Review Article Compte rendu

All in the family: A comparative look at coronaviruses

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Abstract

Background

Coronaviruses, members of the order *Nidovirales,* the largest and most complex of the positive-stranded RNA viruses, have been recognized as important causes of disease in veterinary medicine for nearly a century. In contrast in human medicine, especially until the recent SARS-CoV-2 pandemic, they were unimportant viruses associated with the common cold.

Objective and procedures

This is a brief comparative review of the biology of coronaviral infections emphasizing the commonalities among the various members of the family and considering how the veterinary experience with coronaviruses can inform the response to SARS-CoV-2.

Results

Coronaviruses are perhaps best viewed as mutation machines whose genetic sequences can readily change through genetic drift, recombination, and deletions from a large genome. However, to be of clinical concern, variants must have the perfect set of amino acids in the S protein receptor binding domain *and* in their replication-mediating nonstructural proteins.

Conclusion and clinical relevance

Extensive experience with veterinary coronaviral vaccines suggests that optimal clinical immunity is a tandem of mucosal and systemic responses induced by combination mucosal and parenteral vaccines.

Résumé

Quelle famille : un regard comparatif sur les coronavirus

Contexte

Les coronavirus, membres de l'ordre des *Nidovirales*, le plus grand et le plus complexe groupe de virus à ARN à brin positif, sont reconnus comme des causes importantes de maladie en médecine vétérinaire depuis près d'un siècle. Contrairement à la médecine humaine, en particulier jusqu'à la récente pandémie de SRAS-CoV-2, il s'agissait de virus sans importance associés au rhume.

Objectif et protocole

Nous décrivons ici un bref examen comparatif de la biologie des infections coronavirales mettant l'accent sur les points communs entre les différents membres de la famille et considérant comment l'expérience vétérinaire avec les coronavirus peut éclairer la réponse au SRAS-CoV-2.

Résultats

Les coronavirus sont peut-être mieux considérés comme des machines à mutation dont les séquences génétiques peuvent facilement changer par dérive génétique, recombinaison et suppression d'un grand génome. Cependant, pour être une préoccupation clinique, les variants doivent avoir l'ensemble parfait d'acides aminés dans le domaine de liaison au récepteur de la protéine S *et* dans leurs protéines non structurelles induisant la réplication.

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Conclusion et portée clinique

Une vaste expérience avec les vaccins coronaviraux vétérinaires suggère que l'immunité clinique optimale est un tandem de réponses mucosale et systémique induites par une combinaison de vaccins administrés par voies mucosale et parentérale.

Introduction

In the words of Siegfried Farnon, James Herriot's eccentric, but wise, mentor and partner, *"There's nothing that brings people to their senses like a dead animal."* Bleak, perhaps, but one of the truisms of life. Dead piles of *both* bipedal and quadripedal animals, accumulated as a result of microbial incursions, have put the hominid capabilities for carnage to shame and have literally determined the course of history. The latest grim reaper is coronavirus disease-19 (COVID-19), caused by Severe Acute Respiratory Syndrome (SARS) coronavirus-2 (SARS-CoV-2). In human medicine, aside from the dramatic blips of mortality with SARS, and then Middle East Respiratory Syndrome (MERS), in the last 2 decades, coronaviruses have generally been relegated to the realm of sniffles; several of them being causally implicated in the common cold. In contrast, in veterinary medicine, members of this family of viruses have long been recognized as *bona fide* pathogens, including, for example, poultry infectious bronchitis virus (IBV) and mouse hepatitis virus (MHV), the 2 prototypical coronaviruses (1) that were identified and studied in the 1930s and 1940s respectively, before they were officially designated as coronaviruses.

This is a current snapshot of the coronavirus family, the subject of a rapidly burgeoning literature. It focuses on familial similarities and differences, the basis and likelihood for interspecies infection, and the possibility of cross-reactive immune responses, as these relate to vaccine application in veterinary and human medicine.

The coronaviral family tree: What are the relationships?

Earlier on, when genomic sequencing was tedious if possible at all, viruses were grouped and named primarily on the basis of the way they looked under the electron microscope. The coronavirus family got its name in the 1960s because members looked like a sun and its corona to some imaginative microbiologists (2). Since that time 2 major sequence-based taxonomic reorganizations of the *Coronaviridae* have been done. The most recent re-organization was driven largely by the dramatic emergence of SARS-CoVs and subsequent investigation of coronaviruses in bats. In fact, the emergence of SARS, and now, COVID-19, has led to a chiropteran revolution of sorts; before that the study of bat coronaviruses was largely an arcane academic pursuit in search of funding. Now, some have even mused that all coronaviruses derived from ancestors in bats, since that host has the oldest and most genetically diverse representatives of the family (3).

Currently, the coronaviral family tree comprises 4 main branches or genera: alpha, beta, gamma, and delta, with the alpha- and beta-groups containing the most mammalian pathogens (1) (Table 1). Splitting off from these are smaller branches or dendrites. A cluster of these dendrites originating from the same ancestral branch forms a "clade" (4), a group of related "subspecies" or genotypes. The lengths of the branches between individual variants or genotypes represent the genetic distances, or degree of disparity, simply differences in sequence, or base composition of the RNA. It is within these clades, among the smaller branches where the most interesting and clinically relevant evolution has occurred, a branching process that is ongoing as the viruses continue to mutate and add to the family tree.

Within the *Alphacoronavirus* genus, canine coronaviruses (CCoV) and feline coronaviruses (FCoV) are endemic enteric agents, first recognized in the 1970s (5,6). Each of these has been subdivided into 2 major genotypes, and 2 serotypes, I and II (7,8). There is some persistent confusion in the nomenclature around FCoVs. It is acknowledged that feline infectious peritonitis viruses (FIPVs) are mutants of the feline enteric coronaviruses (FECoVs), the ones that are endemic and horizontally spread. These are 2 biotypes of FCoVs; the latter virulent and the former mostly benign (9). Mustelids, mink and ferrets, have their own enteric coronaviruses, causally associated with diarrhea, that are closely related to FCoV (1). Type I CCoVs and FCoVs are thought to be the progeny of a common unidentified ancestor, and the type II viruses are the products of multiple recombinational events involving unidentified viruses (10). The notorious FIPVs arise continuously in FECoV-infected cats by virtue of mutations in the non-structural protein gene 3c (11) and the S gene (12). In dogs and ferrets, less well-studied variants of their enteric CoVs are causally associated with multi-systemic diseases that have clinical and pathologic similarities to FIP (1,13). To make things even more incestuous, CCoV type II is the progenitor of transmissible gastroenterovirus virus (TGEV) of swine (10), *and* "backcrosses" (through recombination) of TGEV with CCoV type II circulate in dogs (14). More than 30 y ago, mutation, by way of a few deletions in TGEV, gave rise to porcine respiratory coronavirus (PRCoV), a less virulent variant than the "parent" virus with a different tropism, respiratory *versus* enteric (15). Porcine epidemic diarrhea virus (PEDV) (15), first identified in the 1970s in Europe (16), is now an emergent concern in North America. Looking at the "molecular clock" of sequence disparity among PEDV isolates suggests that *all* derived from bats, the North American ones originating within the last decade from bats in southern China (17). The latest bat-derived entrant in the alpha coronaviral enteric sweepstakes, swine acute diarrhea syndrome virus (SADS-CoV), is clinically and pathologically indistinguishable from its cousins, TGEV and PEDV (17), and in a clade all its own (18).

In the *Betacoronavirus* genus, long before COVID-19, even before coronaviruses were recognized as a separate family, some of the first viruses studied in the early 1960s were human cold viruses (19). Many isolates only grew in organ cultures; their study was supplemented with the experimental inoculation

Table 1. Coronaviruses of veterinary importance in Canada and coronaviral vaccines currently licensed in North America.[a]

Virus	Disease syndrome	Vaccine	Comments
Alphacoronavirus			
Canine CoV (CCoV)	Enteric	Inactivated; SQ, IM	Used infrequently
Feline CoV	Enteric, multisystemic (FIPV)	Modified-live; IN	Used infrequently
Transmissible gastroenteritis virus (TGEV)	Enteric	Inactivated; IM, oral	Use declining; decreased prevalence of virus
Porcine respiratory CoV	Respiratory	None	Deletion mutant TGEV; TGEV vaccines cross-protect?
Porcine epidemic diarrhea virus	Enteric	Inactivated; IM	Strain variation affects vaccine efficacy
Betacoronavirus			
Bovine CoV	Enteric, respiratory	Modified-live; IN, oral Inactivated; SQ, IM	Scant published data on efficacy
Canine respiratory CoV	Respiratory	None	CCoV vaccine unlikely to cross-protect
Gammacoronavirus			
Infectious bronchitis virus	Respiratory, multi-systemic	Inactivated; SQ, IM Modified-live; aerosol, spray, IN, intraocular, oral	Strain variation markedly affects vaccine efficacy
Deltacoronavirus			
Porcine delta CoV	Enteric	None	Strain variation likely to affect efficacy

[a] Adapted from reference (62).
CoV — Coronavirus; SQ — Subcutaneous; IM — Intramuscular; FIPV — Feline infectious peritonitis virus; IN — Intranasal.

of human volunteers. One of those culture-adapted prototypical beta-coronaviruses is HCoV-OC43. In the early 1970s, bovine coronavirus (BoCV) was discovered serendipitously during a vaccine trial for prophylaxis against rotaviral diarrhea in calves (20). Molecular clock analyses indicate that HCoV-OC43 descended from BCoV, or a common ancestor around the turn of the 20th century (21). Another likely spawn of BCoV is the canine respiratory coronavirus (CRCoV), which was discovered in outbreaks of "kennel cough" in England in the early 2000s (22,23), but apparently had been circulating in Canada before that time (24).

Preeminent in the genus *Gammacoronavirus* is IBV, first recognized in the 1930s as a respiratory virus (25), and still a bane in poultry production as a polytropic multisystemic pathogen; not just another "cold virus" as its name implies (1,25). There was a war of words concerning the classification and naming of IBV-like viruses, since they infect gallinaceous birds other than just chickens, notably turkeys and pheasants (26). Based on cross-infectability amongst galliforms and ducks, IBV and IBV-like viruses are now named "Avian CoV" (26).

The genus *Deltacoronavirus* is unique; its members infect avian and mammalian species (27). For example, porcine deltacoronaviruses, cause of a recent diarrheic scourge of baby piglets in North America, probably originated in sparrows and leapt into pigs, where it continues to mutate and defy vaccination.

What are some clinical implications of coronaviral virology?

By taxonomic definition, coronaviruses share common features of bio-physicality and replication strategy with varying degrees of conservation amongst the proteins encoded by their very similar genomes (1). Details of coronaviral characteristics are reported in recent textbooks (1) and review articles (28); these are beyond the scope of this review. In brief, coronaviruses are pleomorphic enveloped viruses, and the envelope renders the viruses potentially labile to adversities outside the host, such as detergents, desiccation, UV light, or anything else that disrupts lipid membranes. This has important practical implications for assessment of the role of fomites in transmission, as exemplified in the case of SARS-CoV-2. Testing methods applied can dramatically impact the conclusions that can be drawn from the results. Unfortunately, much of current testing for SARS-CoV-2 and other coronaviruses is done, not with tedious cell culture, but with reverse-transcription polymerase chain reaction (RT-PCR), which is sensitive, but incapable of determining whether the detected RNA is "dead," or in a viable and potentially transmissible virion. Indeed, early in 2021, the most recent data indicate that the initial, PCR-driven concern over fomite transmission of SARS-CoV-2, complete with televised instructions for disinfection of plastic grocery bags, was exaggerated (29). It is now acknowledged that airborne transmission is the primary means of spread of this virus (30). Those data would be viewed as neither novel, nor unexpected by most veterinarians. A high incidence of respiratory coronaviral infections has long been recognized in venues from hen houses to calf barns to kennels (1); venues with high population densities and often poor ventilation. These venues are predisposed to aerosolization of particulates, including viruses. The bottom line is that a positive PCR test should be taken as 1, sample-dependent, grain of salt in the epidemiologic shaker.

The approximately 30 Kb genome is the largest of the positive-stranded RNA viruses and encodes for 4 or 5 structural proteins, including spike (S), envelope (E), membrane (M), nucleocapsid (N) and, in some beta-coronaviruses, the

hemagglutinin esterase (HE). These proteins, most notably the S protein, which is the viral ligand for host receptors, and the primary target for vaccine development (1), have been the traditional centers of attention. Increasingly, there is focus on the larger part of the genome, which flanks and is interspersed between the open reading frames for the structural proteins. This array of less glamorous genes encodes for a "toolkit" of up to 19 nonstructural proteins (NSP) that assist in, and control replication (1,28). For a clinically relevant example, one of the tools in this kit is the essential RNA-directed RNA polymerase, which is being targeted with adenosine nucleoside analogues. Insertion of these analogues into the enzyme cripples it, preventing viral replication (31). One of these drugs, Remdesivir (6) is being applied therapeutically in SARS-CoV-2 infections in humans. It is only occasionally noted that the seminal work validating this approach was first conducted in feline infectious peritonitis virus-infected cats (32).

How do coronaviruses change over time and space?

Coronaviruses, like other single-stranded RNA viruses, exist as "quasi-species" (1). What this means is that a "species" of virus, SARS-CoV-2, for example, is really a population of related viruses (1,33). What dictates this mode of existence is an error-prone replication scheme. In coronaviruses, and other members of the order *Nidovirales* (*nidus,* Latin for nest) replication of the viral RNA is mediated by the virally encoded error-prone RNA polymerase, followed by the transcription of sub-genomic "nests" of mRNAs which are then translated by the cell machinery into cleavable polyproteins or individual proteins (1,28). It has been proposed (34) that there are 3 properties of this replication strategy that contribute to the quasi-specific diversity of these viruses:

i) Genetic drift. The toolkit of coronaviral NSPs contains a unique exoribonuclease RNA proofreading apparatus that provides a certain level of fidelity of RNA replication (35), or less genetic drift, at least *in vitro.* Other RNA viruses, such as influenza virus, do not have this. But this fidelity is compromised *in vivo* when it comes to demographic changes in the quasi-species in the presence of host factors, notably the immune response. It is well-documented that the immunological "pressure" of antibodies drives the evolution of antibody escape mutants of IBV in poultry (36). This process favors S protein variants that are more fit and can outcompete the neutralizing capabilities of a slower moving antibody response. The emergent concern over the effects of escape mutation on the efficacy of SARS-CoV-2 vaccines and therapy with monoclonal antibodies (37) was predicted in the hen house.

ii) Recombination. The dynamics of coronaviral RNA replication concocts a cytoplasmic "soup" of different forms of RNA, including full-length strands and variously sized bits, the sub-genomic transcripts. In cells that are co-infected homologously with another coronavirus, or heterologously with another species of virus, this enhances the chances for recombination and the generation of new variants through genetic shift. Indeed, beyond theory, recombination frequencies as high as 25% have been observed in MHV-infected cells (38).

iii) "Space" for mutations. The relatively large genome of coronaviruses, compared to most other RNA viruses, means that there is more room for mutational errors in progeny viral RNAs without the generation of deleterious or lethal changes. Exemplary of this plasticity is a naturally occurring viable variant of PRCoV that derived from an over 600 nucleotide deletion mutation from the S gene of another PRCoV (39); this gene is usually made up of 4000 bases *in toto!* The bottom line is that coronaviruses are perhaps best viewed as mutation machines with rheostats. They crank along slowly in controlled monocultures of cells in the laboratory but can speed up considerably to fill niches in the multifactorial environment in often dense vertebrate host populations, becoming of Darwin in real time. This concept, well-supported in the veterinary literature, is now "breaking news" in COVID-19 coverage.

Noise in the (mutation) machine: When does a genetic variant become a (new) strain?

The word "strain" inevitably appears in any discussion of viruses and other pathogens. It is especially tossed about in the RNA virus space where it adds confusion to the biology of coronavirus infections. Unfortunately, strain means different things to different people (33). For many molecular virologists and Internet sources, it means "a stable genetic variant," with no mention of phenotype. Such molecular entities are common in quasi-species populations, and now, relatively easily and cheaply detected with high-throughput sequencing (33). A better clinical definition is a stable genetic variant *with* some measurable phenotypic difference, for example, a difference in transmissibility, a recent concern with SARS-CoV-2 (33). Importantly, the change in behavior is not necessarily directly related to number of nucleotides (bases), or even amino acids, that are different between 2 variants (33). Few changes can result in big differences in behavior, as famously recognized in the emergence of canine parvovirus, or yearly point mutational changes in influenza viruses that require vaccine reformulation to achieve better efficacy. The phenomenon of "minor" changes and their phenotypic sequelae, well studied in "animal" coronaviruses such as IBV, MHV, and FCoV, is now the subject of news alerts in SARS-CoV-2 reportage.

What are the determinants of the host range of coronaviral infections?

Generically, productive virus infection of cells depends on both extra- and intra-cellular processes, each of which may contribute to species specificity and target cell tropism. The first step in all viral infections is the interaction between viral capsid or envelope proteins and receptors on the host cell. Like all receptor-ligand interactions that orchestrate life, this is dependent on compositional and conformational arrangements of viral amino acids that favor electrostatic attractions to host cell membrane motifs, peptide or carbohydrate, that constitute a bond (1,28,34). Many cellular receptors for viruses have been recognized and molecularly characterized. Some are biochemical motifs with no apparent specific function, such as sialic acid, that may be widespread among host cells,

or present on only a subset of cells. Others are cell membrane molecules that perform a specific task and are subverted by a virus. Angiotensin converting enzyme-2 (ACE-2), the already infamous receptor for the SARS-CoVs, and aminopeptidase-N, a cell surface metalloprotease and the probable primary receptor for FIPV and TGEV, are exemplary of these (1,28,34). The coronaviruses use both types of receptors, and there is no predictable pattern of receptor usage among or within the genera to aid in remembering the details. The spike or "S" envelope glycoprotein is the major ligand on coronaviruses. It exists as a trimer, each with an S1 and an S2 subunit. The S1 subunit is involved in binding to the host cell; the S2 in fusion with the cell membrane. As with all proteins, there are N-terminal and C-terminal regions or ends, and to further complicate things, both can contain (different) receptor binding domains (RBD), providing for many possibilities in initial virus/host interactions. Seemingly minor differences in the amino acid composition of the RBDs can affect binding affinity to the S protein, manifesting in differences in host species proclivity, or intraspecies transmissibility, the current concern in SARS-CoV-2 infections (1,28,34,37).

It is generally thought that interactions between the S protein and cellular receptors play the major role in determining the host specificity of coronaviruses (34). However, inside the cell, NSP-mediated modulation of viral replication also plays a determinative, but less well-understood role. One of the best studied of these processes is the interplay between the virus and the innate immune system, primarily involving the type-1 interferons (IFN) (40). Like all viruses, the coronaviruses have biochemical motifs that they do *not* share with their vertebrate hosts. These are called pathogen-associated molecular patterns (PAMPs), or "danger signals," because the cell recognizes them as trouble. Double-stranded RNA, formed during replication of corona- and other RNA viruses, is prototypical of these. The danger signals are ligands for another series of receptors, the pattern recognition receptors (PRRs), including TLRs, RIG-1, and MDA5. Engagement of the PAMPs with the PRRs in virtually any infected nucleated cell, initiates various kinase-driven phosphorylation pathways with the final common pathway being production of type-1 interferons. Interferons upregulate hundreds of genes involved in the control of cellular metabolism, many of which affect viral replication, often inhibitively. But, not to be outdone, many viruses including the coronaviruses have evolved mechanisms to inhibit the innate immune response comprising 3 general *modi operandi:* inhibition of IFN induction, inhibition of IFN signaling, and sequestration of PAMPs (40). These involve 1, or often, more of the "tools" in the coronaviral NSP tool kit.

Host-species "jumping:" Is this a cause for clinical concern?

Long before the emergence of SARS-CoV-2, cross-infection of beta-coronaviruses was studied within the BCoV cluster of viruses. At least experimentally, BCoV can infect dogs (41) and a human enteric coronavirus can infect calves (42). However, there is no indication that those variants routinely wander into, and effectively transmit in the aberrant hosts. Similarly, experimentally, MHV can infect rats, and rat coronaviruses can infect mice, but natural cross-infection does not appear to happen even when these rodent hosts are housed in the same facilities (1). Whether or not the alphacoronaviruses, CCoVs and FECoVs, cross-infect to any large extent among carnivore hosts is unclear (43). However, in 1 dramatic episode in the mid-1980s a newly released CCoV vaccine apparently actually contained FCoV. Its use was associated with the death of hundreds of dogs with neurologic and pancreatic lesions (44).

Early in the SARS-CoV-2 pandemic, questions arose about the susceptibility of domestic animals, notably household pets, to the virus (45). Initial studies of the ACE-2 receptor indicated variable sequence identity with the human version among various potential host species. Co-incident epidemiologic studies documented rare, isolated PCR-positivity and rarer disease in cats and dogs, notwithstanding a highly publicized outbreak in captive tigers; the conclusion being that cases in animals were anthropogenic (45). There have been a few prospective experimental SARS-CoV-2 infections in cats and dogs, with the consensus being that cats can develop productive infections of $<$ 14-day duration, transmissible among cats, with minimal, if any, clinical signs, minimal virus-containing lesions in the upper respiratory tract, and seroconversion. Dogs appear to have non-productive, abortive infections without signs or lesions, but with seroconversion (46,47). Ferrets are similar to cats in their overall response to experimental SARS-CoV-2 infection and are a model in vaccine development (46). First in Europe and then in Canada, SARS-CoV-2 infections resulting in moderate to severe disease were reported in farmed mink. Whole genomic sequencing indicated anthropogenic transmission, followed by mutation, and then zoonotic re-transmission (48). It is unresolved whether wild mink, not subjected to the inbreeding, stress, and population densities of farms, would react to SARS-CoV-2 in the same way. Livestock and mice seem not to support infection at all (46).

Altogether, the ongoing examination of a wide variety of animals for evidence of SARS-CoV-2 infection or infectability illustrates constraints on viral fitness in real time. Just because a coronavirus, or any virus, for that matter, is capable of "infecting" a species is not necessarily indicative of a clinical concern; it is more biologically complicated than that. The bottom line is that, as far as variants within a coronaviral quasi-species go, only those with just the right set of amino acids in the S protein RBD *and* in their replication-mediating NSPs are going to be fit enough to *replicate enough* in a new host and, *may,* in the context of enabling host genetic and environmental cofactors, be a cause for clinical concern.

What are the correlates of protective immunity in coronaviral infections?

Before the COVID-19 era, family doctors probably considered corona-viral infections (a cold) mostly an annoyance unworthy of a clinical consult; the immunology of human coronaviral infections was little studied. Not so in veterinary medicine, in which coronaviral immunity has been extensively examined for decades.

At least for the epitheliotropic coronaviruses, such as TGEV, BCoV, CCoV, that cause diarrhea and/or respiratory disease, disease-sparing immune responses fit an overall pattern common to most viral infections: neutralizing antibodies reduce the possibility of infection, whereas cell-mediated responses enhance the chances for recovery (1). In mammals, before SARS-CoV-2, coronaviral immunity was most studied in pigs (1,49). Innate immune responses involving primarily interferons I and III are implicated as rapid "first responders" in enteric porcine coronaviral infections. Predictably, the primary targets of neutralizing lactogenic immunity, first IgG in colostrum, then IgA in milk, and later systemic antibody responses are epitopes on the S protein, primarily in the receptor binding domain (RBD). The S protein is also the target of cytotoxic T-cells, both intraepithelial and systemic CD8+ T-cells. In addition, epitopes on the nuclear protein are also targets for cell-mediated immune responses. A similar constellation of viral epitope targets and active and passive responses can modulate disease in less-extensively studied, bovine and canine corona-viral infections (1).

Are immune responses against coronaviruses cross-protective?

The common occurrence of coronaviral infections raises the question: is there cross-protective immunity among related coronaviruses? The simple answer, based mainly on the outcome of primary infections in the laboratory and the field and the examination of neutralizing antibodies targeting the S protein, is no. This specificity of protective antibody responses was documented, large-scale, in pig barns following the emergence of PEDV in North America in the 2010s. Neutralizing antibodies against TGEV, its alpha-corona-viral cousin, conferred no disease-sparing effect on PEDV (49). Long ago, on the companion animal side, attempts to immunize cats against FIPV with its alpha-coronaviral sibling CCoV ended in failure (43), as did attempts to immunize with another relative, TGEV (50). Even considering responses to infections with different strains of the *same* coronavirus, work conducted more than 30 y ago with MHV infections in mice, the natural host of the virus, demonstrated protective immunity, that, unfortunately, was "strain-specific" presumably related to disparity in the S (then called E2) proteins among strains (51) typical of genetic drift in a quasi-species. A similar strain-dependent phenomenon is operant in FCoV infections in cats (52) and IBV infections in chickens (36,53). In contrast, there is some evidence of cross "strain" protection among rat coronaviruses, although, there, the viral genetics are less well-characterized (54).

One by-product of the urgency to control COVID-19 is a more nuanced answer to the question of cross-protective immunity. Trying to explain the heterogeneity of clinical outcomes to SARS-CoV-2 infections, several studies in human populations documented that 20 to 50% of unexposed individuals have circulating CD4+ (and to a lesser extent CD8+ T-cell) responses to SARS-CoV-2 (55). Furthermore, these responses are equally cross-reactive with responses to several coronaviral common cold viruses, in both alpha and beta genera (55). In other words, there are memory responses from previous exposure to common cold viruses. These observations have several clinically relevant implications that are being further investigated (55). Cross-reactive T-cell memory responses could explain enhanced clinical immunity and amelioration of disease in some SARS-CoV-2-infected individuals, and relatedly, a potential for anamnestic responses to SARS-CoV-2 vaccines in human populations. Conversely, this immunological *déjà vu* could effectuate immune-mediated enhancement of disease following SARS-CoV-2 infection in some individuals, although this possibility is currently considered less likely (55).

A double-edged sword: How does the immune response enhance coronaviral disease?

For coronaviruses that have a cellular tropism beyond just epithelial cells and a tendency to produce multi-systemic disease, such as FIPV, MHV and IBV, and, now, SARS-CoV-2, immune responses are more of a double-edged sword. An "elephant-in-the-room" in the now ubiquitous discussions concerning COVID-19 is the ominous possibility that the immune response to SARS-CoV-2 is the purveyor of pathology rather than protection. This concept that immune-mediated disease is a feature of coronaviral infections is not new to veterinarians, especially the cat-healing ones. The role of immunopathology, notably antibody-dependent enhancement (ADE), in FIP is well-documented, even if still somewhat enigmatic, and, maybe, more pronounced in experimentally induced *versus* naturally occurring disease (56). In the early days of FIPV research in the late 1970s, it was observed that cats with preexisting FCoV-specific antibodies not only did not have disease-sparing effects after experimental challenge, but experienced more severe disease (57). Initially, from a mechanistic standpoint, antigen-antibody complexes and a resultant classic Arthus reaction or type III hypersensitivity reaction were considered the main culprit (52). The resulting vaso-centric inflammatory response certainly explained the ascites typical of the "wet form" of the disease. Then, it was shown that antibodies against the S protein, even neutralizing ones, enhanced the infection of feline macrophages *in vitro* (52). Tropism for subsets of monocyte/macrophage lineage cells is now viewed as the primary phenotypic marker of strain virulence among FCoVs, even though the genetic basis of this remains elusive. In other words, strains of FCoVs, mutants of FECoVs onboard in the intestines, that are likely to cause FIP, are macrophage-tropic. Their entry into those cells is enhanced *via* antibody and/or complement binding to the Fc receptors on those cells. From a pathophysiological standpoint this property is important in at least 2 ways. First, the FIPV-infected macrophages are "Trojan horses" that ferry the viruses from the gut to other organs. Second, macrophages normally play a central role in cytokine-mediated inflammation. Feline infectious peritonitis viruses (FIPVs) hijack this function and put it into overdrive, the result being a version of a cytokine storm. Similarly, MHV (58) and IBV (53) are long recognized as being polytropic and immunomodulatory and can cause multisystemic disease. SARS-CoV-2 is not novel in this regard. However, the role of ADE is less well-established in MHV and IBV-mediated diseases compared to its central

role in FIP; perhaps because FIPVs are primarily macrophage-tropic, whereas the others, including SARS-CoV-2, are not. Alternatively, the explosion of SARS-CoV-2-related research is reiterating the probable role of a dysregulated innate immune response, a big part of the cytokine storm, in pathogenesis (55). This NSP-mediated dysregulation is likely operant in all polytropic coronaviral infections — a double-edged sword, indeed.

Great expectations: What is the best approach to coronaviral immunoprophylaxis?

In veterinary medicine, control of coronaviral diseases through vaccination spans more than 75 y. In the 1950s, one of the first attempts used dead piglets ("feedback") for successful prophylaxis against TGEV (59). This technique has been more recently applied to PEDV (49). It is fitting, then, that from the mammalian perspective, most success and mechanistic understanding of vaccine-induced immunity to coronaviral infections derives from experiences in swine. Virtually all of this is related to successful reduction of enteric diseases, most notably due to TGEV, with a spectrum of platforms from conventional to "high tech" (49). Certainly, working with large numbers in confined populations, with usually defined genetics, and operating on the cusp of tight financial margins over a short feeding/finishing period, has fostered this effort. Similar conditions have applied in the assessment of IBV vaccines in poultry, albeit with the observation of less efficacy due to the strain variation of the virus (53).

In cattle, commercial, combination modified-live oral or intranasal (IN) vaccines, and inactivated parenteral BCoV vaccines have been available for decades and used to control neonatal diarrhea and enhance colostral antibody production, respectively (1). However, there are few published data substantiating their efficacy, especially with regard to current circulating strains. One study provides circumstantial evidence that application of IN vaccines can reduce respiratory disease in feedlot calves, even though BCoV vaccines do not have a label claim for that use (1). The correlates of immunity to respiratory coronaviral infections in cattle, pigs, and dogs remain to be fully characterized. To date, IBV vaccines stand alone in having a legal claim for vaccination against respiratory disease (1,53).

Mucosal (IN) vaccination has also been used as a means of stimulating IgA and of side-stepping immune-mediated enhancement of disease that can result from parenteral vaccination and resultant systemic (IgG) responses. In the 1990s a modified-live FCoV IN vaccine demonstrated reasonable efficacy in a robust FIP challenge model without inducing ADE that had previously been observed after parenteral administration (60). However, its efficacy and utility were controversial (52) and, even though it is still commercially licensed in some countries, it is little used. Nevertheless, this experience with IN vaccination in cats is relevant, should disease enhancement result from injectable SARS-CoV-2 vaccines.

The development of vaccines for SARS-CoV-2 using a range of formats from conventional whole-virus formulations to novel mRNA constructs has proceeded at record speed. Reviews of this effort are already available (61). Implicit in the application of SARS-CoV-2 vaccines is the assumption that induced immune responses will be protective. Indeed, that hope evokes the success of veterinary coronaviral vaccines, mostly those targeting the epithelial-tropic viruses (62). However, in contrast to many veterinary coronaviral vaccines (62), the leading candidates for SARS-CoV-2 immunogens are mRNA, viral-vectored, and subunit constructs that reductively target the S protein, or regions thereof. A major advantage of the mRNA, viral-vectored, or even lower tech modified-live vaccines, *versus* S subunit or, simpler whole-virus inactivated vaccines is a function of antigen presentation (61). The former approaches present antigen *via* both endogenous and exogenous pathways and can more broadly stimulate CMI and antibody, whereas the latter stimulate little if any cytotoxic T-cell responses. However, it is currently unresolved whether the reductionist approach of targeting 1 region of 1 protein will stimulate "mutation-proof" and durable responses. There are already televised data suggesting the contrary, but little acknowledgment or apparent awareness that this is really another *déjà vu* for veterinarians; it has all been experienced before in the long history of IBV vaccines (53). Regrettably, the reductionist approach to vaccination and (monoclonal antibody) therapy (61) with very limited epitopic targeting may be a main driver of SARS-CoV-2 evolution going forward. There is already some preliminary evidence for this epiphenomenon.

Beyond disease-sparing clinical protection, there is much discussion in the SARS-CoV-2 vaccine space about the pot-of-gold possibility of vaccine-conferred sterilizing immunity, i.e., prevention of infection. Again, the veterinary experience with coronaviral vaccines indicates that the likelihood of achieving this with injectable vaccines alone is low, as some level of mucosal immunity in the form of immune-exclusionary IgA, is required to at least partially effectuate sterility. Relatedly, the current news that re-infection with SARS-CoV-2 is possible should not be newsworthy; immune exclusion is transient and less than complete. There are data examining responses to mucosally delivered porcine, bovine, and feline coronaviral vaccines (1,62). However, arguably, it is never-televised IBV immunoprophylaxis that began in the 1940s with "planned exposure" (63) that is most data-rich and instructive in the nascent SARS-CoV-2 vaccine rollout (53). Notwithstanding strain-dependent immunity and application of numerous vaccine technologies, it is a relatively low-tech protocol, heterologous prime-boosting, first with aerosolized modified-live followed by injectable inactivated whole-virus vaccines that remains the best approach to the control of IBV infections and is therefore the industry standard (53). The bottom line is that extensive experience with veterinary coronaviral vaccines (1,62) suggests that optimal clinical immunity is a tandem of mucosal and systemic responses induced by the combination mucosal and parenteral vaccines.

Although talk of the relationships among the coronavirus family usually summons fears of zoonotic infections, the other side of the coin is the potential for cross-protective immunity. Especially in view of the recent documentation of cross-reactive T-cell responses (55), the close relationship among the human cold viruses, HCoV-OC43 and HCoV-HKU1, and BCoV and CRCoV, all *Betacoronaviruses,* or the relatedness among other

cold viruses HCoV-NL63 and HCoV-229E, and FECoV, PEDV, TGEV, and CCoV, all *Alphacoronaviruses,* raises the possibility that repetitive (natural) exposure to corona-viral-infected livestock or companion animals could also engender cross-reactive memory T-cell responses that could affect human owners' responses to SARS-CoV-2 infection or vaccination. Preferential stimulation of cell-mediated *versus* antibody responses takes on additional *gravitas* should ADE be a limiting factor in more conventional approaches to vaccination with, for example, S protein (inactivated) subunit vaccines (55,61). Although the "free vaccination" of natural exposure is difficult to model in the laboratory, the Jennerian approach, effectively using a related pathogen as the (free) vaccine against another, could be discernable in populations if someone bothers to look. This would be a new and profound take on the human-animal bond and is worthy of investigation.

Like *déjà vu* all over again?

In conclusion, the coronaviruses comprise a family of related agents with similarities and differences; the expression of a defining genomic blueprint. There is a pattern of diseases among infected hosts. Generally, they have been associated with enteric and respiratory diseases. But, there can be systemic manifestations of coronaviral infections that are mostly associated with an overwrought immune response. SARS-CoV-2 has really awakened the world to coronaviruses, agents that veterinarians have been dealing with clinically for nearly a century. Minimally, some comparative knowledge of the coronaviral family, and infections in animals can prevent reinvention of the wheel when it comes to control and prophylaxis of SARS-CoV-2.

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