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MOLECULAR EVOLUTION OF CORONA- AND TOROVIRUSES

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Coronaviruses belong to the newly established *Nidovirales*—the second order (in addition to the *Mononegavirales*) in animal virus taxonomy. They comprise a group of enveloped, positive-stranded RNA viruses infecting mammals and birds. The order consists of the bigeneric family *Coronaviridae*, to which the genera *Coronavirus* and *Torovirus* have been assigned, and the monogeneric family *Arteriviridae* (Murphy et al., 1995).

Despite essential differences in genetic complexity and virion architecture between corona-, toro-, and arteriviruses, they show a striking resemblance in genome organization and replication strategy, which have been viewed as an overriding principle by the ICTV (Murphy et al., 1995) and resulted in the present classification. The name *Nidovirales* (from the Latin *nidus*, nest) refers to the 3′ co-terminal nested set of subgenomic viral mRNAs that is produced during infection. Sequence similarities, though mostly restricted to the polyprotein (POL) 1b, from which the replicase-associated proteins are derived, indicate that the *Nidovirales* are phylogenetically related and may have evolved from a common ancestor. Extensive genome rearrangements through heterologous RNA recombination have resulted in a set of viruses that utilize similar replication and transcription mechanisms, but disparate strategies to package their genetic information. For a recent review, see de Vries et al. (1997).

The phylogenetic relationship between viruses in the *Nidovirales* order is not apparent from their morphology. Coronavirions are roughly spherical, 100–120 nm in diameter, and display a typical fringe of 20 nm long petal shaped spikes (peplomers). Some coronaviruses exhibit a second fringe of smaller surface projections about 5 nm in length. Torovirus particles are more pleiomorphic, spherical, oval, elongated and kidney-shaped, measuring 120 to 140 nm in their largest axis. The surface projections of toroviruses closely resemble the coronavirus peplomers. The differences in virion

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architecture become even more apparent when comparing the nucleocapsid structures. Coronaviruses possess a loosely wound, helical inner ribonucleoprotein structure and a capsid of supposedly icosahedral disposition (Risco et al., 1996), while the torovirus nucleocapsid has a compact tubular organization and exhibits kidney, disc or rod shapes in ultra-thin sections (for reviews, see Koopmans and Horzinek, 1994; Snijder and Horzinek, 1993). In view of these observations, it is not surprising that the nucleocapsid proteins (N) of the *Nidovirales* differ considerably both in sequence and in size (50, 19, and 14 kDa for corona-, toro-, and arteriviruses, respectively) and are completely unrelated.

There are also differences in the composition of the viral envelopes. The membrane of coronaviruses invariably contain (i) the 180–200 kDa spike protein (S), (ii) the 25–30 kDa triple-spanning membrane protein M, and (iii) the 9 kDa transmembrane protein E, a minor protein species, but together with M essential for virus assembly. The shorter surface projections of some coronaviruses mentioned above consist of a dimer of a 65 kDa class I membrane protein, the hemagglutin-esterase (HE), sharing 30% sequence identity with the N-terminal subunit of the hemagglutinin-esterase fusion protein (HEF) of influenza C virus. Apparently, this gene was acquired via heterologous RNA recombination, as will be discussed below.

Toroviruses also specify a 180 kDa S and a 26 kDa triple spanning M protein. Although primary sequence similarity is absent, the S and M proteins of toro- and coronaviruses are similar in size and function. There are also structural similarities: the M proteins have the same membrane topology; furthermore, heptad repeats, indicative for a coiled-coil structure first discovered in the spike proteins of coronaviruses, are also found in the torovirus peplomer. Thus, the S and M genes of these viruses may well be phylogenetically related. Toroviruses lack a homologue for the coronavirus E protein, which could indicate a key difference in their mode of assembly. The presence of a third membrane protein, the 65 kDa hemagglutinin-esterase, will be discussed in detail.

FELINE INFECTIOUS PERITONITIS

The members of the genus *Coronavirus* have been assigned to three clusters, with the prototypes mouse hepatitis virus, porcine transmissible gastroenteritis virus (to which also the feline coronaviruses belong) and avian infectious bronchitis virus, based on serological and genetic properties.

When considering molecular evolution, coronaviruses are a showcase of biologic (antigenic) and pathogenetic variability. Feline infectious peritonitis (FIP) is arguably the most enigmatic coronaviral disease, its etiology, pathobiology, and immunology having been explained largely by the Davis group of workers (Pedersen, 1976a, b; Pedersen, 1987; Pedersen et al., 1984). It is infrequent but fatal, involves immune-mediated phenomena such as antibody-dependent enhancement of infection and shows immune-complex induced pathology. The existence of a carrier-state of a harmless enteric precursor virus (conveniently termed feline enteric coronavirus; FCoV) has recently been formally proven (Herrewegh et al., 1997). The sporadic occurrence of the condition is interpreted as the result of *in-vivo* mutants that have acquired macrophage tropism—a hypothesis first formulated by Niels Pedersen.

Feline infectious peritonitis is a progressive, debilitating lethal disease of domestic and wild *Felidae*. The initial signs of naturally occurring FIP are not very charac-

teristic. The affected cats show anorexia, chronic fever, and malaise. Occasionally, ocular and neurological disorders occur. In classical "wet" or effusive FIP these signs are accompanied by a gradual abdominal distension due to the accumulation of a viscous yellow ascitic fluid. The quantity of fluid can vary from a few milliliters to well over a liter.—There is also the "dry" or non-effusive form of FIP where little or no exudate is present. The wet and dry forms of FIP are different manifestations of the same infection. For a recent review, see Groot and Horzinek (1995).

Pathogenesis, Pathology, and Immunity

The key athogenic event leading to FIP is the infection of monocytes and macrophages. However, also the ubiquitous avirulent FCoV strains are not confined to the digestive tract and do spread beyond the intestinal epithelium and regional lymph nodes—so the difference must rather be a quantitative one. In vitro, the virulence of FCoV strains was indeed correlated with their ability to infect cultured peritoneal macrophages, the avirulent ones infected fewer macrophages and producing lower titers than virulent strains (Stoddart and Scott, 1988, 1989). Moreover, the avirulent strains were less able to sustain viral replication and to spread to other macrophages. This is no black-and-white phenomenon, rather a gradual transition, as the course of FIP is not uniform.

Humoral immunity is not protective: FCoV-seropositive cats that are experimentally challenged develop an accelerated, fulminating course of the disease, leading to the 'early death' syndrome. Clinical signs and lesions develop earlier, and the mean survival time is reduced as compared to seronegative cats. Direct evidence for the involvement of antibodies was obtained from the transfusion of purified FCoV anti-IgG into cats which developed accelerated FIP upon experimental challenge (Pedersen, 1987).

Though the vascular and perivascular lesions in FIP are thought to be immune-mediated, there is uncertainty about the actual pathogenetic mechanism. Some vascular injury may be attributed to immune-mediated lysis of infected cells: FIPV-infected white blood cells were detected in the lumen, intima, and wall of veins and in perivascular locations. Furthermore, inflammatory mediators such as cytokines, leukotrienes, and prostaglandines that are released by infected macrophages could play a role in the development of the perivascular pyogranulomas. These products could induce vascular permeability changes and provide chemotactic stimuli for neutrophils and monocytes. In response to the inflammation, the attracted cells may release additional mediators and cytotoxic substances; the monocytes would also serve as new targets for FIPV. The result would be enhanced local virus production and increased tissue damage.

Other observations point towards an immune complex (ICX) pathogenesis. Deposition of ICX and subsequent complement activation may cause an inflammatory response that extends across blood vessel walls. The resulting vascular damage would permit leakage of fluid into the intercellular space and lead to the accumulation of thoracic and abdominal exudate. The morphologic features of the vascular lesions (necrosis, polymorphonuclear cell infiltration associated with small veins and venules) indicate an Arthus type reaction. The lesions contain focal deposits of virus, IgG, and C3. Moreover, complement depletion and circulating ICX are found in cats with terminal FIP (Jacobse-Geels et al., 1980, 1982). Although FIP viruses do not infect T-cells, depletion and programmed cell death (apoptosis) has been observed in lymphoid

organs of infected cats. Apoptosis was mediated by the ICX present in the serum and ascitic fluid of diseased cats (Haagmans et al., 1996).

Gross FIP lesions appear as multiple grayish-white nodules (<1 to 10 mm) in the serosal membranes, liver, lungs, spleen, omentum, intestines, and kidneys. Microscopic lesions consist of disseminated foci of necrosis and pyogranulomatous inflammation, frequently located around smaller vessels. These lesions are characterized by accumulations of fibrin and necrotic debris, and by perivascular infiltrations of macrophages, neutrophils, and lymphocytes. Histologically, disseminated perivascular pyogranulomatous inflammation and exudative fibrinous serositis in the abdominal and thoracic cavities are characteristic of the disease.

Persistent Infections and Biotypes

The fatal scenario leading to FIP may be as follows: a kitten suckled by its seropositive queen is protected by colostral antibody during the first few weeks. As the maternal antibodies wane, mucosal protection ebbs away and during an episode of maternal FCoV shedding the kitten is infected. A bout of diarrhea may be the only sign this has happened. It now develops an active immunity, but not a sterilizing one in most cases: virus and antibodies continue to co-exist in the kitten's organs, and an efficient cell-mediated immunity keeps infected macrophages and monocytes in check. In a small, socially stable cat community this animal can stay healthy.

Problems emerge when the kitten is experiencing any situation of stress, to be equated with immune suppression. Infections with FeLV or FIV are unmistakably immunosuppressive (Poland et al., 1996); Egberink et al., unpublished observations), but density (numbers of cats per surface unit), geographic change (displacement into a new environment), and other territorial factors (e.g. change in group hierarchy, dominance) are now becoming more and more important—in view of the declining prevalence of retrovirus infections. The failing immune surveillance allows the coronaviral quasispecies cloud of mutants to expand, and more macrophage-tropic mutants emerge in this stochastic process. Amongst them are some that reach high titers and outcrowd the moderate ones. This is the point when immune pathogenesis starts.

Crucial to this pathogenetic model is the evidence that coronaviruses indeed persist in individual cats—a plausible assumption based on clinical and epidemiological observations, but until recently unconfirmed. The Utrecht group has studied FCoV persistence and evolution in a closed cat breeding facility where serotype I FCoV infection was endemic (Herrewegh et al., 1995, 1997). The serotype I is represented by biotypes that are prevalent in most geographic areas, are poorly released from infected cells and usually cannot be cultivated; serotype II FCoVs are less common in the field but best-studied, and are antigenically related with canine coronaviruses.

Using the reverse transcriptase polymerase chain reaction (RT-PCR), viral RNA was detected in the feces and/or plasma of 36 out of the 42 cats tested. Of five cats, identified as FCoV shedders during an initial survey, four had detectable viral RNA in the feces when tested 111 days later. To determine whether this was due to continuous re-infection or to viral persistence, two cats were placed in strict isolation and fecal virus shedding was monitored. In one cat, shedding was found for up to seven months. The other animal was sacrificed after 124 days in order to identify the sites of virus replication. While viral genomic RNA was found in most organs tested, mRNA was detected only in the ileum, colon, and rectum. In these tissues, single FCoV-infected cells were identified by immunohistochemistry in a background of uninfected tissue.

These findings provide the first formal evidence that FCoV causes chronic enteric infections (Herrewegh et al., 1997).

To assess FCoV heterogeneity in the breeding facility and to study viral evolution during chronic infection, samples from individual cats were characterized by RT-PCR amplification of selected regions of the viral genome followed by sequence analysis. Phylogenetic comparison of nucleotides 007 to 146 of ORF7b to corresponding sequences obtained for independent European and American isolates indicated that the viruses in the breeding facility form a clade and likely have originated from a single founder infection. Comparative consensus sequence analysis of the more variable region formed by residues 079 to 478 of the S gene revealed that each cat harbored a distinct FCoV quasispecies. Moreover, FCoV appeared to be subject to immune selection during chronic infection (Herrewegh et al., 1997).

The combined data support a model in which chronically infected carriers perpetuate the endemic infection. Virtually every cat born to the breeding facility becomes infected, indicating that FCoVs are spread very efficiently. Infected cats, however, appear to resist superinfection by closely related FCoVs (Herrewegh, Ph.D.thesis Utrecht, 1997).

While the conversion of innocuous enteric coronaviruses to the lethal, FIP-inducing mutants is probably due to minute changes in one or more non-structural genes (which still need to be identified), we have recently shown that large chunks of genetic information may be shuttled between them. When studying the type II FCoV strains 79-1146 and 79-1683 (employed in most studies due to their satisfactory growth in vitro) we have found that they result from homologous RNA recombination events between FCoV type I strains and canine coronaviruses (CCV). In both cases, one template switch took place between the S and M genes, giving rise to recombinant viruses which encode a CCV-like S protein and the M, N, 7a and 7b proteins of FCoV type I (Motokawa et al., 1995; Vennema et al., 1993). Additional cross-over sites were mapped in the ORF1ab frameshifting region of strain 79-1683 and in the 5′-half of ORF 1b of strain 79-1146; this shows that the type II FCoVs have arisen from double recombination events (Herrewegh, Smeenk, Horzinek, Rottier, and de Groot 1997, unpublished results).

Serologic studies had suggested that the type II FCoVs are more closely related to CCV than to type I strains. Thus immunodominant neutralisation epitopes shared by the S proteins of CCV and FCoV type II are absent in the S of FCoV type I. The fact that the template switch occurred at different sites in strains 79-1146 and 79-1683 can only mean that they have arisen from separate recombination events. FCoV type I and CCV are the presumptive parental viruses (Motokawa et al., 1996), but the host species where the recombination took place is unknown. Cats can be experimentally infected with CCV (Barlough et al., 1984; McArdle et al., 1990, 1992; Stoddart et al., 1988), but whether CCV and FCoV readily cross species barriers in the field remains to be determined.

Two models can explain the recombination events from which the type II viruses resulted. In the most simple scenario, the recombination may have involved only two parental virus strains, with RNA replication initiating on a type I FCoV template of either negative or positive polarity (Liao and Lai, 1992), polymerase-switching to a CCV template, followed by a switch-back. Alternatively, a more complicated scheme can be envisaged in which a CCV-FCoV hybrid, arisen from a single template switch, spread into the cat population and in turn engaged in additional recombination events with (an)other type I FCoV strain(s). As explained above, FCoV type I viruses cause

chronic enteric infections that may last for at least seven months (Herrewegh et al., 1997). Conceivably, persistence of FCoV would raise the odds of double infections to occur.

In general, a recombinant virus, in order to emerge and to establish itself in the field, needs not just to be viable but to have a selective advantage. Thus, the uptake of CCV sequences by the type II FCoVs may have led to increased fitness as compared to the type I FCoV. Which of the acquired genes provided the selective advantage is yet unknown. Studies on other coronaviruses have shown that the S protein plays a crucial role in eliciting protective immunity. Moreover, genetic characterization of FCoVs isolated from persistently infected cats suggested that S is subject to antigenic drift, implying that this protein is a prime target for the immune system during chronic infection. From this work, it also appeared that FCoV-infected cats develop resistance against FCoV superinfection, at least by antigenically closely related strains. Perhaps the acquisition of a CCV spike by a type I FCoV resulted in an antigenic shift, allowing the recombinant virus to escape host and/or herd immunity. However, the acquired CCV sequences may have also provided a growth advantage. In contrast to type I FCoVs, type II strains replicate efficiently in tissue culture cells and produce 50–100 fold higher titers of extracellular virus (Pedersen et al., 1984).

Both type II strains studied have retained FCoV sequences from the 5′ and 3′ end of the FCoV type I genome. One interpretation is that these FCoV sequences are required for efficient replication in the cat, and that recombinant viruses, arisen from a single template switch, are selected against. It is particularly intriguing that both strains 79-1146 and 79-1683 have retained the FCoV type I ORF1a. The POL1a polyprotein gives rise to a number of cleavage products of unknown function (reviewed in de Vries et al., 1997), some of which may be involved in specific virus-host interactions. Further genetic characterization of type II FCoVs and mapping of recombination sites will provide insight into these issues and hopefully increase our understanding of coronavirus pathobiology and evolution.

TOROVIRUSES IN UNGULATES

Thus far, two torovirus species are officially recognized: a bovine torovirus (BoTV, originally named Breda virus), evidenced in the feces of diarrheic calves (Woode, 1982), and an equine torovirus (ETV, formerly described as Berne virus), which was isolated in cell culture from rectal swabs of a horse (Weiss et al., 1983). There is serological evidence for the existence of toroviruses in other mammals (Brown et al., 1987; Weiss et al., 1984) and man (Beards et al., 1984, 1986; Brown et al., 1987, 1988; Duckmanton et al., 1997; Jamieson et al., 1998; Koopmans et al., 1993, 1997; Krishnan and Naik, 1997; Lacombe et al., 1988; Van Kruiningen et al., 1993).

During a serosurvey in Switzerland, we had already detected ETV-neutralizing antibodies in the sera of 91 out of 112 pigs tested (Weiss et al., 1984). Furthermore, several authors have reported torovirus-like particles in the feces of swine (Penrith and Gerdes, 1992; Scott et al., 1987; Woode, 1987). However, in negatively stained preparations for electron microscopy, torovirions are often pleiomorphic and may appear as spherical, oval, elongated or kidney-shaped particles, carrying either a single or a double fringe of surface projections. Without additional immunological and molecular confirmation torovirions are difficult to distinguish from coronaviruses and other viral—and even non-viral—fringed particles.

A Porcine Torovirus

In a recent paper (Kroneman et al., 1998) we presented formal evidence for the existence of a porcine torovirus (PoTV). By using a reverse-transcriptase polymerase chain reaction (RT-PCR) targeted to the 3′-nontranslated region of the genome, toroviral RNA was detected in the feces of piglets. Moreover, torovirions were identified in these samples by immunoelectron microscopy. Virus shedding, as monitored by RT-PCR, started shortly after weaning and lasted for 1 or more days. Comparative sequence analysis of the N-gene indicates that PoTV is a novel torovirus closely related to, but clearly distinct from, BoTV and ETV.

The virus was detected in the feces of piglets; in fresh fecal samples, PoTV particles appeared elongated, measuring 120 nm in length and 55 nm in width. Two types of surface projections were observed, the longer of which being club-shaped, 18–20 nm in length, and most likely representing oligomers of the S protein (Horzinek et al., 1986). The shorter spikes were 6 nm long and presumably represent the HE. Surface projections of this size are also seen in BoTV, but are absent in ETV (Cornelissen et al., 1997) where the HE gene is truncated at its 5′ end (Snijder et al., 1991). Preliminary observations from RT-PCR amplification and sequence analysis indicate that (like BoTV) PoTV contains an intact HE gene (A. Kroneman, Utrecht, unpublished data).

Comparative sequence analysis of the toroviral N-genes showed that BoTV and ETV are closely related, with 81% sequence identity in this region. In contrast, PoTV shared only 68% sequence identity with the N-genes of the other two viruses. The 3′ non-translated regions (NTR) of PoTV, BoTV, and ETV are highly conserved, with sequence identities of about 88%. We conclude that PoTV is antigenically and genetically related to, but clearly distinct from the bovine and equine representatives of the torovirus genus and should therefore be considered as a new member species.

In a heterotypic *in vitro* neutralization assay, >80% of the adult sows in the Netherlands turned out to be positive for torovirus antibody; we had reported similar observations for Switzerland (Weiss et al., 1984). In pigs, torovirus infections are obviously as common and widespread as in cattle and horses. Piglets are infected shortly after weaning, when protection by maternal antibodies and/or lactogenic immunity has waned. Virus shedding in the feces, as monitored by RT-PCR, lasted between one to nine days, which suggests that PoTV can cause acute enteric infections.

The high percentage of seropositive animals and the time of infection shortly after weaning, when maternal immune protection has waned, is indicative for PoTV endemicity. The virus would persist on a farm because of the continuous presence of susceptible piglets and reinfection of partly immune animals. Also chronically infected carriers may exist, as has been demonstrated for other *Nidovirales* such as coronaviruses (Crouch et al., 1985; Herrewegh et al., 1995) and arteriviruses (for a review, see Plagemann and Moennig (1992). A more sensitive nested RT-PCR targeted to the conserved 3′ NTR may allow the identification of long-term shedders among the adult pig population.

Recombination in Toroviruses

RNA viruses are genetically very flexible—they lack the proofreading mechanisms that operate during DNA replication. When RNA genomes are replicated, nucleotide substitutions occur at a high frequency, thereby allowing swift adaptation to selective pressure. For some viruses, homologous RNA recombination, i.e. genetic

exchange between closely related RNA molecules, functions as a correction mechanism counteracting genetic drift and Muller's ratchet (Chao, 1990). At the same time, it allows the rapid horizontal spread of advantageous mutations (for a review on RNA recombination, see Lai, 1992). Heterologous RNA recombination involves non-related RNA molecules and provides a means to acquire blocks or "modules" of new genetic information either from other viruses or from the host (Goldbach and Wellink, 1988; Strauss and Strauss, 1988, 1991; Zimmern, 1987).

One remarkable example of heterologous recombination is that of the coronavirus hemagglutinin-esterase (HE) gene. In addition to the spike- (S), membrane (M), envelope (E), and nucleocapsid protein (N), some coronavirus species possess a fifth structural protein, HE (for a review, see Brian et al., 1995). This 65 kDa class I membrane protein shares 30% amino acid identity with the HE-1 subunit of the hemagglutinin-esterase fusion protein (HEF) of influenza C virus (ICV), a negative-stranded RNA virus with a segmented genome (Brian et al., 1995; Luytjes et al., 1988). It has been speculated that coronaviruses have captured the HE-module from ICV or a related virus during a mixed infection (Luytjes et al., 1988). Presumably, this event occurred after coronavirus speciation, since the HE gene is present only in the genomes of viruses related to mouse hepatitis virus (MHV), and is absent in the antigenic coronavirus clusters represented by FIPV and infectious bronchitis virus (IBV) (Luytjes, 1995). Both in ICV and in coronaviruses, the HE displays an acetylesterase activity specific for *N*-acetyl-9-*O*-acetylneuraminic acid. The ICV HEF serves as a receptor-binding/receptor-destroying protein (Herrler et al., 1988; Vlasak et al., 1987) and it has been suggested that the coronavirus HE has similar functions (Parker et al., 1989; Vlasak et al., 1988).

We have reported HE-like sequences to occur also in the genome of ETV (Snijder et al., 1991). The equine isolate is the only torovirus that can be grown in tissue culture and is therefore the best studied. The 3′-most 15 kb of its 25–30 kb genome have been sequenced, identifying the genes for POL1b, S, M, and N (Snijder and Horzinek, 1995). In addition, a non-functional open reading frame, ORF4, was found. Translation of this ORF yielded a polypeptide sequence 30% identical to the C-terminal 142 amino acids of the HE(–1) proteins of ICV and coronavirus. These findings have led to the hypothesis that the HE may be part of the toroviral standard gene repertoire, and that ETV is a mutant that has lost part of its HE gene during tissue culture adaptation (Snijder et al., 1991).

During the recent genetic characterization of a BoTV strain (Breda 2), which is antigenically closely related to ETV, evidence has been provided that it carries an intact, functional gene for an HE homologue, and that the toroviral HE is a structural protein. Its origin is unknown. Although corona- and toroviruses are related, they apparently acquired their HE genes through separate heterologous RNA recombination events. This is best illustrated by the fact that the HE genes are located at different positions in their genomes, Snijder and Horzinek, 1995; Snijder et al., 1991; Cornelissen et al., 1997). It is also of interest that the HE sequences of ICV, corona- and toroviruses are evolutionary equidistant and that several amino acid motives, conserved in the HE(–1) of ICV and torovirus, have been lost in the coronavirus HE (Cornelissen et al., 1997). We have offered the hypothesis that ICV, coronaviruses and toroviruses each have acquired the HE-module independently from yet another source, perhaps by recombination with a host mRNA.

In general, recombinant viruses will be lost from the viral population unless the acquired genetic information results in a gain of fitness. Apparently, the HE provides

a considerable selective advantage, at least during the natural infection. The role of the ICV HEF in receptor binding and entry is well-established (Herrler et al., 1988), but the function of the HE-proteins of corona- and toroviruses is not exactly known. For coronaviruses, receptor-binding and membrane fusion is mediated by the S-protein (Cavanagh, 1995). It has been suggested that the coronavirus HE may serve as an additional receptor-binding protein (Parker et al., 1989; Vlasak et al., 1988). However, recent findings suggest that MHV-infection cannot be mediated by HE alone, but requires the interaction of S with its receptor (Gagneten et al., 1995). Perhaps, HE does not play a role in viral entry but at an even earlier step of the infection. ICV, corona-, and toroviruses infect the epithelial cells of the respiratory and enteric tract. These cells are protected by a mucus layer, a gel formed by non-covalent interactions between large, highly hydrated glycoproteins, that can be up to 400 □m thick (for a review, see Mantle and Allen, 1989). This mucus layer thus presents a formidable barrier that has to be traversed before adsorption to the host cell can occur. The HE proteins may mediate viral adherence to the intestinal wall through the specific, yet reversible binding to mucopolysaccharides. In fact, the process of binding to 9-*O*-acetylated receptors, followed by cleavage and rebinding to intact receptors could, theoretically, even result in virus migration through the mucus layer and thereby not only facilitate the infection but also virus release and spread. Clearly, the role of the HE proteins during the infection of corona- and toroviruses deserves further study. More generally than just in the *Nidovirales*, they may be the result of molecular evolution by conferring a selective advantage to viruses that must penetrate through mucous layers, which shielding their epithelial targets in the respiratory, gastrointestinal, and genital tracts.

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