FCoV-23 INTERNATIONAL RESEARCH CONSORTIUM (IRC)
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Position Statement: Classification, Surveillance & Diagnosis of Feline Coronaviruses and FIP

Background: Feline coronavirus (FCoV) is the cause of feline infectious peritonitis (FIP), one of the most impactful infectious diseases in feline medicine. Based on clinical presentation, two distinct viral biotypes have been proposed to account for the remarkable pathogenic properties attributed to FCoV: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) (1). Based on *in vitro* neutralization tests, FCoV has also long been known to exist as two distinct serotypes (I and II), with the possibility of antibody-dependent enhancement (ADE) of infection (2, 3). However, recent information on this highly complex disease and agent has prompted a re-evaluation of these traditional paradigms, which may now be considered outmoded.

Coronaviruses exist across a wide range on animal species. They are enveloped RNA viruses, with a large genome size. As an RNA virus, the replication machinery is error-prone; however, a proof-reading enzyme (Endo U) limits the rate of mutation for coronaviruses. The replication machinery generates deletions that are used as part of the distinctive template switching/copy-choice strategy of the virus, whereby genomic deletions produce the sub-genomic RNAs necessary to produce the viral structural and non-structural proteins (4). The coronavirus replication strategy also allows an extensive level of recombination between homologous genomes, greatly increasing the evolutionary capacity of the viruses. As an enveloped virus, FCoV (as with all coronaviruses) is relatively environmentally labile; however, robust data on virus stability is lacking. The viral envelope contains the prominent spike protein (S), which gives coronaviruses their name.

Classification

Serotypes. Historically, it was appreciated that sera from certain cats with FIP or infected with FCoV failed to cross-react/cross-protect with sera from other cats, leading to the concept that the virus exists as two distinct serotypes. This concept was reinforced by the isolation of monoclonal antibodies to what is now known as FCoV-2 (see below), whereby distinct differences in spike protein antigenicity were apparent.

Biotypes. Once FCoV was recognized as the cause of FIP, it became clear that the virus existed as two distinct pathogenic forms, which became known as biotypes. The virus is readily transmitted in a form that usually causes little-to-no disease signs; this form is generally referred to as low-pathogenicity FECV, which can be shed in the feces of cats for long periods of times and/or at high viral loads, based on PCR testing (low Ct values). In some cats (5-10%), a variant of the virus is selected following an internal mutation or deletion to cause FIP. This highly pathogenic virus is generally referred to as FIPV. FIPVs are thought to be robustly macrophage-tropic, accounting for the disease signs seen in cats where this pathogenic variant arises. FIP can present as either effusive (wet) or non-effusive (dry) – with or without neurological involvement or ophthalmic signs.

Genotypes. Based on early sequencing methodologies, it quickly became apparent that the spike proteins of the two FCoV serotypes were highly genetically distinct - typically showing only approximately 50% sequence identity/similarity. To demonstrate these differences more effectively they have been categorized as being in distinct genetic clades (A and B), but with the advent of next-generation sequencing technologies they are better referred to as distinct genotypes (FCoV-1 and FCoV-2). While both genotypes can be associated with FIP (i.e. FIPVs may be FCoV-1 or FCoV-2 in origin), the majority of clinical cases are caused by FCoV-1 viruses. FCoV-2 viruses are typified by recombination with canine coronaviruses (CCoV) and contain a CCoV spike gene as the result of recombination with an FCoV-1 backbone. FCoV-2 viruses have recently risen to prominence based on the emergence of FCoV-23, the cause of a large ongoing and deadly FIP outbreak in Cyprus. It is important to note that based on conventional taxonomic classification, all FCoVs (as well as CCoVs and certain coronaviruses of pigs) are a single virus species, *Alphacoronavirus-1* (Genus Alphacoronavirus, sub-genus Tegacovirus, member species *Alphacoronavirus suis*.);
see, https://ictv.global/report/chapter/coronaviridae/coronaviridae/alphacoronavirus.

Surveillance

Our information on FCoV geographic distribution and sequence diversity has been driven by clinical need in relation to FIP cases, and by individual research projects; as such, information is currently very patchy and may not reflect the abundance of either symptomatic or asymptomatic FCoV-1 and FCoV-2 infections. Genomic sequence information is limited; while certain individual genes, e.g. the viral polymerase (RdRp) are typically analyzed, a recent study only found 40 complete genomes (17 FIPV and 23 FECV) in sequence databases, the vast majority of which were FCoV-1, with very few FCoV-2 (5). Spike gene sequences are often under-represented in available datasets. Geographical distribution may simply reflect pockets of activity by individual research groups. Thus, the true scale of FCoV in relation to disease burden and transmissibility remains uncertain and is almost certainly under-reported, and is poorly classified.

Diagnosis

Feline coronavirus infections causative for FIP are difficult to diagnose and often rely on clinical assessment. “Wet” FIP is typified by the presence of straw-colored effusion, with high protein levels (A/G ratio <0.8) being predictive. The Rivalta test is easy to perform but of limited utility. Dry FIP can be diagnosed based on histopathology and FIP immunohistochemistry on tissue biopsies (see below), or may also be a diagnosis of exclusion for neurological signs and eye changes.

Serological analysis will be important to show evidence of past exposure to the virus, but can be highly variable for clinical diagnosis; titers (even rising titers) are not diagnostic of ongoing FCoV infection or FIP disease, and based on the distinct antigenicity of the spike would be expected to be highly subtype-specific.

PCR-based analysis is widely used and can demonstrate viral shedding, but as with serological titers, RNA levels can be highly variable. Most available tests do not discriminate between subtypes or biotypes; despite initial assertions, mutation in spike gene residue 1058/1062 (e.g. M1058L) is no longer considered predictive of FIP, but may be connected to the ability of the virus to spread systemically (6, 7). PCR-based analysis for ascites fluid can be predictive for cases of ‘wet’ FIP, but sensitivity can be limited, particularly where cats are on anti-virals. Sampling and PCR analysis of other fluids antemortem (e.g. cerebral spinal

fluid) can also be highly predictive for FIP. Molecular diagnosis of FIP likely requires next-generation sequencing approaches that are currently in development.

The gold standard for diagnosis remains immunohistochemistry (IHC); current tests typically use antibodies reactive to the viral nucleocapsid (N) protein and recognize both FCoV-1 and FCoV-2. However, IHC may not have high sensitivity with false-negatives possible; this can be improved through the use of RNA probes (*in situ* hybridization, or ISH).

Call to Action/Recommendations

We propose:

- The use of the nomenclature "FCoV-1"; instead of serotype I, or clade A; as in (8).
- The use of the nomenclature "FCoV-2"; instead of serotype II, or clade B; as in (8).
- This is more consistent with naming conventions with other viruses.
- The use of the term low pathogenicity ("low-path") FCoV; instead of FECV, or less virulent FCoV as in (9).
- The use of the term highly pathogenic ("high-path") FCoV; instead of FIPV, or FIP-associated FCoV as in (9).
- This is more consistent with naming conventions with other viruses, especially as the internal mutation between FECV and FIPV is still not fully understood,
- Formally the term virulence measures quantifiable parameters of disease, which are still lacking for FIP/FCoV.
- Sequencing-based approaches are strongly encouraged and need to be developed, and technology widely disseminated for diagnosis/risk assessment/epidemiology; as in (10).
- In the short term, more specific PCR probes are needed (e.g. for FCoV-23).
- For serology studies, more available antibodies are needed for both FCoV-1 and FCoV-23.
- For pathology studies, use of RNA-based probes for histochemistry is strongly encouraged; as in (11)
- Whether available anti-viral drugs perform similarly across the range of FIP cases caused by FCoV-1, FCoV-2 or FCoV-23 is unknown. Routine genotyping of virus is needed to address this question.
- Similarly, development of a universal FCoV vaccine will require a global effort to build a robust database of full genome sequences.

I sign in support of the above position statement:

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