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Investigation of cross-neutralization between different coronavirus species

Coronavírusok közti keresztneutralizáció vizsgálata

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1. List of Abbreviations

Abbreviation	Definition
CoVs	Coronaviruses
SARS-CoV	Severe acute respiratory syndrome coronavirus
MERS-CoV	Middle East respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus disease-19
TGEV	Transmissible gastroenteritis virus
PRCV	Porcine respiratory coronavirus
PEDV	Porcine epidemic diarrhoea virus
SADS-CoV	Swine acute diarrhoea syndrome-coronavirus
PHEV	Porcine hemagglutinating encephalomyelitis virus
PDCoV	Porcine deltacoronavirus
HCoV	Human coronavirus
IBV	Infectious bronchitis virus
MHV	Murine hepatitis virus
mRNA	Messenger RNA
ORFs	Open reading frames
N protein	Nucleocapsid protein
M protein	Membrane protein
E protein	Envelope protein
S protein	Spike protein
RBD	Receptor-binding domain
APN	Aminopeptidase N
HA	Hemagglutinating
HE protein	Hemagglutinin-esterase protein
BCoV	Bovine coronavirus
ACE2	Angiotensin-converting enzyme 2
TMPRSS2	Transmembrane serine protease 2
gpE2	Envelope glycoprotein
DPP4	Dipeptidyl serine protease-4

ELISA	Enzyme-linked immunosorbent assay
CCIF	Cell culture immunofluorescent
CCoV	Canine coronavirus
FIPV	Feline infectious peritonitis virus
TCoV	Turkey coronavirus
FIP	Feline infectious peritonitis
FeCoV	Feline coronavirus
DAD	Diffuse alveolar damage
SeCoV	Swine enteric coronavirus
IFN	Interferon
NNK	Nemzeti Népegészségügyi Központ
NÉBIH	Nemzeti Élelmiszerlánc-biztonsági Hivatal
DMEM	Dulbecco’s Modified Eagle Medium
FBS	Foetal bovine serum
PBS	Phosphate buffer saline
TCID50	Median Tissue Culture Infectious Dose
CPE	Cytopathogenic effects
VNT	Virus neutralisation test

2. Introduction

Coronaviruses (CoVs) have a wide host range in both mammalian and avian species. In the veterinary world CoVs infect a variety of livestock, poultry, and companion animals, causing economically important diseases in these animals. Some CoVs were originally found as enzootic infections, limited only to their natural animal hosts, but have crossed the animal-human species barrier and adapted to humans. These diseases are not zoonotic, because adopted viruses cannot infect other animals, not even the original host.

In the last two decades novel CoVs have been on the forefront of large human disease outbreaks, including severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. In 2019 a novel respiratory coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), or as the disease is more commonly known as coronavirus disease 19 (COVID-19), led to an ongoing pandemic and public health problem.

After the emergence of SARS-CoV and MERS-CoV, our outlook has expanded in both human medicine and veterinary medicine on CoVs as zoonotic pathogens, that can severely affect human health. The improvement of our comprehension of the basic principles of coronavirus replication and pathogenesis were accelerated. Furthermore, the remarkable speed and technological progress of coronavirus research, that began after the discovery of SARS-CoV-2 has led to our rapidly growing understanding of this newly emerging pathogen and of its associated disease, COVID-19.

Pigs have commonly been used as a model for human disease pathogenesis. They are large animals with similar physiology and immunology to humans, they are also the natural host of Transmissible gastroenteritis virus (TGEV) and Porcine respiratory coronavirus (PRCV). Models have been created to investigate SARS-CoV-2 in mice, ferrets, hamsters, and non-human primates, but these are not natural host species for infection and cannot always correlate with human disease. Pigs are the natural host for many porcine coronaviruses and are also similar to humans anatomically. Better understanding of the relationship between human and animal coronaviruses, their capacity for cross-species transmission, and the sharing of genetic information may facilitate improved prevention and control strategies for future emerging zoonotic coronaviruses.

In this investigation, we are interested in whether the antibodies of human coronavirus, SARS-CoV-2 can cross neutralise the porcine virus, TGE virus, and we use serological methods to test this hypothesis. If this hypothesis is found to be true, we can then demonstrate that there is a level of cross protection between these two viruses which belong to different groups within the Coronaviridae family.

Neutralisation of a virus is the loss of infectivity through reaction of the virus with a specific antibody. Cross-neutralisation in this research refers to the ability of antibodies, produced against a virus (SARS-CoV-2), to block the site or sites that are used to enter the target cell of a different virus (TGEV). In this research, we perform a virus neutralisation test to investigate our hypothesis. The TGE virus and the human serum which contained antibodies against SARS-CoV-2 were mixed together under appropriate conditions and then inoculated into cell culture. The neutralisation can be evaluated by the lack of reactions such as cytopathogenic effects.

3. Literature review

3.1 Coronavirus classification and structural properties

CoVs belong to the Coronaviridae family of the Nidovirales order. The Coronaviridae family of viruses are enveloped, positive single-stranded RNA viruses grouped into four genera: alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus. There are currently six coronaviruses known to infect pigs, including alphacoronaviruses: TGEV, PRCV, porcine epidemic diarrhoea virus (PEDV), and swine acute diarrheoa syndrome-coronavirus (SADS-CoV); one betacoronavirus: porcine hemagglutinating encephalomyelitis virus (PHEV); and a deltacoronavirus: porcine deltacoronavirus (PDCoV). TGEV, PRCV, and PHEV have been known to infect pigs for decades, while PEDV, PDCoV, and SADS-CoV are seen as emerging CoVs. Emerging porcine CoVs of the alphacoronavirus and deltacoronavirus genera display an increased susceptibility for interspecies transmission [35].

There are also coronaviruses that affect humans, as seen within the past decades. Three highly pathogenic coronaviruses have emerged into the human population as the result of spillover events from animals that can cause severe respiratory illness: SARS-CoV, MERS-CoV, and as of 2019, SARS-CoV-2 [10].

The lengths of CoVs range from 27,317 nt for human coronavirus (HCoV)-229E to 31,357 nt for murine hepatitis virus-A59, confirming the coronavirus genome as the largest known among RNA viruses. Coronaviruses have non-structural proteins involved in proteolytic processing, genome replication, and transcription. They are encoded within the 5'- proximal two-thirds of the genome on gene 1, and the structural proteins are encoded within the 3'-proximal one-third of the genome. The genes for the major structural proteins in all coronaviruses occur in the 5' to 3' order as S, E, M and N [7].

CoVs have commonality with other positive-sense RNA viruses in that the genome is infectious when transfected into tolerant host cells. This was originally demonstrated for TGEV, infectious bronchitis virus (IBV), and murine hepatitis virus (MHV). The genome is responsible for a variety of functions during infection. In the initial stages, it acts as a messenger RNA (mRNA) that is translated into the huge replicase polyprotein. The whole synthesis of which depends on a ribosomal frameshifting event. The only translation product that comes from the genome is the replicase; all downstream open reading frames (ORFs) are conveyed

from sub-genomic RNAs. After this process the genome then provides a template for replication and transcription. Subsequently, the genome is involved in assembly, as progeny genomes are integrated into progeny virions [22]. The genome is packed inside a helical capsid formed by the nucleocapsid protein (N) and further surrounded by an envelope. There are at least three structural proteins that are associated with the viral envelope. The membrane protein (M), the envelope protein (E), and the spike protein (S). The M and E proteins contribute to virus assembly [15].

The shape of the viral envelope is determined by the M protein and is also the most abundant structural protein. Research has demonstrated that the M protein is a dimer, a protein complex that consists of two identical molecules linked together. Its role is known to promote membrane curvature and also bind to the nucleocapsid. The N and M protein together stabilise the nucleocapsid, as well as the internal core of virions, and help complete the viral assembly. The M and E proteins create the viral envelope and this in turn begins the production and release of virus-like particles. The N protein solely binds to the RNA genome. The N protein is also involved in viral assembly and budding; this ends in complete virion formation [21]. The E protein is integrally important in the virus replication cycle. Recombinant CoVs that have low levels of the E protein result in reduced viral titres, crippled viral maturation, or yield propagation incompetent progeny. This shows how the E protein is important in virus production and maturation [27].

The S protein mediates virus entry into host cells, it also determines the viral host range and tissue tropism and a major inducer of host immune responses [15]. As the S protein is the main target of neutralising antibodies when infection occurs it is also in direct examination for therapeutic and vaccine design [33]. The S protein forms large protrusions from the virus surface, giving coronaviruses the appearance of the crown formation.

The S protein is made up of three segments: a large ectodomain, a single-pass transmembrane anchor, and a short intracellular tail [15]. The ectodomain contains both the receptor-binding domain (RBD) and the domains involved in fusion, rendering it the pivotal protein in the CoVs entry process. The S protein can be divided into two functionally distinct subunits: the globular S1 subunit is involved in receptor recognition, whereas the S2 subunit facilitates membrane fusion and anchors the S protein into the viral membrane [11]. The S protein is cleaved by host proteases at the S2 subunit which is thought to activate the protein for membrane fusion via extensive irreversible conformational changes. The entry of CoVs in the cells in the host is a

complicated action that needs the concerted process of receptor-binding, as well as proteolytic processing of the S protein to allow virus- cell fusion [33]. For cross-species adaptation of CoVs to infect different hosts, it appears one of the main determinants is the S protein, which stated above, mediates the entry of the virus into host cells and determines tissue tropism. The structure of the S protein subunits differs between the CoVs genera. For alphacoronaviruses spike proteins, two independently folding domains have been assigned in the S1 subunit. These domains can interact with host cell's surface molecules, an N-terminal domain, and the C-terminal domain. Contrary to betacoronaviruses, these two receptor-interacting domains in alphacoronavirus spikes are separated by some 275 residues, which may fold into one or more separate domains.[11].

Specially in TGEV, the S protein mediates the binding of the virus to the cell surface by specific interaction with porcine aminopeptidase N (APN). This is the cellular receptor for TGEV. In addition, the S protein is probably also responsible for the fusion between the viral and the cellular membranes. In TGEV the S protein has hemagglutinating (HA) activity, whereas this is not seen in PRCV. The absence of HA activity from PRCV suggests that the sialic acid binding site is located in the segment of the S protein of TGEV that is deleted in the comparable protein of PRCV. The presence of HA activity in TGEV, which is an enteropathogenic virus, and the absence of HA activity in PRCV, which is a respiratory variant, may indicate that the sialic acid binding seen in TGEV causes it's enteropathogenic nature. The sialic acid has been demonstrated to serve as a ligand for the binding of TGEV to erythrocytes in the gastrointestinal tract. TGEV is able to recognise N-acetylneuraminic acid, though it binds more efficiently to N-glycolylneuraminic acid, another type of sialic acid [14].

Some coronaviruses also encode an envelope-associated hemagglutinin-esterase protein (HE). The viruses that are encoded with the HE protein are organised into the betacoronavirus genera [15]. The HE protein is defined as an accessory protein, and it makes up the fourth constituent of the membrane envelope. The HE protein forms a second set of small spikes located as an understory among the S protein spikes. It was first detected as a hemagglutinin in PHEV and Bovine coronavirus (BCoV). It was thought that because of the hemagglutinating property of the HE protein, that this protein may duplicate or replace the role that is now given to the S protein. This was disproven with research about MHV-BCoV, which are chimeric viruses. It found that in BCoV infection, with the absence of the S protein and the presence of the HE protein alone, could not initiate infection in tissue culture. There are two suggested ways that the HE protein may act during coronavirus replication. It could aid the S protein in virus entry

to the host cells as a cofactor. Furthermore, it may prevent aggregation of progeny virions and aid with travel of CoVs through extracellular mucosa [22].

3.2 The role of structural proteins in viral entry to the cell

The structural proteins of CoVs play an important role in the viral entry to the host cell. The M protein is critical for combining essential viral components into new virions during morphogenesis. The N protein joins with the viral genome and M protein to express genome packaging into new viral particles. The ion channel is formed in the viral membrane by the E protein, which also is involved in viral assembly.

To complete viral entry, the S protein is required, as it binds to the target receptors of the host cell, and initiates fusion with the cell membrane. This process can be further broken down, involving the S1 and S2 domains of the S protein within the RBD. The S1 engages with the host receptor and the S2 controls successive membrane fusion, to allow the virus to enter the cytoplasm of the host cell. To activate the S protein, to achieve these processes, proteolytic cleavage must occur at two sites on the S protein. The first cleavage site on the S protein is at the S1/S2 boundary. The proteolytic cleavage leads to structural reconfiguration in the S2 domain, which reorganises it in a prefusion configuration. The cleavage site at S2' changes, which drives coalescence of the viral and cellular membranes. This allows the release of the N protein coated RNA genome into the cytoplasm of the host cell.

Different CoVs target unique receptors and proteases, as well as subcellular sites of S protein cleavage. In SARS-CoV and SARS-CoV-2 infections, the receptor targeted on the host cell by the S protein is angiotensin-converting enzyme 2 (ACE2) [10]. In PRCV infection of pigs, the target receptor is APN, and similarly in TGEV infection the target receptor is also APN. This is because PRCV is a mutation of TGEV [30]. APN is a cell-surface, zinc-binding protease that assists in the digestion of small peptides in the epithelia of the respiratory and enteric tract. It is also found in human neural tissue that is susceptible to HCoV-229E. In experimental studies of TGEV receptor activity, it was found that the use of specific APN inhibitors, or the mutation disruption of the catalytic site of porcine APN did not affect the TGEV receptor activity, and in turn did not prevent infection. This demonstrates that APN is not required for initiation of infection. The specificity of receptors can be noted, and the receptor activities of APN are not exchangeable. Human APN cannot act as a receptor for TGEV, and porcine APN cannot act as

a receptor for HCoV-229E. However, it has been proposed that porcine APN can allow cellular entry of PEDV, although it has also been suggested that the major receptor of PEDV most likely differs from that for TGEV. This is due to the fact the two viruses are able to grow in mutually exclusive sets of cell lines, derived from different species. An exception to the general rule of APN being a receptor for alphacoronaviruses is the revelation that HCoV-NL63 does not use human APN to trigger infection. Interestingly, it uses the same receptor as SARS-CoV: ACE2. It may suggest why HCoV-NL63 causes a much milder respiratory disease than that of SARS-CoV and SARS-CoV-2. Another topic for research is why two very different zinc-binding, cell surface peptidases APN and ACE2 act as receptors for such a large number of different CoVs. At present, this is seen as an interesting coincidence, though perhaps, in the future, more research can be completed about this area of CoVs [22].

ACE2 is a peptidase found in most organs, and in particularly a large amount in the epithelia of the lung and small intestine. The role of ACE2 is to hydrolyse angiotensin II. When the receptor binding of the ACE2 occurs, the S protein of SARS-CoV and SARS-CoV-2 cleaved and are activated by the host cell-surface protease transmembrane serine protease 2 (TMPRSS2) at the aforementioned cleavage sites, S1/S2 and S2'. This leads to membrane fusion of the virus and the host cell. Some studies suggest that SARS-CoV-2 may have a pre-cleavage event at S1/S2 cleavage site. This suggests that SARS-CoV-2 may only require cleavage at the S2' site which would increase the rate of the membrane fusion process. This increased potentiation of the membrane fusion process could relate to the increased transmissibility and expanded tropism of SARS-CoV-2, and also somewhat explain the bat-to-human spillover of the CoVs. This shows the importance of the S protein in cellular viral entry by CoVs [10].

3.3 Cross protection

TGEV, PEDV, and PDCoV all induce gastroenteritis in pigs, each with similar clinical signs and pathogenesis. Necrosis of the infected intestinal epithelial cells causes villous atrophy and malabsorptive diarrhoea. Profuse diarrhoea, frequently combined with vomiting that results in dehydration, leads to the death of piglets. Strong immune responses following natural infection protect against subsequent homologous challenges, however, these viruses display no cross-protection [31].

Cross protection can be seen between TGEV and PRCV. PRCV is the mutant form of TGEV, where a naturally occurring spike gene deletion occurred, first recognised in the 1980s. PRCV and TGEV vary in tissue tropism. PRCV has an affinity to the respiratory system, whereas TGEV affects the gastrointestinal system [30]. Pigs infected with PRCV produce antibodies that can cross-neutralise TGEV. The point mutation of the S protein of PRCV led to the loss of two antigenic sites. This can be seen by the lack of reactivity with monoclonal antibodies. The other antigenic sites are not affected by the deletion, and this explains the serological relatedness between PRCV and TGEV [14]. This demonstrates some level of cross protection between PRCV and TGEV infection [20]. The immune response induced by TGEV and PRCV cannot be differentiated by a seroneutralisation test in a cell culture. However, it can be differentiated by monoclonal antibodies as many of the epitopes located on the C and D domains of the envelope glycoprotein (gpE)2 of TGEV are not found on the gpE2 of PRCV. TGEV and PRCV share a close antigenic relationship [6].

A recently discovered porcine enteric coronavirus is SADS-CoV. This novel virus contains 95% of the same genome as bat coronavirus HKU2, which confirms that it originated from bats. The clinical signs of SADS-CoV are very similar to other enteric coronavirus of pigs, but there is no cross-reaction between it and TGEV, PEDV or PRCV. By proving that there is no cross-reaction between viruses, this proposes that there is no cross-protection between viruses either [18].

The S protein of human coronavirus SARS-CoV-2 induces the largest specific antibody response. The other proteins, N, M and E also produce antibodies. SARS-CoV-2 and SARS-CoV are considerably similar, with 76% amino acid sequence homology. They also both depend on ACE2 as an attachment receptor, as well as the TMPRSS2 protease for priming [4]. The ACE2 receptor is used by SARS-CoV-2 to gain access into human cells as the receptor site for the S protein [28]. The S protein RBD, within the S1 subunit, binds to the ACE2

receptor which triggers a conformational change in the S protein that consequently initiates membrane fusion events with the host cell [19]. The virus entry process into the host cell is an important factor in determining the virus tropism and also influences the level of viral infection. This can change due to the high rate of mutations in CoVs. This is seen in the mechanism in which CoVs change their specificity or binding affinity for a specific receptor. For instance, CoV-NL63, SARS-CoV and SARS-CoV-2 use ACE2. A difference can be seen between the SARS species and CoV-NL63, since CoV-NL63 leads to mild respiratory illness, probably because of its low affinity interaction with the ACE2 receptor. MERS-CoV is also a betacoronavirus, but it uses dipeptidyl peptidase-4 (DPP4) as a receptor, which is involved in glucose metabolism, apoptosis and the immune system [28]. This demonstrates that not all betacoronaviruses use the same receptors for viral entry into the host cells.

If cross-reactivity with SARS-CoV-2 is a common feature of SARS-CoV antibodies, then humans who have been infected with SARS-CoV may still maintain SARS-CoV-2 reactive antibodies. In recent studies it has been noted that there is a partial cross-reactivity between SARS-CoV and SARS-CoV-2. Neutralisation experiments preformed in these specific studies found that antibodies that previously neutralised against SARS-CoV were less likely to be strongly cross-reactive with SARS-CoV-2. This may be explained by our understanding of rapidly evolving viruses, much like influenza and lentivirus e.g. HIV, where it is noted that neutralising antibodies are more likely to bind to highly variable epitopes lying in the host-interacting surfaces of viral proteins. This is seen in the specific antibodies produced by the S protein, but no cross reaction has been verified between the N, E, M structural proteins. Further research, to determine the evolutionary forces that are shaping the continued change of the SARS-CoV-2 S protein in terms of widespread antibody-based immunity, may make it possible to forecast mutations that could result in more virulent strains of CoVs [4].

3.4 Antigenic cross-reactivity

The antigenic relationship of porcine CoVs are complex, and include intergenus, and intragenus cross-reactivity. Some research demonstrates cross-reactivity between TGEV (Millar strain), and current US PEDV strains. This cross-reactivity is similar to the N protein mediated antigenic cross-reactivity between SARS-CoV and alphacoronaviruses of porcine, canine and feline origin [35]. Dominant antigenic cross-reactions among alphacoronavirus species members have been proven using a variety of immunoassays, for example: virus neutralisation assay, enzyme-linked immunosorbent assay (ELISA), and western blotting. This confirms their allocation as a single species.

Both PEDV and TGEV are members of the alphacoronavirus genera and share similar pathogenesis, clinical signs, and pathological lesions in new-born piglets, as previously mentioned. Previous studies suggest no serological reaction between TGEV and PEDV. However, in research completed by *Lin et al.* showed that hyperimmune TGEV Millar strain of pig antisera cross-reacted with all PEDV strains at lower cell culture immunofluorescent (CCIF) antibody titres, although TGEV Purdue strain did not cross-react. Additionally, assessment of a panel of TGEV monoclonal antibodies supported at least one conserved epitope on the N protein that contributed to this cross-reactivity [17].

Research has demonstrated cross-reactivity between SARS-CoV and other animal CoVs which focused to define the region of the N protein and to decipher the boundaries of the cross-reactive antigenic sites. The determination of cross-reactivity between SARS-CoV and other CoVs groups was completed by ELISA. The research specifically included two porcine CoVs: TGEV strains (Purdue-P115 and Miller-M6) and PRCV-ISU1, as well as canine coronavirus (CCoV)-UCD1, feline infectious peritonitis virus (FIPV)-79-1146 and HCoV-NL63, since cross-reactivity had previously been confirmed. For betacoronaviruses, they investigated bovine coronavirus varieties and for gammacoronaviruses, they investigated IBV-Massachusetts and turkey coronavirus (TCoV-IN).

The levels of cross-reactivity between the SARS-CoV antigen and the alphacoronaviruses antisera varied. TGEV-P115 and TGEV-M6 antisera demonstrated higher levels of reactivity with SARS-CoV, compared to lower levels of reactivity between SARS-CoV and PRCV, SARS-CoV and FIPV, and SARS-CoV and CCoV antisera. No reactivity was detected between SARS-CoV and gammacoronaviruses, IBV and TCoV-IN or betacoronaviruses, BCoV.

In completion of the research, it was confirmed that TGEV and PRCV antisera from pigs cross-react with SARS-CoV using ELISA and Western blot analysis. Also concluded, in this research, is that cross-reactivity is not a common feature for all alphacoronaviruses and SARS-CoV. This was demonstrated by the lack of cross-reactivity between SARS-CoV and HCoV-NL63. The N and S structural proteins and immunogens were important areas of assessment, with regards to cross-reactivity, in this research [32].

3.5 Coronavirus mutation

RNA viruses, like CoVs usually have high mutation rates [10]. An example of this is the mutation of TGEV, porcine enteric virus. A novel virus was discovered in 1983, that was similar molecularly to TGEV although presents differently through the clinical outcome. PRCV, the mutation of TGEV, affects the respiratory system of pigs. The main molecular difference between these two viruses is a large deletion in the S protein. This deletion/mutation of the S protein occurred naturally in the environment. The PRCV has a deletion of 224th amino acids in the European strain or the 227th amino acid in the American strain, and also diverges in the antigenic domains C and B in the S protein. These deletions changed the tissue tropism of the virus from the enteric tract tissue to the respiratory tract tissue. The close molecular similarity between the viruses induces a cross-neutralisation between TGEV and PRCV. The antibodies induced by PRCV infection can neutralise both TGEV and PRCV. PRCV antibodies are now thought to be a natural vaccine against TGEV [1].

In recent research, it has been noted that PRCV targets non-ciliated and non-mucus-producing cells. This demonstrates the change in tissue tropism of the virus [30]. PRCV is unable to bind to sialic acid, therefore it can't bind to the receptors in the gastrointestinal tract, but the deletion does not prohibit it from binding to the APN, which is the TGEV host receptor. PRCV also had deletions in accessory gene 3; this shows a loss of both or either 3a and 3b proteins. These affect in vivo tissue tropism and subsequent clinical disease. Similar changes in tissue tropism of other CoVs have been observed. For example, MHV, IBV and SARS-CoV, have all been associated with deletion in the S protein.

The clinical picture of PRCV presents as a mild subclinical infection but can also occur together with secondary infections which may result in more severe pneumonia. This clinical picture resembles that of human coronavirus, SARS-CoV. To understand the more severe clinical

cases of coronavirus infection, we study the mechanisms of emerging severe coronavirus diseases of livestock and humans. PRCV cellular tropism within the lung consists of pneumocytes, alveolar macrophages, and bronchiolar epithelium. A major area where SARS-CoV-2 and PRCV differ is that in SARS-CoV-2 infection a vascular tropism is seen, and this leads to the severe disease presentation of Covid-19. Lesions of PRCV seen in pigs in the upper respiratory tract, such as microscopic exudative rhinitis, were similar to SARS-CoV-2 in humans and animal models, or swine influenza. Studying the porcine model of coronavirus infection allows new insights into understanding the pathogenesis and immune control of human coronaviruses, such as SARS-CoV-2.

Animals are both reservoirs and natural hosts of several important zoonotic respiratory viral diseases for example, influenza and coronavirus. Pigs especially have natural infections of both respiratory and enteric coronaviruses. Pigs also exhibit similar anatomical, physiological, and immunological similarities to humans. Investigating the process of porcine viral diseases is an interesting model to aid our understanding of human viral diseases, with a specific focus in respiratory diseases as this has been our focus since the SARS-CoV-2 pandemic [13].

3.5.1 Coronavirus mutation – feline coronavirus

Mutation can also be seen similarly in feline Coronaviruses. Feline infectious peritonitis (FIP) is caused by feline coronavirus (FeCoV), which is a member of the genus alphacoronavirus. This group also contains TGEV and PRCV. FeCoV has two serotypes; serotype 1 has a high prevalence clinically, causing disease and cannot be propagated easily by cell culture, whereas serotype 2 can be propagated by cell culture, but has a much lower prevalence clinically. There are also two biotypes within each serotype, each causing different disease presentations. The current understanding of FIPV is that it is the outcome of genetic mutation of FeCoV. Unfortunately, the exact mutation of the FeCoV biotype is not known. From our existing knowledge of CoVs and other RNA viruses, we know that the replication process of these viruses are error prone. The most current hypothesis is that it may be a mutation involving the 3c and 7b genes that cause FIPV. The coronavirus S protein of FeCoV is involved in receptor binding (S1) and fusion (S2), and as stated above, dictates cellular tropism of the virus. The mutation within the S protein involving changes of the proteolytic cleavage site can lead to distinct alterations in disease outcome and progression [16]. The most valuable understanding of the mutation is the shift in tissue tropism from enterocytes to macrophages or monocytes. This change results in different disease progression, from a mild to moderate enteric disease

into a fatal systemic disease of cats. The overall picture of this coronavirus mutation is similar to that of TGEV and PRCV [23].

3.6 Comparative pathogenic characteristics of SARS-CoV, SARS-CoV-2 and PRCV

The pathogenesis of SARS-CoV-2 is yet to be fully understood due to the novel nature. It is likely to share similar pathogenic or immunopathologic features of SARS-CoV. SARS-CoV-2 has a tropism for both the upper and lower respiratory tract. In studies by *Saif et al.* based on ex vivo cultures of human bronchus and lung and in nonhuman primate (cynomolgus macaque), it has been seen that SARS-CoV-2 has the ability to infect ciliated epithelial cells more efficiently, lining the nasal and bronchial mucosal epithelium. In comparison, SARS-CoV may be less efficient in viral shedding and inter-person direct contact or aerosol transmission, compared to SARS-CoV-2, due to the increased tissue tropism to the nasal mucosa of SARS-CoV-2.

Similar to SARS-CoV and SARS-CoV-2, PRCV has a tropism for both the upper and lower respiratory tract. PRCV can also occasionally be detected in the small intestine, tracheobronchial lymph nodes, and blood in infected pigs, but as we know this is not the main tissue tropism of PRCV. In pigs that were infected with PRCV, antigens were found in type 1 and 2 pneumocytes, and to a lesser extent, epithelial cells of the nares, trachea, bronchi, bronchioles, and sporadically alveolar macrophages. Another similarity between PRCV, SARS-CoV, and SARS-CoV-2 is it increased inflammatory response found in the lung tissue of patients or animals infected with these CoVs, including IFN-α, TNF-α, IL-6, IFN-γ and IL-2. The innate cytokine response that appears in the lung tissue in early stages of PRCV infected pigs inhibit initial viral replication, and modulate Th1/Th2 response, with the latter enhancing B-cell responses. This effectively leads to secretion of virus neutralising antibodies [26]. T lymphocytes are a major source of cytokines, which allow the recognition of foreign pathogens through antigen specific receptors on their cell surface [5].

There are some pathogenic features of PRCV that differ from SARS-CoV and SARS-CoV-2. In pigs infected with PRCV, and humans infected with SARS-CoV and SARS-CoV-2, most have mild or subclinical disease and recover. In individuals severely infected with SARS-CoV, SARS-CoV-2, the respiratory disease may lead to complicated cytokine storms and multiple organ failure, which can have irreversible changes. In studies with non-human primates, SARS-

CoV and SARS-CoV-2 caused severe diffuse alveolar damage (DAD) and pulmonary edema this is because of extensive destruction of type 1 pneumocytes, lining the alveoli. In pigs infected with PRCV, it is seen to cause only bronchoalveolitis and airway plugging. These changes are caused because of an ambulation of necrotic cells and inflammatory cells in the bronchial and bronchoalveolar lumens. This is a distinct area of difference between PRCV, and SARS-CoV, and SARS-CoV-2. Multiorgan failure, seen in patients with SARS-CoV-2, is unlikely to occur in pigs infected with PRCV, as there is less systemic proinflammatory response in PRCV, which is consistent with a milder, subclinical infection. In COVID-19 infection in humans, we see neutrophilia, an increased neutrophil frequency in the infection sites of blood and lungs, which is not seen in PRCV infection of pigs. It is clear that there are similarities between the human model of SARS and the porcine model of PRCV, but the major differences can be seen in the severity of the diseases, as PRCV is mostly a mild or subclinical disease, whereas SARS-CoV and SARS-CoV-2 can cause a more severe disease in humans. We must also consider that we are not including coinfections, e.g., bacteria, other viruses, and immunosuppressive factors that can ultimately exacerbate disease both in humans and animals [26].

3.7 Coronavirus recombination

Recombination can be defined as an exchange of genetic material between at least two separate viral genomes. Recombination occurs when two or more viruses co-infect a single cell and exchange segments, often giving rise to viable hybrid progeny [34]. RNA viruses such as CoVs have a high frequency of homologous and nonhomologous RNA-RNA recombination. This has been described to occur among selected and unselected markers during the course of infection. Most research surrounding the topic of recombination has been established with MHV, however this trait of high rate of recombination can be denoted to the coronavirus family as a whole. Recombination has been recognised in other CoVs in all genera, including: TGEV, FIPV, BCoV, and IBV. For RNA recombination to occur, it must possess three mechanistic requirements, according to the most supported model. Firstly, the RNA polymerase must pause during synthesis. Secondly, a new template must be in physical proximity to the RNA strand. Thirdly, some property of the new template must allow the transfer of the incipient RNA strand and after this the continuation of RNA synthesis must occur [22].

The recombination of CoVs occurs when many viruses of the same family are simultaneously maintained in nature resulting in novel viruses. Between the years 2007 and 2014, a newly circulating swine enteric coronavirus was discovered. The newly recombinant swine coronavirus contained the backbone of TGEV and PEDV spike gene. It was allocated the new name of swine enteric coronavirus (SeCoV). This chimeric virus is thought to have resulted from a recombination event. This was confirmed by RT-PCR with primers spanning possible recombination sites and analysis of overlapping reads from next generation sequencing. It is not known yet if novel recombinant viruses pose a threat in terms of virulence and tissue tropism and increased research may be needed to completely understand this area [2].

3.8 Immune response

The innate immune response is the body's first line of defence against foreign substances, in this case against coronaviruses. Innate immune response in terms of cell mediated effector mechanisms include natural killer cells, and soluble effects such as type I interferons (IFN). These both play an important role in the body's defence against viral infection. Interferons are a group of cytokines which induce resistance to viral infection, but also have been recognised as proinflammatory molecules. Interferons act in both the innate and adaptive immune response of the body. In research, IFN have been seen to be increased in mucosal and lung secretions in experimental infection of pigs with PRCV. Similarly, with TGEV infection of piglets, a high and early increased level of IFN has been characterised in intestinal secretions and other organs. The high level of IFN production at the early stage of infection in pigs shows that IFN inducers may demonstrate an effective way in controlling coronavirus infection, as the IFN mediate antiviral and immunomodulatory effect within the immune response [9].

Some studies, researching TGEV show that the M protein plays a direct role in the production of αIFN, by the leukocytes in porcine blood. However, the significance of this remains unclear in correlation to TGEV immunity [25].

3.9 Interspecies transmission

Around 70% of novel human pathogens originate from animals. In recent times, major outbreaks of these pathogens have been a result of RNA viruses, including CoVs. This is as a result of their ability to mutate at an increased rate compared to other types of pathogens. Also, they have the capability for unique genetic changes as previously mentioned, such as recombination and mutation [8]. CoVs infect a wide range of human and animal hosts. Some coronaviruses have even shown zoonotic transmission from animal to human.

The knowledge we learn from coronavirus transmission and replication in animals not only aids our work in the veterinary field but also the work towards the One health perspective. In recent years, newly emerging coronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2 have been seen across the world. Research into these viruses have shown a close genetic relationship with bat coronaviruses, which were transmitted through an intermediate host. Such intermediate hosts have included: civet cat, dromedary, and pangolin, for SARS-CoV, MERS-CoV, and SARS-CoV-2 respectively. Interspecies transmission has been noted from birds and bats to pigs. Originally the natural hosts of deltacoronaviruses were wild birds, but we know now that they can also infect pigs, such is as in cases of PDCoV. In an experimental environment, to has also been observed that PDCoV can also infect chickens, with shedding and seroconversion also observed. Interspecies transmission of CoVs, such as SARS-CoV2 and PDCoV, despite being from different genus, could be used as a model, and give practical information, for further zoonotic viruses and animal coronavirus in the veterinary world [23].

Bats have been investigated as a crucial natural reservoir for emerging viruses. They have several exclusive ecological, immunological, biological, and genetic features that make them a suitable reservoir for newly emerging viruses. Viruses are obligate intracellular pathogens; it is understood that viruses have many independent evolutionary origins not dissociable from co-evolution of their hosts. Bats have a high biodiversity which in turn makes them an important source of novel viruses for interspecies transmission. CoVs have three major factors that allow for interspecies transmission. These include their high estimated rate of mutation, at nearly 2×10^{-6}, this is exceptional even among RNA viruses. They use a ‘copy-choice’ mechanism during their RNA replication which allows for a high rate of RNA recombination. This has been seen in both HCoV-HKU1 and HCoV-OC43, as well as animal CoVs such as bat-SARS-CoV, and bat-CoV-HKU9. Finally, CoVs have the largest genome of all RNA

viruses which gives way to increased resilience in modifying genes. The remarkable characteristics of CoVs, diversity of species and genotypes, ability of high frequency mutation and recombination, and their genetic structure uniquely correlate to interspecies transmission and the emergence of novel CoVs into both the human and animal population [8].

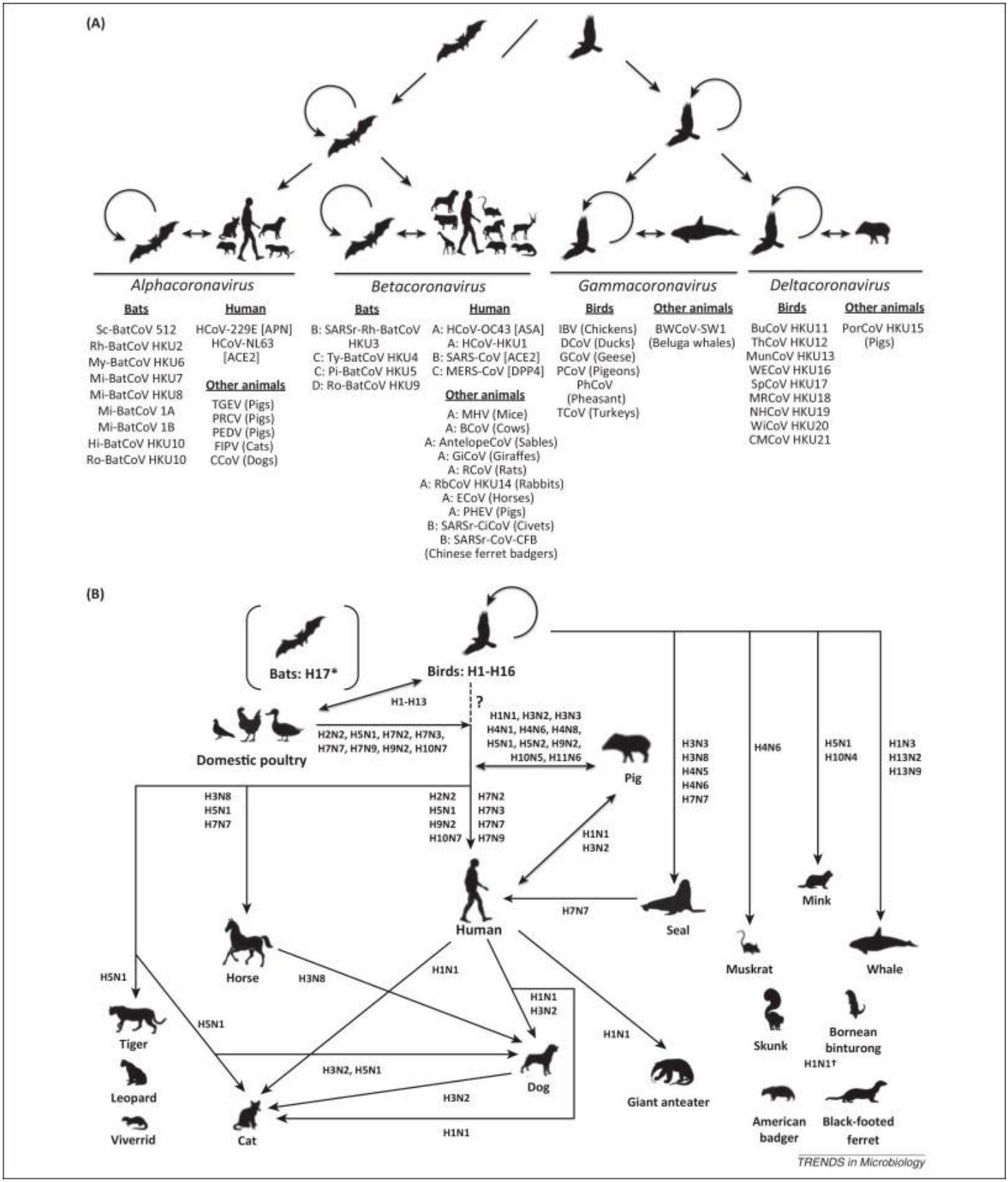

Figure 1 Bats and birds as probable gene sources for the evolution of ***(A)*** *coronaviruses and* ***(B)*** *influenza viruses, based on epidemiological, virological, and phylogenetic evidence. 'A', 'B', 'C', and 'D' represent groups A, B, C, and D in* Betacoronavirus [8].

4. Method and materials

4.1 Materials

Human Blood Serum

Human blood serum samples (a total of 1211) were collected from CMC Déli Klinika, (-Kuny Domokos Street 13-15, Budapest). The samples were collected by Dr. István Jankovics from the Nemzeti Népegészségügyi Központ (NNK, the National Public Health Centre) and stored by Dr. Béla Dénes from Nemzeti Élelmiszerlánc-biztonsági Hivatal (NÉIBH, the National Food Chain Safety Office). The samples were part of a national COVID-19 examination.

The serum samples were tested for the antibody produced against the SARS-CoV-2 virus using an ELISA test. A brief anamnesis was collected with each sample containing information including age, gender, COVID-19 result (positive/negative), vaccination status (vaccinated/ unvaccinated), if clinical signs were present etc. We did not receive this data for our study so that it would not influence the outcome of the results.

TGE Virus

The TGE virus was isolated by Dr. Tamás Tuboly in Canada. It was stored at -80 °C in the University of Veterinary Medicine, Budapest.

Cell Culture

We used STE/62 cell culture which is a modified porcine kidney cell culture with Dulbecco's Modified Eagle Medium (DMEM) and 5% Foetal Bovine Serum (FBS) to support the growth of the cells and an antibiotic antimycotic solution (SIGMA-Aldrich, 1 ml cell culture medium contain 10 U penicillin, 10 μg streptomycin and 25 ng amphotericin B). The DMEM and FBS were both from Lonza. The cell culture was made by Dr. Attila Cságola and stored at -80°C.

Neutral Red Staining

We dissolved 0.5 g neutral red powder (VWR) in 50 ml double distilled water. Then we dissolved 0.45 g sodium chloride (VWR) in 10 ml double distilled water. After that we mixed both solutions and filtered them. This procedure is according to the VMR manuscript.

Phosphate Buffer Saline

Neutral phosphate buffer saline (PBS) containing: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8mM KH_2PO_4 in 1 litre distilled water. This solution was then sterilised in the autoclave.

4.2 Method

Preparation of cell culture

10,000 STE/62 cells were added into each well on a 96-microtiter plate (VWR) containing 200 µl DMEM with 5% heat inactivated FBS. The plate was incubated at 37 °C with 5% CO_2 concentration. After 3 days, the cells had grown to 80-85% of the plate. That is the percentage at which the cell culture was ready for use.

Preparation of virus solution, virus titration

Aim: to prepare a standard amount of virus is necessary to determine the antibody content of the serum.

To prepare the virus solution we must first determine the Median Tissue Culture Infectious Dose (TCID50) of the TGE virus. A two-fold dilution of concentrated TGE virus which was added to DMEM and FBS in a 96-microtiter plate. This was incubated at 37 °C with 5% CO_2 concentration for 3 days. After the incubation period, the cells were inspected for cytopathogenic effects (CPE). The dilution at which 50% of the wells showed CPE was used to calculate the TCID50 of the virus sample.

Calculation of TCID50/ml for virus titration using method of Reed and Muench for serum virus neutralisation test.

$$\frac{TCID50}{ml} = \frac{\left((\%infected\ at\ dilution\ immediately\ above\ 50\%) - (50\%)\right)}{(\%infected\ at\ dilution\ immediately\ above\ 50\%) - (\%infected\ at\ dilution\ immediately\ below\ 50\%)}$$

Inoculation of cell culture

60 µl serum sample and 60 µl TGE virus solution was mixed and added to the cell culture (1:64 dilution). The mixture was incubated at 37 °C (without 5% CO_2 concentration) for 1 hour. We removed the cell medium by Pipetting. We pipetted 100 µl DMEM without FBS (but contained antibiotic antimycotic solution) and 100 µl of our mixture. This was incubated at 37 °C with 5% CO_2 concentration for 3 days. We then evaluated the test using neutral red staining: we removed the virus containing medium and washed the plate 3 times in PBS. Then we added 50 µl staining and 50 µl PBS. When the result was positive, the test was repeated. In the repeated

test we used 120 µl, 2-fold diluted serum sample. The sample was diluted with DMEM. This was mixed with 120 µl TGE virus solution (1:64 diluted). Then incubated at 37 °C (without 5% CO_2 concentration) for 1 hour. 50 µl from this mixture was added to the 96 well plate, containing 10000 STE/62 cells. The first sample was concentrated and the last was 1:128 dilution. *Figure 2* shows the layout of the first plate. When repeating the test, we used a double measurement of 100 µl. We evaluated the test after 3 days with neutral red staining.

A positive result is determined by the lack of cytopathogenic effect in the modified porcine kidney cell culture. The highest dilution of serum that prevents infectivity establishes the neutralising antibody titre.

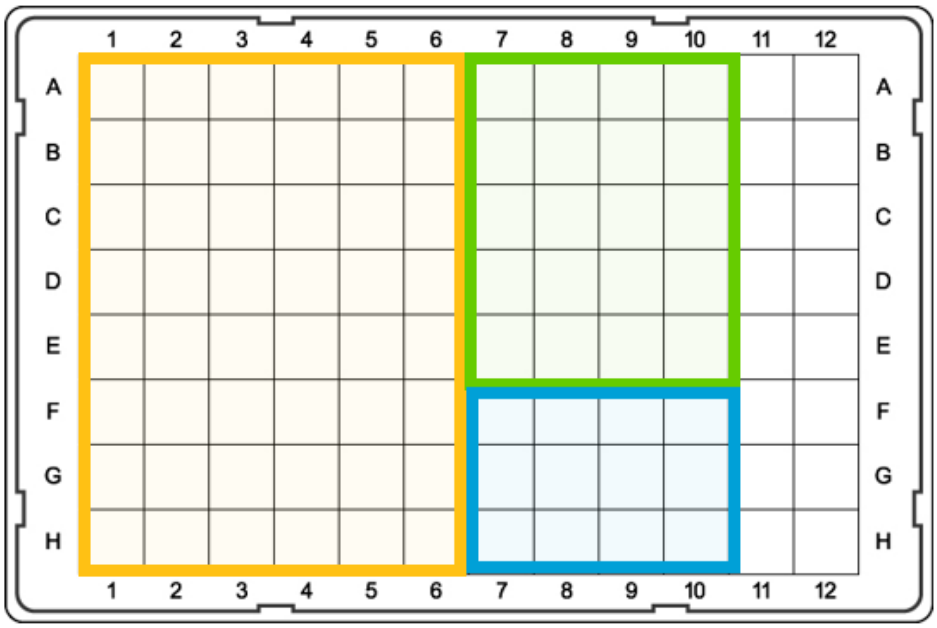

Figure 2 Example layout 96-microtiter plate for virus neutralisation test. The area presented in yellow is the serum (assay) part also known as the positive control; it contains cell culture, virus(constant), serum (diluted) and cell culturing media (DMEM and FBS). The area presented in green is the virus control, also known as the negative control. It contains cell culture, virus in a 2-fold dilution and cell culturing media (DMEM and FBS). The area presented in blue is the cell control. It contains cell culture and cell culturing media (DMEM and FBS).

5. Results

Virus titration

The virus Median Tissue Culture Infectious Dose was 1:64. We stored this stock dilution in 50ml centrifuge tubes (VWR) at -80 °C.

The cytopathogenic effects were already visible on the first day but were only read on the third day. We selected the sample where two of the four measurements gave positive, and two negative results.

Virus neutralisation

The result with the concentrated serum sample was positive in 51 cases of the total 1211 samples. This is 4.2%. Female samples were half of the positive cases (25), and 26 cases were male. *Table 1*. Shows the age distribution of positive cases.

Table 1. Age distribution of positive cases

Date of Birth	**Female**	**Male**	**Total**
2004-2022	0	1	1
1997-2003	1	1	2
1987-1996	3	6	9
1977-1986	12	7	19
-1976	9	11	20
Total	25	26	51

The results of the double measurement of the positive cases are shown in *Table 2.* The highest titre is 1:32, but two others also reached a titre of 1:16. The 'C' in *Table 2* represents samples that could only be found to be positive at a concentrated dilution. Eight samples showed positive results at a concentrated dilution, these samples are not counted as positives in the final result. The results of the double measurement of the positive cases are shown in *Table 2.*

Table 2. Results of the double measurement of the positive cases

Plate	**Sample 1**		**Sample 2**		**Sample 3**		**Sample 4**		**Sample 5**		**Sample 6**	
1	4	4	8	4	**2**	**4**	**2**	**4**	8	8	8	4
2	**2**	**4**	4	4	**2**	**2**	8	8	8	4	**4**	**2**
3	8	4	4	4	**2**	**2**	**2**	**2**	4	4	**C**	**2**
4	32	32	**C**	**C**	8	8	4	4	8	16	8	8
5	8	16	8	4	**2**	**4**	4	4	**4**	**2**	**2**	**4**
6	4	4	**C**	**C**	**C**	**C**	**4**	**2**	**8**	**C**	**C**	**C**
7	16	16	8	16	4	4	8	4	8	8	8	8
8	**C**	**C**	8	8	8	8	8	4	**C**	**C**	**C**	**C**
9	**C**	**C**	**2**	**8**	16	16						

6. Discussion

Our results demonstrate that 51 concentrated serum samples were positive, out of 1211 samples. This is 4.2% of 1211 samples examined. There was equal positivity between male and female samples. We did not have information about the environment, history, or other factors that could have influenced the results of the human serum samples. This information could have been useful to know whether the people in the investigation had a connection with pigs in their environment.

TGE virus is an alphacoronavirus, while SARS-CoV-2 is a member of betacoronavirus genus. Among viruses of different genera, cross neutralisation is impossible, but a match between epitopes is conceivable [17].

It is known that human seasonal coronaviruses can have an effect on immunity against SARS-CoV-2. The 'common cold' CoVs such as HCoV-NL63 and HCoV-HKU1 etc., widely circulate in the human population and are responsible for mild self-limiting respiratory symptoms [29].

SARS-CoV-like bat viruses circulate in Europe, and some bat alphacoronavirus have a zoonotic potential [3]. Therefore, based on our investigation, we investigated the possible neutralising effect of the antibody content of coronaviruses in human serum to that of coronaviruses of animals. My investigation was just a part of the larger ongoing research group project conducted in the Immunology Department of the University of Veterinary Medicine Budapest, Hungary.

We were surprised that there were positive samples among the human serum that were investigated. As there should be no cross-reactivity between CoVs of alpha and beta genera. We do not know whether the humans involved in the investigation have a connection with pigs or bats, for example, humans who work with pigs as a veterinarian or farm worker. If there is a connection, the pig virus, TGEV, could generate IgG production in humans. Our opinion is that a large proportion of the human population do not have a connection with pigs, because the serum samples came from a private clinic in Budapest. This could mean the population of people whom the samples were collected from come from an urban location and wouldn't have contact with pigs in their daily lives.

In the results, the highest titre was 1:32, it is high enough to elicit a highly positive response. In research completed by *Irsara et al.* a titre of at least 1:4 gave a positive result in a human virus neutralisation test [12].

Based on our results, 29 samples out of 51 gave a positive result with a titre if at least 1:4, and this has also been proven to be positive based on the human tests. (In *Table 3*, the Sample 2 on Plate 9 is evaluated as negative, because the difference of the two measurement was bigger than 1 titre). This is above half of the positive samples and 2.39% of all samples investigated. Three of the samples gave a minimum 1:16 titre, which elicits a highly positive result. *Table 3* presents the results seen in *Table 2* in relation to the 1:4 titre. As seen in *Table 3* the positive sign (+) represents a titre of at least 1:4, a positive result. The negative sign (-) represents a titre below 1:4, a negative result. The double positive sign (++) represents a titre above 1:16, a highly positive result.

Table 3 Final results of the virus neutralisation test of double measurement of positive samples

Plate	**1**	**2**	**3**	**4**	**5**	**6**
1	+	+	-	-	+	+
2	-	+	-	+	+	-
3	+	+	-	-	+	-
4	++	-	+	+	+	+
5	+	+	-	+	-	-
6	+	-	-	-	-	-
7	++	+	+	+	+	+
8	-	+	+	+	-	-
9	-	-	++			

We do not know the exact reason currently why the human samples cross-neutralised the TGE virus in these cases. TGEV does not provide cross-protection against other coronaviruses, except PRCV. The well-known human coronavirus, SARS-CoV-2 belongs to the betacoronavirus genus. BCoV is a betacoronavirus similar to SARS-CoV-2. BCoV is known to be closely related to human seasonal coronaviruses [36].

Our results show that 2.39% of the human serum samples containing antibodies produced against SARS-CoV-2 showed cross-neutralisation with the porcine virus, TGEV. The reason at this time is unknown and further research into these results should be completed to

understand the extent of this investigation. Factors including the history of the people in this examination may play a crucial role in understanding why these results were found.

There are a limited number of alphacoronaviruses that can infect humans (NL63 and 229E strains). These viruses can cause the seasonal flu in winter. Both seasonal CoVs, and the TGE virus contain a tyrosine-based sorting signal in the spike protein. This protein is important for receptor binding and membrane fusion. The sorting signal is important in intracellular retention of alphacoronaviruses. Tubulins interact with porcine and human S proteins of the genus alphacoronavirus and support successful assembly and release of infectious viral particles [24]. It can partially explain the cross-neutralisation we found in our investigation, but the structure of these three viruses are different in this genomic section as seen in *Table 4*.

Table 4. Last 39 amino acid stretches of coronavirus cytoplasmic domains linked to green fluorescent protein.

TGEV	CCCSTGCCGCIGCLGSCCHISCSRRQFENYEPIEKVHVH
HCoV-NL63	CLSTGCCGCCNCLTSSMRGCCDCGSTKLPYYEFEKVHVQ
HCoV-229E	LCCCSTGCCGFFSCFASSIRGCCESTKLPYYDVEKIHIQ
SARS-CoV	CCMTSCCSCLKGACSCGSCCKFDEDDSEPVLKGVKLHYT

We can see from the information provided in *Table 4* that the function of the protein is the same, but the gene segments do not match [24].

In conclusion, we do not know the connection between TGEV and human coronaviruses. Perhaps, there is a common alphacoronavirus that is not yet discovered, and it may have a relationship with these viruses, similar to the pattern seen between BCoV and human OC43 or CCoV. With further research in this area and new generation genome segment analysis, these viruses can be fully understood and finally it might answer this open-ended question.

7. Abstract in English

Coronaviruses are highly variable RNA viruses. They are distributed worldwide and infect humans and a variety of animals. They have caused major outbreaks globally, including SARS-CoV, MERS-CoV, and SARS-CoV-2. Coronaviruses can also be detrimental to animal health and can cause huge economic impacts in animal production. Investigating the relationship between animal and human coronaviruses may provide valuable information for both human and veterinary medicine.

In this research, we investigate whether antibodies of human coronavirus, SARS-CoV-2 can cross neutralise the porcine virus, TGEV and whether there is an antibody in human blood that can neutralise the swine virus. The serological method used to investigate the cross-reactivity for this investigation was virus neutralisation test. TGEV is an alphacoronavirus and SARS-CoV-2 is a betacoronavirus. The virus neutralisation test was evaluated with neutral red staining. A positive result was determined by the lack of cytopathogenic effects in the cell culture. The highest dilution of serum that prevented infectivity established the neutralising antibody titre. The virus neutralisation test is effective to evaluate serological cross-reactivity between the antibodies of SARS-CoV-2 and TGEV that may correlate with cross-protection between these two viruses.

The results of this investigation showed that 51 samples of the total 1211, gave positive results. Based on our results, 29 samples out of 51 gave a positive result with a titre of at least 1:4. The titre of 4:1 was proven by human virus neutralisation tests in external research. The final result showed that 2.39% of all samples were positive. This means 29 samples cross-neutralised with the TGE virus.

8. Absztrakt

Az RNS tartalmú coronavírusok rendkívül variábilisak. Világszerte elterjedtek, és az emberek mellett számos állatfajt megfertőznek. Járványkitöréseket okoztak mindenütt a világon, így a SARS-CoV, a MERS-CoV és a SARS-CoV-2 az emberek körében. A coronavírusok az állatokat is megbetegíthetnek, ezzel óriási gazdasági károkat okozhatnak. Az állati és emberi coronavírusok kapcsolatának vizsgálata értékes információkkal szolgálhat a humán- és állatgyógyászat számára egyaránt.

Ebben a kutatásban azt vizsgáljuk, hogy a humán SARS-CoV-2 antitestek képesek-e semlegesíteni a sertések TGEV-t, illetve van-e olyan ellenanyag az emberi vérben, mely képes neutralizálni a sertésvírust. Ebben a vizsgálatban a keresztreaktivitás vizsgálatára használt szerológiai módszer a vírusneutralizációs teszt volt. A TGEV egy alfacoronavírus, a SARS-CoV-2 pedig egy bétacoronavírus. A vírusneutralizációs tesztet neutrálvörös festéssel értékeltük. A pozitív eredményt a citopatogén hatások hiánya határozta meg a sejttenyészetben. A neutralizációs titer az a legmagasabb szérumhígítás, mely a vírus fertőzőképességet akadályozza meg. A vírusneutralizációs teszt hatékonyan értékeli a SARS-CoV-2 ellenanyagok és a TGEV antigénjei közötti szerológiai keresztreaktivitást, amely összefüggésben állhat a két vírus közötti keresztvédelemmel.

Vizsgálataink során 1211 savómintát néztünk meg, ebből 51 mutatott szeropozitivitást. Az 51 minta hígításával 29 minta esetében kaptunk pozitív eredményt úgy, hogy pozitívnak a legalább 1:4-es titert vettük alapul. A pozitivitást mutató titer értékét egy humán SARS-CoV-2 vírusneutralizációs teszt alapján vettük alapul. A végeredmény alapján az összes minta 2,39%-a pozitív eredményt adott. Ez azt jelenti, hogy ezekben az esetekben a vérsavóban kersztneutralizáló ellenanyagokat találtunk TGE vírusra.

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