesions in the nasal turbinates, trachea, kidney, gonads, oviduct, lungs and airsacs. Lesions can also be found in the gastrointestinal tract, bursa of Fabricius and cecal tonsils.

4. Avian coronavirus infectious bronchitis virus

The IBV virion has a typical coronavirus pleomorphic shape with the characteristic club-shaped surface projections designated spikes (Perlman et al., 2008). The enveloped virus particle is heat labile, unstable at extreme pH, and sensitive to most disinfectants. The viral genome is 5' capped, with a poly-A tail and the gene organization is 5'UTR-1a/1ab-S-3a-3b-E-M-5a-5b-N-3'UTR.

The 5' and 3' UTRs contain structural motifs that are involved with interactions between the UTRs as well as with viral encoded replicase proteins and possibly host proteins (Li et al., 2008). The UTRs play a key role in viral RNA transcription and replication. The 1a and 1ab ORFs encode polyproteins that are post-translationally cleaved into 15 nonstructural proteins (Nsps) by two viral proteases, a papain-like protease (PLP) and the main protease (Mpro) or 3C-like protease (van Hemert et al., 2008). In contrast to the alpha and betacoronaviruses, which have 16 nsps, nsp1 is lacking in gammacoronaviruses (see Table 1).

The 1ab polyprotein is translated through a – 1 frame-shift at nucleotide position 12,466 (in IBV/Mass41/41, AY851295) that occurs approximately 20–40% of the time (Imbert et al., 2008). The Nsp 2 has been shown to play a role in facilitating de novo IBV protein synthesis by blocking protein kinase R (PKR) phosphorylation of eukaryotic initiation factor 2 (eIF-2alpha), which shuts down protein synthesis. In addition, Nsp 2 induces expression of GADD34, a component of the protein phosphatase 1 (PP1) complex, which dephosphorylates eIF-2alpha (Wang et al., 2009). The Nsp 3 contains multiple domains including an acidic domain, an ADP-ribose 1 phosphatase, the papain-like protease (PLP), a Y domain and a transmembrane domain (Imbert et al., 2008; Neuman et al., 2008). The PLP of IBV and, interestingly, also SARS-CoV, is orthologous to PLP2 of other coronaviruses like mouse hepatitis virus (MHV). The PLP1 in IBV is truncated whereas in SARS-CoV it is missing altogether (Barretto et al., 2005; Ziebuhr et al., 2007). The PLP is responsible for cleaving between Nsps 2–3 and 3–4 and has been shown to contain deubiquitinating and interferon antagonism activities (Clementz et al., 2010; Frieman et al., 2009; Lindner et al., 2005). The Nsp 4 is a membrane spanning protein and along with Nsps 2 and 6 is thought to anchor the viral

replication complex in double membrane vesicles at the golgi (Graham et al., 2008). Nonstructural protein 5 contains Mpro, a cysteine protease with a Cys-His catalytic dyad (Ziebuhr, 2006). The Mpro is responsible for cleaving Nsps 4 through 16. Nonstructural proteins 6 through 10 are membrane-localized proteins some of which have RNA binding activity and likely play a role in formation of the viral replication complex (Johnson et al., 2010; Kumar et al., 2007; Tangudu et al., 2007; Zhai et al., 2005; Ziebuhr, 2006). Nonstructural protein 11/12 is the RNA-dependent RNA-polymerase (RdRp), Nsp 13 contains the RNA helicase, Nsp 14 contains an exonuclease domain, Nsp 15 has an endoribonuclease domain, and Nsp 16 is a methyltransferase (Graham et al., 2008; Ivanov et al., 2004; Ziebuhr, 2005, 2006).

Structural protein genes are located at the 3' end of the genome. The S glycoprotein is post-translationally cleaved in IBV into S1 and S2 subunits. The S1 subunit contains a receptor-binding domain responsible for virus attachment to host cells and the S2 subunit, which is noncovalently attached to S1 has a trans-membrane domain that spans the viral membrane and anchoring the spikes to the virion. The S glycoprotein is also involved in virus and cell membrane fusion and entry into the host cell, and it contains epitopes that induce neutralizing antibodies. Variability in the S1 gene is responsible for the multiple different genetic and antigenic types of the virus (see Fig. 1). The E protein is an integral membrane protein and the M protein is a membrane spanning protein. Both are involved with virus assembly. The N protein binds to the viral genome, plays a role in viral RNA replication and in virion assembly.

It is well documented that multiple serotypes of IBV exist that do not cross-neutralize (Fabricant, 1998). Genetic information for the S gene strongly suggests that different serotypes, as defined by neutralizing antibodies, exist for many other coronaviruses. However, they have only been reported for feline coronavirus (FCoV) types I and II, and there is some evidence that two serotypes may exist for SARS-CoV (Decaro et al., 2009; Hohdatsu et al., 1991). In addition to multiple serotypes of IBV, there are countless variant viruses, some of which may also fail to cross react in a virus neutralization test. Multiple different serotypes with little or no cross protection makes control of IBV, which is primarily through the use of attenuated live vaccines, extremely challenging.

5. Genetic diversity; mutations and recombination

Avian coronavirus IBV creates genetic diversity through rapid replication and large population sizes coupled with a high mutation rate and recombination. Mutations include substitutions, which are the result of a high error rate and limited proof reading

Table 1
Nonstructural proteins (Nsps) and their function.

| Protein | Function |
|-----------|---|
| Nsp 1 | Amino terminal protein lacking in IBV (present in alpha and beta coronaviruses) inhibition of cellular mechanisms including translation and IFN signaling |
| Nsp2 | Shuts down host protein synthesis by blocking phosphorylation of eIF-2alpha |
| Nsp 3 | Papain-like proteases PLP1 (nonfunctional in IBV and SARS-CoV) and PLP2 cleave Nsps 2–3 and 3–4 |
| Nsp 4 | Membrane spanning, anchors replication complex in double membrane vesicles |
| Nsp 5 | Cysteine protease (Mpro) cleaves Nsps 4–16 |
| Nsp 6 | Membrane-localized protein in double membrane vesicles |
| Nsp 7 | Double membrane vesicle protein, RNA binding |
| Nsp 8 | Double membrane vesicle protein, primase |
| Nsp 9 | Double membrane vesicle protein, replication complex |
| Nsp 10 | Double membrane vesicle protein, replication complex |
| Nsp 11/12 | RNA-dependent RNA-polymerase |
| Nsp 13 | RNA helicase |
| Nsp 14 | ExoN, RNA synthesis proof reading and repair |
| Nsp15 | Endoribonuclease |
| Nsp 16 | Methyltransferase, RNA cap formation |

capability of the viral RdRp, and insertions and deletions, caused by recombination events or by RdRp stuttering or slippage. Broad genetic diversity likely facilitates survival of the virus in a constantly changing environment.

5.1. Mutation

The average rate of synonymous mutation in all coronaviruses including IBV is approximately 1.2×10^{-3} substitutions/site/year (Hanada et al., 2004; Holmes, 2009). The rate of other RNA viruses with smaller genomes can be as high as 1×10^{-1} substitutions/site/year. The difference is presumably due to the presence of a 3' to 5' exoribonuclease (ExoN) domain in Nsp 14, which contains similarities to host proteins involved in proofreading and repair (Minskaia et al., 2006; Snijder et al., 2003). Using an nsp 14-ExoN mutant SARS-CoV, Eckerle et al. (2010) showed impaired growth and a 21-fold increase in mutation rate for the mutant virus compared to wild type. That data and their previous work with a mouse hepatitis virus coronavirus (MHV-CoV) ExoN mutant (Eckerle et al., 2007), confirms that ExoN, which is conserved in all coronaviruses, does contribute to the fidelity of the viral RdRp. High fidelity of the polymerase results in a higher “error threshold” and likely permits the virus to maintain a large genome size (Holmes, 2009).

The emergence of new strains and serotypes of IBV is largely due to the accumulation of mutations in the S gene over time as opposed to recombination events. This is thought to be the primary method of cross-species transmission and was shown to lead to the emergence of SARS-CoV (Holmes and Rambaut, 2004; Hon et al., 2008).

5.2. Recombination

Recombination can reduce mutational load, create genetic variants that may be very different from parental strains, and result in the emergence of new strains (Worobey and Holmes, 1999). Recombination has been reported in many coronaviruses (Brooks et al., 2004; Lee and Jackwood, 2000; Magiorkinis et al., 2004; Thor et al., 2011). Recombination hot spots, or regions of the viral genome with higher incidences of recombination breakpoints, have been reported in IBV. Hot spots (see Fig. 2) tend to lie immediately upstream of the S glycoprotein gene, as well as in nsp 2, nsp 3, and nsp 16 (Armesto et al., 2009; Hagemeyer et al., 2010; Thor et al., 2011). Recombination in the nonstructural proteins associated with the RdRp can alter replication efficiency of the virus, which can in turn affect pathogenicity.

Because the S glycoprotein gene is involved in host cell attachment and contains viral neutralizing epitopes, recombination in the S glycoprotein gene can result in the emergence of new strains or serotypes of the virus as well as new viruses capable of causing disease in other host species. The emergence of new variants and serotypes of IBV, whether the result of mutations or recombination, occurs continuously (Jackwood et al., 2005). Recombination directly leading to the emergence of a new avian coronavirus in turkeys (TCoV) was recently documented. The S gene of IBV recombined with an unknown virus, which altered its pathotype (respiratory to enteric) and host specificity (chicken to turkey),

resulting in the emergence of TCoV (Jackwood et al., 2010). Data from that study showed that the full-length genomes of IBV and TCoV are greater than 86% similar whereas the spike glycoproteins are less than 36% similar with clear recombination break points at the 3' end of gene 1ab (nucleotide position 20,173) immediately upstream of the S gene and in the 3' end of the spike gene (nucleotide position 23,849) (Jackwood et al., 2010). It is likely that the unknown virus contributing S gene sequences in the emergence of TCoV is another avian coronavirus, since the only evidence of a gamma coronavirus recombining with either an alpha or beta coronavirus is the mosaic composition reported for the SARS-CoV (Zhang et al., 2004).

6. Vaccination and the evolution of IBV

Control of infectious bronchitis virus in commercial poultry is through the use of live attenuated and killed vaccines. Since little or no cross-protection occurs between different serotypes or different variants of the virus, a variety of different virus antigenic types are used in vaccines. Typically more than one immunization is given and multivalent vaccines containing two or more antigenic types are used in an attempt to provide broad protection. It is not uncommon for vaccines to induce only partial protection against varied antigenic types of the virus, which allows some viruses to continue to replicate and persist in a flock. Presumably replication of the virus in a vaccinated host provides it with an opportunity to further adapt and evade the immune response (Gandon and Day, 2008; Gandon et al., 2001).

The use of modified live vaccine can result in faster evolutionary rates in IBV. Mutation rates for the 793/B IBV type where vaccines against that specific serotype were not used have been estimated to be 3×10^{-3} substitutions/site/year (Cavanagh et al., 1998), whereas the GA98 virus, which emerged in the face of vaccination with the closely related DE072 type was reported to have a mutation rate of 1.5×10^{-2} substitutions/site/year (Lee and Jackwood, 2001). However, caution must be exercised when estimating mutation rates of IBV antigenic types when live attenuated vaccines against that same virus type are used. Mutation rates for Mass and Conn IBV types, where live vaccines against those types are commonly used, were estimated to be 1.9×10^{-3} and 1.5×10^{-4} nucleotide changes/site/year, respectively (McKinley et al., 2011). Reisolation of vaccine viruses that are continuously introduced into the field and circulating in birds for a relatively short period of time likely contributed to the apparently low mutation rates.

7. Transmission and emergence of new viruses

Emergence of new variants or serotypes of IBV is dependent on the ability of existing strains to be transmitted from one individual to another. The average number of new infections caused by one infected individual (reproductive number, R), is a measure of the sustainability of a virus in the host population. For $R < 1$, the chain of transmission is not sustainable and the disease slowly dies out. For $R > 1$, the number of infected hosts increases, which in some cases can result in an epidemic. Eventually the pool of susceptible hosts is sufficiently depleted (or intervention measures are imple-

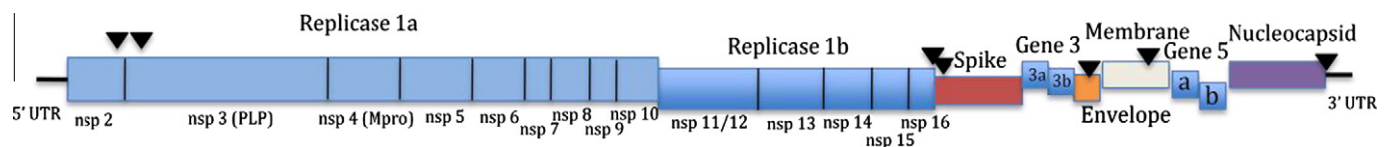


Fig. 2. The coronavirus avian infectious bronchitis virus genome (not drawn to scale) showing locations of 5' and 3' UTRs and coding regions. Black triangles (▼) indicate recombination hot spots within the genome.

mented) and R drops to below 1. The reproductive number for the situation where all hosts are susceptible (basic reproductive number, R_0) is a measure of the transmissibility of a given pathogen. The R_0 value for IBV has been estimated to be 19.95, which is extremely high (comparable to highly transmissible human diseases such as measles) and reflects the highly infectious nature of this virus (de Wit et al., 1998). Vaccination with a homologous antigenic type of the virus was found to reduce R_0 to 0.69 indicating that proper and careful vaccination with a well-matched vaccine is an effective control strategy for IBV (de Wit et al., 1998).

Transmission of a virus from one individual to another individual often involves a bottleneck where only a small number of virions are transmitted. Severely restricted transmission bottlenecks have been predicted for HIV, where studies suggest that new infections are started by a single virion (Abrahams et al., 2009). In contrast, a more recent study on equine influenza virus showed that although the consensus sequence did not vary, genetically distinct subpopulations of the virus were transmitted to a new susceptible host, suggesting a somewhat less restrictive transmission bottleneck (Murcia et al., 2010).

Theoretically, transmission through a restrictive bottleneck followed by rapid expansion of the number of virus particles in the host can take a rare virus subpopulation and make it the predominant type, potentially leading to the emergence of a new genotype. Whether this is the mechanism behind the emergence of new types of IBV is not known because there exists little *in vivo* experimental data on bottlenecks and expansion of viruses transmitting and replicating in their natural animal host. There is a substantial body of experimental studies in the laboratory, especially using bacteriophages, that have addressed a variety of evolutionary factors including transmission bottlenecks and the dynamics of adaptation, (reviewed in (Elena and Sanjuan, 2007)). However, *in vivo* experiments in animal hosts have usually involved only two or a few individuals, which makes predicting viral evolution as a virus is transmitted through several hosts (i.e. over many transmission events) extremely difficult (Bouma et al., 2009; Coffey et al., 2008; Hoelzer et al., 2010; van der Goot et al., 2005). While studies are being performed to try to bridge the gap between the laboratory and nature, it is a challenging task. Consequently, there is a critical need for studies that utilize multiple-host chains of transmission similar to those used *in vitro*, but where host to host transmission events involve natural infections, in an experimental setting that allows for accurate measurements of viral evolution at critical time points. Understanding transmission and development of viral genetic diversity in a natural setting will hopefully elucidate the mechanisms behind the emergence of different genetic and antigenic types of IBV, allowing us to prevent, or at least to better predict the emergence of new coronavirus diseases. The rapid evolution of IBV makes this virus an excellent model to study viral genetic diversity and the mechanisms behind the emergence of coronaviruses in their natural host.

8. Mathematical modeling of coronavirus dynamics and evolution

Mathematical models, on their own or in combination with experimental data, have proven to be useful for understanding various aspects of viral dynamics and evolution. Examples of topics that have received significant modeling attention are the estimation of transmissibility (R_0) (Kenah, 2011; Roberts, 2007), the evolution of drug resistance (Handel et al., 2009; Lipsitch et al., 2007; Temime et al., 2008; Wodarz and Nowak, 2000), the impact of bottlenecks (Campos and Wahl, 2009; Elena et al., 2001; Escarmis et al., 2006; Handel and Bennett, 2008; Manrubia et al., 2005; Novella et al., 2008), and the impact of different fitness landscapes

on evolutionary trajectories (Antia et al., 2003; Clune et al., 2008; Handel and Rozen, 2009).

Some of these mathematical modeling approaches have been applied to coronaviruses, specifically the SARS-CoV. Early in the SARS epidemic, data was used to estimate the reproductive number and the impact of potential control strategies (Lipsitch et al., 2003; Riley et al., 2003). Other modeling studies investigated aspects such as the likely route of transmission of SARS-CoV (Yu et al., 2004), the impact of contact structure (Meyers et al., 2005), the impact of control measures that were implemented (Wallinga and Teunis, 2004) and the impact of travel on possible future SARS outbreaks (Ruan, 2009). With regard to evolutionary questions, models have been used to estimate the timing of the emergence of the common ancestor of the SARS-CoV epidemic strain (Lu et al., 2004) and to better understand its reservoir (Song et al., 2005).

Other coronaviruses, including IBV, have to our knowledge not been modeled, but a combination of the experimental approaches outlined above with appropriate models seems to be a promising direction to gain general insights into evolutionary dynamics, as well as to help with the further development of effective IBV control strategies. The recent availability and reduced cost of quantitative, deep-sequencing methods should aid in providing data in the future that will be ideally suited for mathematical and statistical modeling to estimate relevant parameters, and predict transmission dynamics.

9. Diagnostic and control challenges

Compared to other coronaviruses, multiple serotypes and variant viruses that continue to adapt and change appears to be unique to IBV, and makes diagnosis and control of IBV extremely challenging. Traditional virus neutralization tests conducted in embryonated eggs are expensive and time consuming, taking weeks or months to type only a few viruses. Currently, molecular typing tests that involve RT-PCR amplification of the S1 gene followed by nucleotide sequence analysis is being used to inexpensively determine the genetic type of a number of viruses in a short period of time. In addition, since the S1 gene of inactivated viruses can be amplified and sequenced, viruses can be imported (with the proper permits) from abroad. This along with an explosion of genetic data available in GenBank (<http://www.ncbi.nlm.nih.gov>) has allowed genetic comparison of IBV isolates from all over the world, significantly improving epidemiological data and attempts to control the disease (Callison et al., 2001).

Identification of the IBV type causing an outbreak in commercial poultry is necessary to be able to choose the appropriate vaccine(s) capable of inducing a protective immune response. Because only a few vaccine types, and in many countries only one vaccine (Mass type H120) can be used, variant viruses persist and outbreaks continue to occur (Gelb et al., 1991; Ignjatovic et al., 2006; Jackwood et al., 2005, 2009). Evolutionary rates for IBV are so rapid that even live attenuated vaccines undergo selection and mutation following only a single round of infection in the host. One study compared reisolated vaccine viruses with the original vaccine virus administered to the bird and found that selection of subpopulations as well as mutations were occurring in the S1 gene (McKinley et al., 2008). Another study found distinct virus subpopulations in the S1 gene were selected when chickens were vaccinated with the Ark-DPI strain of IBV (van Santen and Toro, 2008). Further analysis found that virus subpopulations were different in the microenvironment of distinct tissues of the host (Gallardo et al., 2010). These data indicate that different selection pressures leading to evolution of the virus during replication in the host can result in an even higher degree of virus diversity.

10. Summary

A wide variety of genetic and antigenic variants of avian coronavirus IBV, coupled with continuous emergence of new types, make this virus an ideal model to study viral evolution. Multiple infected hosts, high replication within hosts, and high mutation rates, lead to an extraordinarily diverse population of virus particles, which facilitates the evolution of the virus. Determining the size of bottlenecks during transmission, and the changes in virus genetic diversity during single and multiple infection cycles and how these affect transmission is important for evaluating the role of drift and selection on evolution, which is critical for the control of outbreaks of infectious bronchitis as well as other known coronaviral diseases. In addition, that knowledge is an important step in understanding the evolution of RNA viruses, which will make it possible to take a proactive approach to preventing the emergence of new coronaviral diseases.

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