Title: **Feline Infectious Peritonitis: challenges and promises of coronavirus RNA detection.**

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ABSTRACT:

Our perspective article covers major findings concerning Feline Infectious Peritonitis (FIP) - a fatal coronaviral disease of cats. In the context of FIP pathogenesis, we outline disease signalment and focus on the challenges and promises of FIP invoking coronavirus RNA detection. In particular, we outline critical aspects of coronavirus RNA replication and biogenesis. We infer the replicative intermediates of feline coronavirus may constitute an underappreciated factor triggering the progression of the maladaptive immune response underlying FIP pathogenesis.

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Feline Infectious Peritonitis (FIP) is a terminal disease resulting from infection with entero-tropic Feline Coronavirus (FCoV). Considering FIP as a transmissible disease of cats, there are two major antigenically distinct serotypes of Feline Coronavirus involved: most common FCoV type I and FCoV type II (Uzal et al. 2016). Below we outline the events and processes underlying the above diversity in the context of critical biogenesis steps in coronaviral replicative cycle.

Coronaviruses, including FIP pestilent inhabiting feline niche, are undoubtedly the largest and most complex RNA viruses. Encapsidated and infectious RNA genome consists of a single (+) strand RNA molecule, approaching 27-33kb in length (Hegemeijer et al. 2012; Hartenian et al. 2020). Most of the region following the untranslated portion at 5' end of coronaviral genomic RNA encodes two polyprotein precursors translated of one open reading frame disjointed by programmed ribosomal frameshift (Plant et al. 2008). Encoded polyprotein precursors referred to as ORF1a and ORF1b, together occupy about two-thirds of the RNA genome. In a coronaviral replication cycle ORF1 (i.e. replicase encoding ORF) translate directly of infectious (+) RNA strand - immediately after uncoated coronaviral RNA enters a cell. ORF1 translates into a viral replicase RNA-dependent RNA-polymerase (and several other non-structural proteins with RNA modifying functions – including RNA helicase, exoribonuclease, uridylate specific endoribonuclease, and ribose 2'-O-methyltransferase - required for the completion of coronavirus replication) proteolytically cleaved of a fairly large polyprotein precursor. RNA-dependent RNA-polymerase (along with auxiliary proteins providing RNA modifying functions) is critical for the synthesis of complementary anti-genomic (-) strand RNA. Anti-genomic (-) RNA strand constitutes the essential replicase co-factor required for infectious (+) RNA strand synthesis (and for the synthesis of viral mRNAs, as well as minor subgenomic coronaviral RNAs of both positive and negative polarities; see Hegemeijer et al. and Hartenian et al. for details and relevant references).

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This mode of copying of the genetic information is common to all non-reverse transcribing RNA viruses and immediately suggest the presence of intermediate dsRNA in the replicative cycle.

In addition to RNA-dependent RNA-polymerase i.e. replicase genes, RNA genome embeds 8-10 open reading frames encoding for other coronaviral proteins. The other than ORF1 coronaviral genes, located toward the 3' portion of the genome, encode for structural capsid proteins and few other proteins referred to as accessory, are translated after the completion of synthesis of (-) RNA strands (both major anti-genomic cRNA strand and minor, subgenomic RNAs of negative polarity (Hegemeijer et al. 2012; Hartenian et al. 2020)). While coronaviruses may have evolved machinery ensuring the replicative fidelity, because of the large genome size; the RNA replication involving anti-genomic cRNA strand synthesis (with the inevitable formation of dsRNA intermediates) is regarded as an error-prone process, resulting in replicative infidelities, hence contributing factor promoting coronaviral variant diversity. The replicative infidelities associated with RNA replication involving dsRNA intermediates are also known to contribute to the process of RNA-RNA recombination. It has been proposed that the mechanisms of genomic RNA-RNA recombination promote exchange and mediate shuffling of the coronaviral RNAs between replicating molecules (Terada et al. 2014). Indeed, coronavirus diversity seems also to origin from frequent replication-associated genomic RNA-RNA recombinations expectedly increasing the variability of replicating RNA molecules (Hartenian et al. 2020).

The genomic structure of FCoV and its pernicious FIP-invoking derivative is typical for the family of coronaviruses. In terms of the overall genomic architecture, where kinship is based on the RNA sequence homology, FIP causing feline coronavirus appears related and most similar to coronaviruses infecting canids. As represented by branches radiating at the top of the cladogram

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shown in (Fig.1), most of the FIP virus genomes clearly form a distinct clade within feline coronaviruses. In contrast, coronaviruses infecting other species, (where genomes of canine isolates cluster with porcine coronaviruses) are represented by branches radiating at the bottom of the tree. Only four annotated isolates appear as side branches radiating at intermediate positions – located in the middle of the presented cladogram – positioned between distinct FIP/FCoV feline coronavirus clade and canine coronavirus branches. Those four annotated feline coronavirus genomes represent type II FIP/FCoV isolates. This pattern of branching is consistent with the accepted perspective that the later type II FCoV represents a product of the inter-specific RNA-RNA recombination between the aforementioned type I feline coronavirus with canine coronavirus (Herrewegh et al. 1998; Terada et al. 2014; Jaimes et al. 2020).

Circulating feline coronaviruses are ubiquitous in the environment; still minimally resistant to environmental damage - easily inactivated by most of the disinfectants and common detergents. Primary FCoV infection is largely subclinical or only associated with transient (usually mild) enteric symptoms (and reviewed elsewhere Addie et al. 2009, 2019; Pedersen 2014a: 2014b; Uzal et al. 2016). Infections with mostly benign entero-tropic pathotypes of feline coronaviruses are prevalent in the cat population. There seems to be strong evidence that FIP causing coronavirus is not transmitted horizontally; instead, the pathogen evolves independently within each cat that eventually develops symptoms of infectious peritonitis. An increase in pathogenicity, in that case, observed as FIP is assumed to be balanced by the loss of infectivity. The pathomechanism of FIP, sporadic transmissible disease of wild and domesticated felids, seems insufficiently defined. According to the accepted perspective, FIP is a systemic form of immune-mediated idiosyncratic reaction invoked by coronavirus replication, with poorly defined proximal disease triggers.

The detailed pathomechanism of FIP remains cryptic. Clinical picture is not reconciled

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easily with a modality of the immune response observed upon feline coronavirus replication. The disease may manifest in two extreme forms: effusive and non-effusive (the later referred also as a granulomatous or 'dry' form), depending on the type and the intensity of the adverse immune reactions. In the course of infection, acquired entero-tropic coronavirus replicates initially within the epithelial cells lining the enteric mucosa. The replication of the entero-tropic coronavirus limited to the subpopulation of cells implicates the replicase genes expressed first from infectious (+) RNA strands released from coronavirus capsids are restricted to the intestinal epithelium. Therefore, at that stage translated coronaviral RdRp will engage with genomic (+) RNA and synthesize antigenomic cRNA copy – an essential replicase co-factor required for (+) strand amplification and completion of the coronaviral replicative cycle – in a mode confined within the alimentary tract. That early stage of infection is consistent with enteric coronavirus transmission and explains how the shedding of the infectious (+)RNA strand embedded in coronavirus capsids – into the intestinal lumen occurs.

However, in a small proportion of infected cats mostly innocuous enteric coronavirus acquires hyper-replicative properties resulting in systemic spread of pervasive, FIP-invoking RNA. After crossing the intestinal wall the spread of pervasive RNA invoking FIP is accompanied by adverse immuno-pathological response involving many organs. Again for poorly defined reasons, factors, or conditions enteric coronavirus infection elicits a strictly maladaptive immune-mediated response of an inflammatory type. Quoting after Uzal et al. '*A requirement for the development of FIP is likely the capacity of the virus to replicate within monocytes of the host.*' Clearly, the replicative cycle proceeds trough complementary-RNA intermediate regardless of the cell type supporting coronavirus replication. Expectedly both genomic and subgenomic cRNA replicons are required for the completion of the replication cycle, hence the replicative byproducts consisting of dsRNA will form in the infected mononucleated phagocytes. During the systemic spread, feline

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coronavirus is localized in the liver, spleen, lymph nodes, and wherever else macrophages are found. The release of the virus infecting mononucleated phagocytes results in the release of coronaviral molecules acting as epitope presenting antigens. Nonetheless, clinically observed is a form of adverse immune reaction that leads to the formation of the excess immunoglobulin complexes. The infection course is thought to depend on the type and strength of the maladaptive immune reaction triggering the inflammatory response rather than particular feline coronavirus subtype. In the infection course, as outlined above, coronavirus affected cats develop either effusive ('wet') or non-effusive ('dry') forms of FIP, dominated by symptoms resulting from vasculitis and pyogranulomatous inflammation respectively. The factors or conditions involved in wet/dry transition presently remain elusive. Similarly, factors determining when and where benign enteric coronavirus evolves into pervasive FIP-invoking RNA, remain covert.

The most common, effusive form (case presented in Fig. 2 and Fig. 3) is therefore regarded as a transmissible inflammatory condition of visceral serosa and omentum. Coronavirus induced serositis with vascular and perivascular lesions may manifest in many organs. Exudation into the peritoneal cavity - signalment hallmark of FIP – severe abdominal distention rarely poses diagnostic doubts. Additionally, pleural effusion may be present. The palpable peritoneal fluid accumulation may be confirmed with diagnostic imaging. Definitive diagnosis requires abdominal cavity punction. Up to a liter of coronavirus RNA containing exudate could be drawn from a peritoneal cavity of a single cat affected with an effusive form of FIP (Fig.4). The aspirated fluid is typical of high specific-gravity (1.017–1.047), aseptic, and protein-rich (often above 50g/l), and of moderate cellularity (Hsieh &Burney 2014). Further, the aspirate is typically translucent, opaque fluid, from light yellowish to gold in color (Fig.5), intrinsically viscous, it may contain strands of fibrin, and may appear flocculent.

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The dominant symptom of ascites may be accompanied by other symptoms not pathognomonic for FIP. In particular, dyspnea can be present in cats with pleural involvement. Disease signalment may also involve apathy, malaise, fatigue, inappetence, weight loss, fluctuating antibiotic irresponsive fever, jaundice, in addition to lymph node enlargement, ocular manifestations (Fig. 6), and neurological symptoms. Leukocytosis and neutrophilia are concluded with routine hematology counts. Hyperbilirubinaemia, hypergammaglobulinemia, and altered albumin-to-globulin (A:G) ratio are concluded with serum biochemistry. Effusive FIP must be differentiated from non-coronaviral peritonitis (e.g. bacterial).

In contrast non-effusive i.e. granulomatous FIP, a disease of insidious onset, poses a considerable diagnostic challenge. Most of the time only a presumptive diagnosis can be reached. Symptoms caused by granuloma formation are grossly non-specific; rather reflect the malfunction of the particular coronavirus affected organs (involving: hepatic icterus, nervous system symptoms, renal symptoms, with concomitant fever - present singly or in combination). None of the above symptoms justifies the legitimate diagnosis of the non-effusive form of coronaviral FIP. Considerably altered albumin-to-globulin ratio (A:G) may be indicative, but may also be observed in other, unrelated disorders (below). Intravital diagnosis may involve granuloma biopsy (of lymph nodes or kidneys) followed by histopathology. However, due to technical impediments, the intravital granuloma collection (given the variable spatiotemporal formation of granulomas) could be challenging and expectedly biased toward false-negative results. Until recently (and still of considerable concern), the conclusive diagnosis could only be ascertained post-mortem (Dunbar et al. 2019).

Altered albumin-to-globulin ratio, consisting of concomitant hypoalbuminemia with hypergammaglobulinemia, is a common feature in both FIP forms. Hypergammaglobulinemia,

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however, seems not directly related to elevated feline coronavirus antibody titers (Paltrinieri et al.1998), foreshadowing the non-specific maladaptive immune-complex mediated process underlying FIP pathogenesis. Serologically circulating antibodies recognizing feline coronavirus epitopes appear detectable also in clinically unaffected cats (hence reasonable doubts concerning the clinical utility of serological FIP examinations). The other relevant indication is based on certain confirmed FIP cases that appear seronegative. Together, the consensus concerning causality between the systemic spread of FIP coronavirus and the type of immune-complex mediated lesions observed in the disease course is lacking. Clinically gammopathies could be mistaken for the neoplastic process, as could granulomas may mimic a neoplasm.

Interestingly FIP, coronaviral disease of cats, bears considerable similarity to murine disease caused by naturally contracted MHV observed in IFN-γ (-/-) deficient mice (France et al. 1999). MHV induced pleuro-peritonitis in mice with targeted disruption into interferon-γ gene can be regarded as an important model for serosal inflammation in coronaviral feline infectious peritonitis. Moreover, both feline and murine coronaviruses infection result in either enteric or systemic disease, where RdRp-dependent replication in macrophages is considered a key event in the pathogenesis of coronaviral infection.

Above considerations prompted our interests in developing an improved methodologies for detection of pervasive FIP invoking coronavirus-derived RNA species present in diseased felids. While encapsidated (+)RNA strand is present in coronavirus virions it is possible that increased load of coronavirus derived dsRNAs – themselves formed of coronavirus replicative intermediates detectable in infected cats – may contribute to the observed immunopathology.

The sampling of the effusions is regarded as the most useful diagnostic step, especially in

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cases of effusive form, providing the ample of diagnostic material for the detection of coronaviral RNA (Addie et al 2019). Provided our early efforts in cDNA synthesis involved the implementation of recombinant *Thermus thermophilus* (rTth) DNA Polymerase I (Myers et al. 1991; used instead of retroviral reverse transcriptases) for reverse transcription step in amplification reactions of SL-RNA isolated from non-coronaviral enteric pathogen (Magalska A. 2000) were productive (Kapulkin WJ et al. 2016), we next turned our attention toward the detection of RNAs isolated from viral pathogens. Above methodologies were immediately successful in attempts of detection of feline coronaviral RNA in the ascitic fluid of naturally infected cats presented to us clinically (Kita P. 2002; also independently validated on canine distemper virus samples described in Jozwik A. 2004). Since both forward and reverse primers are included in the reverse transcriptase step, so both sense (+) RNA and anti-genomic complementary (-) strand RNA may initiate cDNA amplifications. This step has the advantage of simplicity in clinical settings; the entire reaction is confined to a single tube which minimizes the risk of cross-contamination and other errors of mishandling. However, in spite of initial enthusiasm, we (Kita et al. 2002) and others (Vennema et al. 1998; Pedersen 2012; 2014) collectively failed to discern between mostly avirulent FCoV and pathogenic FIP invoking feline coronavirus strains.

Since our early efforts focused on the detection of FIP-coronavirus RNA were reported and embedded into clinical guidelines (Addie et al. 2004) below we present certain relevant advances and formulate further suggestions.

***i.)* FIP virus RNA detection.** Since the detection of anti-genomic (-) strand could be taken as *bona fide* evidence for coronaviral RdRp activity, we recommend that two separate strand-specific RT-reactions are initiated. Ideally, this could be done in a genome-wide manner (with 12-24 primer pairs sparsed across genomic RNA), discriminating between (+) strand genomic RNA and

antigenomic cRNA (-) strand. This approach has been validated with several other than coronavirus RNA viruses including Respiratory Syncytial virus (RSV), Influenza virus, and Hepatitis C virus (HCV) preparations (Kapulkin WJ, Belino P, Godzik P, Szkoda T, et al. unpublished observations). We further note additional primer pairs may be required to discern polyadenylated coronaviral mRNAs and negative-sense subgenomic replicons derived from FIP virus RNA. Moreover, we remark, the terminal 5' and 3' end sequences (not accessible with conventional amplification format) may need to be determined to fully evaluate the diversity of circulating FIP replicons. This could be achieved with a T4 RNA ligase-based viral RNA ligation used for circularization or concatenation of the terminal 5' and 3' ends of coronaviral replicons (as described by Wang et al. 2014, perceivably including the prior removal of 7-methyl guanosine-caps). While aware above steps may generate additional costs (precluding the routine diagnostics), given the strand stoichiometry favors (+) strand synthesis in the coronaviral replicative cycle, we collectively suggest the ratio of (+) and (-) RNA strands may have a diagnostic value.

ii.) **Approaches toward quantification of FIP RNA load.** Quantitative RT-PCR reactions are presently regarded as gold-standard (Claassen J. 2005) in viral RNA quantifications. However, qRT-PCR approaches seem to suffer from a number of limitations (Chetverina et al. 2002), hence Molecular Colony Technique (MCT) has been proposed as a convenient diagnostic alternative (Chetverina & Chetverin 2015). Molecular Colony Technique is a nucleic acid amplification carried in the RT-PCR compatible semi-solid format; typically a thin gel layer sufficiently flattened to provide 2-D readout. Given the spatial separation of amplification products, viral nucleic acids amplified in such settings form distinct foci comprised each of many copies descended of the initial RNA molecule. An accurate representation of sampled coronavirus RNA load could be quantified by simply counting the number of observed amplification foci reflecting the FIP RNA titer. MCT should be useful in clinical settings when samples often contain a massive excess of other target and

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non-target nucleic acids and when the presence of viruses other than coronavirus is expected. Indeed combinatorial FIP infections have been reported, in cats involved with Feline Immunodeficiency virus (FIV) and Feline Leukemia virus (FeLV), or other concomitant infectious agents complicating the disease picture.

***iii.)* FIP coronavirus RNA sequencing.** In recent years PCR primer-targeted sequencing of FIP variants resulted in the characterization of polymorphisms tentatively associated with the systemic spread of coronavirus infection (reviewed in Pedersen 2014). However, sequence-level FIP variant characterization appears to lack the expected clear consensus (Pedersen et al. 2012) concerning variants specifically involved with pathogenesis (Addie et al. 2019). Several RT-PCR limitations may contribute; first and foremost entire coronavirus genome may not be amplified in a single reaction. Additionally, error-prone RNA replication of the coronavirus genome may result in an increase of the variant diversity (perceivably expected during the systemic spread of the pervasive FIP RNA at a hyper-replicative disease stage). While PCR-MCT may overcome some of those limitations, and enrich for some variants disfavoured by in vitro amplification reactions, recent technological advances in RNA sequencing and computational data analysis (Dolja &Koonin 2018, with relevant references therein) hold the promise of unbiased detection of coronaviral RNAs in complex samples. In a recent example, the metagenomic analysis identified the pangolin niche for zoonotic coronavirus (Wahba et al. 2020, with relevant discussion and references on metagenomic virome analysis). Collectively, advances in sequence-based technology make the implementation of unbiased FIP RNA detection methods into clinical sequencing increasingly feasible, providing a mean of evaluation for loads of (+) and (-) RNA strands. Hereby we foresee unbiased detection of FIP replicons should be clinically informative particularly in the settings when multiple samplings are collected in a disease course to monitor FIP progression.

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***iv.)* Feline coronavirus-derived D-RNA or DI-RNA?** Perhaps a most confounding aspect of FIP pathogenesis and one so far under-explored FIP research direction concern the detection of atypical coronavirus-derived subgenomic replicons in diseased felids. Atypical RNA species smaller than genomic RNA have been described to emerge in certain coronaviruses other than FIP: including Murine Hepatitis virus (MHV) Makino et al. 1989; avian Infectious Bronchitis virus (IBV) Penzes et al. 1996; and Transmissible Gastro-Enteritis virus (TGE) Masters &Perlman 2013. These smaller RNA species are collectively referred to as defective RNAs (D-RNA) or defective-interfering RNAs (DI-RNA). D-I-RNA (defective-or-interfering RNAs) are efficiently a form of parasitic RNA-replicons, replicating at the convenience of coronaviral replicase, involving the synthesis of replicative dsRNA intermediates. The emergence of D-I-RNA may interfere with coronavirus replication hence may confer variable disease susceptibility. Sequence-based diagnostic technologies will expectedly facilitate the discovery and aid in the characterization of atypical subgenomic FIP replicons as well as permit for feline D-I-RNA characterization. Feline coronavirus-derived D-RNA and/or DI-RNA emergence during infection and its association with FIP progression remains still pending and an open question.

Significance and perspective

In this article we argue that feline coronavirus derived dsRNA act as mediator (or modulator) of idiosyncratic reaction underlying FIP pathogenesis. Our article outlines critical biogenesis steps of coronaviral replication perceivably involved in dsRNA formation in FIP pathogenesis. While coronaviral agents may limit dsRNA formation, e.g. imposing strand-specific RNA secondary structures, coupling RNA degradation with RNA strand sequestration, or some other intricate means (Kindler &Thiel 2014; Nelemans &Kikkert 2019), the products of (+) and (-)

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RNA strand collapse can constitute a key, while still underappreciated, event in FIP pathogenesis.

Therefore, conceivably FIP-borne dsRNA, of either genome or sub-genome size, may constitute one of the key proximal pro-inflammatory mediators relevant in the coronaviral disease of cats. This is because of well recognized side effects, known to occur after parenteral administration of double-stranded RNAs acting as a potent inducer of the interferon response. Induction of the interferon response pathway by dsRNA involve the recognition and sensing of viral replicative intermediates by several components of the innate immune system. The innate immune recognition of dsRNA has been extensively studied and characterized (Hilleman et al. 1971; Fire A. 2005; Gantier &Williams 2007; Nellimarla &Mossman 2014 and elsewhere in cited literature) albeit in animal systems other the felids, hence not specifically covered here. Of note, FIP treatment with the available type of recombinant feline interferon accounts for viable therapeutic modality, of some reported curative potential (Ishida et al. 2004). Collectively, we propose replicative intermediates of feline coronavirus consisting of dsRNA may constitute previously unforeseen pro-inflammatory mediator or modulator relevant in maladaptive immune response triggering the progression of observed immunopathology.

Induction of the endogenous feline innate immune-response pathways by coronaviral replicative intermediates corroboratively assumes to engage in dsRNA sensing pathways characterized in other mammals. Innate immune dsRNA response observed in viral infections (Kawai &Akira 2006) typically consider sensing of replicative intermediates (regarded as pathogen-associated-patterns, PAMP) by certain types of pattern recognition receptors (PRR) - a dedicated dsRNA sensing structures confined mostly to subcellular compartments supporting the coronaviral replication. While coronavirus replication is confined to intracellular structures assembled FIP virus may also be released by the lysis of the infected feline cells (Uzal et al. 2016). Extracellular viral

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and monocytic antigens were indeed seen in the foci with scattered intercellular necrosis (Paltrinieri et al.1998), suggesting the necrotic process may have affected FIP coronavirus infected cells. This type of disordered release of encapsidated coronavirus (+) RNA strand would expectedly guide an inadvertent release of dsRNA replicative intermediates into the extracellular compartment. Moreover, other mechanisms leading to eventually lytic processes involving FIP virus-infected cells may efficiently contribute to the extracellular release of coronavirus dsRNA. Here, we infer the release of undegraded coronaviral replicative intermediates (consisting of complementary (+) and (-) RNA strands) into the extracellular compartment may plausibly constitute the critical pathogenic event underlying the systemic spread of feline coronavirus clinically presenting as FIP.

The extracellular exposure of coronavirus-derived dsRNA rises yet another unanticipated possibility. The particular previously under-explored pathway concern dsRNA acting as a remarkably efficient antigen engaging the detectable humoral immune response (Bonin et al. 2000). Indeed experimental immunization (via parenteral administration of certain dsRNA formulations) was shown to elicit specific types of anti-dsRNA antibodies in rabbits (Schwartz &Stollar 1969; Kitagawa &Okuhara 1980), mice, rats, guinea-pigs, dogs, and baboons (Cunnington &Naysmith 1975). Moreover, several distinct murine monoclonal anti-dsRNA antibodies were developed (Schönborn et al. 1991) presumably recognizing unique dsRNA epitopes. Those anti-dsRNA antibodies were used to confirm the demonstrable reactivity against the number of RNA viruses (Stollar et al. 1970; Silverstein &Schur 1970; Stollar &Stollar 1970; Miller et al. 1975; Weber et al. 2006; Son et al. 2015) replicating through dsRNA intermediates (i.e. complementary RNA strand synthesized by RNA-dependent-RNA-polymerase). Markedly themselves instructive examples of the practical use of anti-dsRNA antibody reactivities include certain, other than feline, coronaviral agents (Comar et al. 2019; Hackbart et al. 2020 – with the relevant discussion on dsRNA epitopes). In particular, Hackbart et al. study provides innovative approaches involving dsRNA-antibody

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immunoprecipitation followed by RNA sequencing clearly applicable to FIP coronaviral.

In that context dsRNA-antibody formation in response to extracellular exposure of dsRNA has not been considered in coronavirus infection. However, circulating dsRNA-reactive antibodies were seen in certain autoimmune cases reported in humans and mice (Schur &Monroe 1969; Koffler et al. 1974; Payne &Kalmakoff 1975; Winfield et al. 1975; Klassen et al. 1979). Elevated levels of anti-dsRNA antibodies could be suggestive of the previous dsRNA exposures, hence represent an unperceived factor contributing to coronavirus pathogenesis. If confirmed, broadly reactive circulating anti-dsRNA antibodies may explain the mechanisms that trigger maladaptive immune-complex mediated reactions observed in FIP progression. Perceivably undegraded dsRNAs can act as multivalent ligands for itself dimeric anti-dsRNA antibodies resulting in extracellular formation of ribonucleoprotein nets consisting of anti-dsRNA antibodies complexed with exposed coronavirus-derived dsRNAs. This may explain immune-mediated systemic reactions involving vicious antibody response resulting in maladaptive immune-complex formation. Provided antibodies confer opsonizing properties, formation of dsRNA containing, circulating, immune-complexes may explain certain aspects of macrophage involvement in FIP – suggesting a novel mechanism for engulfment of dsRNA. Preferential replication in macrophages, by the same token, perceivably could be a factor in the seemingly paradoxical phenomenon known as Antibody-Dependent-Enhancement (ADE) postulated in certain viruses (Kulkarni 2019) including FIP invoking feline coronavirus and coronaviruses infecting man (Negro 2020; Paltrinieri et al. 2020). Postulated opsonizing properties of circulating anti-dsRNA antibodies may mediate viral RNA engulfment by monocytes, hence underly certain unexplained features of maladaptive ADE activity (Tirado et al. 2004), implicating the role of viral replicative dsRNA intermediates in that process.

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Together, in an absence of the direct evidence, we suggest that certain viral replicative intermediates i.e. coronaviral dsRNA may elicit anti-dsRNA antibody response resulting in the formation of circulating dsRNA containing immune-complexes. Viral dsRNAs complexed with anti-dsRNA immunoglobulins may confer opsonizing properties (either direct or through interactions with certain complement components) mediating dsRNA phagocytosis. Acting as opsonins, circulating anti-dsRNA immunoglobulins, mediating macrophage invasion can lead to maladaptive activation of several components of the immune system. Since either genomic or subgenomic replicative intermediates (including DI-RNA replicons) of either partial or perfect complementarity could serve as engulfment cargo, immunological mischief can be envisioned. Elevated levels of anti-dsRNA immunoglobulins could be a contributing factor explaining the variability in the response to viral infection. Klassen et al. hypothesized *'it is possible that spontaneous production of anti-dsRNA in mammals may be caused by "immunization" with viral nucleic acid'*. In our view, this unsuspected possibility needs to be considered in terms of both clinical and scientific relevance. Notably, postulated 'immunization' with viral nucleic acids resulting in the formation of anti-dsRNA immunoglobulin could be perceived as an inter-specific phenomenon; previous exposure to certain viral-borne dsRNA can have a measurable effect on subsequent infection with unrelated dsRNA producing viral agents. More broadly, we suggest the release of nucleic acids into extracellular space could represent an unrecognized event in pathogenesis of viral infections, here specifically postulated as a proximal trigger of idiosyncratic reaction observed in FIP progression.

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Feline Infectious Peritonitis: challenges and promises of coronavirus RNA detection.

FIGURES

Figure 1. Feline Infectious Peritonitis/Feline coronavirus whole-genome alignment presented as the radial tree of distance (Fast Minimum Evolution method; default parameters applied over BLAST homology search with gb NC_002306.3). Feline coronavirus/FIP isolates cluster at the top of the presented tree. Canine coronavirus isolates appear distant as separate branches at the bottom of the tree, along with PRCV (porcine coronavirus) clade.

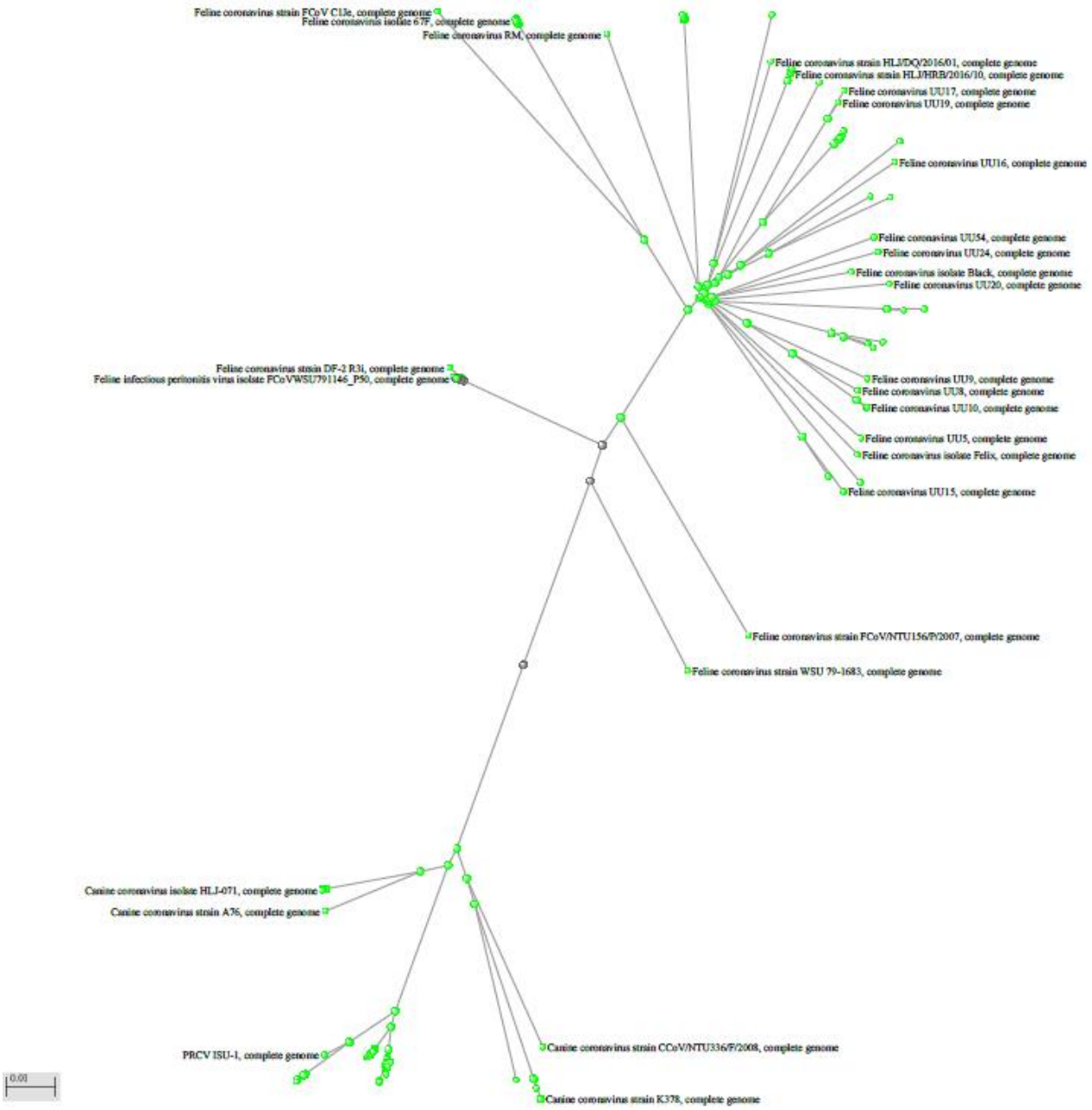

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Figure 2. Clinically presented coronaviral FIP ascites. Symmetric distention in abdominal region.

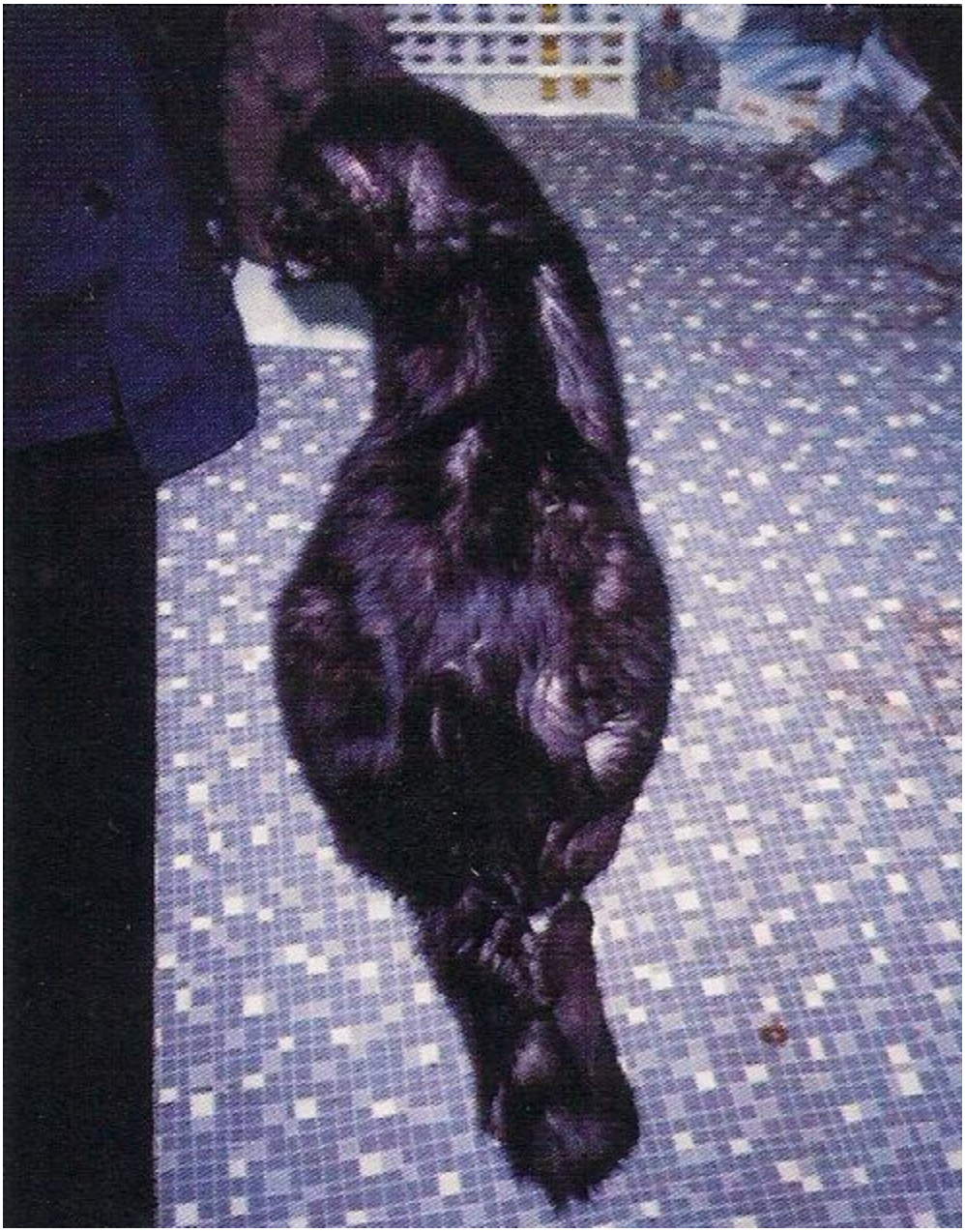

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Figure 3. Clinically manifested coronaviral FIP ascites. Lateral presentation (same cat as in Fig.2). Severe abdominal distention.

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Figure 4. Up to a liter of coronavirus RNA containing exudate could be drawn from the peritoneal cavity of a single cat affected with an effusive form of FIP.

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Figure 5. Clinically manifested coronaviral FIP ascites. The typical appearance of coronavirus RNA containing effusions (see text for details) aspirated with a 20ml syringe.

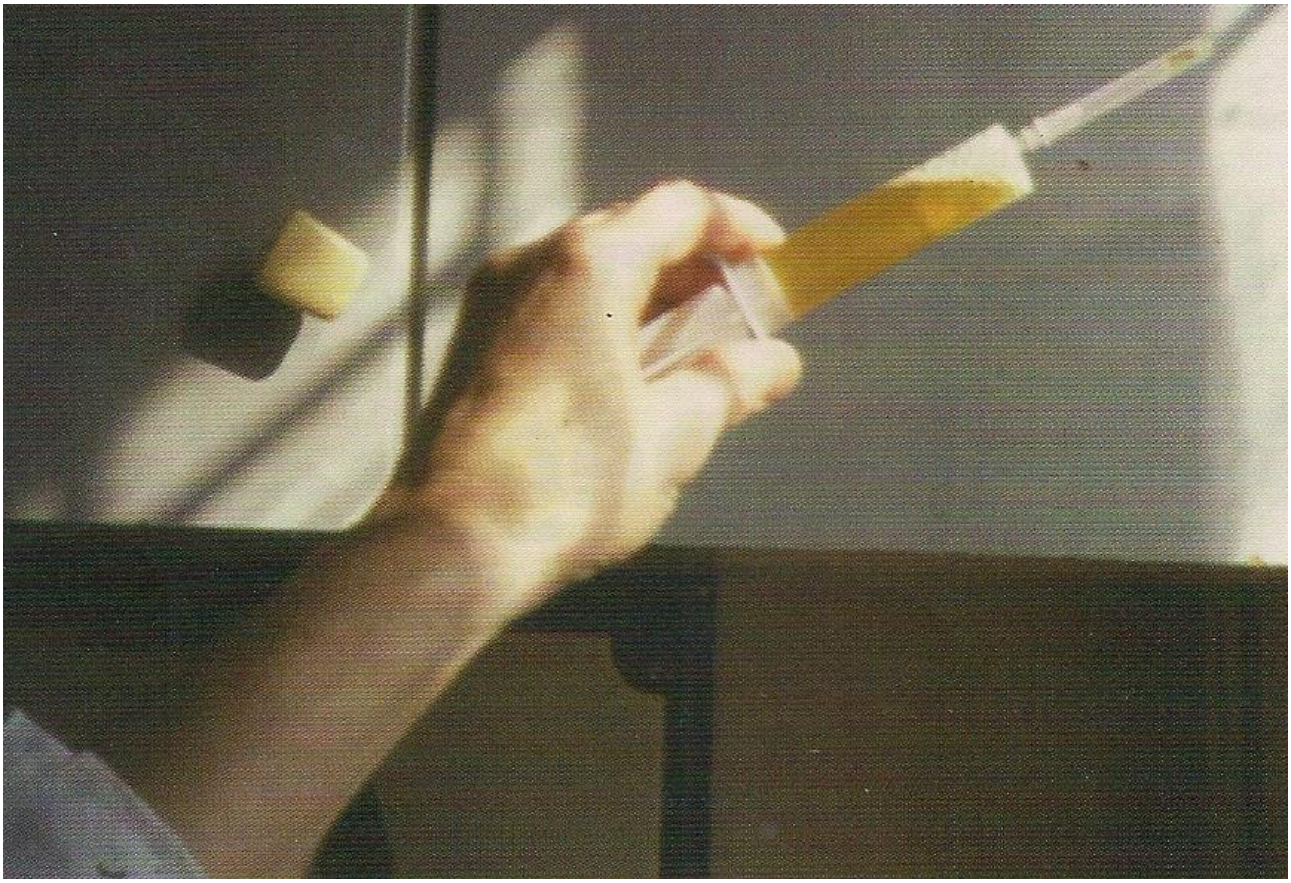

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Figure 6. Ocular manifestations of FIP.