Effektivität von oral verabreichtem GS-441524 zur Behandlung von Katzen mit feliner infektiöser Peritonitis

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ABKÜRZUNGSVERZEICHNIS

%	Prozent
x	mal
®	registered trademark (registrierte Warenmarke)
±	Plus-Minus
ABCD	European Advisory Board On Cat Diseases
ALT	Alanin-Aminotransferase
AP	alkalische Phosphatase
bp	Basenpaar
COVID-19	coronavirus disease 2019 (Coronavirus-Krankheit-2019)
CRFK	Crandell-Rees feline kidney
CT-Wert	cycle-threshold-Wert (Zyklusschwellenwert)
EEG	Elektroenzephalographie
et al.	et alii (und andere)
FCoV	felines Coronavirus
Fc-Rezeptor	fragment-crystalline-Rezeptor
FECV	felines enterales Coronavirus
FIP	feline infektiöse Peritonitis
FIPV	FIP-verursachendes Virus
FISS	felines Injektionsstellen-assoziiertes Sarkom
FHS	felines Hyperästhesiesyndrom
HIV	humanes Immundefizienz-Virus
IFN	Interferon
IFT	Immunfloureszenz-Test
Ig	Immunglobuline
IHC	Immunhistochemie
IU	international unit (internationale Einheit)
kg	Kilogramm
l	Liter
KG	Körpergewicht
M1058F	Substitution Phenylalanin statt Methionin an Position 1058 des Spike-Proteins des felinen Coronavirus
M1058L	Substitution Leucin statt Methionin an Position 1058 des Spike-Proteins des felinen Coronavirus
mg	Milligramm
ml	Milliliter
MIS-C	multisystem inflammatory syndrome in children (multisystemisches Entzündungssyndrom bei Kindern)
MRT	Magnetresonanztomographie
PARR	polymerase chain reaction for antigen receptor rearrangements (Polymerase-Kettenreaktion für Antigen-Rezeptor-Gen-Rearrangement)
PCR	polymerase chain reaction (Polymerase-Kettenreaktion)
q24h	alle 24 Stunden
RNA	ribonucleic acid (Ribonukleinsäure)
RT-PCR	reverse transcriptase polymerase chain reaction (Reverse-Transkriptase-Polymerase-Kettenreaktion)
RT-qPCR	real-time reverse transcriptase polymerase chain reaction (quantitative Reverse-Transkriptase-Polymerase-Kettenreaktion)
S	spike
S1060A	Substitution Alanin statt Serin an Position 1060 des

	Spike-Proteins des felinen Coronavirus
SAA	Serum Amyloid A
SARS-CoV-2	severe acute respiratory syndrome coronavirus type 2 (schweres-akutes-respiratorisches-Syndrom-Coronavirus Typ 2)
SDMA	symmetrisches Dimethylarginin
SC	subkutan (unter die Haut)
µMol	Mikromol
z. B.	zum Beispiel

I. EINLEITUNG

Die feline infektiöse Peritonitis (FIP) ist eine häufig vorkommende, unbehandelt tödlich verlaufende Infektionskrankheit bei Katzen (CAVE et al., 2002). Das feline Coronavirus (FCoV) aus der Familie der Coronaviridae ist ein einzelsträngiges Ribonukleinsäure- (RNA-) Virus (LAI & CAVANAGH, 1997), welches in 2 Pathotypen vorkommt, die auch als felines enterales Coronavirus (FECV) und als FIP-verursachendes Virus (FIPV) bezeichnet werden (PEDERSEN et al., 1981a; PEDERSEN et al., 1981b; PEDERSEN et al., 1984). FCoV gelangt durch oronasale Übertragung in den Körper und vermehrt sich im Darmepithel. RNA-Viren, und somit auch FCoV, sind für eine Fehleranfälligkeit bei der Replikation prädisponiert; dies manifestiert sich in einer hohen Mutationsrate (PEDERSEN, 2009; PEDERSEN et al., 2009; WOO et al., 2010; DESMARETS et al., 2016). So entsteht das FIP-verursachende FCoV durch Mutationen des wenig pathogenen FCoV (POLAND et al., 1996; VENNEMA et al., 1998); etwa 5,0 – 12,0 % der FCoV-infizierten Katzen in Mehrkatzenhaushalten entwickeln FIP (ADDIE & JARRETT, 1992; ADDIE et al., 1995). In der Tierarztpraxis werden Katzen mit FIP meist mit unspezifischen Symptomen, wie Anorexie, Fieber, Gewichtsverlust und gestörtem Allgemeinbefinden, vorgestellt (ADDIE et al., 2009). Als Folge einer Vaskulitis entwickeln Katzen mit FIP häufig Körperhöhlenergüsse (SPARKES et al., 1991; ADDIE et al., 2009; TSAI et al., 2011; RIEMER et al., 2016). Etwa ein Drittel der erkrankten Katzen weisen neurologische Symptome auf (KENT, 2009) und etwa ein weiteres Drittel zeigen Augensymptome wie Iridozyklitis und Chorioretinitis (PEDERSEN, 2009; KIPAR & MELI, 2014; PEDERSEN, 2014; DOENGES et al., 2017; ABCD, 2021; ABCD, 2022).

Unbehandelt verläuft FIP immer tödlich. Die mittlere Überlebenszeit ohne Therapie beträgt 9 Tage (RITZ et al., 2007). Hoffnung gibt ein neues antivirales Medikament namens GS-441524. GS-441524, die aktive Substanz von Remdesivir (1′-cyanosubstituiertes Adenin-C-Nukleosid-Ribose-Analogon), besitzt in der Zellkultur eine starke antivirale Aktivität gegen Coronaviren wie zum Beispiel (z. B.) das Schwere-akute-respiratorische-Syndrom-Coronavirus Typ 2 (SARS-CoV-2), und ist molekularer Vorläufer des pharmakologisch aktiven Nukleosid-Triphosphat-Moleküls (WEBER, 2022). Dieses dient als alternatives Substrat und RNA-Ketten-Terminator der viralen RNA-Polymerase (CHO et al., 2012;

MURPHY et al., 2018) und wird während der Replikation anstelle von Adenosin in die RNA eingebaut. Dadurch kommt es zum Kettenabbruch und damit zur Hemmung der viralen Replikation (AMIRIAN & LEVY, 2020). *In vitro* konnte eine hervorragende Effektivität von GS-441524 gegen FCoV bestätigt werden (MURPHY et al., 2018; DOKI et al., 2022). Auch *in vivo* wurde die Effektivität von GS-441524 zur Therapie der FIP nachgewiesen (MURPHY et al., 2018; PEDERSEN et al., 2019).

GS-441524 ist nicht zugelassen; es gibt kein legales und unter kontrollierten Herstellungsbedingungen hergestelltes Präparat auf dem veterinärmedizinischen Markt. Da das Medikament nicht zugelassen ist, darf es von Tierärzten in Deutschland und den meisten anderen Ländern mit Ausnahme von England und Australien nicht angewendet werden. Diese Tatsache verleitet Katzenbesitzer dazu, die antiviralen Medikamente über den Schwarzmarkt ohne Qualitätskontrolle zu beziehen und die Katzen selbst, ohne tierärztlichen Rat, zu behandeln. Viele verzweifelte Katzenbesitzer beziehen GS-441524 illegal online (JONES et al., 2021). In England und Australien können an FIP erkrankte Katzen aufgrund eines speziellen Apothekengesetzes legal mit GS-441524 behandelt werden, welches von BOVA Specials UK Ltd, London, legal und kontrolliert hergestellt und auch vertrieben wird. Da dieses Präparat nicht zugelassen ist, kann es nicht nach Deutschland importiert werden.

Ziel des Übersichtsartikels war es, einen Überblick über verschiedene Therapieansätze in der Vergangenheit und über aktuelle und erfolgsversprechende Studien zur Therapie der FIP zu geben. Weiterhin enthält der Artikel einen Ausblick auf Möglichkeiten zum legalen Einsatz dieser wirksamen antiviralen Medikamente.

Ziele der verschiedenen Studien dieser Arbeit war es, die Wirksamkeit eines der auf dem Schwarzmarkt angebotenen Medikamente namens Xraphconn® (Mutian, China; aktive Substanz: GS-441524) zur oralen Behandlung von FIP in Bezug auf die Überlebensrate, die Entwicklung klinischer und labordiagnostischer Parameter, den Verlauf der FCoV-Viruslasten im Blut, Erguss und Kot, den Verlauf des anti-FCoV-Antikörpertiters sowie Nebenwirkungen der Therapie zu ermitteln. Zusätzlich sollte die antiviral wirksame Substanz des Studienmedikamentes identifiziert werden (**Publikation 1 und 2**). Des Weiteren wurden Blut, Erguss und Kot der behandelten Katzen auf das Vorhandensein von Spike-Gen-Mutationen untersucht (**Publikation 2**). Proben (Blut und Kot) der Partnerkatzen, die mit den

behandelten Katzen zusammenlebten, wurden auch auf fäkale FCoV-RNA-Ausscheidung getestet (**Publikation 2**). Eine der behandelnden Katzen verstarb bei einem Autounfall und wurde obduziert. Die Ergebnisse wurden in einem Fallbericht zusammengefasst. Ziel des Fallberichtes war, den klinischen Verlauf unter und nach Therapie zu beschreiben und zu untersuchen, ob postmortal in den Geweben Veränderungen vorhanden waren, die auf FIP oder eine vollständige Heilung nach der Behandlung mit GS-441524 hinwiesen und nach Resten von FCoV-RNA und FCoV-Antigen zu suchen (**Publikation 3**). Darüber hinaus wurden die behandelten Katzen über einen Zeitraum von einem Jahr in einer Langzeitbeobachtung weiterverfolgt, um eventuelle Rückfälle nach erfolgreicher Therapie oder Nebenwirkungen erkennen zu können (**Publikation 4**).

II. Publikation 1: Übersichtsartikel

Optionen zur Therapie der felinen infektiösen Peritonitis – früher und heute

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Optionen zur Therapie der felinen infektiösen Peritonitis – früher und heute

Options for treatment of feline infectious peritonitis – previously and today

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Schlüsselwörter:

FIP, Behandlung, antivirale Chemotherapie, GS-441524, FCoV, felines Coronavirus

Zusammenfassung:

Die feline infektiöse Peritonitis (FIP) ist eine der häufigsten Infektionskrankheiten bei Katzen und verläuft unbehandelt tödlich. Bisher gibt es in Deutschland keine legal verfügbare wirksame Therapie. Therapieoptionen reichen von der symptomatischen Therapie (z. B. Glukokortikoide, Propentofyllin) über immunmodulatorische Ansätze (z. B. Interferone, Polyprenyl-Immunstimulanz) bis hin zur antiviralen Therapie mit einem Protease-Inhibitor (z. B. GC376) oder Nukleosid-Analoga (z. B. GS-441524, Remdesivir). Die symptomatische Therapie führt nicht zur Heilung der FIP, sondern nur zu einer kurzzeitigen Verbesserung der klinischen Symptome bei wenigen Katzen. Auch eine immunmodulatorische Therapie stellte sich als wenig erfolgversprechend heraus. Die antiviralen Medikamente GS-441524 und GC376 waren in mehreren Studien hochwirksam und konnten das Leben vieler an FIP erkrankten Katzen retten. Beide Wirkstoffe sind aktuell in Deutschland nicht zugelassen und können von Tierärzten nicht legal angewendet werden. Katzen dürfen aktuell nur in Großbritannien und Australien legal mit GS-441524 therapiert werden. GS-441524 wird daher von Katzenbesitzern in vielen anderen Ländern über den Schwarzmarkt bestellt und in Eigenregie angewendet. Dieser Artikel gibt eine Übersicht über verfügbare Therapieoptionen und einen Ausblick zur legalen Anwendung wirksamer antiviraler Medikamente.

Key words:

FIP, therapy, antiviral chemotherapy, GS-441524, FCoV, felines Coronavirus

Abstract:

Feline infectious peritonitis (FIP) is one of the most common infectious diseases in cats and is fatal if untreated. So far, there is no legally available effective treatment in Germany. Therefore, treatment options include only symptomatic treatment (e.g. glucocorticoids, propentofylline), immunomodulatory approaches (e.g. interferons, polyprenyl immunostimulant), antiviral chemotherapy with protease inhibitors (e.g. GC376) or nucleoside analogues (e.g. GS-441524, remdesivir). Symptomatic treatment does not cure FIP; it only can lead to a short-term improvement of clinical signs in a few cats. Immunomodulatory treatment is not very promising. On the other hand, the antiviral compounds GS-441524 and GC376 were highly effective in several studies and could save the lives of many cats suffering from FIP. However, both agents are currently not licensed and cannot be legally used by veterinarians in Germany. Cats can only be legally treated with GS-441524 in Great Britain and Australia. In other countries, GS-

441524 is imported by cat owners via the black market and used by the owners. This article provides an overview of available treatment options and an outlook on the legal use of effective antiviral drugs.

Einleitung

Bei der felinen infektiösen Peritonitis (FIP) handelt es sich um eine häufig vorkommende, unbehandelt tödlich verlaufende Infektionskrankheit bei Katzen [1]. Die mittlere Überlebenszeit ohne Therapie beträgt 9 Tage [2]. FIP wird verursacht durch eine Infektion mit dem felinen Coronavirus (FCoV). FCoV gehört der Familie der Coronaviridae an und ist ein einzelsträngiges RNA-Virus [3-5]. FCoV existiert in 2 Pathotypen, die manchmal auch als felines enterales Coronavirus (FECV) und als FIP-verursachendes Virus (FIPV) bezeichnet werden [6-8]. Durch oronasale Übertragung gelangt FCoV in den Körper, vermehrt sich im Darmepithel und wird hauptsächlich fäkal-oral übertragen. Jedoch nur 5,0 – 12,0 % der FCoV-infizierten Katzen in Mehrkatzenhaushalten entwickeln FIP [9, 10]. FCoV, ein RNA-Virus, ist für eine Fehleranfälligkeit bei der Replikation prädisponiert; dies spiegelt sich in einer hohen Mutationsrate wieder [11-14]. Nach spontanen Mutationen des wenig pathogenen FCoV entsteht das hochpathogene FIP-verursachende FCoV [15, 16]. Mutationen können in verschiedenen Genen auftreten [17]. Verschiedene Mutationen wurden in der Vergangenheit mit dem Auftreten von FIP assoziiert, wie Mutationen in den Genen des S-Proteins, 3C-Proteins und des akzessorischen Proteins 7b, wobei Mutationen im Gen des Spike-Proteins die wichtigste Rolle zu spielen scheinen [17, 18]
Am häufigsten werden Katzen mit FIP in der Tierarztpraxis mit unspezifischen Symptomen, wie Anorexie, Fieber, Gewichtsverlust und Störung des Allgemeinbefindens, vorgestellt [17]. Früher wurde FIP in verschiedene „Formen“ (trockene, feuchte, okuläre und neurologische Form) eingeteilt. Nach dem aktuellen Wissensstand ist diese Einteilung hinfällig. Katzen mit Symptomen der „feuchten Form“ (Erguss thorakal und/oder abdominal) entwickeln ebenso Veränderungen, die der „trockenen Form“ zugeteilt werden (z. B. pyogranulomatöse Läsionen, Perivaskulitis) und umgekehrt entwickeln Katzen mit der „trockenen“ Form häufig mit der Zeit Ergüsse. Ergüsse entstehen als Folge einer Vaskulitis [17, 19-21]. Etwa ein Drittel der an FIP erkrankten Katzen weisen neurologische Symptome auf [22], und etwa ein Drittel der Katzen ohne Erguss haben auch Augensymptome, wie Iridozyklitis und Chorioretinitis [23-25].
FIP kann nicht anhand eines einzigen Tests diagnostiziert werden [26, 27]. Die Diagnose FIP wird gestellt unter Beachtung verschiedener Kriterien, wie Signalement (z. B. junges Alter, Rassekatze), Herkunft (z. B. Katze aus Mehrkatzenhaushalt), Vorbericht (z. B. möglicher Stressor, wie Kastration), klinische Symptome (z. B. Umfangsvermehrung des Bauches), labordiagnostische Veränderungen (z. B. Hypergammaglobulinämie, proteinreicher und zellarmer Erguss) sowie zusätzlich mithilfe eines Nachweises von FCoV-RNA (möglichst

quantitativ aus Erguss, Blut, Liquor) oder mit Nachweis einer FIP-assoziierten Mutation mittels reverse-Transkriptase-Polymerase-Kettenreaktion (RT-PCR) oder eines Nachweises von FCoV-Antigen mittels Immunhistochemie (IHC) aus veränderten Organen. Das Expertengremium European Advisory Board On Cat Diseases (ABCD) hat hierzu einen Diagnose-Baum entwickelt, an dem sich Tierärzte orientieren können [24].
Unbehandelt verläuft FIP immer tödlich. In der Vergangenheit wurden viele Therapiestudien durchgeführt, größtenteils ohne jeglichen Erfolg. Hoffnung geben Studien, in denen neue antivirale Medikamente zum Einsatz kamen und sich als effektiv erwiesen. Leider sind diese antiviralen Medikamente nicht zugelassen und in Deutschland nur illegal zu beziehen. Dies stellt Besitzer und Tierärzte vor ein ethisches Dilemma.
Ziel dieses Artikels ist es, einen Überblick über verschiedene Therapieansätze (in der Vergangenheit und heute) und über aktuelle Studien zur Therapie der FIP (Zusatzmaterial Tabelle 1), sowie einen Ausblick auf legale Möglichkeiten mit wirksamen antiviralen Medikamenten zu geben.

1. Symptomatische Therapie

Eine symptomatische Therapie führt nicht zur Heilung der FIP, sondern lediglich zur kurzzeitigen Verbesserung der klinischen Symptome und Verbesserung der Lebensqualität.

1.1. Glukokortikoide

Glukokortikoide (empfohlen wird z. B. bei Erguss: Dexamethason, 1 mg/kg, q24h, für 7 Tage (oder bis kein Erguss mehr vorhanden ist) intrathorakal oder intraperitoneal, anschließend 2 – 4 mg/kg, q24h, PO mit anschließender Dosisreduktion; ohne Erguss: Prednisolon, anfangs 2 mg/kg, q24h, PO mit anschließend langsamer Dosisreduktion) werden als immunsuppressive und antiinflammatorische Medikamente zur Unterdrückung der immun-mediierten Veränderungen bei FIP eingesetzt. Hierdurch wird aber lediglich eine kurzzeitige Verbesserung der klinischen Symptome (bei wenigen Katzen über Monate), aber keine Heilung erzielt. Zur tatsächlichen Effektivität von Glukokortikoiden bei Katzen mit FIP gibt es keine kontrollierten Studien [2, 28].

1.2. Zytostatika

Zytostatika, wie Chlorambucil oder Cyclophosphamid, zielen, wie Glukokortikoide, auf eine Immunsuppression der Katze ab und können in Kombination mit Glukokortikoiden (diese werden dann in der Dosis reduziert) verabreicht werden. Kontrollierte Studien liegen nicht vor [17].

1.3. Meloxicam

NSAIDs (non-steroidal anti-inflammatory drugs), z. B. Meloxicam werden als antiinflammatorische Medikamente eingesetzt. In einem Fallbericht wurde eine Katze mit FIP (Diagnosestellung: IHC) mit Meloxicam (0,05 mg/kg, q24h, PO) über einen Zeitraum von 119 Tagen in Kombination mit zusätzlicher unterstützender Therapie behandelt und überlebte 787 Tage [29]. NSAIDs dienen jedoch lediglich zur Unterdrückung der Symptome und können FIP nicht heilen.

1.4. Ozagrelhydrochlorid

Der Thromboxan-Synthetase-Hemmer Ozagrelhydrochlorid (Kissei Pharmaceutical, Matsumoto, Japan), welcher die Thromboxan-A2-Synthese und somit die Thrombozytenaggregation hemmt [30], wurde bei 2 Katzen mit FIP-Verdacht (keine definitive Diagnose) eingesetzt. Katze 1 erhielt eine Kombination aus Ozagrelhydrochlorid (5 mg/kg, q12h, PO) und Prednisolon (2 mg/kg, q24h, PO). Zwei Wochen nach Start der Therapie verbesserten sich die Symptome der Katze deutlich. Die Therapie mit Ozagrelhydrochlorid wurde nach 12 Monaten beendet und die Katze blieb weitere 6 Monate gesund. Die 2. Katze erhielt die doppelte Dosis Ozagrelhydrochlorid (10 mg/kg, q12h, PO), ebenfalls in Kombination mit Prednisolon. Auch bei dieser Katze konnte bereits nach 12 Tagen eine deutliche Besserung der Symptomatik verzeichnet werden. Die Therapie mit Ozagrelhydrochlorid wurde bei dieser Katze nach 9 Monaten aufgrund von Nasenbluten gestoppt. Nach Absetzen des Medikamentes kam es bei dieser Katze zu einem Rezidiv und die Katze verstarb 2 Monate später [31]. Ob tatsächlich Ozagrelhydrochlorid oder eher das begleitend eingesetzte Prednisolon die Besserung der Symptome erzielte, bleibt unklar. Kontrollierte Studien fehlen.

1.5. Propentofyllin

Propentofyllin wurde ebenfalls zur symptomatischen Therapie eingesetzt. Durch Propentofyllin sollte die durch FIP verursachte Vaskulitis und Fibrinogensynthese durch Unterdrückung der Tumornekrosefaktor-(TNF-) α-Synthese reduziert werden [32]. In einer placebokontrollierten Doppelblind-Studie mit 23 Katzen mit FIP (Diagnosestellung: Histologie und/oder IHC) erhielten 7 Katzen Propentofyllin (18 – 25 mg/kg, q12h, PO, für 7 Tage). Es konnte jedoch weder eine signifikante Verringerung der TNF-α-Konzentration, der Ergussmenge noch eine Verbesserung weiterer gemessener Parameter, inklusive des Blutbildes und Serumchemie, gezeigt werden. Es gab keinen statistisch signifikanten Unterschied in der Überlebenszeit der therapierten Katzen gegenüber Katzen der Placebogruppe (n=16) [28].

2. Immunmodulatorische Therapie

Die Idee hinter einer immunmodulatorischen Therapie ist die Stimulation der zellvermittelten Immunantwort und eine Reduktion der überschießenden reaktiven humoralen Immunantwort. Dadurch soll die durch die FIP hervorgerufene Entzündungsreaktion abgeschwächt werden.

2.1. Polyprenyl-Immunstimulanz

Polyprenyl-Immunstimulanz, ein in den USA zugelassenes veterinärmedizinisches Präparat, bestehend aus einer Mischung aus phosphorylierten, linearen Polyisoprenolen, soll laut Herstellerangaben wirksam gegen eine Reihe von Viren, wie z. B. das feline Herpesvirus, sein und zu einer Hochregulation der Biosynthese der mRNA von Th-1-Zytokinen über Toll-like-Rezeptoren führen. Dadurch könnte Polyprenyl-Immunstimulanz bei Erkrankungen, die mit einer Unterdrückung der zellulären Immunität einhergehen, eine positive Wirkung erzielen [33, 34].

Polyprenyl-Immunstimulanz (1 mg/kg, q12h, PO; 3 mg/kg, q84h, SC) kam in einer Fallserie bei 3 Katzen mit FIP-Verdacht zum Einsatz. Katze 1 wurde 14 Monate nach Therapiestart euthanasiert; in der Sektion konnte FIP mittels Histopathologie bestätigt werden. Katze 2 (Diagnosestellung: IHC) und Katze 3 (keine definitive Diagnose) waren nach 24 bzw. 28 Monaten noch am Leben und bei gutem Allgemeinbefinden [35]. In einer Folgestudie wurden 60 Katzen mit FIP-Verdacht (alle ohne Erguss, FIP meist nicht bestätigt) mit Polyprenyl-Immunstimulanz (3 mg/kg, q56h, PO) behandelt. 16/60 Katzen überlebten > 100 Tage, davon 8/60 Katzen > 200 Tage, und davon 4/60 Katzen > 300 Tage. Die Überlebenszeit der Katzen, die nicht gleichzeitig eine Therapie mit Glukokortikoiden erhielten, war signifikant länger [36]. Weitere Studien mit kontrollierten Einschlusskriterien sind jedoch notwendig, um beurteilen zu können, ob durch Polyprenyl-Immunostimulanz die Progression der FIP tatsächlich verlangsamt werden kann [35, 36].

2.2. Propionibacterium acnes

Propionobacterium acnes, ein anaerobes Bakterium, welches eine immunmodulierende Wirkung auf die humorale und zelluläre Immunantwort besitzt [37], stellte sich in einer sehr alten experimentellen, placebokontrollierten Studie bei 74 Katzen mit FIP bei alleiniger Verabreichung (0,4 mg/Katze oder 4 mg/Katze, q84h, IP oder IV, für 14 Tage, anschließend 1x pro Woche für 21 – 28 Tage) als nicht wirksam dar [38]. Allerdings verstärkte es die Wirkung von humanem Interferon-α (s. u.).

2.3. Humanes Interferon-α

Interferone wurden ebenfalls zur immunmodulatorischen Behandlung von Katzen mit FIP eingesetzt. Interferone sind Zytokine, die eine immunmodulatorische, antihumorale und antivirale Wirkung aufweisen können [38].

Interferon-α wird als Antwort auf virale Infektionen gebildet und ist *in vitro* gegen FCoV wirksam [39]. Rekombinantes humanes Interferon-α (rHuIFN-α) wurde in einer Dosis von $10^4/10^6$ IU/kg, q24h, IM, für 8 Tage bei 29/74 Katzen mit experimentell induzierter FIP allein oder in Kombination mit *Propionibacterium acnes* eingesetzt. Zwar gab es keinen Unterschied hinsichtlich der Mortalität bei behandelten versus unbehandelten Katzen; die Katzen, die nach Infektion mit mutiertem FCoV mit der hohen Dosis von rHuIFN-α (10^6 IU/kg) allein oder in Kombination mit *Propionibacterium acnes* behandelt wurden, hatten jedoch eine signifikant längere Überlebenszeit (Verlängerung der durchschnittlichen Überlebenszeit um 1 – 3 Wochen) im Vergleich zu den unbehandelten Katzen. [38]. Eine Kombination von rHuIFN-α mit *Propionibacterium acnes* war dabei wirksamer als rHuIFN-α allein.

Katzen entwickeln jedoch 3 – 7 Wochen nach subkutaner Injektion neutralisierende Antikörper gegen das humane Interferon-α, die zu einer Unwirksamkeit führen [40]. Aus diesem Grund können humane Interferone nicht über einen längeren Zeitraum subkutan verabreicht werden. Seitdem felines Interferon-ω für den tiermedizinischen Markt in vielen Ländern verfügbar ist, wird humanes Interferon-α in diesen Ländern nicht mehr eingesetzt.

2.4 Felines Interferon-ω

Rekombinantes felines Interferon-ω (reFeIFN-ω) (10^6 U/kg, q24h, SC, bis zur Remission, dann 1x pro Woche) wurde erstmals in einer klinischen Studie mit 12 Katzen mit Erguss (FIP nicht bestätigt) in Japan in Kombination mit Glukokortikoiden eingesetzt. 4/12 Katzen überlebten weniger als einen Monat; 4/12 Katzen überlebten 2 – 5 Monate; 4/12 Katzen (zwischen 6 und 16 Jahre alt) überlebten > 2 Jahre. Die Autoren schlussfolgerten daher, dass die Progression der FIP bei älteren erkrankten Katzen verlangsamt werden könnte [41]. In einem Fallbericht wurde eine Katze mit okulären FIP-Symptomen (Diagnosestellung: Mutations-PCR aus Lymphknotenaspirat) im Anschluss an eine 50-tägige antivirale GS-441524-Therapie mit reFeIFN-ω (10^6 U, q24h, PO) behandelt und befand sich 6 Monate nach Diagnosestellung in anhaltender Remission [42]. In einer placebokontrollierten Doppelblindstudie wurde die Effektivität von reFeIFN-ω (10^6 U/kg, q24h, SC, über 8 Tage, dann 1x pro Woche) bei 37 Katzen (21 Katzen erhielten reFeIFN-ω; 16 Katzen ein Placebo-Präparat) mit nachgewiesener FIP (Diagnosestellung: Immunfluoreszenz) untersucht. Alle Katzen erhielten als symptomatische Therapie zusätzlich Glukokortikoide (Dexamethason oder Prednisolon) und Antibiotika. Es konnte kein signifikanter Unterschied zwischen den beiden Gruppen (IFN-

Gruppe versus Placebo-Gruppe) in der Überlebenszeit (median 9 Tage) oder Lebensqualität gezeigt werden. ReFeIFN-ω war also in dieser kontrollierten Studie nicht wirksam [2].

3. Antivirale Chemotherapie

Antivirale Medikamente greifen in den viralen Replikationszyklus ein und führen zur Hemmung der Virusvermehrung [43].

3.1. Ribavirin

Ribavirin (1-β-D-ribo-furanosyl-1,2,4-Triazol-3-carboxamid) ist ein Nukleosid-Analogon mit einem breiten antiviralen Spektrum [44, 45]. In einer Studie wurden 24 spezifisch pathogenfreie Katzen mit experimentell induzierter FIP 18 Stunden nach Inokulation mit dem antiviralen Medikament Ribavirin (16,5 mg/kg, q24h, PO, IM oder IV, für 10 – 14 Tage) therapiert. Im Vergleich zu einer Kontrollgruppe zeigten Katzen, die mit Ribavirin therapiert wurden, schwerere klinische Symptome (z. B. Gewichtsverlust), eine kürzere mittlere Überlebenszeit und massive Nebenwirkungen (hämorrhagische Läsionen in Gastrointestinaltrakt, Gehirn, Leber, Lunge, Herz, Diaphragma und Subkutis). Als Versuch, die Toxizität zu verringern, wurde Ribavirin in Lecithin-enthaltende Liposomen inkorporiert; jedoch blieb eine antivirale Wirksamkeit auch in diesem Versuch aus und alle Katzen verstarben. Somit ist Ribavirin nicht zur Therapie der FIP und generell nicht als antivirales Medikament für Katzen geeignet [46].

3.2. Mefloquin

Mefloquin, ein Nukleosid-Analogon, welches die zytopathischen Effekte von FCoV reduziert [47], wird zur Prophylaxe und Behandlung von Malaria beim Menschen eingesetzt. Mefloquin erwies sich *in vitro* gegen FCoV als wirksam und zeigte keinen zytotoxischen Effekt [48]. In einer Studie zur Pharmakokinetik von Mefloquin wurde das Medikament klinisch gesunden Katzen oral verabreicht. Als Nebenwirkungen wurden Erbrechen nach Eingabe ohne Futter sowie ein Anstieg der Konzentration von symmetrischem Dimethyl-Arginin (SDMA) gezeigt [49].

Obwohl noch keine klinischen Studien vorliegen, wird Mefloquin in Australien zur FIP-Therapie eingesetzt. Mefloquin ist preislich günstiger als andere antivirale Medikamente wie z. B. Remdesivir oder GS-441524 (s. u.) und könnte eine weiterführende Therapieoption nach Start mit einem Nukleosid-Analogon darstellen, beispielsweise wenn ein ganzer Zyklus mit GS-441524 finanziell nicht möglich ist [50, 51]. Kontrollierte Studien sind dringend nötig.

3.3. Itraconazol

Itraconazol, ein Antimykotikum, agiert als Inhibitor der Cholesterinsynthese und des Cholesterintransports, und führt zur Anreicherung von Cholesterin in den Zellen, und dadurch zur Hemmung der FCoV-Replikation [52-54]. *In vitro* wurde die Kombination von GS-441524 mit Itraconazol auf eine synergistische antivirale Wirkung untersucht. Itraconazol kann die antivirale Wirkung von GS-441524 und damit die Hemmung der Replikation von FCoV verstärken [55].

Itraconazol (50 mg/Katze, q24h, PO, für 30 Tage) wurde zusammen mit Adalimumab (einem monoklonalen Antikörper gegen den humanen TNF-alpha, 10 mg/Katze, q12h, IV, für 4 Tage) bei 3 Katzen mit experimentell induzierter FIP eingesetzt. Zwei Katzen zeigten eine Verbesserung der klinischen Symptome und labordiagnostischen Parameter, wie einen Anstieg der Anzahl der Lymphozyten. Die 3. Katze dieser Studie wurde aufgrund ausbleibender Besserung euthanasiert. Es wurden keine Nebenwirkungen nachgewiesen [56]. In einem Fallbericht wurde Itraconazol (10 mg/kg, q12h, PO für 38 Tage) bei einer 3 Monate alten, FIP-verdächtigen Katze mit Erguss (Diagnosestellung: FCoV-PCR aus Erguss) in Kombination mit Prednisolon (1 mg/kg, q24h, für 18 Tage) eingesetzt. Der Erguss nahm unter Therapie ab. Ab Tag 22 traten erneut neurologische Symptome auf und die Katze erhielt erneut Prednisolon. 38 Tage nach Start der Therapie wurde die Katze aufgrund eines Status epilepticus euthanasiert. In der Obduktion wurde die Diagnose FIP mittels IHC bestätigt [57]. Generelle Nebenwirkungen von Itraconazol bei Katzen sind Hypersalivation, Erbrechen, Durchfall, Anstieg der Aktivität der Leberenzyme; in seltenen Fällen auch Ikterus [58]. Kontrollierte Studien bei Katzen mit FIP sind nötig, um die Wirksamkeit einschätzen zu können. Möglicherweise eignet es sich zur Kombinationstherapie mit anderen antiviralen Medikamenten.

3.4. Molnupiravir

Molnupiravir (EIDD-2801), ein Prodrug des Nukleosid-Analogons B-D-N4-Hydroxycytidin [59], ist *in vitro* gegen FCoV wirksam [60, 61]. Bei 26 Katzen mit FIP-Verdacht wurde in einer retrospektiven Studie Molnupiravir (durchschnittlich 12,8 – 14,7 mg/kg, q12h, PO) über einen Zeitraum von 84 Tagen angewendet; 10/26 Katzen hatten vorher bereits einen Therapiezyklus mit GS-441524 erhalten. 24/26 Katzen waren zum Zeitpunkt der Veröffentlichung krankheitsfrei, eine Katze wurde euthanasiert und eine weitere Katze wurde aufgrund eines Rückfalls erneut therapiert. Daher scheint Molnupiravir erfolgsversprechend zu sein. Bei einer Dosis von > 23 mg/kg waren Faltohren, abgebrochene Schnurrhaare, sowie eine hochgradige Leukopenie als Nebenwirkungen vorhanden [62]. Kontrollierte Studien sind dringend nötig.

3.5. GC376

Coronaviren besitzen eine virale 3C- oder 3C-like-Protease, die während der Replikation für die Spaltung viraler Polyproteine in funktionelle, strukturelle oder nicht-strukturelle Virus-Proteine verantwortlich und daher für die Replikation von Coronaviren erforderlich ist. Die virale Protease bietet somit einen guten Angriffspunkt für antivirale Medikamente, sogenannte Proteaseinhibitoren [63-67]. Auch *in vitro* konnte eine Wirksamkeit des Proteaseinhibitors GC 376 gegen das FIPV bestätigt werden ([68]. In einer Studie wurde der Proteaseinhibitor GC376 (5 – 10 mg/kg, q24h, SC, für 14 – 20 Tage) bei 8 spezifisch pathogenfreien Katzen (Alter 8 – 10 Monate) mit experimentell induzierter FIP angewendet. Zwei Katzen wurden aufgrund der Schwere ihrer klinischen Symptome 4 und 7 Tage nach Beginn der antiviralen Behandlung euthanasiert. Die 6 verbliebenen Katzen zeigten eine rasche Verbesserung der Symptomatik und bis zu 8 Monaten keine Anzeichen eines Rückfalls. Die Viruslast im Erguss der verbliebenen Katzen nahm signifikant ab [68].

In einer Feldstudie wurden 20 Katzen mit und ohne Erguss (Diagnosestellung: Signalement, Historie, klinische und labordiagnostische Veränderungen, Bestätigung der FIP mittels qRT-PCR aus Erguss oder veränderten Organen) mit GC376 (15 mg/kg, q12h, SC) behandelt. Katzen mit neurologischen Symptomen wurden ausgeschlossen. Der Gesundheitszustand der ersten 5 Katzen besserte sich schnell, sodass die Therapie nach 14 Tagen beendet wurde. Jedoch kam es bei allen 5 Katzen zu einem Rückfall (1 – 7 Wochen nach Therapieende). Die nachfolgenden Katzen wurden über einen längeren Behandlungszeitraum (21 – 84 Tage) therapiert. Bei diesen Katzen verbesserten sich die klinischen Symptome rapide; 24 – 48 Stunden nach Beginn der Therapie waren die Katzen fieberfrei bei gleichzeitig verbessertem Appetit, verbesserter Aktivität und Gewichtszunahme. Aszites war bereits nach 2 Wochen nicht mehr vorhanden. Auch okuläre Symptome verbesserten sich innerhalb von 48 Stunden und waren nach 1 Woche nicht mehr erkennbar. Dennoch hatten 13/20 Katzen einen Rückfall (innerhalb von 1 – 7 Wochen der Erst- oder Wiederholungsbehandlung/en); 8 davon wurden aufgrund schwerer neurologischer Symptome und 5 aufgrund nicht-neurologischer Symptome euthanasiert. Nebenwirkungen der Therapie traten vor allem durch die subkutane Injektion in Form von Irritationen an den Einstichstellen auf, wie z. B. Schwellungen und Alopezie. Bei den 4 juvenilen Katzen (Alter bei Therapiebeginn: zwischen 3,3 – 4,4 Monate) trat verzögertes Wachstum und Durchbrechen der permanenten Zähne (Ausbleiben oder verzögerter Zahnwechsel) auf [65].

Leider sind bereits Resistenzen gegen den Protease-Inhibitor GC376 beschrieben. Eine Katze der Studie von Pedersen et al. (2018) (Diagnosestellung: klinische und labordiagnostische Veränderungen, Signalement, Anamnese, Erguss) wurde nach zweimaliger Therapie mit GC376 (1. Therapiezyklus: 10 – 30 mg/kg, q12h, SC, für 9 Wochen; 2. Therapiezyklus: 7,5 – 15 mg/kg, q12h, SC, für 84 Tage) aufgrund eines zweiten Rückfalls euthanasiert. Diese Katze

überlebte insgesamt 236 Tage nach Therapiestart. Die FCoV-3C-like-Protease aus Gewebe der euthanasierten Katze wurde mit der FCoV-3C-like Protease aus Erguss (vor Therapiebeginn) verglichen und zeigte 3 Aminosäureveränderungen (N25S, A252S und K260N). Viren mit der Aminosäureveränderung N25S zeigten eine bis zu 1,68-fache geringere Empfindlichkeit gegenüber GC376. Dies lässt auf eine Resistenz schließen [69]. Auch eine *in-vivo*-Studie zeigte, dass FCoV in der Lage ist, in Anwesenheit von GC376 zu mutieren und eine Resistenz gegen GC376 zu entwickeln. GC376 ist unwirksam gegen diese resistenten Mutanten [70]. Dies beweist, dass ein restriktiver Einsatz dieser potenten antiviralen Medikamente wichtig ist.

3.6. Remdesivir

Remdesivir (GS-5734) ist ein Prodrug, das nach Verabreichung in der Leber zu GS-441524 umgewandelt wird. Im Gegensatz zu seiner aktiven Form (GS-441524) muss Remdesivir als Injektion verabreicht werden, da es oral nicht wirksam ist [71]. Remdesivir ist in der Humanmedizin zugelassen und wird zur Therapie von COVID-19 eingesetzt [72]. Es gibt Hinweise, dass Remdesivir bei Katzen mit FIP zur Heilung führen kann; allerdings gibt es noch keine prospektiven kontrollierten Studien [73].

Ein Fallbericht aus Südafrika beschreibt die Therapie einer Katze mit FIP mit Erguss (Diagnosestellung: IHC aus Erguss-Zellpellet) mit Remdesivir. Die Katze erhielt über einen Zeitraum von 80 Tagen Remdesivir (4,9 – 5,6 mg/kg, q24h, IV und SC), in den ersten 3 Tagen intravenös, anschließend bis zum Ende der Therapie subkutan. Bereits nach einer Woche konnte eine deutliche Verbesserung der Symptome festgestellt werden; nach 80 Tagen war die Katze klinisch gesund. Auch 7 Monate nach Ende der Therapie, zum Zeitpunkt der Veröffentlichung, war die Katze klinisch unauffällig [74]. In Australien wurden laut Homepage der Tierklinik Walkervillevet bereits ca. 30 an FIP erkrankte Katzen (Diagnosestellung nicht bekannt) erfolgreich mit dem umgewidmeten Remdesivir für 84 Tage behandelt [75]. Ein neues Therapieprotokoll aus Großbritannien schlägt eine Kombination mit Remdesivir als Injektionen (10 – 20 mg/kg (Dosierung variierend je nach klinischen Symptomen), q24h, IV für 3 – 4 Tage, evtl. Weiterführung SC bis Tag 7 – 14) und anschließender Weiterführung der Therapie mit dem nicht-lizensierten GS-441524 in Tablettenform (10 – 20 mg/kg, q12-24h, PO) vor [50]. Remdesivir soll laut Homepage der Tierklinik Walkervillevet gut verträglich sein. Folgende unerwünschte Nebenwirkungen wurden gemeldet: vorübergehende lokale Irritation an der Injektionsstelle, Brennen bei der Injektion, Entwicklung oder Verschlimmerung eines Pleuraergusses in den ersten 48 Stunden der Behandlung sowie eine für mehrere Stunden nach der intravenösen Verabreichung anhaltende Apathie und Übelkeit. Über einen Anstieg der Alanin-Aminotransferase (ALT)-Enzymaktivität wurde ebenfalls berichtet [50].

Eine australische Fallsammlung beschreibt die Therapie mit Remdesivir an 26 Katzen mit diagnostizierter FIP (Diagnosestellung nicht bekannt). Die Katzen erhielten Remdesivir (in den ersten 4 Tagen 10 – 15 mg/kg, q24h, SC oder IV; anschließend 6 – 15 mg/kg, q24, SC oder Umstellung auf das oral verfügbare GS-441524) für 84 Tage. 22/26 Katzen überlebten 6 Monate nach Start der Therapie; 3 Katzen starben innerhalb von 48 Stunden; ein Follow-up der 4. Katze ist nicht beschrieben. Bei 11 Katzen wurde die Therapie verlängert; 3 Katzen wurden aufgrund eines Rückfalls der Symptome erfolgreich erneut therapiert. Auch die Katzen dieser Studie zeigten Reizungen an den Injektionsstellen [76].

Eine weitere Fallsammlung beschreibt die Therapie mit Remdesivir von 25 Katzen mit diagnostizierter FIP (Diagnosestellung: Veränderung klinischer und labordiagnostischer Parameter, Bildgebung, Zytologie, IHC). Die Katzen erhielten initial Remdesivir (10 – 20 mg/kg, q24h, IV, für 2 – 9 Tage). 5/25 Katzen wurden 1 – 13 Tage nach Therapiestart euthanasiert. Die 20 restlichen Katzen zeigten bereits nach 2 – 5 Tagen eine deutliche Besserung. Bei 2/25 Katzen wurde die Therapie mit Remdesivir subkutan fortgesetzt; 8/12 Katzen wurden nach 4 – 68 Tagen auf das orale GS-441524 (Dosis äquivalent zu Remdesivir) umgestellt; 10/20 erhielten direkt nach der initialen intravenösen Therapie mit Remdesivir oral GS-441524. Bei der subkutanen Injektion von Remdesivir wurden lokale Hautreaktionen und Schmerzen beobachtet.. 2/5 Katzen wurden aufgrund aufgetretener neurologischer Symptome euthanasiert. Zum Zeitpunkt der Veröffentlichung hatten 10 Katzen den 84-tägigen Behandlungszyklus mit Remdesivir und GS-441524 erfolgreich abgeschlossen und befanden sich in Remission [77].

Ein großer Nachteil von Remdesivir ist, dass die Lösung nach Anmischen nur für kurze Zeit (bei Raumtemperatur bis zu 24 Stunden; gekühlt bis zu 48 Stunden) haltbar ist [78]. Demnach müssen große Mengen an Medikamentenresten verworfen werden, wenn nicht genügend Katzen gleichzeitig therapiert werden. Dies erhöht die ohnehin extremen Kosten in der Anschaffung, sodass die Therapie mit Remdesivir für viele Besitzer nicht möglich ist.

3.7. GS-441524

GS-441524, die aktive Substanz von Remdesivir (1′-cyanosubstituierte Adenin-C-Nukleosid-Ribose-Analogon) besitzt in der Zellkultur eine starke antivirale Aktivität gegen bestimmte RNA-Viren, wie z. B. schweres-akutes-respiratorisches-Syndrom-Coronavirus (SARS-CoV), und ist molekularer Vorläufer des pharmakologisch aktiven Nukleosid-Triphosphat-Moleküls [79]. Es dient als alternatives Substrat und RNA-Ketten-Terminator der viralen RNA-abhängigen RNA-Polymerase [80, 81] und wird während der Replikation anstelle von Adenosin in die RNA eingebaut, woraufhin es zum Kettenabbruch und damit zur Hemmung der viralen Replikation kommt [82]. *In vitro* konnte eine hervorragende Effektivität von GS-441524 gegen FCoV gezeigt werden [55, 80, 83].

GS-441524 ist nicht zugelassen; es gibt also kein legal und unter kontrollierten Herstellungsbedingungen hergestelltes Präparat auf dem veterinärmedizinischen Markt. Da das Medikament nicht zugelassen ist, darf es von Tierärzten nicht empfohlen oder angewendet werden. Katzenbesitzer behelfen sich online über Katzenforen, um an GS-441524-Präparate aus nicht-kontrollierten Produktionen zu gelangen [84]. In Großbritannien und Australien können Katzen mittlerweile aufgrund eines anderen Apothekengesetzes legal mit dem Nukleosid-Analogon therapiert werden. GS-441524 wird von BOVA Specials UK Ltd, London, in Großbritannien und Australien legal und kontrolliert hergestellt und auch vertrieben. Da es jedoch keine Zulassung hat, darf es nicht nach Deutschland importiert werden.

3.7.1. Experimentelle Studien

In einer Studie wurden 12 spezifisch pathogenfreie Katzen (Alter 6 – 9 Monate) experimentell mit mutiertem FCoV infiziert. 10/12 Katzen entwickelten FIP-typische Symptome und wurden mit GS-441524 behandelt (2 – 5 mg/kg, q24h, SC, für 14 Tage). Die klinischen Symptome besserten sich innerhalb von 3 Tagen. Zwei Katzen zeigten 4 bzw. 6 Wochen nach der initialen Therapie erneut FIP-Symptome und wurden für weitere 2 Wochen mit GS-441524 behandelt. Alle Katzen waren bis zum Zeitpunkt der Veröffentlichung in Remission (> 8 Monate nach Infektion). Als Nebenwirkungen wurden Schmerzen an der Einstichstelle beschrieben [80].

3.7.2. Retrospektive Fallbeschreibungen

In einem Fallbericht wurde GS-441524 (8 mg/kg, q24h, PO für 50 Tage) zur Therapie einer Katze mit okulären FIP-Symptomen (Diagnosestellung: Mutations-PCR aus Lymphknotenaspirat) eingesetzt. Die Katze erhielt unterstützend zur Therapie der Uveitis Prednisolon systemisch, mit anschließend topischer Weiterbehandlung. Innerhalb weniger Wochen zeigten sich eine deutliche Gewichtszunahme, Verbesserung der okulären Symptome sowie Verbesserungen in den labordiagnostischen Parametern. Symmetrisches Dimethylarginin (SDMA) war unter Therapie erhöht; ob dieser Anstieg auf die Therapie mit GS-441524 zurückzuführen ist, ist nicht bekannt, da zu Beginn der Therapie kein SDMA gemessen wurde. Nach Abschluss der Behandlung mit GS-441524 sank der SDMA-Wert wieder ab. Im Anschluss an die antivirale Therapie erhielt die Katze felines reFelFN-ω. Zum Zeitpunkt der Publikation befand sich die Katze in anhaltender Remission [42].

In einer retrospektiven Multicenterstudie aus China wurden klinische Veränderungen und Risikofaktoren für FIP anhand Daten von 127 Katzen mit FIP-Verdacht (keine sichere Diagnose) ausgewertet. 88/127 Katzen konnten über einen längeren Zeitraum begleitet werden. 30/88 erhielten in Eigenverantwortung der Besitzer als nicht-lizensiertes antivirales Medikament entweder GS-441524 (2 – 4 mg/kg, q24h, Darreichungsform nicht bekannt, für 28 Tage) und/oder GC376 (6 – 8 mg/kg, q24h, Darreichungsform nicht bekannt, für 28 Tage).

29/30 Katzen konnten geheilt werden, 1/30 Katzen zeigte nach der Behandlungsdauer von 4 Wochen einen Rückfall und musste euthanasiert werden [85].

An einer aktuellen Umfrage des College of Veterinary Medicine der Ohio State University, Columbus, nahmen 393 Katzenbesitzer teil, die ihre an FIP erkrankten Katzen (keine sichere Diagnose) selbstständig mit einem der nicht-lizensierten GS-441524-Präparate aus dem Internet behandelten. Die Katzen wurden mit 2 – 16 mg/kg, SC, über 84 Tage therapiert. 348/393 (88,5 %) Katzen erhielten eine 84-tägige Behandlung; bei 45/393 (11,4 %) Katzen wurde die Behandlung um durchschnittlich 28 Tage verlängert. Innerhalb einer Woche konnten 88,2 % der Besitzer eine Verbesserung der klinischen Symptome feststellen. Zum Zeitpunkt der Veröffentlichung waren 97 % der Katzen noch am Leben; 54 % galten als geheilt und 43 % befanden sich in einer 12-wöchigen „Wartezeit" nach Ende der Therapie. Bei 12 % der Katzen kam es zu einem Rückfall; 3 % der Katzen verstarben. Nebenwirkungen wurden nach den subkutanen Injektionen in Form von Schmerzen oder Wunden an den Einstichstellen bemerkt. Die Katzenbesitzer hatten von ihrem Tierarzt direkt (15 %) oder indirekt (12 %) Informationen zu der nicht zugelassenen Therapie erhalten; 30 % der Besitzer gelangten über eine Online-Recherche und 23 % über Social-Media-Kanäle an das Medikament. [84].

In einer japanischen Studie wurde GS-441524 bei 141 Katzen mit Erguss und diagnostizierter FIP (mittels qualitativer RT-PCR aus Blut, Aszites oder Pleuraerguss) getestet. GS-441524 wurde in einer Dosis von 5 mg/kg, q24h, PO oder SC für 84 Tage verabreicht. Von den 141 Katzen überlebten 116 Katzen. Die Blutwerte der verstorbenen Katzen wurden mit denen der überlebenden Katzen verglichen. Die Bilirubinkonzentration war bei überlebenden im Gegensatz zu den verstorbenen Katzen signifikant niedriger. Nur 1/7 Katzen mit einer Bilirubinkonzentration > 4,0 mg/dl überlebte. Diese Studie schlägt daher Bilirubin als prognostischen Faktor und Prädiktor für die Wirksamkeit der Therapie mit GS-441524 vor [86].

In einer retrospektiven Studie wurden 42 Katzen mit FIP (Diagnosestellung: Histopathologie, IHC, RT-PCR, Mutations-PCR, Veränderungen klinischer und labordiagnostischer Parameter) in Bezug auf den Verlauf des akute-Phase-Proteins Alpha-1-Acid-Glykoprotein (AGP) und dessen prognostische Aussagekraft untersucht. AGP war bei 40/42 Katzen vor Therapie erhöht. 15/42 Katzen erhielten GS-441524, die anderen Katzen erhielten unterschiedliche Medikamente (22/42 Katzen: felines Interferon-ω, 1/42 Katzen: Polyprenyl-Immunstimulanz, 1/42 Katzen: Kokosnussöl plus Abziehen des Ergusses, 2/42 Katzen: Fettstammzell-Therapie, 1/42: unbekannte Therapie) in unterschiedlichen Dosierungen. 13/42 Katzen verstarben. 29/42 Katzen überlebten bis zum Zeitpunkt der Publikation. Bei 26 dieser Katzen normalisierten sich die AGP-Werte innerhalb von 13 Tagen bis 16 Monaten. Die Autoren schlussfolgerten, dass das Absinken der AGP-Werte in den Referenzbereich auf eine Genesung hinweist [87].

3.7.3. Prospektive, kontrollierte Studien

In einer Studie wurde die Effektivität von GS-441524 (initial 2 mg/kg, q 24h, SC) bei 31 Katzen mit FIP (Diagnosestellung: Signalement, Anamnese, klinische und labordiagnostische Veränderungen, Ergussanalyse, RT-PCR und IHC) über einen Zeitraum von 84 Tagen untersucht. Katzen mit neurologischen und okulären Symptomen wurden von der Studie ausgeschlossen aufgrund von Bedenken einer Undurchlässigkeit der Blut-Hirn-Schranke und der Blut-Augen-Schranke für GS-441524 [88]. Bei Katzen, die nicht auf die initiale Dosis ansprachen oder erneut Symptome während des Therapieverlaufs entwickelten, wurde die Dosis auf 4 mg/kg erhöht. 26/31 Katzen wurden über 12 Wochen behandelt. Die Katzen sprachen gut auf GS-441524 an und waren innerhalb von 12 – 36 Stunden fieberfrei. Der Appetit, das Gewicht und die Aktivität der Katzen nahmen zu. Abdominale und pleurale Ergüsse waren nach 7 – 14 Tagen verschwunden. 25/26 Katzen befanden sich in anhaltender Remission zum Zeitpunkt der Veröffentlichung. Eine Katze verstarb an einer vermutlich nicht mit der FIP assoziierten Herzproblematik. Milde Nebenwirkungen, wie vorübergehende lokale Hautreaktionen, wurden bei 16/26 Katzen beobachtet. Die Reaktionen traten in den ersten 4 Wochen der Behandlung auf. Eine Katze entwickelte im Therapieverlauf erhöhte Konzentrationen an Harnstoff und SDMA. Nach Absetzen der Therapie normalisierten sich diese beiden Werte [88].

In einer Fallserie wurden 4 Katzen mit neurologischen und/oder okulären Symptomen der FIP mit GS-441524 (5 – 10 mg/kg, q24h, SC, für 98 – 133 Tage) behandelt. Alle 4 Katzen zeigten ein Ansprechen auf die Therapie. 3/4 Katzen lebten zur Zeit der Veröffentlichung der Studie jeweils 528, 516 und 354 Tage nach Therapiestart ohne klinische Symptome. Eine Katze wurde nach 216 Tagen nach zweimaliger Behandlung mit GS-441524 aufgrund von wiederholten neurologischen Rückfällen euthanasiert. Der Therapieerfolg wurde per Magnetresonanztomographie, Liquor-Analyse und Augen-Bildgebung (mittels Fourier-domain optical coherence tomography sowie *in vivo* confocal microscopy) überprüft. Auch die Behandlung neurologischer und/oder okulärer Symptome der FIP scheint erfolgreich zu sein, wenn höhere Dosierungen gegeben werden [89].

Eine Studie an der Medizinischen Kleintierklinik der LMU München untersuchte ein chinesisches Präparat namens Xraphconn® des Herstellers Mutian (Mutian Life Sciences Limited, Nantong, China) zur oralen Therapie der FIP. Neben pflanzlichen Inhaltstoffen sollten die Tabletten laut Hersteller eine GS-441524-ähnliche Substanz (MT-0901) enthalten. Das Medikament wurde mittels Massenspektrometrie und Kernspinresonanzspektroskopie analysiert; als aktive Substanz konnte schließlich GS-44152 identifiziert werden. Die Studie untersuchte die Effektivität von oral verfügbarem GS-441524. In Zellkultur konnte eine hervorragende Effektivität von GS-441524 nachgewiesen werden. Für den Nachweis der Effektivität des Medikamentes (5 – 10 mg/kg, q24h, PO, für 84 Tage) *in vivo* wurden 18 Katzen

mit diagnostizierter FIP (Diagnosestellung: Vorbericht, typische klinische und labordiagnostische Parameter und positive Mutations-PCR und/oder IHC), eingeschlossen. Katzen ohne neurologischen und/oder okulären Symptomen erhielten laut Herstellerinformationen 5 mg/kg der aktiven Substanz. Katzen mit neurologischen und oder/okulären erhielten die doppelte Dosis. Neueste Analysen des Medikamentes deuten jedoch darauf hin, dass eine Tablette Xraphconn® mehr GS-441524 enthält als vom Hersteller offiziell angegeben (persönliche Kommunikation J. Horak). In den ersten 7 Tagen der Studie wurden die Katzen stationär aufgenommen und intensivmedizinisch betreut. Die Katzen erhielten keine standardisierte Begleittherapie, sondern wurden individuell je nach auftretender Symptomatik unterstützend therapiert (z. B. Infusionstherapie, antiemetische, fiebersenkende Therapie). Anschließend wurden die Katzen in die häusliche Pflege übergeben und zu 4 weiteren Kontrolluntersuchungen (Tag 14, 28, 56, 83) vorgestellt. Das Allgemeinbefinden sowie die klinischen und labordiagnostischen Parameter verbesserten sich rapide (je Parameter bereits ab Tag 1 – 28 signifikanter Unterschied zu Tag 0). Die Ergussmenge und Viruslast im Erguss nahmen stetig ab. Auch neurologische Symptome verbesserten sich kontinuierlich. Die Therapie mit GS-441524 verursachte nur milde Nebenwirkungen. Eine Katze entwickelte am letzten Tag der Therapie eine moderate Heinz-Body-Anämie. Diese Katze wurde mit S-Adenosyl-Methionin behandelt und die Erythrozytenzahl normalisierte sich; und die Heinz-Bodies verschwanden. 11/18 Katzen zeigten unter Therapie erhöhte Leberwertenzymaktivitäten, 2/11 Katzen mit deutlicher Erhöhung (ALT >350 IU/l, AP >350 IU/l). Diese Katzen wurden mit Silymarin, einem Mariendistel-Extrakt, therapiert und die Leberenzymaktivitäten normalisierten sich. 14/18 Katzen zeigten eine Lymphozytose, möglicherweise als Regeneration der oftmals bei Katzen mit FIP auftretenden Lymphopenie. 11/18 Katzen hatten eine Eosinophilie, vermutlich im Sinne einer Heilung [90]. Alle 18 Katzen schlossen den Behandlungszyklus über 84 Tage erfolgreich ab und waren zum Zeitpunkt der Veröffentlichung klinisch gesund. Diese Studie konnte eine Überlebensrate von 100 % erzielen, die einerseits auf die hohe Wirksamkeit des Medikamentes, aber auch auf eine zu Beginn stattfindende intensivmedizinische Betreuung zurückzuführen ist [83].

In der soeben beschriebenen Studie verstarb eine der behandelten Katzen 164 Tage nach Ende der 84-tägigen Behandlung bei einem Autounfall und konnte obduziert werden. Die postmortalen Untersuchungen (Obduktion, Histopathologie, IHC, quantitative FCoV-RT-PCR) zeigten, dass die orale Behandlung mit GS-441524 zur vollständigen Heilung der FIP-assoziierten Veränderungen und zur vollständigen Elimination von FCoV (mittels IHC und quantitativer RT-PCR) aus allen Geweben geführt hatte [91]. Die übrigen Katzen der Studie wurden nach Ende der Therapie weiterhin alle 12 Wochen zu Follow-up Untersuchungen vorgestellt. Die Behandlung mit GS-441524 erwies sich als wirksam und die Katzen waren

innerhalb des Beobachtungszeitraumes von einem Jahr Rückfallfrei. Zwei Katzen entwickelten leichte neurologische Symptome (vereinbar mit einem felinen Hyperästhesie-Syndrom). Ob milde neurologische Erscheinungen eine Nebenwirkung der GS-441524-Therapie sein könnten oder mit einem "long-FIP-Syndrom" verbunden sind, muss noch weiter untersucht werden [92].

Eine Studie untersuchte die Wirksamkeit von GS-441524 in Kombination mit GC376. In die Studie eingeschlossen wurden 46 Katzen mit FIP-Verdacht (Diagnosestellung: FCoV-qPCR in Erguss und Lymphknotenaspiraten) mit Erguss (36 Katzen) und ohne Erguss (10 Katzen). Die Katzen wurden über einen Zeitraum von 28 Tagen therapiert (Gruppe 1: GS-441524 5 mg/kg, q24h, SC und GC376 20 mg/kg, q12 h, SC; Gruppe 2: GS-441524 2,5 mg/kg, q24h, SC und GC376 20 mg/kg, q12 h, SC; Gruppe 3: GS-441524 2,5 mg/kg, q24h, SC und GC376 10 mg/kg, q12 h, SC; Gruppe 4: GS-441524 5 mg/kg, q24h, SC und GC376 10 mg/kg, q12 h, SC). 45/46 Katzen überlebten; 43/46 Katzen waren nach dem 28-tägigen Therapiezyklus klinisch gesund; 2/46 Katzen erst nach einem längeren Therapiezeitraum (49 – 84 Tage). Zehn Monate später, zum Zeitpunkt der Publikation, waren alle 45 Katzen symptomfrei und am Leben [93].

3.7.4. Therapie der FCoV-Ausscheidung

In einer Studie wurde untersucht, ob durch die Therapie mit GS-441524 die fäkale Ausscheidung von FCoV zu stoppen ist. 29 FCoV-ausscheidende Katzen wurden über 7 Tage mit GS-441524 (2,0 – 4,0 mg/kg, q24h, PO) behandelt. Am Ende der 7 Tage schieden 21/22 Katzen, die GS-441524 in einer Dosis von 4 mg/kg erhielten, kein FCoV mehr aus. Ein großer Nachteil der Studie ist, dass nicht bei allen Katzen Follow-up-Kotuntersuchungen durchgeführt wurden. Eine Katze zeigte innerhalb von 9 Tagen eine Reinfektion [94]. Für die sichere Detektion von fehlender FCoV-Ausscheidung sind Untersuchungen mehrerer Kotproben (mindestens 3) in ausreichenden Intervallen (1 Woche – 1 Monat) erforderlich [95]. Eine höhere Reinfektionsrate ist anzunehmen. Zudem wurden Reinfektionen bei Katzen mit FIP nach erfolgreicher Elimination unter Therapie bereits beschrieben [96]. Während der antiviralen Behandlung mit GS-441524 nahm die fäkale FCoV-Ausscheidung der Studienkatzen der Studie an der Medizinischen Kleintierklinik der LMU München deutlich ab; die meisten Katzen (17/18; 94 %) schieden bereits ab Tag 4 der Behandlung kein FCoV mehr über den Kot aus. Bei einer Katze wurde 83 Tage nach Beginn der Behandlung (und 80 Tage nach FCoV-Ausscheidungsstopp) eine erneute Ausscheidung festgestellt. Eine Reinfektion durch die Partnerkatze ist wahrscheinlich. Bei 2 Katzen wurde am Tag 0 keine Ausscheidung festgestellt, 2/3 Katzen schieden erst nach Ende des stationären Aufenthaltes FCoV über den Kot aus. Diese beiden Katzen lebten mit Partnerkatzen zusammen [96]. Der Status des Fehlens einer FCoV-Ausscheidung kann also nur kurzfristig erreicht werden. Daher ist eine

Gabe von GS-441524 bei Katzen ohne FIP kontraindiziert und nicht zu empfehlen. Eine prophylaktische Gabe kann zu viralen Resistenzen führen, wie es für den Protease-Inhibitor GC376 bereits beschrieben wurde [69,70]. Nur Katzen mit nachgewiesener FIP (Diagnosestellung: Anamnese, Klinik, Labor, IHC oder RT-PCR) sollten die neue Therapie erhalten. Ziel in einem Multikatzenhaushalt sollte weiterhin sein, den FCoV-Infektionsdruck und das Übertragungsrisiko (durch z. B. gründliche Hygiene, ausreichende Anzahl an Katzentoiletten, wenn möglich Freigang) zu senken [17].

Schlussfolgerung und Ausblick

Das wirksamste Medikament zur Therapie der FIP ist ohne Zweifel GS-441524 und daher Mittel der Wahl. Leider ist das Medikament in Deutschland nicht zugelassen und über den Schwarzmarkt erhältliche, nicht zugelassene und unkontrolliert hergestellte Präparate dürfen von Tierärzten nicht angewendet werden. Dies veranlasst Katzenbesitzer dazu, das Medikament ohne tierärztliche Unterstützung anzuwenden [84]. Die illegal angebotenen Medikamente werden in nicht-kontrollierten Produktionen hergestellt, und es besteht keine Garantie für die Zusammensetzung (Reinheit und Konzentration des Wirkstoffes). Die Zulassung von GS-441524 wäre dringend erforderlich, um eine legale Therapie dieser tödlichen Krankheit unter veterinärmedizinischer Obhut mit entsprechenden Kontrolluntersuchungen zur Verlaufskontrolle zu ermöglichen. Auch wäre somit die dringend notwendige intensivmedizinische Betreuung in den ersten Tagen der Behandlung gesichert. Regelmäßige Tierarztbesuche sind zudem wichtig für die Kontrolle des Therapieerfolges und Detektion potentieller Nebenwirkungen und um eine unterstützende symptomatische Behandlung an die Bedürfnisse der einzelnen Katzen anzupassen. Grundsätzlich sollte vor Therapiestart mit einem antiviralen Medikament eine gesicherte Diagnose der Erkrankung FIP vorliegen. Die Behandlung von Katzen mit FIP sollte nicht allein den Besitzern überlassen werden. Aktuell gibt es für Tierärzte nur wenige legale Optionen, um FIP zu therapieren. (1) Eine Therapie mit umgewidmetem Remdesivir ist legal möglich, aber unter den derzeitigen Bedingungen extrem teuer. (2) Eine Überweisung der Katze zu einem Tierarzt nach Großbritannien ist in den meisten Fällen unrealistisch. (3) Eine Teilnahme an einer wissenschaftlichen Therapiestudie. Um die nicht absehbare Zeit bis zur Zulassung von GS-441524 oder von GC376 zu überbrücken, müssen Therapiestudien in Deutschland genehmigt werden um den an FIP erkrankten Katzen eine legale und tierärztlich kontrollierte Therapie zu ermöglichen.

Interessenskonflikt

Es besteht keinerlei Interessenskonflikt. Dieser Artikel wurde ohne finanzielle Unterstützung der Autoren seitens einer im Manuskript erwähnten Firma erstellt.

Zusatzmaterial Tabelle 1: Optionen zur Therapie der felinen infektiösen Peritonitis

Supplementary Table 1: Options for the treatment of feline infectious peritonitis

Wirkstoff	Präparat	Wirk-mechanismus	Dosierung	Studien						Einschätzung der Wirksamkeit aus Sicht der Autoren
				Autoren	Art der Studie	Anzahl der Katzen	Diagnose	Neben-wirkungen	Outcome	
symptomatisch										
Prednisolon/ Dexamethason	verschiedene tiermedizinische Präparate erhältlich	immunsuppressiv	bei Erguss: Dexamethason IT/IP: 1 mg/kg, q24h, für 7 Tage (oder bis kein Erguss mehr vorhanden), anschließend 2 – 4 mg/kg, q24h, PO, dann Dosisreduktion ohne Erguss: 2 mg/kg, q24h, PO, dann Dosisreduktion	keine kontrollierten Studien						keine Heilung, kurzzeitige Verbesserung der klinischen Symptome bei wenigen Katzen
Chlorambucil	Leukeran®[1] (Aspen Germany, GmbH, München, Deutschland)	immunsuppressiv	keine veröffentlichten Studien							keine Heilung
Cyclophosphamid	Endoxan®[1] (Baxter Oncology GmbH, Halle, Deutschland)									
Meloxicam	Metacam®[3] (Boehringer Ingelheim, Ingelheim, Deutschland)	antiinflammatorisch	0,05 mg/kg, q24h, PO	Hugo et al., 2015 [29]	Fallbericht	1	IHC	k. A.	Überlebenszeit bis 787 Tage nach Erstvorstellung	weitere Studien notwendig
Ozagrel-hydrochlorid	DOMENAN®[2] (Kissei Pharmaceutical, Matsumoto, Japan)	Thromboxan-Synthetase-Hemmer Reduktion Thrombozyten-aggregation und Zytokinfreisetzung	5 – 10 mg/kg, q12h, PO, für 270 – 360 Tage	Watari et al., 1998 [31]	Fallserie	2	keine definitive Diagnose	Epistaxis	Überlebenszeit zwischen 3 und 6 Monate nach Ende der Therapie, Rezidiv bei 1/2 Katzen	nicht zu empfehlen

Propentofyllin	Karsivan®[3] (MSD Tiergesundheit; Unterschleißheim, Deutschland)	Reduktion der TNF-α-Synthese, Vaskulitis und Fibrinogen-synthese	18 – 25 mg/kg, q12h, PO, für 7 Tage	Fischer et al., 2011 [28]	placebo-kontrollierte Doppelblind-studie	23 (davon 7)	Histologie, IHC	k. A.	keine Wirksamkeit, kein signifikanter Unterschied in Überlebenszeit (median 8 Tage) oder Lebensqualität	nicht zu empfehlen
immunmodulatorisch										
Polyprenyl-Immunstimulanz	Polyprenyl Immunstimulant™[4] (VetIMMUNE, SASS & SASS, Inc., Oak Ridge, Tennessee, USA)	immun-modulatorisch	1 – 3 mg/kg, q12h oder q84h, PO oder SC, für 150 bis > 810 Tage	Legendre et al., 2009 [35]	Fallserie	3	2/3 Katzen IHC, 1/3 keine definitive Diagnose	k. A.	Überlebenszeit 14 Monate – 2 Jahre nach Diagnosestellung	keine Heilung, aber Verlängerung der Überlebenszeit
			3 mg/kg, q56h, PO zur Dauertherapie	Legendre et al., 2017 [36]	prospektive Studie	60	keine definitive Diagnose	k. A.	Überlebenszeit 100 – 1829 Tage nach Therapiestart	
Propionibacterium acnes	veterinär-medizinisches Präparat nicht mehr erhältlich	immun-modulatorisch	0,4 – 4 mg/Katze, q84h, IP oder IV, für 14 Tage, anschließend 1x pro Woche für 21 – 28 Tage	Weiss et al., 1990 [38]	experimentelle placebo-kontrollierte Studie	74 (davon 18)	experimentell induzierte FIP	k. A.	bei alleiniger Verabreichung keine Wirksamkeit	nicht zu empfehlen
rekombinantes humanes Interferon-α	nur für Forschungszwecke	immun-modulatorisch + antiviral	10^4/10^6 IU/kg, q24h, IM, für 8 Tage	Weiss et al., 1990 [38]	experimentelle placebo-kontrollierte Studie	74 (davon 29)	experimentell induzierte FIP	k. A.	in Kombination mit *Propionibacterium acnes*: signifikant längere Überlebenszeit (1 - 3 Wochen)	nicht zu empfehlen
felines Interferon-ω	Virbagen® Omega[3] (Omega, Virbac, Bad Oldesloe, Deutschland)	immun-modulatorisch + antiviral	10^6 U/kg, q24h, SC, bis zur Remission, dann 1x pro Woche	Ishida et al., 2004 [41]	prospektive Studie	12	keine definitive Diagnose	k. A.	Überlebenszeit 1 Monat bis > 2 Jahre	keine Wirksamkeit
			10^6 U/kg (0,1 ml/kg), q24h, SC, für 8 Tage, dann 1x pro Woche	Ritz et al., 2007 [2]	placebo-kontrollierte Doppelblind-studie	37 (davon 21)	IFA	k. A.	keine Wirksamkeit, kein signifikanter Unterschied Überlebenszeit (median 9 Tage) oder Lebensqualität	
antiviral										
Ribavirin	Ribavirin-ratiopharm®[1] (ratiopharm GmbH, Ulm, Deutschland)	Nukleosid-Analogon	16,5 mg/kg, q24h, PO, IM oder IV, für 10 – 14 Tage	Weiss et al., 1993 [46]	experimentelle placebo-kontrollierte Studie	50 (davon 24)	experimentell induzierte FIP	hämorrhagische Läsionen in GIT-Trakt, Gehirn, Leber, Lunge, Herz, Diaphragma und Subkutis	keine Wirksamkeit; schwerere klinische Symptome als Kontrollgruppe	kontraindiziert

Mefloquin	Lariam® [2] (Roche, Millers Point, NSW, Australien)	Hemmung des zytopathischen Effektes (Antiparasitikum)	20 – 25 mg, q24h, PO für 84 Tage	Mejias et al. 2021	keine klinischen Studien					*in vitro* wirksam, evtl. weiterführende Therapieoption nach Start mit einem Nukleosid-Analogon, kontrollierte Studien notwendig
Itraconazol	Itrafungol® [3] (Virbac, Bad Oldesloe, Deutschland)	Inhibitor der Cholesterin-synthese und des Cholesterin-transports (Antimykotikum)	50 mg/Katze, q24h, PO, für 30 Tage	Doki et al., 2020 [56]	experimentelle Studie	3	experimentell induzierte FIP	k. A.	Besserung der Klinik, 1/3 euthanasiert	möglicherweise zur Kombinations-therapie mit anderen antiviralen Medikamenten geeignet, kontrollierte Studien notwendig
			10 mg/kg, q12h, PO für 38 Tage; (in Kombination mit Prednisolon)	Kameshima et al., 2020 [57]	Fallbericht	1	PCR	k. A.	Euthanasie nach 38 Tagen	
Molnupiravir	Lagevrio® [2] (Merck Sharp & Dohme Limited, London, UK)	Nukleosid-Analogon	12,8 – 14,7 mg/kg, q12h, PO für 84 Tage	Roy et al., 2022 [62]	retrospektive Studie	26	keine definitive Diagnose	Dosis > 23 mg/kg: gefaltete Ohren, abgebrochene Schnurrhaare, hgr. Leukopenie	24/26 in Remission zum Zeitpunkt der Veröffentlichung	vielversprechende Wirksamkeit, kontrollierte Studien notwendig
GC376	in Deutschland nicht zugelassen (wird derzeit von ANVIVE, Long Beach, California, USA, entwickelt; noch keine Zulassung)	Proteaseinhibitor	5 – 10 mg/kg, q24, SC, für 14 – 20 Tage	Kim et al., 2016 [68]	experimentelle Studie	8	experimentell induzierte FIP	k. A.	Überlebenszeit bei 6/8 Katzen bis 8 Monaten, 2/8 Katzen euthanasiert	bewiesene Wirksamkeit, weitere Studien notwendig
			15 mg/kg, q12h, SC für 14 – 84 Tage	Pedersen et al., 2018 [65]	prospektive Studie	20	Signalement, Anamnese, klinische und labor-diagnostische Veränderungen, PCR	lokale Irritationen an den Einstichstellen, verzögertes Wachstum und Durchbrechen der permanenten Zähne	13/20 Katzen Rückfall (innerhalb von 1 – 7 Wochen der Erst- oder Wiederholungs-behandlung/en)	
Remdesivir	Veklury® [1] (GILEAD Sciences Ireland UC, Carrigtohill, Irland)	Nukleosid-Analogon	4,9 – 5,6 mg/kg, q24h, IV und SC für 80 Tage	Bohm et al., 2022 [74]	Fallbericht	1	IHC	k. A.	in Remission zum Zeitpunkt der Veröffentlichung	Hinweise, dass Remdesivir für die Therapie der FIP geeignet ist, kontrollierte Studien notwendig
			10 – 20 mg/kg, q24h, IV für 3 – 4 Tage, Weiterführung SC bis Tag 7 – 14, anschließend Therapie mit GS-441524 (10 – 20 mg/kg, q12-24h, PO)	Mejias et al., 2022 [50]	Erfahrungs-bericht	k. A.	k. A.	Anstieg der ALT-Enzymaktivität	k. A.	

			10 – 15 mg/kg, q24h, SC oder IV, für 4 Tage, anschließend 6 – 15 mg/kg, q24h, SC oder Umstellung auf GS-441524 PO für 84 Tage	Coggings et al., 2022 [76]	Fallsammlung	26	nicht bekannt	Reizungen an den Injektionsstellen	22/26 überlebten 6 Monate nach Start der Therapie, 3/26 starben innerhalb von 48 Stunden, 3/26 wurden aufgrund eines Rückfalls der Symptome erfolgreich erneut therapiert	
			10 – 20 mg/kg, q24h, IV, für 2 – 9 Tage, Weiterführung der Therapie mit GS-441524	Green et al., 2022 [77]	retrospektive Studie	25	klinische und labor-diagnostische Veränderungen, Bildgebung, Zytologie, IHC	Reizungen an den Injektionsstellen	Studie noch nicht abgeschlossen	
GS-441524	in Deutschland nicht zugelassen in Großbritannien und Australien über Apotheke BOVA (BOVA Specials UK Ltd, London, UK) beziehbar	Nukleosid-Analogon	2 – 5 mg/kg, q24h, SC, für 14 Tage	Murphy et al., 2018 [80]	experimentelle Studie	10	experimentell induzierte FIP	Reizungen an den Injektionsstellen	2/10 Rückfall (4 – 6 Wochen nach Therapieende), anschließend erneuter Therapiezyklus, 10/10 in Remission > 8 Monate nach Infektion	hervorragende Wirksamkeit, Mittel der Wahl zur Therapie der FIP
			2 – 4 mg/kg, q24h, SC, für 84 Tage	Pedersen et al., 2019 [88]	prospektive Studie	31	Signalement, Anamnese, klinische und labor-diagnostische Veränderungen, Ergussanalyse, PCR, IHC	1/31 Katzen: erhöhte Konzentrationen an Harnstoff und SDMA, Reizungen an den Injektionsstellen	8/31 Rückfall (Ø 23 Tage nach Therapieende), 24/31 in Remission (zum Zeitpunkt der Veröffentlichung, 1/31 Euthanasie aufgrund Herzerkrankung	
			8 mg/kg, q24h, PO für 50 Tage	Addie et al., 2020 [42]	Fallbericht	1	PCR	SDMA-Erhöhung	Katze in Remission (6 Monate nach Diagnosestellung)	
			5 – 10 mg/kg, q24h, SC, für 98 – 133 Tage	Dickinson et al., 2020 [89]	Fallserie	4	keine definitive Diagnose	k. A.	3/4 geheilt, Beobachtungszeit zw. 354 – 528 Tage, 1/4 nach 216 Tagen (nach zweimaliger Behandlung mit GS-44152) euthanasiert	
			GS-441524: 2 – 4 mg/kg, q24h, für 28 Tage;	Yin et al., 2021 [85]	retrospektive Studie	30	keine definitive Diagnose	k. A.	29/30 in Remission, 1/30 Rückfall und Euthanasie	

			oder GC376: 6 – 8 mg/kg, q24h Darreichungsform nicht bekannt							
			5 mg/kg, q24h, PO oder SC für 84 Tage	Katayama et al., 2021 [86]	retrospektive Studie	141	PCR	k. A.	116/141 in Remission	
			2 – 16 mg/kg, SC für 84 Tage	Jones et al. 2021 [84]	retrospektiv (Umfrage)	393	keine definitive Diagnose	Reizungen an den Injektionsstellen	380/393 in Remission zum Zeitpunkt der Umfrage	
			5 – 10 mg/kg, q24h, PO, für 84 Tage	Krentz et al., 2021 [83]	prospektive Studie	18	PCR/IHC	ggr. NW: 1/18 Heinzbody-Anämie, 11/18 Eosinophilie, 11/18 Erhöhung der Leberenzym-aktivitäten	18/18 in Remission zum Zeitpunkt der Veröffentlichung	
			5 – 10 mg/kg, q24h, PO, für 84 Tage	Krentz et al., 2022 [91]	Fallbericht	1	IHC	ggr. NW	Unfalltod nach 164 Tagen nach Ende der Therapie: Katze zu 100 % virusfrei	
			unterschiedliche Dosierungen, q24h, IV oder PO, für 45 – 184 Tage	Addie et al., 2022 [87]	retrospektive Studie	15	Histopathologie, IHC, RT-PCR	k. A.	Überlebenszeit zwischen > 6 Monate und > 2 Jahre zum Zeitpunkt der Veröffentlichung	
			2,5 – 5 mg/kg, q24h, SC für 28 Tage (in Kombination mit GC376 10 – 20 mg/kg, q12h, SC)	Lv et al., 2022 [93]	prospektive Studie	46	keine definitive Diagnose	Schmerzen bei der Injektion	45/46 in Remission, 2/46 mussten länger therapiert werden (7 – 12 Wochen), Überlebenszeit 10 Monate zum Zeitpunkt der Publikation	

[1] humanmedizinisches Präparat, in Deutschland zugelassen

[2] humanmedizinisches Präparat, im Ausland zugelassen

[3] tiermedizinisches Präparat, in Deutschland zugelassen

[4] tiermedizinisches Präparat, im Ausland zugelassen

IT = intrathorakal, IP = intraperitoneal; PO = per os; SC = subkutan; IV = intravenös; qXh = alle X Stunden; IHC = Immunhistochemie; PCR = Polymerasekettenreaktion; IFA = Immunfluoresenz-Assay; k. A. = keine Angabe; GIT =Gastrointestinal-Trakt; ALT = Alaninamino-Transferase; hgr. = hochgradig; ggr. = geringgradig; NW = Nebenwirkung; SDMA = symmetrisches Dimethylarginin

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III. PUBLIKATION 2: ORIGINAL-PUBLIKATION I

Curing cats with feline infectious peritonitis with an oral multi-component drug containing GS-441524

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Article

Curing Cats with Feline Infectious Peritonitis with an Oral Multi-Component Drug Containing GS-441524

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Abstract: Feline infectious peritonitis (FIP) caused by feline coronavirus (FCoV) is a common dis-ease in cats, fatal if untreated, and no effective treatment is currently legally available. The aim of this study was to evaluate efficacy and toxicity of the multi-component drug Xraphconn® in vitro and as oral treatment in cats with spontaneous FIP by examining survival rate, development of clinical and laboratory parameters, viral loads, anti-FCoV antibodies, and adverse effects. Mass spectrometry and nuclear magnetic resonance identified GS-441524 as an active component of Xraphconn®. Eighteen cats with FIP were prospectively followed up while being treated orally for 84 days. Values of key parameters on each examination day were compared to values before treatment initiation using linear mixed-effect models. Xraphconn® displayed high virucidal activity in cell culture. All cats recovered with dramatic improvement of clinical and laboratory parameters and massive reduction in viral loads within the first few days of treatment without serious adverse effects. Oral treatment with Xraphconn® containing GS-441524 was highly effective for FIP without causing serious adverse effects. This drug is an excellent option for the oral treatment of FIP and should be trialed as potential effective treatment option for other severe coronavirus-associated diseases across species.

Keywords: FIP; feline coronavirus; FCoV; treatment; therapy; antiviral chemotherapy; Xraphconn®; GS-441524; Mutian

1. Introduction

Coronaviruses comprise a large family of RNA viruses that infect a wide variety of mammalian and avian hosts, causing severe disease in some of them [1–3]. Their high diversity causes the continuous emergence of new coronavirus variants that can have changed target cell tropism and/or host spectrum and lead to interspecies or zoonotic transmission; recent examples of the latter are Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and the most recent Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causing Coronavirus Infectious Disease 2019 (COVID-19) [4–7]. The newly emerging coronavirus-associated diseases raise awareness of the potential risks of highly virulent coronavirus infections in humans with close contact to animals harboring coronaviruses [8]. Shifts in tissue or cell tropism resulting in changes in virulence are distinctive features of coronaviruses. The best prototypic example for such a virulence change is the feline coronavirus (FCoV), which occurs as two different biotypes [9–12]. Only a small proportion (7–14% in multi-cat environments) of cats infected with FCoV, which is very common among multi-cat populations, develops the fatal disease feline infectious peritonitis (FIP) [13], triggered by spontaneous mutation of FCoV, thus gaining tropism for monocytes/macrophages in individual cats [14–19]. All cats with FIP either die or have to be euthanized without availability of effective treatment. The median survival time of untreated cats is only eight to nine days [9,20]. Previous controlled treatment trials with antiviral compounds either showed limited efficacy, such as the use of feline interferon-omega [9], or were too toxic for cats, such as ribavirin [21–23]. In most countries, such as in Europe and the United States, there is currently no effective licensed treatment option for cats with FIP, although unlicensed compounds have been imported and used by cat owners. It is important that veterinarians consult their local regulations and laws before embarking on the use of any unlicensed antiviral agents for the treatment of FIP due to variation in laws governing the veterinary profession and veterinary medicines worldwide.

However, a few studies have been published recently in which compounds not yet licensed showed extraordinarily promising results [24–27]. The most recent promising treatment for cats with FIP is the use of the nucleoside analogue GS-441524 [24,25,28], which is the active form of the prodrug remdesivir [29]. Currently, remdesivir is only conditionally licensed to treat human patients with severe COVID-19 symptoms [30–32]. Cats in these prospective studies received GS-441524 subcutaneously for various time periods and owners were instructed by social media groups to inject the compounds likewise for a period of 12 weeks or longer. Subcutaneous injections over such a long time period are difficult to perform by owners, can be very painful for cats due to the low pH of these unlicensed and uncontrolled preparations, and moreover could be associated with feline injection site sarcomas (FISS) [33]. Thus, oral compounds would be advantageous. Indeed, one case of successful treatment of a single cat with FIP (showing ocular signs but no effusion) with an oral preparation called Mutian (Mutian 200, Nantong Biotechnology, Nantong, China) has been reported [26]. Mutian was administered orally every 24 h (q24h) for 50 days and the cat received additional symptomatic treatment as well as feline interferon-omega. Within a few weeks of treatment, the cat showed marked gain in body weight and improvement of ocular signs as well as hematological and biochemical parameters. In two recent retrospective studies, cats were also treated with oral antivirals. In one, a large-scale online survey was addressed to cat owners who treated their cats with suspected FIP with an antiviral drug to collect their experiences, e.g., improvement of symptoms, potential relapses, and supportive therapy during the treatment [34]. In the other retrospective study from China, the disease course of 127 cats with suspected FIP was followed up; of these, 24 were treated with GS-441524 (although the route of administration was not mentioned) and only one cat relapsed [35]. However, to the authors' knowledge, there are no published prospective studies on the oral treatment of cats with highly suspected or confirmed FIP with antiviral compounds. If the efficacy of an oral treatment in cats with a deadly disease like FIP could be consistently proven, this could

also open up new avenues for future research investigating its effect in other severe coronavirus-associated diseases, such as COVID-19. Furthermore, due to its similarities of clinical features, the FIP-cat model could serve as natural model for elucidating pathophysiological features and potential treatment approaches in severe systemic inflammatory coronavirus-associated syndromes [6].

Therefore, the aim of this study was to prospectively evaluate the efficacy of an oral multi-component drug, first in vitro and then as oral treatment, in cats with naturally occurring FIP in a clinical trial, by determining survival rate, improvement of clinical and laboratory parameters, reduction of viral loads, and measurement of anti-FCoV antibodies. Additionally, potential adverse effects were recorded and the chemical structure of the active ingredient within the multi-component drug was identified by means of structural chemistry analysis.

2. Materials and Methods

2.1. Compound

The compound Xraphconn® was used for the in vitro study as well as in the cats. It was provided by Mutian Life Sciences Limited in 2 batches (Supplementary File S1). According to the tablet insert, the active ingredient (referred to as MT-0901 in the package insert) was available at a concentration of 2.5 mg or 10 mg tablet, respectively. Other ingredients according to the tablet inserts were, among other substances, Radix scrophulariae, Platycodon grandiflorum, Phyllostachys pubescens, Forsythia suspensa, and Anemarrhena asphodeloides.

2.2. In Vitro Efficacy of the Multi-Component Drug

Efficacy of Xraphconn® was first evaluated in an established cell culture model. Crandell-Rees Feline Kidney (CRFK) cells (Collection of Cell Lines in Veterinary Medicine CCLV, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany) were grown and maintained in Eagle's minimal essential medium (MEM; Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum (FCS; Biochrom) and kept at 37 ± 1 °C under a 5% CO_2 atmosphere. Feline coronavirus RVB-1259 (a serotype I FCoV strain) was propagated once in CRFK cells in MEM as described above.

For the application on cells, a tablet (50 mg) said to contian 2.5 mg of the active ingredient was crushed using a mortar and 5 mL MEM or dimethyl sulfoxide (DMSO) was added. Suspensions were cleared by syringe filtration (Millex-GP syringe filter, pore size 0.22 µm). Subsequently, a logarithmic dilution was performed with both suspensions (1:10 up to 1:100,000), mathematically corresponding to an active ingredient concentration ranging from 50 µg/mL to 5 ng/mL at the assumption that the yield of extraction was 100%.

After exposure of the cells to the different dilutions of the filtrated tablet suspension, cell viability measurement using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay (Cell Proliferation Kit; Roche, Basel, Switzerland) was performed. Briefly, CRFK cells were seeded on a 96-well plate and different compound dilutions were added after 24 h. Cells were incubated for another 24 or 48 h, respectively. Subsequently, 10 µL MTT was added and cells were incubated for another 4 h. After incubation, solubilization solution was added and the enzymatic reaction was measured spectrophotometrically on the next day.

To evaluate the efficacy of Xraphconn® in vitro, CRFK overnight cell cultures were infected with FCoV at a multiplicity of infection (MOI) of 0.01. After infection, wells were incubated at 37 ± 1 °C under a 5% CO_2 atmosphere for 60 min and then washed with phosphate-buffered saline (PBS). Fresh culture medium (MEM supplemented with 5% FCS) containing different Xraphconn® dilution levels was added. Finally, supernatants were collected at 24 or 48 h post infection. All tests were carried out as quadruplicate measurements.

Total RNA was extracted from all supernatants using the Nucleo-MagVet kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions in an elution volume of 100 µL. Feline coronavirus RNA was detected by reverse transcriptase polymerase chain

reaction (RT-PCR) as described below. To calculate the half maximal effective concentration (EC_{50}), the viral RNA load for virus-infected non-treated cells was set to 100% and RNA values obtained for treated cells were normalized to this value; EC_{50}-values were calculated by a non-linear regression analysis using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA).

2.3. Patients of the Prospective In Vivo Study

This study complied with the German guidelines for prospective studies and was approved by the Government of Upper Bavaria (reference number 55.2-2532.Vet_02-20-52) and by the ethical committee (reference number 261-19-03-2021) of the Centre for Clinical Veterinary Medicine of the LMU Munich. In addition, owners of the cats also gave their written consent to participate. Twenty cats were originally enrolled in the study (Figure 1). Inclusion criteria were (1) diagnosis of FIP, (2) body weight of at least 2 kg, (3) negative test results for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infection, and (4) absence of other severe diseases. Cats were excluded if (1) they were in a severe moribund or comatose condition at the time of presentation (as in such condition oral medications cannot be expected to be reliably absorbed), (2) it was not possible to administer tablets to the cats, or (3) owners were not compliant to either medicate their cats properly or to attend appointments.

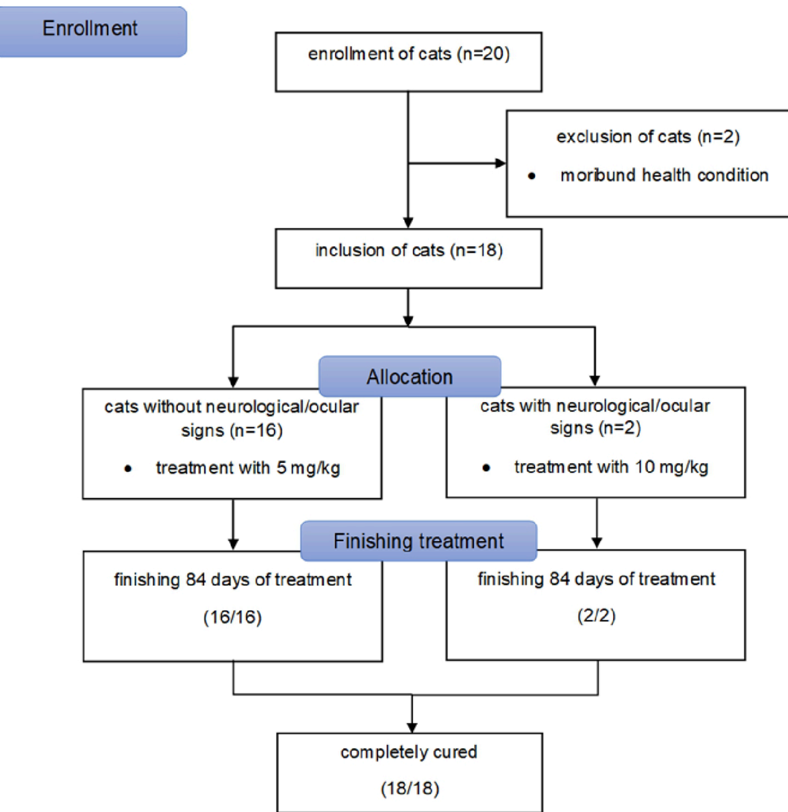

Figure 1. Flow diagram illustrating enrollment, inclusion, allocation process to high-dose treatment (10 mg/kg) for cats with neurological/ocular signs or low-dose treatment (5 mg/kg) for cats without neurological/ocular signs (based on the package inserts, Supplementary File S1), and outcome of cats in the study.

A diagnosis of FIP was made (Table 1) if (1) virus was detected directly, either by immunohistochemistry detecting FCoV antigen within macrophages of altered organs [36,37] and/or by detection of a mutated strain of FCoV in effusion, blood, or an organ fine needle aspirate by a commercial RT-PCR and analysis targeting FCoV spike gene mutations leading to spike protein substitutions M1058L and S1060A (IDEXX laboratories, Ludwigsburg, Germany) in combination with (2) clinical and clinicopathological abnormalities considered typical for FIP [38] being present in the cats. Body weight was measured using a baby scale (AE Adam MTB 20 baby scale, Felde, Germany). The weight limit was set at 2 kg to comply with guidelines for blood volume sampling in cats according to the Gesellschaft für Versuchstierkunde—Society of Laboratory Animal Science [39]. Presence of anti-FIV antibodies and FeLV antigen was tested by the referring veterinarian or at presentation with either a point-of-care test or at a commercial laboratory. Absence of other severe diseases was determined by obtaining a complete history and physical examination as well as by performing an abdominal ultrasound in all cats on the day of entering the study; in addition, echocardiography was performed in cats with a thoracic effusion and a full neurological examination was performed in cats with neurological signs. Presence of mild disease (such as intestinal parasite infestation, otitis externa, rhinitis, chronic gingivostomatitis) did not lead to exclusion (Table 1). One cat had mild renal azotemia at the time of inclusion (Table 1). It was reasonable to suspect that these renal changes were caused by FIP and therefore the cat was included in the study. Of the 20 cats that were originally enrolled into the study, two cats were subsequently excluded due to their severe moribund health condition (Figure 1). Thus, 18 cats finally entered the study. Cats were then allocated to either high-dose treatment (10 mg/kg) if they had neurological/ocular (n = 2) or low-dose treatment (5 mg/kg) if they had no neurological/ocular signs (n = 16).

The age of the 18 cats (Table 1) ranged between 4.7 and 56.5 months (median 7.7 months) and 15 cats (83.3%) were younger than one year. Most cats (11/18; 61.1%) were European Shorthair (ESH); the others belonged to different breeds. The majority of cats was male (12/18; 66.6%) with 5 of them neutered. Of the female cats (6/18; 33.3%), 3 were neutered.

2.4. *In Vivo Study Design*

Cats were treated orally with Xraphconn® for 84 days (day 0 to day 83). The dosage of the compound was chosen based on described studies with GS-441524 [24,28] and was based on the information of the tablet package insert on the concentration of the active component in the tablet. Thus, cats were treated with 5 mg/kg of the active component per os (PO) q24h if they had no neurological and/or ocular signs and at 10 mg/kg PO q24h if they had neurological and/or ocular signs. Dosage of Xraphconn® tablets was adjusted daily to the determined weight and tablets were always administered at the same time on an empty stomach. Half an hour after tablet administration, food was provided to the cats.

Table 1. Cats participating in the study, including signalment, number of additional cats in the household, method of diagnosis of feline infectious peritonitis (FIP), FIP-associated signs, Xraphconn® treatment dose, other diseases at the start of and developing during treatment, adverse effects, and additional symptomatic therapy.

Cat	Age (Months)	Sex	Breed	Additional Cats in the Household (Number)	Diagnosis of FIP [1]	FIP-Associated Cardinal Signs	Dosage (mg/kg q24h)	Adverse Effects and Duration (on Days of Treatment)	Other Unrelated Diseases before Treatment	Other Unrelated Diseases Developing during Treatment	Additional Symptomatic Therapy
cat 1	6.0	male neutered	ESH	yes (1)	immunohisto-chemistry (eye)	ocular signs	10 mg/kg	lymphocytosis (2–end[2])			fluid therapy [3], metamizole [4]
cat 2	6.3	male intact	ESH	yes (1)	immunohisto-chemistry (eye)	neurologic signs, ocular signs	10 mg/kg	lymphocytosis (0–end)	surgical wound infection after eye enucleation		fluid therapy, antibiotics [5], buprenorphine [6]
cat 3	9.8	male neutered	ESH	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	increased liver enzyme activity (4–14)		renal mineraliza-tion	fluid therapy, antibiotics, maropitant [7], mirtazapine [8]
cat 4	7.2	male intact	ESH	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (2–end), eosinophilia (14–56)			fluid therapy, metamizole-sodium, antibiotics, maropitant, mirtazapine
cat 5	6.4	female intact	ESH	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (0–end), increased liver enzyme activity (4–83), eosinophilia (14–end)			fluid therapy, antibiotics

Table 1. *Cont.*

Cat	Age (Months)	Sex	Breed	Additional Cats in the Household (Number)	Diagnosis of FIP [1]	FIP-Associated Cardinal Signs	Dosage (mg/kg q24h)	Adverse Effects and Duration (on Days of Treatment)	Other Unrelated Diseases before Treatment	Other Unrelated Diseases Developing during Treatment	Additional Symptomatic Therapy
cat 6	10.7	male neutered	ESH	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (83–end)			antibiotics, mirtazapine
cat 7	4.7	male intact	Siamese	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion, thoracic effusion	5 mg/kg	lymphocytosis (0–end), increased liver enzyme activity (4–83), eosinophilia (14–end)	chronic gingivo-stomatitis		fluid therapy, antibiotics, silymarin [9]
cat 8	6.4	male intact	Maine Coon	no	RT-PCR detecting S gene mutations (effusion)	thoracic effusion	5 mg/kg	lymphocytosis (0–14), eosinophilia (14–83)	chronic gingivo-stomatitis		fluid therapy, antibiotics, oxygen cage [10]
cat 9	8.9	male neutered	ESH	yes (3)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (28–end), increased liver enzyme activity (4–83), eosinophilia (28–end)			fluid therapy, antibiotics, mirtazapine, silymarin
cat 10	39.1	female neutered	ESH	yes (3)	immunohisto-chemistry (lymph node)	abdominal effusion	5 mg/kg	eosinophilia (0–end)	intestinal parasite infestation (*Giardia* spp., treated with fenbenda-zole)		

Table 1. *Cont.*

Cat	Age (Months)	Sex	Breed	Additional Cats in the Household (Number)	Diagnosis of FIP [1]	FIP-Associated Cardinal Signs	Dosage (mg/kg q24h)	Adverse Effects and Duration (on Days of Treatment)	Other Unrelated Diseases before Treatment	Other Unrelated Diseases Developing during Treatment	Additional Symptomatic Therapy
cat 11	56.5	female neutered	ESH	yes (3)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (0–14), increased liver enzyme activity (4–97)			fluid therapy, antibiotics
cat 12	11.7	male neutered	Birman	yes (1)	immunohisto-chemistry (lymphnode), RT-PCR detecting S gene mutations (effusion)	thoracic effusion	5 mg/kg	lymphocytosis (2–end), increased liver enzyme activity (28–56), eosinophilia (28–83)			fluid therapy, antibiotics, buprenorphine
cat 13	28.8	female intact	Maine Coon	yes (9)	RT-PCR detecting S gene mutations (effusion)	thoracic effusion	5 mg/kg	eosinophilia (28–end)	rhinitis		fluid therapy, antibiotics
cat 14	7.5	male intact	ESH	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg			intestinal parasite infestation (*Giardia* spp., treated with fenbendazole)	fluid therapy, antibiotics, maropitant, mirtazapine, buprenorphine, pregabalin [11]
cat 15	7.6	male intact	Maine Coon	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (2–56), eosinophilia (2–28)		distorsion on right forelimb	fluid therapy, antibiotics, buprenorphine, meloxicam [12]

Table 1. *Cont.*

Cat	Age (Months)	Sex	Breed	Additional Cats in the Household (Number)	Diagnosis of FIP [1]	FIP-Associated Cardinal Signs	Dosage (mg/kg q24h)	Adverse Effects and Duration (on Days of Treatment)	Other Unrelated Diseases before Treatment	Other Unrelated Diseases Developing during Treatment	Additional Symptomatic Therapy
cat 16	8.9	female neutered	BSH	yes (1)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (7–14), increased liver enzyme activity (2–14)	chronic gingivo-stomatitis		fluid therapy, mirtazapine
cat 17	7.7	male intact	Scottish Fold	yes (1)	RT-PCR detecting S gene mutations (effusion)	thoracic effusion	5 mg/kg	lymphocytosis (2–end), eosinophilia (28–end)	otitis externa	pyothorax	fluid therapy, antibiotics
cat 18	7.6	female intact	ESH	yes (2)	RT-PCR detecting S gene mutations (effusion)	abdominal effusion	5 mg/kg	lymphocytosis (0–end), eosinophilia (2–end)			fluid therapy, antibiotics

mg, milligram; kg, kilogram; q24h, every 24 h; ESH, European shorthair; RT-PCR, reverse transcription polymerase chain reaction; S, spike; BSH, British shorthair; spp., species. [1] diagnosis of FIP: FIP confirmed: positive IHC plus consistent histopathology; FIP very likely: positive RT-PCR and analysis for FCoV spike mutation. [2] end: until the end of the observation period. [3] fluid therapy with Ringer's lactate with potassium supplementation at 20 mval/L for dehydration at an individual dosage calculated by rehydration and maintenance needs. [4] metamizole 30 mg/kg intravenously (IV) for treatment of fever (body temperature > 40.5 °C) as a single injection. [5] antibiotics (i.e., amoxicillin/clavulanic acid 20 mg/kg q8h IV or per os (PO); trimethoprim sulfadiazin 20 mg/kg q12h IV or PO; marbofloxacin 2 mg/kg q24h IV; pradofloxacin 6 mg/kg q24h PO; ampicillin 12.5 mg/kg q8h IV for treatment of secondary suspected or proven bacterial infection (neutrophilia with left shift or continuously high body temperature). [6] buprenorphine 0.01 mg/kg q8h IV for treatment of pain. [7] maropitant 1 mg/kg q24h IV for treatment of gastrointestinal signs, such as vomiting and anorexia. [8] mirtazapine ointment q24h for appetite stimulation. [9] silymarin 20 mg/kg q12h PO for 10 days and then 20 mg/kg q24h PO for treatment of increased liver enzymes. [10] oxygen cage for support in cases of dyspnea in cats with massive thoracic effusion. [11] pregabalin 2 mg/kg q12h PO when buprenorphine was not sufficient to control pain. [12] meloxicam 0.1 mg/kg q24h PO for 1 day and then 0.05 mg/kg q24h PO for treatment of orthopedic pain.

All cats were hospitalized for the first 8 days (day 0 to day 7). During this time, cats were under intensive surveillance and medical care by board-certified specialists in internal medicine and intensive care for 24 h per day, and all diagnostic procedures, supportive measures, and symptomatic treatments were applied as necessary (Table 1). On day 0, before treatment with Xraphconn®, a history and complete physical examination as well as hematological and clinical chemistry parameters were obtained in each cat. Several parameters were evaluated during the first 8 days and at several time points during the rest of the treatment period (Table S1). This included physical examination (including measurement of body weight and determination of the modified Karnofsky's score (Table S2) (day 0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 56, 83), abdominal ultrasound (day 0, 4, 7, 14, 28, 56, 83), hematology and clinical chemistry (including symmetric dimethylarginine (SDMA) and serum amyloid A (SAA)) (day 0, 2, 4, 7, 14, 28, 56, 83), and measurement of blood viral load (day 0, 2, 4, 7, 14, 28, 56, 83), effusion viral load (in case of effusion accessible for drainage), and serum anti-FCoV antibodies (day 0, 7, 14, 28, 56, 83). As long as the cats had abdominal or thoracic effusions, abdominal or thoracic ultrasound and if possible abdominocentesis or thoracocentesis, respectively, were performed daily, and fluid was stored for determination of viral load.

The Karnofsky's score modified for cats by Hartmann and Kuffer (1998) was used to evaluate the general condition and well-being of the cats (Table S2) using a classification from 0% (dead) to 50% (normal health condition) [40,41]. Hematology was performed using an automatic analyzer (Cell-Dyn 3500, Abott Laboratories, IL, USA). Differential blood count was additionally performed manually on blood smears exposed to Haema Quick Staining/Diff-Quick staining if hematology parameters were abnormal. Serum biochemistry parameters were measured by an automatic analyzer (Hitachi 911, Roche, Grenzach-Wyhlen, Germany), SDMA was analyzed at IDEXX Diavet AG (Bäch, Switzerland) using a high-throughput immunoassay, and SAA was determined using a latex agglutination turbidimetric immunoassay reaction (LZ Test SAA, Eiken Chemical Co., Ltd., Tokyo, Japan) on a cobas® c 501 clinical chemistry analyzer (Roche Diagnostics AG, Rotkreuz, Switzerland).

Cats were discharged on day 7 (unless clinical condition required prolonged inpatient care), and owners continued daily administration of the study drug. Owners received personalized instructions, including videos, on how to administer the tablets. They were instructed to call in at any time in case of problems. For the remaining period, owners had to closely monitor their cats at home and remain in close contact with the study team. Cats were not allowed outdoors during the treatment period. However, 17/18 cats had between 1 and 9 partner cats (most of which were allowed to go outside) in the same household with close contact (Table 1). Owners had to keep a daily diary for recording their cats' clinical parameters and body weight to guarantee optimal drug dosing at all times.

2.5. FCoV Viral Load in Blood and Effusion

Feline coronavirus RNA load was determined on several days in blood and effusion samples (as long as present). Samples were stored at −80 °C and analyzed collectively by RT-quantitative PCR (RT-qPCR). Viral total nucleic acids (TNA) were extracted from 200 µL of effusion or 100 µL ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood using the MagNa Pure 96 (Roche Diagnostics AG, Rotkreuz, Switzerland) and the MagNA Pure 96 DNA and Viral NA SV Kit (Roche Diagnostics AG, Risch-Rotkreuz, Switzerland) according to the manufacturers' instructions with an elution volume of 100 µL. For all samples, the viral nucleic acid (NA) plasma external lysis SV 4.0 protocol was applied. For each batch of extractions, negative controls were run in parallel to check for cross-contamination.

A previously published RT-qPCR assay was used to detect the FCoV 7b gene [42]. The methods were adapted as follows: primer and probes [42] were used with the AgPath-ID™ One-step RT-PCR kit (Applied Biosystems, Rotkreuz, Switzerland). The mastermix consisted of 1X RT-PCR buffer, 1.0 µL Array Script reverse transcriptase and

AmpliTaq Gold DNA polymerase, 300 nM forward primer (FCoV2), 900 nM reverse primer (FCoV1), 300 nM probe (FCoVp), and nuclease-free H_2O was added to a final volume of 20 µL. All RT-qPCR assays were run with 5 µL of TNA in a final volume of 25 µL. Positive and negative controls were run in parallel using a ABI 7500 Fast instrument (Applied Biosystems). All samples were tested for absence of inhibition. A FCoV RNA standard curve was run in parallel to determine the viral RNA copy number.

2.6. Anti-FCoV Antibodies

Antibodies against FCoV were measured on days 0, 7, 14, 28, 56, 83. Serum samples were stored at −80 °C and analyzed by an indirect immunofluorescence assay (IFA) as previously described [43–46]. Briefly, cat samples were tested at dilutions of 1:25, 1:100, 1:400, 1:1600, and 1:6400. The fluorescein isothiocyanate (FITC) conjugated secondary antibody (rabbit anti cat IgG (H + L) (Nordic-MUbio, Sustern, Netherlands; LuBio Sience GmBH, Luzern, Switzerland) was diluted at 1:40. Slides were prepared using porcine kidney cells (PD-5 cells) infected with transmissible gastroenteritis virus (TGEV, Purdue strain). The antigen preparations were tested for the absence of contaminating viruses by RT-qPCR and qPCR as previously described [45]. A positive control (aliquoted serum sample of a FCoV-antibody-positive field cat) and a negative control (aliquoted serum from a specific pathogen free FCoV-antibody-negative cat) were run with each slide.

2.7. Characterization of the Active Ingredient in the Multi-Component Drug

Characterization of the active ingredient was performed by mass spectrometry (MS) and nuclear magnetic resonance (NMR). For details regarding these analyses, refer to the supporting information (Supplementary Text S1).

2.8. Data Analysis

Data were analyzed using R statistical language (version 4.0.3; R Core Team, 2020). Due to the repeated measures for an individual animal on multiple days, all variables were evaluated using linear mixed-effects models, with an individual animal as a random effect. The following model assumptions were always checked: (1) the normality of residuals was evaluated by the Shapiro–Wilk normality test, (2) the homogeneity of variances was evaluated with Levene’s test, (3) the heteroscedasticity (constancy of error variance) was evaluated with Breusch-Pagan test. In case assumptions were violated, a robust linear mixed-effects regression (RLMER) was applied; RLMER computes weighted estimates via Design Adaptive Scale approach and thus, solves heteroskedastic and non-normally distributed residuals by assigning lower weights to outliers and other contaminations. All contrasts (differences) between particular days were assessed after model-fitting by the estimated least-squares marginal means with the Bonferroni *p*-value correction for multiple comparisons. For comparison of the number of cats with effusion per day, pairwise Fisher’s tests were performed also with the Bonferroni *p*-value adjustment for multiple testing. Results with a p-value ≤ 0.05 were considered statistically significant. A nonparametric bootstrap with replacements that does not assume normality was used for obtaining and visualizing means and 95% confidence limits of blood and effusion viral loads.

3. Results

3.1. Efficacy of the Multi-Component Drug In Vitro

The cytotoxic effect of the multi-component drug Xraphconn® solubilized in MEM or DMSO was examined on CRFK cells using an MTT assay. After 24 and 48 h of treatment, the Xraphconn® solution exhibited no major cytotoxicity at a dilution of 1:100, independent of the applied solvent. Therefore, Xraphconn® solubilized in MEM at 1:100 and successive log dilutions were used for the antiviral efficacy testing. For this purpose, FCoV (MOI of 0.01) replication in Xraphconn®-treated and non-treated CRFK cells was measured by RT-qPCR, and FCoV replication inhibition by Xraphconn® (concentration 1:100) was significant, as viral RNA levels in treated cells were drastically reduced compared to the non-treated

controls. This dilution corresponds to a concentration of 5 µg/mL of the active compound in the solution. For FCoV-infected CRFK cells, the calculated EC_{50}-value of the Xraphconn® solution was 1.65 µg/mL (Figure 2).

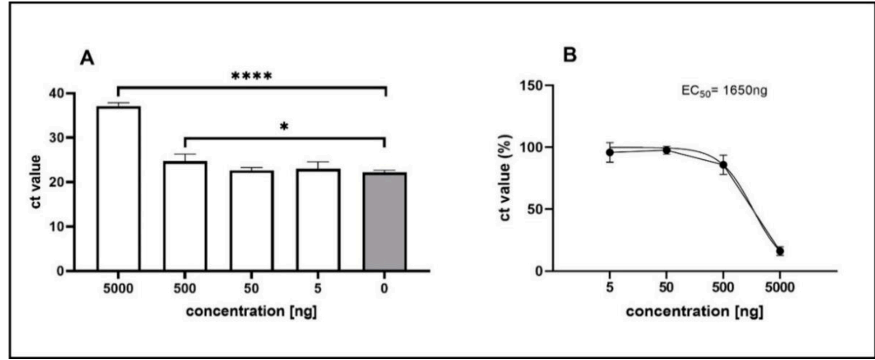

Figure 2. Feline coronavirus (FCoV) replication inhibition by Xraphconn®. (**A**) FCoV cycle threshold (ct) values in supernatants collected from infected Crandell-Rees Feline Kidney (CRFK) cells (multiplicity of infection (MOI = 0.01) treated with the indicated active compound concentrations were collected at 24 h post infection ($n = 4$). Significance levels compared to the results for untreated cells were determined by the Bonferroni's multiple comparisons test and are indicated as follows: *, $p \leq 0.05$; ****, $p < 0.0001$. (**B**) Data from four biological replicates were used to calculate the half maximal effective concentration (EC_{50}) value by non-linear regression analysis.

3.2. Efficacy of the Multi-Component Drug in Cats With FIP

All 18 cats completely recovered clinically within the 84 days of treatment. No relapse occurred, and at the time of publication (99 days after the last cat had finished its treatment course), all cats were still alive. With one exception, all cats were healthy and asymptomatic at the end of the treatment period. The only cat that showed any clinical signs at the end of treatment, as well as at the time of publication, was the cat presenting with renal azotemia at time of inclusion in the study (cat 3). This cat recovered clinically from all other signs related to FIP, but developed unilateral renal mineralization, which was first observed on day 21 of treatment. Renal azotemia improved with fluid therapy and overall remained stable during the treatment period. On day 168, three months after the end of treatment, the cat had developed unilateral ureteral obstruction with hydronephrosis and mild ascites, which most likely occurred secondary to the obstruction and was unrelated to FIP.

In all treated cats, clinical and laboratory parameters improved constantly and significantly during the treatment (Figures 3, Figure 4, Figure 5 and S1). Of the 18 cats, 17 were discharged from the hospital after the first 7 seven days with a modified Karnofsky's score of at least 45% (with 50% being equivalent to completely healthy) (Figure 3A); one cat had to stay hospitalized for 11 days due to the development of pyothorax. All cats gained body weight rapidly (Figure 3B) and had normal body temperatures soon after treatment initiation (Figure 3C) and the amount of effusion decreased markedly (Figure 3D), as did the number of cats that still had effusion (Figure S1). In addition, all clinicopathological parameters (hematological and clinical chemistry parameters including SAA) improved steadily (Figure 4A–H) with significant differences in the parameters by day 1 to day 28 when compared to day 0 (before treatment). Remarkably, in most of the cats (15/18), viral RNA could be detected in blood before treatment, but blood viral loads decreased massively in all cats by day two to four after treatment initiation (Figures 5A and 6A). By day 14, viral RNA was no longer detected in the blood of the cats (Figures 5A and 6A), indicating that all had cleared detectable viremia. All accessible effusions tested RT-qPCR-positive at the start of the study (Figures 5B and 6B). Later on, viral RNA was detectable in all but three effusion samples (Figure 5B). However, these three cats had smaller volumes of effusion collected and evaluated by RT-qPCR. All cats had serum anti-FCoV antibodies at the

initiation of the study, most of them with high antibody titers (14/18 with titers ≥1:1600). Anti-FCoV antibody titers declined in 14/18 cats, in some cats starting as early as 28 days after the initiation of treatment (Figures 5C and S2). In none of the cats was an antibody titer increase observed.

3.3. Adverse Effects of the Multi-Comoonent Drug in Cats

No serious adverse effects were observed during Xraphconn® treatment (Table 2) and therefore in none of the cats did treatment have to be discontinued. Mild Heinz body anemia (with 19.8% of red blood cells containing Heinz bodies, hematocrit 32.5% (reference range: 33–44%)) was observed in one cat on the last day of the Xraphconn® treatment (day 83). The cat was treated with S-adenosyl-methionine (Table 1) and the hematocrit normalized (to 34.7% on day 100) and Heinz bodies decreased (to 8.8% on day 126). Lymphocytosis was seen in 14/18 (77.7%) cats; three of those already had moderate, and two of those severe, lymphocytosis before treatment initiation (Table 2). In most of the cats, however, lymphocytosis was only mild to moderate. One cat (before treatment, the cat had a lymphocyte count at the lower end of the reference range (1.1×10^9/L)), however, developed a massive lymphocytosis on day 83 (40.7×10^9/L). Cytology of a blood smear revealed a high number of predominantly small lymphocytes, isolated large or reactive lymphocytes with dark blue cytoplasm. To rule out chronic lymphocytic leukemia, a PCR for Antigen Receptor Rearrangements (PARR) analysis was performed, which was consistent with a reactive lymphoid cell population. The majority of cats (11/18; 61.1%) developed eosinophilia during the treatment period, but in all cats this was only mild ($<2.0 \times 10^9$/L). Fecal examinations were performed in 10/11 cats with eosinophilia to exclude parasite infestation. In one cat, *Giardia* spp. infection was present before start of the Xraphconn® treatment, and the cat was treated with fenbendazole; in another cat, *Giardia* spp. infection was detected by fecal examination on day 56 (this cat had no eosinophilia; fecal examination was performed because the partner cat suffered from *Giardia* spp. infection). An increase in liver enzyme activity was noted in 11/18 cats between day 2 and day 83, but was mostly only mild to moderate. An increase of alanine aminotransferase (ALT) activity was observed in 7/18 (38.9%) cats; of those, two cats had a severe ALT activity increase and were treated with silymarin. An increase in alkaline phosphatase was seen in 8/18 cats, but was only mild (seven cats) to moderate (one cat). No renal toxicity was detected. With one exception, SDMA remained within the reference range throughout the whole treatment period. One cat had renal azotemia, which was already present before treatment initiation with increased creatinine, urea, and SDMA. None of the cats developed azotemia or showed an increase in creatinine of more than 26.4 µmol/L in the non-azotemic range within 48 h and none of the cats developed oliguria, and thus, there was no evidence of acute renal toxicity [47]. Mild prerenal azotemia (with isolated urea concentration elevation between 11.6–12.7 mmol/L) was detected in four cats, but those cats had not been fasted before blood sampling.

3.4. Characterization of the Main Active Ingredient in the Multi-Component Drug

Evaluation of the structure of the active component in Xraphconn® by Ultra-High-Performance-Liquid Chromatography Electro-Spray QTRAP Mass Spectrometry (UHPLC-ESI-QTRAP-MS/MS) using Multiple Reaction Monitoring with Information Dependent Acquisition and Enhanced Product Ion Scan (MRM-IDA-EPI scan) provided a MS/MS fragment pattern that was practically identical to that of a control measurement performed with GS-441524 (Figure 7).

As LC-MS/MS could not distinguish between a furanose ring of GS-441524 and a pyranose ring and a ring opening during collision-induced decay (CID) would provide very similar MS-fragmentation patterns of the two compounds, further structure elucidation could only be performed via NMR spectroscopy.

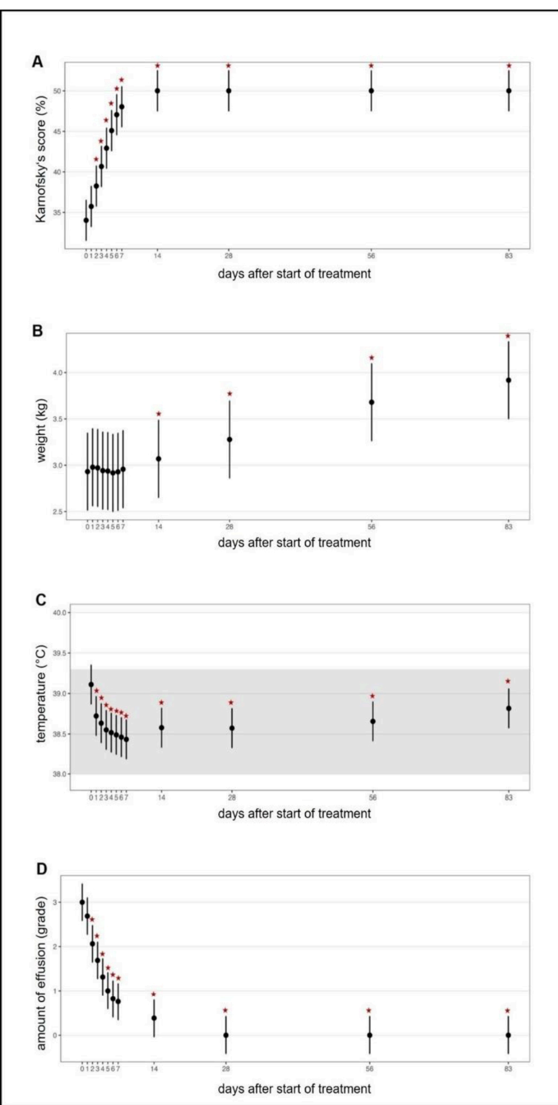

Figure 3. Timeline visualizing improvement of clinical parameters throughout the study course. Figures show average predictive values and 95% confidence intervals of each parameter. Grey shading marks the reference ranges of the parameters. Red asterisks mark significant difference ($p \leq 0.05$) of the parameters on different days of treatment when compared to day 0 (before treatment) measured by a linear mixed-effects model (for temperature) and by robust linear mixed-effects models. (**A**) Karnofsky's score modified for cats. (**B**) Body weight. (**C**) Body temperature. (**D**) Amount of effusion subjectively evaluated during abdominal/thoracic ultrasound and paracentesis (grades 0 (no fluid) to 4 (massive effusion)).

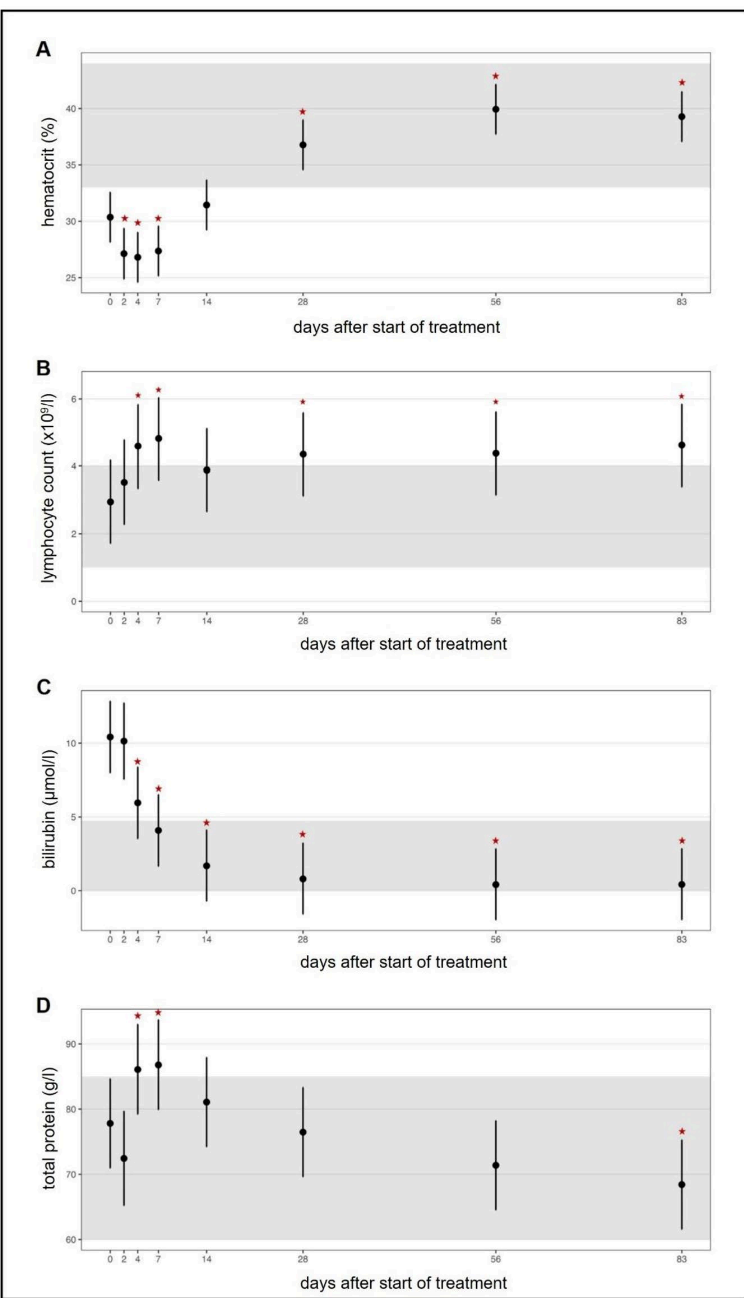

Figure 4. *Cont*.

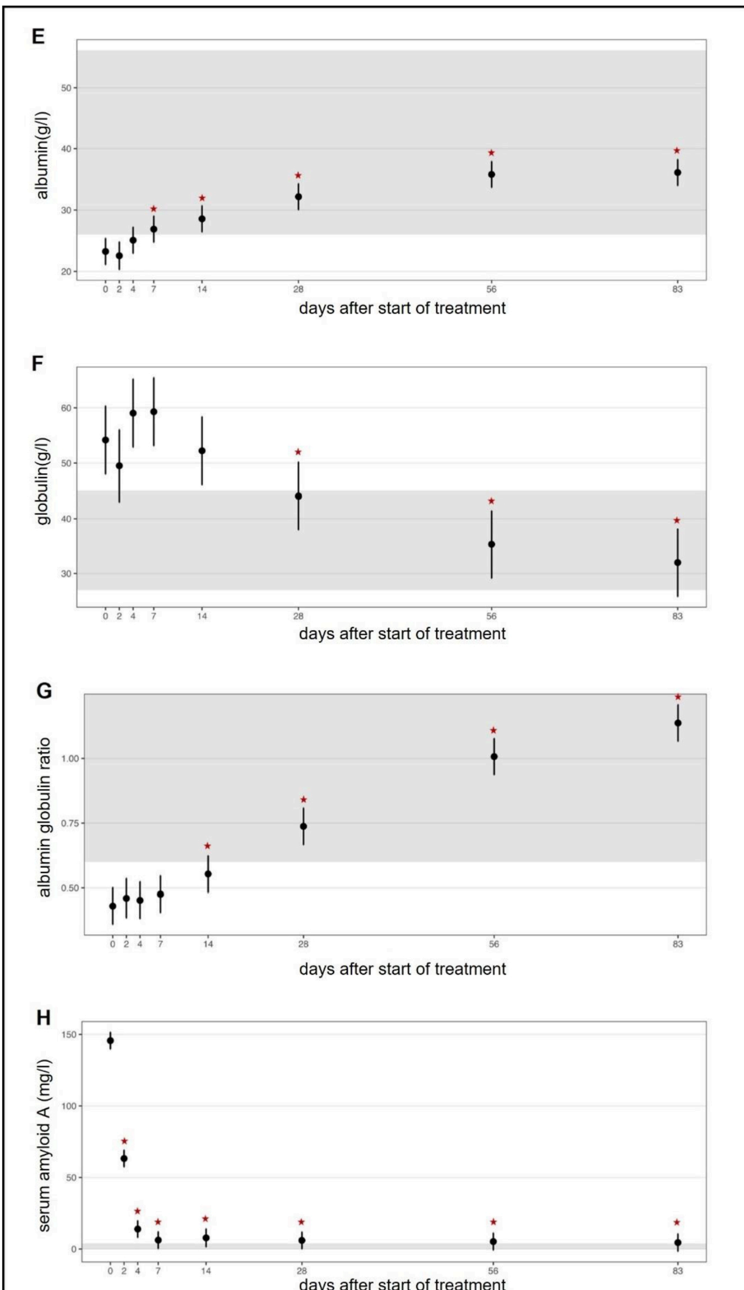

Figure 4. Timeline visualizing improvement of clinicopathological parameters throughout the study course. Figures show average predictive values and 95% confidence intervals of each parameter. Grey shading marks the reference ranges of the parameters. Red asterisks mark significant difference ($p \leq 0.05$) of the parameters on different days of treatment when compared to day 0 (before treatment) measured by a linear mixed-effects model (for albumin) and by robust linear mixed-effects models. (**A**) Hematocrit. (**B**) Lymphocyte count. (**C**) Bilirubin concentration. (**D**) Total protein concentration. (**E**) Albumin concentration. (**F**) Globulin concentration. (**G**) Albumin/globulin ratio. (**H**) Serum amyloid A (SAA) concentration.

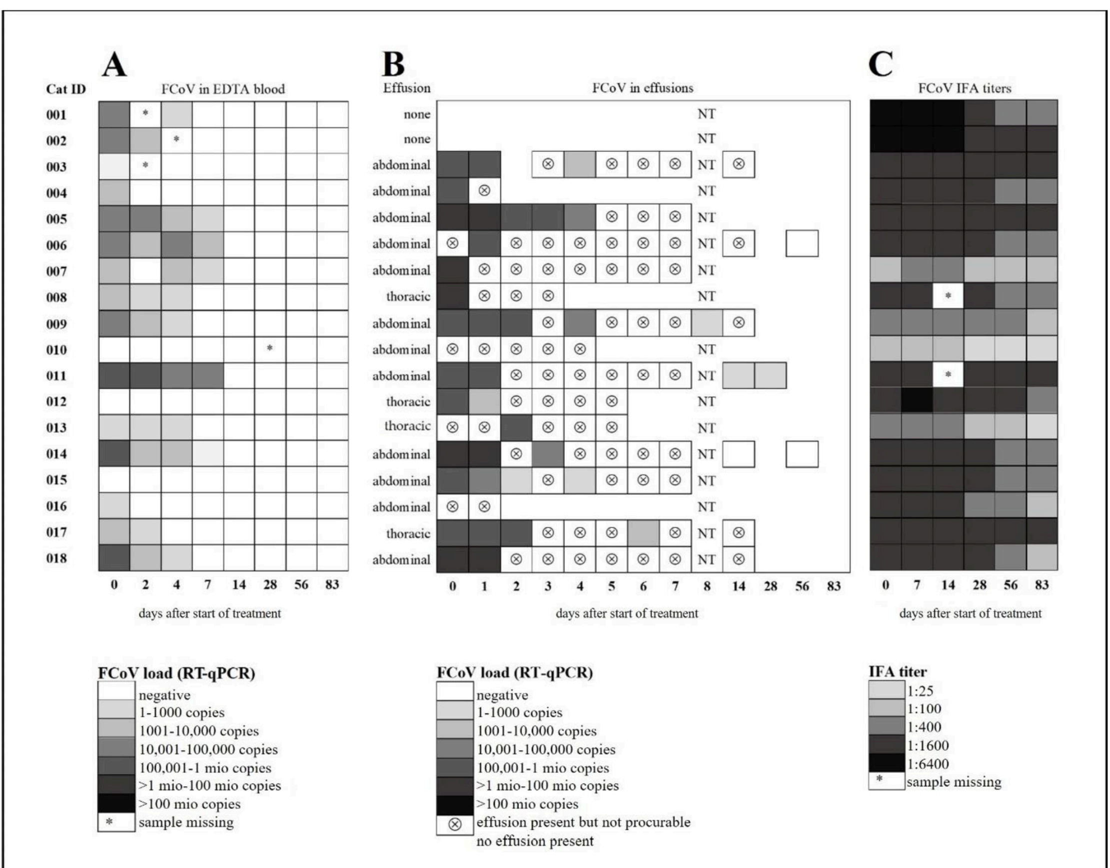

Figure 5. Feline coronavirus (FCoV) viral RNA loads in blood and effusion samples and serum anti-FCoV antibody titre measurements. (**A**) FCoV RNA loads in EDTA anticoagulated blood. (**B**) FCoV RNA loads in effusions. (**C**) Serum anti-FCoV antibody titres. FCoV RNA loads were determined by quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) (**A**,**B**). Antibody titers were determined by indirect immunofluorescence assay (IFA). NT, not tested.

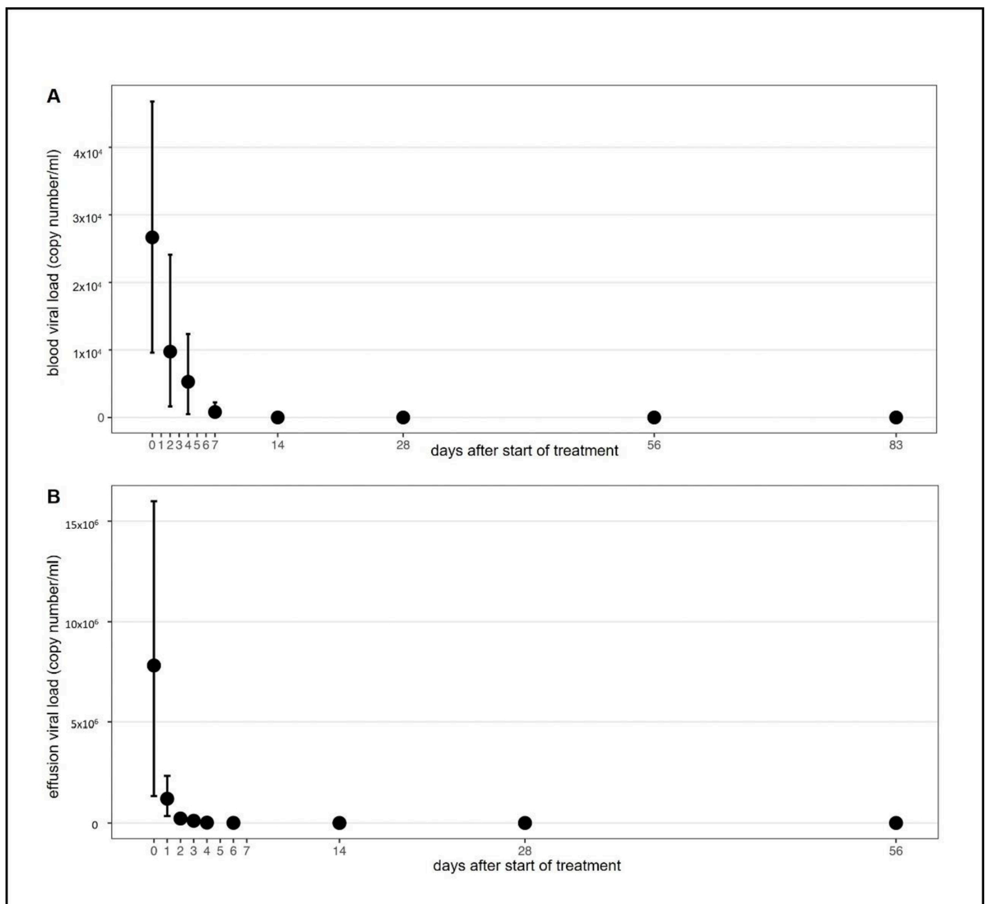

Figure 6. Viral load in blood and effusion throughout the study course. Figures show visualization of data using nonparametric bootstraps. (**A**) FCoV RNA loads in EDTA anticoagulated blood. (**B**) FCoV RNA loads in effusions.

Table 2. Adverse effects of the Xraphconn® treatment, grades of adverse effects, day of first appearance, and respective symptomatic treatment. [1] Heinz body formation: mild (5–9.9% of red blood cells), moderate (10–25% of red blood cells), severe (>25% of red blood cells); [2] lymphocytosis: mild (4–7.9 $\times$ 10^9/L), moderate (8–15 $\times$ 10^9/L), severe (>15 $\times$ 10^9/L) [3] was already present on day 0: lymphocytosis: 5/14 cats; eosinophilia: 1/11 [4] eosinophilia: mild (0.6–1.9 $\times$ 10^9/L), moderate (2–10 $\times$ 10^9/L), severe (>10 $\times$ 10^9/L); [5] increased liver enzymes: increased alanine aminotransferase (ALT) activity: mild (ALT < 200 IU/L), moderate (ALT 200–350 IU/L), severe (ALT >350 IU/L); increased alkaline phosphatase (AP): mild (AP < 200 IU/L), moderate (AP 200–350 IU/L), severe (AP > 350 IU/L).

Adverse Effect		Number of Cats	Grade	Median Day of First Appearance (Range)	Symptomatic Treatment
Heinz body formation [1]		1/18	moderate	83	S-adenosyl-methionine
Lymphocytosis [2]	14/18 [3]	4/14	mild	4.5 (2–83)	no
		6/14	moderate	1 (0–28)	no
		4/14	severe	1 (0–2)	no
Eosinophilia [4]	11/18 [3]	11/11	mild	14 (0–28)	no
increased liver enzyme activity [5]	11/18	8/11	mild	28 (2–83)	no
		1/11	moderate	4	no
		2/11	severe	4	silymarin

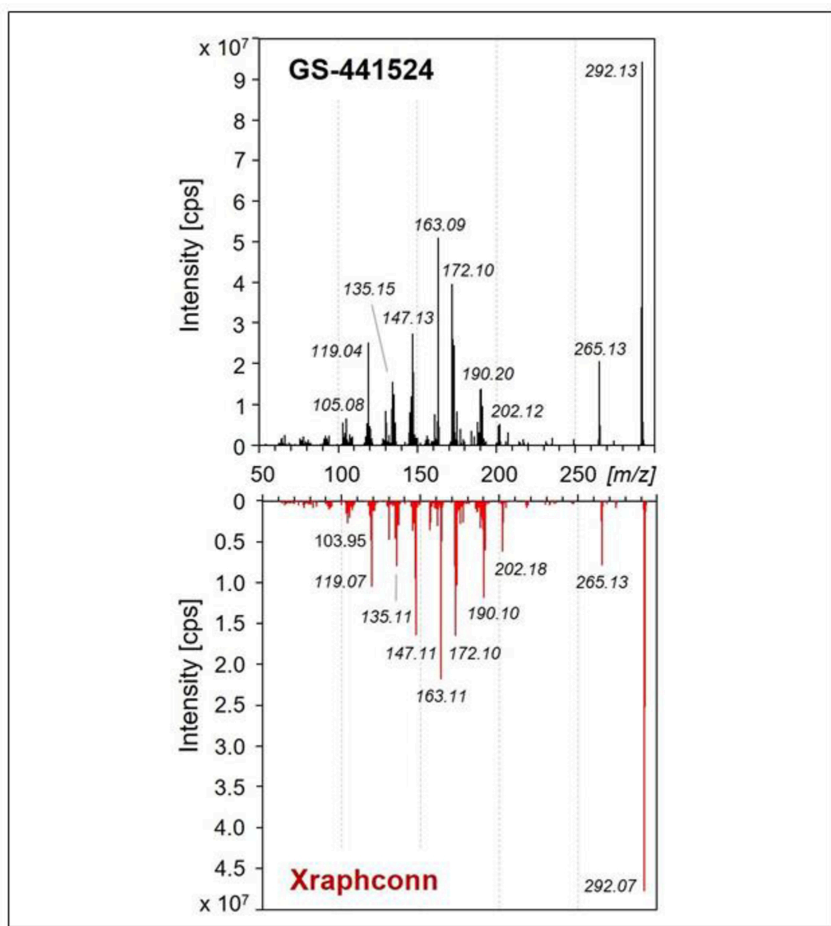

Figure 7. Comparison of the Ultra-High-Performance-Liquid Chromatography Electro-Spray QTRAP Mass Spectrometry (UHPLC-ESI-QTRAP-MS/MS) spectra of GS-441524 and the active component of Xraphconn® extracted from the tablet. The compounds in both test solutions were not only isobaric with [M + H]+ m/z 292.1040, but exhibited identical fragmentation spectra. Mass spectra were generated at a collision energy of 50 eV with positive ionization using the Multiple Reaction Monitoring with Information Dependent Acquisition and Enhanced Product Ion (MRM-IDA-EPI) scan mode. Cps, counts per second.

In NMR, starting from a 2D correlated spectroscopy (2D-COSY) spectrum [48], all non-exchangeable protons and exchangeable hydroxyl protons of the major compound in the sample could be assigned. The observed HO-5′ (with COSY cross peaks to both H-5′ protons) and absence of HO-4′ are characteristic for a furanose ring, while in a pyranose ring, the opposite is expected. The Heteronuclear Single Quantum Coherence (HSQC) spectrum [49] provided assignment of carbon signals for CH2 and CH groups in the molecule, exploiting J-coupling interaction over the proton-carbon chemical bond. Quaternary carbons were assigned by 3-bond J-coupling interaction between protons and carbons in the Heteronuclear Multiple Bond Correlation (HMBC) spectrum [50]. The cross-peak of H-2′ to CN in this HMBC spectrum allowed to unambiguously determine the position of the cyano-group in the molecule. Obtained 13C chemical shifts (Figure 8) and patterns of 1H signals (Table S3) correspond to what is reported on GS-441524 [51]. Differences in 1H chemical shifts can be attributed to different sample conditions for pure

GS-441524 in fully deuterated dimethyl sulfoxide (DMSO-d6) relative to the extract of Xraphconn® in a DMSO-d6/D2O mixture including salts and other impurities.

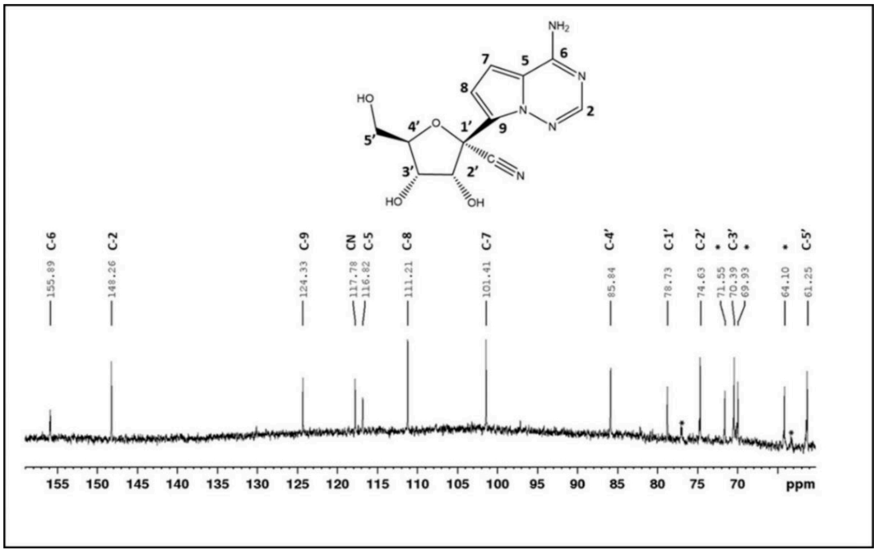

Figure 8. 13C spectrum of the analyzed sample. Labels refer to the assignment of carbons in the active component of Xraphconn® depicted above. All signals above 100 ppm belong to the cyano-group and nucleobase in the identified compound. Some additional signals of uncharacterized impurities were observed below 80 ppm (indicated with *). Ppm, parts per million.

4. Discussion

In this first prospective controlled treatment trial using an oral antiviral compound in field cats with FIP, it could be clearly demonstrated that oral treatment with GS-441524 -containing Xraphconn® displays a striking efficacy. All 18 treated cats showed a very swift response to the treatment with rapid improvement of clinical and clinicopathological parameters leading to full and relapse-free recovery. Without treatment, virtually all cats suffering from FIP die, making FIP one of the most lethal diagnoses in the cat population, instead, the survival rate in the present study for the 18 cats was 100% at the time of publication. In addition, viral loads decreased in both blood and effusion (where present and available for RT-qPCR) within a short period of time after treatment initiation, and none of the cats was blood FCoV RNA-positive after day 14 demonstrating an enormous response to treatment. Also, antibody titers decreased significantly during therapy. The in vivo efficacy also mirrors the in vitro data, where GS-441524 containing Xraphconn® also showed excellent efficacy and induced a rapid decrease of viral loads in FCoV-infected CRFK cells.

All clinicopathological parameters that are typically altered in cats with FIP improved significantly within a few days [37]. Typically, total protein, globulin, and bilirubin concentrations are elevated in cats with FIP, while albumin concentration, albumin/globulin ratio, hematocrit, and lymphocyte counts are decreased [52,53]. All these values normalized within a short period of time with Xraphconn® treatment. Body temperature was elevated in some cats, but decreased to the reference range during the first few days in the clinic. However, temperature was generally higher during control visits when compared to the first days in the clinic, which might be explained by the stress during transportation. Also, SAA concentrations rapidly and significantly decreased upon treatment in all cats. SAA is a major acute phase protein in cats, and SAA expression has been shown to be increased in cats with FIP, indicating the presence of a severe inflammatory response [54–57]. The marked reduction in SAA concentrations in response to treatment reflects the clinical

efficacy of the multi-component drug Xraphconn® and suggests a remarkable attenuation of the hyperinflammatory response seen in cats with FIP.

Structural chemical analysis to identify the active components of the multi-compound drug by using mass spectrometry and NMR identified GS-441524 as the single adenine C-nucleoside ribose analogue extracted from Xraphconn®. No other similar nucleoside analogues were detected, although it cannot be excluded that there might be additional active ingredients present in Xraphconn®.

Although a very good efficacy of GS-441524 upon subcutaneous administration has been demonstrated in an experimental study and in cats in the field before [25,28], the present study demonstrated an even higher efficacy, as all of the 18 treated cats were apparently cured when the drug was administered orally. There are several potential reasons for these favorable results. First, additional substances to the GS-441524, such as herbal preparations, in the multi-component drug Xraphconn® might increase efficacy with some of these substances exhibiting synergistic effects. Second, only cats with a confirmed diagnosis or at least very likely diagnosis of FIP were included in the present study and therefore, there was little chance that cats with other severe diseases were mistakenly treated with the drug. Third, all cats received intensive medical care 24/7 as well as symptomatic treatment, especially during the first critical seven days, including in-depth diagnostic procedures and individualized supportive treatment in a highly specialized university hospital.

This is the first time that oral (instead of subcutaneous) treatment was applied to cats with FIP in a controlled study. In the past, oral treatment was used successfully in a case report in one cat with neurological FIP [26]. The study of Jones and coworkers (2021) "unlicensed GS-441524-like antiviral therapy can be effective for at-home treatment of feline infectious peritonitis" [34] was an online survey study that drew its findings from testimonials from private owners who had provided independent and non-standardized as well as non-supervised therapy to their cats. Similarly, the study of Yin and coworkers (2021) "a retrospective study of clinical and laboratory features and treatment on cats highly suspected of feline infectious peritonitis in Wuhan, China" [35], was a retrospective study and did not have any prospective aspect. Thus, these studies were not prospective and therefore are not comparable to the current study.

Oral treatment has several advantages. In previous studies with subcutaneous injections of GS-441524, more than half of the cats developed injection site reactions [25]. In such inflammatory reactions could trigger development of FISS. In addition, handling of subcutaneous injections is difficult and challenging for owners and can be very painful for the cats causing injection reactions. In the present study, all 18 cats treated orally remained cooperative throughout the entire treatment period and the owners were able to successfully administer the tablets.

The study protocol set a fixed dose for each participating cat (10 mg/kg for cats with neurological/ocular signs and 5 mg/kg for cats without neurological/ocular signs). The package insert specified the concentration of the active component within the tablets and the dosages applied to cats were based on this information and on the previous published studies [24,28]. However, the exact amount of GS-441524 within the Xraphconn® tablets was not verified in the present study, but all cats were apparently cured and no major adverse effects were reported, it has to be assumed that the dose given in the package insert was correct. Individualized dosing of cats, as sometimes recommended by social media groups, based on laboratory parameters, such as globulin concentrations, did not appear necessary or indicated in the current study. In addition, a standardized additional symptomatic therapy for each cat as advised by social media groups, e.g., tablets for liver protection, was not necessary. The present study clearly demonstrates the importance of adapting symptomatic treatment according to the problems and needs of the individual cats. Unrelated problems can occur, such as a renal mineralization in one of the cats in the current study, which would not have been detected if owners had treated their cats at home without veterinary supervision, which can lead to significant problems, independent of

FIP, without adequate diagnosis and therapy. Thus, FIP treatment should not be left in the hands of owners alone, and legal approval of the drug in veterinary medicine is urgently needed. Whether a shorter treatment duration would be possible and successful, and to aid in the reduction of high costs, remains to be determined. In the present study, no cat was viremic by day 14 after starting treatment and viral levels in effusions were similarly reduced. It might therefore be interesting to investigate the efficacy of a shorter treatment period in a well-controlled study.

Two cats had to be excluded from the study due to their severe moribund condition. In the present study, cats were only included after extensive efforts were made to obtain a diagnosis of FIP. This was performed by applying an individualized multi-step diagnostic approach, including extensive physical examination, basic clinicopathological data, diagnostic imaging, and methods for direct virus detection (immunohistochemistry and/or spike gene mutation analysis with RT-PCR). A diagnosis of FIP cannot be obtained easily by one diagnostic test, since clinical and pathological abnormalities and imaging findings are not pathognomonic, and the detection of viral RNA by RT-PCR with or without mutation analysis, is not diagnostic for FIP [36]. Thus, a multimodal diagnostic approach is highly advisable; however, this is time-consuming, and disease progression in the meantime might preclude treatment success in some cats with severe disease. Nevertheless, in the face of cost and emergence of viral drug resistance, the aim should still be to be as confident as possible that a cat is truly suffering from FIP before starting antiviral treatment, and this can only be done by veterinarians, again highlighting the importance of having veterinary involvement in FIP diagnosis and treatment.

The viral RNA loads in accessible abdominal or thoracic effusions decreased in all cats over the study period, and looking at the blood viral RNA loads, it is assumed that viral RNA was also cleared from the thoracic and/or abdominal compartments. The Xraphconn® treatment was therefore beneficial in reducing viral RNA loads in effusions and blood and cured abdominal and/or thoracic effusions in cats with FIP. A remarkably high percentage of cats with FIP in this study (83%) were viral RNA-positive in blood upon entering the study. In other studies in cats with confirmed FIP, detection of viral RNA in blood was rather a rare event [53,58–61]. Thus, the notion that viremia will likely usually be over by the time clinical signs of FIP appear [42], has to be reconsidered in view of the current data. Most cats in the present study with FIP had remarkably high antibody titers, which decreased in most but not all cats over time.

The adverse effects reported with the Xraphconn® treatment in the present study were considered to be acceptable and not serious, and treatment did not need to be discontinued in any of the cats. No gastrointestinal signs were caused by oral administration. Previous studies using GS-441524 have mainly reported adverse effects related to subcutaneous injection (e.g., pain reactions, superficial skin lesions, skin ulceration). A decrease in hematocrit, total protein, and albumin during the first days of treatment in the clinic likely occurred due to blood sampling and might be a dilutional effect due to fluid therapy, but was fully reversible. The most pronounced adverse effect seen in the present study was a lymphocytosis that occurred in 14/18 cats. Significant abnormalities in hematological parameters have not been reported as adverse effects in association with GS-441524 treatment in field cats so far [24–26,28]; however, a marked increase in the lymphocyte count was also seen after treatment of cats with experimental FIP [28]. Lymphocytosis could have been caused by excitement to which cats are very sensitive, or by antigen stimulation. While younger cats generally have higher lymphocyte counts, the changes were too pronounced to be explained only by young age. The changes observed for lymphocyte counts in response to treatment appear to be of particular interest. Though this study did not aim to characterize the host immune response in detail, it is interesting to observe that mild to moderate lymphocytosis was seen in >75% of cats following treatment initiation, while SAA concentrations displayed a rapid and sustainable decline in all participating animals. One cat developed a massive lymphocytosis on day 83 (40.7×10^9/L) and PARR-based exclusion of clonality and thus malignancy suggested a reactive lymphoid population. To

some extent, these lymphocyte responses remind the clinician of features seen in Immune-Reconstitution Inflammatory Syndrome (IRIS) [62]. One could speculate that cytokines produced by monocytes upon FCoV infection could cause both a reduction of lymphocytes and an impaired immune response to FCoV. Lymphopenia is a common feature [53,63] and negative prognostic factor [53] in cats with FIP, caused by increased apoptosis of B and T cells induced by tumor necrosis factor alpha expression [64–68]. As a consequence, a rebound in the immune response could occur after the cytokine effect fades. Additionally, FCoV-infected cats without FIP were shown to exhibit B and T cell hyperplasia [66,69,70] and increased numbers of circulating T cells [63]. It would thus be of particular interest to further characterize these immunologic features and the cellular immune response against FCoV before and after treatment.

Eosinophilia was noted as another effect of the Xraphconn® treatment in this study. Parasitic infestation was ruled out in most cats, and there was no obvious reason for the eosinophilia. Eosinophil counts were not specifically evaluated in previous studies [24,25,28]. Interestingly, an increase in the eosinophil count has also been found in human patients recovering from COVID-19 and has been proposed as a marker for a favorable outcome [71–73]. Whether there was a hypersensitivity reaction resulting in eosinophilia due to treatment in the current study needs to be clarified in further research.

One cat developed mild Heinz body anemia on the last day of therapy. Heinz body formation occurs as a result of oxidative damage to hemoglobin and can result in hemolysis [74]. In cats, increased Heinz body formation has previously been shown to be associated with the administration of certain drugs and components, such as propofol [75], acetaminophen [76], or propylene glycol [77]. Therefore, it seems possible that there is an association between the repeated administration of Xraphconn® and Heinz body formation in this cat. Further studies should include the quantification of Heinz body formation during antiviral treatment in order to clarify a possible causal relationship.

An increase of liver enzyme activity was noted in some cats, but was primarily of a mild to moderate scale. Since GS-441524 is metabolized in the liver, this metabolism could possibly lead to an increased metabolic rate with increased liver enzyme activity. Increases in liver enzyme activity have been reported as adverse effects with this multi-component compound treatment before in an unpublished observation [78]. In the present study, renal toxicity did not occur, which is in agreement with one previously published study [25]. Symmetric dimethylarginine values, considered to be a very sensitive parameter for kidney function, remained within the reference range throughout the treatment. Only one cat with thoracal and abdominal effusion had an SDMA of 18 µmol/L (reference range 0–18 µmol/L) on day 0 before treatment, likely due to impaired perfusion of the kidneys secondary to FIP, but values normalized after treatment was started. Another single cat had renal azotemia and increased SDMA values, which were already present at inclusion into the study. Therefore, it was reasonable to suspect that renal changes before entering the study were caused by FIP. However, in the further course of the study, the cat developed unilateral renal mineralization and mild pyelectasia, which progressed to ureteral obstruction, most likely caused by a ureterolith, after the end of treatment. The cat did not show overt clinical signs related to kidney disease except for the development of a very small amount of ascites. Given that azotemia was already present before the start of Xraphconn® treatment, it seems unlikely that the renal changes represent adverse effects, but rather were the consequence of progressive kidney disease unrelated to the Xraphconn® treatment. In a previous study [25], one cat treated with GS-441524 showed a progressive increase in urea and sudden rise in SDMA concentration during repeated rounds of treatment and as a precaution, it was decided to stop treatment. However, urine specific gravity measurement was not reported in that study, so the presence of renal azotemia, as compared to prerenal azotemia, could not be confirmed. Additionally, urea and SDMA concentrations returned to normal after discontinuation of treatment [25]. In a case report of a cat with FIP treated with the same multi-component compound as in the current study, an increased SDMA was noted during treatment, which normalized after

drug discontinuation, although it was not confirmed whether this was as a consequence of antiviral treatment [26].

The question arises as to whether remdesivir, which is licensed for the treatment of humans with severe COVID-19, could be useful for treatment of FIP in cats, Remdesivir has been used by veterinarians in Australia to treat cats with FIP, although published studies are not yet available. GS-44152 is the main metabolite of remdesivir, a prodrug of the nucleoside analogue, which has been used with limited success in patients with acute COVID-19 [29,79]. Remdesivir needs to be applied by injection since it has no acceptable oral bioavailability. In contrast, oral admission of GS-441524 was shown to be effective in mice against COVID-19 [29,80]. Another advantage of GS-441524 over remdesivir appears to be its reduced liver toxicity allowing dose escalation to ensure effective treatment of systemic coronavirus infections [81]; GS-441524 is directly converted into the active nucleoside triphosphate in liver and lung tissue.

The results of the present study can also be viewed from a broader perspective. In the context of the COVID-19 pandemic, pediatricians worldwide have been confronted with a new disease in children, a syndrome associated with SARS-CoV-2 infection and referred to as multisystem inflammatory syndrome in children (MIS-C). When comparing clinical features of FIP with MIS-C, remarkable parallels can be found [5,6]. MIS-C, like FIP, is a hyperinflammatory immune response, primarily seen in the children [5,6]. Affected children initially show often only gastrointestinal signs, while in the course of the disease, further symptoms appear, such as persistent fever, ascites, pleural, and pericardial effusion [5,82,83], all of which are also commonly found in cats with FIP. Therefore, FIP might provide a useful natural model for insights into the pathogenesis and immunology of MIS-C, but also into potential treatment options pending further studies [84]. Finally, studying FIP in cats and the host response to treatment could serve as an interesting natural model for further elucidating features of MIS-C in children. The model could provide critical insights into pathophysiology and immunology of these similar clinical entities and might also help to explore new potential treatment options for this severe SARS-CoV-2-associated disease.

The major limitation of this study was that no untreated control group was included. However, leaving cats with FIP untreated is not justifiable for ethical reasons, and the short median survival time in cats with FIP of eight to nine days clearly has been demonstrated before [9,20]. Thus, without treatment, almost all cats would die within a very short time frame. Another limitation was that the potential efficacy of the additional components in the tablets (other than GS-441524) was not determined in the present study, and measuring any antiviral efficacy of the other components, once successfully identified and purified, would be an interesting additional approach in the future. Due to the oral application of the treatment, cats in a moribund condition could not be included in the study. The two cats that were excluded from the study were in a severely moribund end-stage condition. An oral drug would likely not have had any effect in such a condition, due to the low metabolic rate and the fact that oral medications are hardly absorbed from a likely inactive intestinal tract. Therefore, the cats were not included in the study and instead euthanized for humane reasons. Also, the small sample size of the current study is an additional limitation.

5. Conclusions

In conclusion, this study is the first prospective treatment trial clearly demonstrating that cats suffering from this tragic and fatal disease can be cured with oral treatment. Unfortunately, the drug is not currently legally available for veterinary use in many countries, forcing well-meaning owners to self-diagnose and treat their cats based on judgment of non-veterinary lay people and social media groups. Thus, there is an urgent need for respective official bodies and industry to work towards a swift licensing process of the drug so that it can be legally used by veterinary experts to offer supervised treatment to cats suffering from FIP.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/v13112228/s1, File S1: Package inserts of Xraphconn®. (A) Package insert of first batch. (B) Package insert of second batch. Table S1: Schedule of examinations performed in all cats (days on which examinations took place are marked in grey). FCoV, feline coronavirus; [1] ultrasound and paracentesis: if abdominal or thoracic effusion was present, additional ultrasounds of abdomen (and/or thorax) were performed daily from day 0 to day 7 to evaluate fluid amount and obtain fluid samples; as long as effusion was present, effusion viral load was determined in addition. [2] including symmetric dimethylarginine (SDMA) and serum amyloid A (SAA). Table S2: Karnofsky's score modified for cats by Hartmann and Kuffer (1998) to evaluate the general condition and well-being [40]. Text S1: Characterization of the active ingredient in Xraphconn®. Table S3: 1H and 13C chemical shifts in ppm for the nucleoside analogue extracted from Xraphconn® compared to data reported on pure GS-441524 in fully deuterated dimethyl sulfoxide (DMSO-d6). nr, not reported; splitting pattern of proton signals (s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; app q, apparent quartet). Figure S1: Percentage of cats with effusion during study course. The bars represent percentage of cats with effusion on each examination day. The grey areas represent the proportion of cats in which effusion was present but not available for paracentesis. The green areas represent the proportion of cats in which effusion could be obtained but was feline coronavirus (FCoV) RNA-negative. The orange areas represent the proportion of cats in which effusion could be obtained and was RNA-positive. Figure S2: Anti-feline coronavirus (FCoV) antibody titers throughout the study course. Figure showing predictive values and 95% confidence intervals of antibody titer. Samples were tested at dilutions of 1:25, 1:100, 1:400, 1:1600 and 1:6400. Red asterisks mark significant difference ($p \leq 0.05$) of the parameters on different days of treatment when compared to day 0 (before treatment) measured by a robust linear mixed-effect model.

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IV. PUBLIKATION 3: ORIGINAL-PUBLIKATION II

Fecal feline coronavirus RNA shedding and spike gene mutations in cats with feline infectious peritonitis treated with GS-441524

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Article

Fecal Feline Coronavirus RNA Shedding and Spike Gene Mutations in Cats with Feline Infectious Peritonitis Treated with GS-441524

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Abstract: As previously demonstrated by our research group, the oral multicomponent drug Xraphconn® containing GS-441524 was effective at curing otherwise fatal feline infectious peritonitis (FIP) in 18 feline coronavirus (FCoV)-infected cats. The aims of the current study were to investigate, using samples from the same animals as in the previous study, (1) the effect of treatment on fecal viral RNA shedding; (2) the presence of spike gene mutations in different body compartments of these cats; and (3) viral RNA shedding, presence of spike gene mutations, and anti-FCoV antibody titers in samples of 12 companion cats cohabitating with the treated cats. Eleven of the eighteen treated FIP cats (61%) were shedding FCoV RNA in feces within the first three days after treatment initiation, but all of them tested negative by day 6. In one of these cats, fecal shedding reoccurred on day 83. Two cats initially negative in feces were transiently positive 1–4 weeks into the study. The remaining five cats never shed FCoV. Viral RNA loads in feces decreased with time comparable with those in blood and effusion. Specific spike gene mutations linked to systemic FCoV spread were consistently found in blood and effusion from treated FIP cats, but not in feces from treated or companion cats. A new mutation that led to a not yet described amino acid change was identified, indicating that further mutations may be involved in the development of FIP. Eight of the twelve companion cats shed FCoV in feces. All but one of the twelve companion cats had anti-FCoV antibodies. Oral treatment with GS-441524 effectively decreased viral RNA loads in feces, blood, and effusion in cats with FIP. Nonetheless, re-shedding can most likely occur if cats are re-exposed to FCoV by their companion cats.

Keywords: FIP; FCoV; shedding; viral loads; spike gene mutations; therapy; treatment; Xraphconn®; feces; sequencing; reinfection

1. Introduction

Feline coronaviruses (FCoVs) are enveloped RNA viruses with a single-stranded, almost 30-kb long, non-segmented genome of positive polarity. They belong to the family Coronaviridae; to the order Nidovirales; together with canine coronavirus (CCoV) and transmissible gastroenteritis virus (TEGV) of pigs, to the subfamily Coronavirinae; to the genus Alphacoronavirus; to the subgenus Tegacovirus and to the species Alphacoronavirus 1 [1]. FCoVs show high mutation rates upon replication, leading to the formation of viral quasispecies, and thus, multiple genetic virus variants related by mutations [2].

FCoVs are endemic in cats and the prevalence of infection is very high, up to 90%, especially in multi-cat environments [3–6]. FCoVs are transmitted horizontally via the fecal–oral route, leading to infection of enterocytes [7,8]. In most of the cases, enteric infection only induces mild enteritis, mostly without clinical signs. However, 4–5% of adult FCoV-infected cats and 5–10% of kittens in multi-cat environments develop feline infectious peritonitis (FIP) [9,10], a fatal FCoV-induced immune-mediated disease characterized by granulomatous vasculitis and perivasculitis [11]. FIP develops when highly virulent FCoVs (FIP-associated FCoVs) arise by mutations from less virulent FCoVs within an individual FCoV-infected cat [12]. This process starts with mutations conferring the ability to infect a broadened target cell spectrum, including monocytes/macrophages, allowing for systemic infection [13]. The key features of FIP development are the activation of infected monocytes/macrophages and the ability of the mutated strains to effectively and sustainably replicate in these target cells [14–17]. Systemically infected healthy cats can carry low amounts of virus in different organs [18,19]; however, different studies have shown that in cats suffering from FIP, viral replication in blood and viral loads in tissues are generally significantly higher [18,20–22].

Cats start shedding FCoV in feces as early as two days after experimental infection, and continuously shed for at least two weeks, after which shedding declines and becomes intermittent [8,19,23]. Upon natural infection, cats can either develop a persistent infection and shed the virus in feces continuously or intermittently for a long time, or eliminate the infection and stop virus shedding [8,24,25]. Furthermore, cats can become repeatedly infected with the same or a different virus strain, resulting in intermittent life-long shedding [19,26–29]. In multi-cat environments, the number of persistent shedders and the overall shedding frequency represent risk factors for the development of FIP in individual cats [7]. The enterocytes in the colon are the main site of FCoV persistence [23,25,29]. The virus can also be found in tissue macrophages, even in the absence of viremia. Therefore, in infected animals, viremia and FIP can develop even after clearance of the virus from the intestine [23].

Anti-FCoV antibodies are detected in the blood as early as one week after experimental infection [19,25,30]. However, antibodies do not seem to be able to confer immunity, as antibody-positive cats can be re-infected and/or develop FIP. High antibody titers are associated with FCoV shedding in feces [8,26,31,32]. Moreover, there is evidence of a positive correlation of shedding frequency as well as shedding intensity with antibody titers [4,33,34]. However, persistent FCoV shedders can also be antibody-negative, and on the other hand, antibody-positive cats can be negative in four sequentially collected fecal samples [4]. Furthermore, although in one study, all 15 included cats with FIP had high anti-FCoV antibody titers, there was much overlap with titers of cats that were presented for any reason to a veterinary hospital, so individual titers alone were of no value for the diagnosis of FIP [35].

Both viral genetic determinants and the host immune system are likely to play roles in the development of FIP [14,36,37], but once FIP is diagnosed, the outcome so far has always been fatal. Several antiviral and immunomodulating FIP treatment regimens have been investigated (for a comprehensive review, see [38]). Thus far, the most promising results were obtained using not yet licensed compounds: a 3C-like protease inhibitor and the nucleoside analog GS-441524 [39–42]. An unlicensed but commercially available oral multi-component drug called Mutian®, Mutian X®, or Xraphconn® (Nantong Biotechnology,

Nantong, China) containing the nucleoside analog GS-441524 [43] proved to be effective in stopping fecal FCoV shedding in naturally infected animals and was successfully applied together with feline interferon omega for the treatment of a cat with non-effusive FIP uveitis [44,45]. We recently published a prospective study using this orally formulated compound (Xraphconn®, Mutian Life Sciences Limited, Nantong, China) containing GS-441524 for the treatment of FIP. All 18 cats with FIP were cured and showed massive improvement in clinical and laboratory parameters within the first few days of treatment without serious adverse effects [43].

The aims of the present study were to further investigate the outcome of these 18 cats with FIP treated with Xraphconn® [43] by (1) determining viral RNA shedding in feces during treatment; (2) comparing fecal viral RNA loads with those in other compartments, i.e., blood and effusion; and (3) comparing viral loads with the presence of anti-FCoV antibodies. Furthermore, viral RNA-positive samples were analyzed to detect FCoV spike gene mutations that lead to the amino acid substitution M1058L and S1060A [46]. Finally, samples from cats living together with the treated FIP cats (companion cats) were tested for viral RNA shedding (including determination of viral sequences) in feces and anti-FCoV antibodies in blood to further investigate the potential for re-exposure of treated cats.

2. Materials and Methods

2.1. In Vivo Study Design and Patients

Eighteen cats with FIP were enrolled in the prospective treatment study: inclusion criteria and study set-up were previously described in detail [43]. Cats were treated orally with Xraphconn® (a multicomponent drug containing GS-441524 [43]) for 84 days (day 0 to day 83). The drug was applied at 5 mg/kg per os (PO) q24h in cats without neurological and/or ocular signs (low dose treatment; 16 cats) and at 10 mg/kg PO q24h in cats with neurological and/or ocular signs (high dose treatment; two cats). All cats were hospitalized for the first 8 days (day 0 to day 7). After discharge, the owners continued daily administration of the compound [43]. Cats were not allowed outdoors during the treatment period. However, some cats from the same households with close contact with the treated FIP cats (17/18 treated cats lived with between 1 and 9 companion cats) had outside access (Table 1). From some of these companion cats, a fecal sample and a serum sample were collected during or after the treatment period of the FIP cats to monitor for infection of these companion cats and virus shedding in the household (Table 1).

Table 1. Cats treated in the study (T1–T18), signalment, signs associated with feline infectious peritonitis (FIP), and information on companion cats in the household (C1–C18) (table partially adapted from [43]).

Identification of Treated FIP Cats	Age (Months)	Sex [1]	FIP-Associated Cardinal Signs [2]	Number of Companion Cats in the Same Household	Identification of Companion Cats with Samples Available	Collection Time Point (Day of Collection) [3] of Fecal and Serum Samples from Companion Cats
T1	6	mn	OS	1	C1 [4]	145
T2	6	mi	NS + OS	1	C2	143
T3	10	mn	AE	1		
T4	7	mi	AE	1	C4	135
T5	6	fi	AE	1	C5	83
T6	11	mn	AE	1		
T7	5	mi	AE + TE	1		
T8	6	mi	TE	0		
T9	9	mn	AE	3		
T10	39	fn	AE	3		
T11	57	fn	AE	3	C11A, C11B, C11C	83
T12	12	mn	TE	1	C12	83
T13	29	fi	TE	9		
T14	8	mi	AE	1	C14	56
T15	8	mi	AE	1	C15	56

Table 1. *Cont.*

Identification of Treated FIP Cats	Age (Months)	Sex [1]	FIP-Associated Cardinal Signs [2]	Number of Companion Cats in the Same Household	Identification of Companion Cats with Samples Available	Collection Time Point (Day of Collection) [3] of Fecal and Serum Samples from Companion Cats
T16	9	fn	AE	1	C16	56
T17	8	mi	TE	1		
T18	8	fi	AE	2	C18	56

[1] mn: male neutered; mi: male intact; fn: female neutered; fi: female intact. [2] OS: ocular signs; NS: neurological signs; AE: abdominal effusion; TE: thoracic effusion. [3] Days after treatment start in treated FIP cats. [4] Companion cats were labeled "C" and with the number of the corresponding treated cat. For cats C1, C2, and C4, samples were only available after the end of the treatment of cats T1, T2, and T4.

2.2. Sample Collection for Determination of Viral Loads and Anti-FCoV Antibody Titers

From the 18 treated FIP cats, blood and, if present and accessible, thoracic and abdominal effusions were collected on days 0, 2, 4, 7, 14, 28, 56, and 83 for viral RNA load determination. Serum samples were collected on days 0, 7, 14, 28, 56, and 83 to measure anti-FCoV antibodies, as described previously [43]. In addition, for the present study, fecal samples were collected from the 18 treated FIP cats on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 56, and 83. Both voided fecal samples and fecal swabs were collected. Fecal swabs were taken if no voided feces were available.

In companion cats, fecal samples for viral RNA loads and serum samples for antibody titer quantification were collected once at different time points (Table 1). All samples were stored at −80 °C until analysis.

2.3. Anti-FCoV Antibody Titers

Serum samples from treated FIP cats and companion cats were analyzed by an indirect immunofluorescence assay (IFA) as previously described [47–49]. Cat samples were tested at dilutions of 1:25, 1:100, 1:400, 1:1600, and 1:6400. A positive control (aliquoted serum sample from an anti-FCoV antibody-positive field cat) and a negative control (aliquoted serum from a specific pathogen-free anti-FCoV antibody-negative cat) were run with each slide.

2.4. FCoV RNA Loads in Feces, Blood, and Effusions

FCoV RNA load was determined in blood, fecal, and effusion samples by RT-qPCR. Briefly, viral total nucleic acids (TNA) were extracted from 200 µL of effusion or 100 µL EDTA anti-coagulated whole blood or 200 µL of fecal samples. For fecal samples, depending on the collected material, approximately 0.05 g of feces was dissolved in 1 mL of 1X sterile phosphate buffered saline, pH 7.4 (PBS; Gibco, Life Technologies Ltd., Paisley, UK), or fecal swabs were dissolved in 400 µL of PBS; fecal material was processed as described [19]. TNA were extracted using the MagNA Pure 96 (Roche Diagnostics AG, Rotkreuz, Switzerland) and the MagNA Pure 96 DNA and Viral NA SV Kit (Roche Diagnostics) according to the manufacturer's instructions, with an elution volume of 100 µL. For all samples, the viral NA plasma external lysis SV 4.0 protocol was applied. For each batch of extractions, negative controls were run in parallel to check for cross-contamination.

A previously published real-time RT-qPCR assay was used to detect the FCoV 7b gene [50]. The methods were adapted as described previously [43]. All RT-qPCR were run with 5 µL of TNA in a final volume of 25 µL. Positive and negative PCR controls were run in parallel using an ABI7500Fast instrument (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Fecal samples were run neat and diluted 1:5 in nuclease free water to detect possible RT-qPCR inhibition. An FCoV RNA standard curve was run in parallel to determine the viral RNA copy number. The FCoV RNA standard was produced as follows: a NotI linearized pCR™II-TOPO® TA vector containing the sequence of the target assay was in vitro transcribed using the Large Scale SP6 Transcription Kit (Novagen, Juro

supply, Lucerne, Switzerland), followed by purification by the RNeasy Mini Kit (Qiagen AG, Hombrechtikon, Switzerland). In vitro transcribed RNA was quantified, the RNA copy number was calculated as described [51], and 10-fold serial dilutions were prepared in PCR-grade water with 30 µg/mL of carrier rRNA (Sigma-Aldrich, Merk, Darmstadt, Germany). The RNA was stored in 50 µL aliquots at −80 °C until use.

2.5. Sanger Sequencing to Detect Spike Gene Mutations in Fecal Samples, Blood, and Effusions

FCoV RT-qPCR-positive samples underwent conventional RT-PCR amplifying part of the spike gene potentially containing FCoV mutations that lead to protein substitutions M1058L and S1060A [46]. Shortly, the FCoV-UCD1-S.3022 forward and FCoV-UCD1-S.3636 reverse primer were used for a first amplification (amplicon 615 bp) using the one-step RT-PCR Kit (SuperScript III RT/Platinum Taq Mix, Invitrogen, Thermo Fisher Scientific). The reaction composition and cycling conditions were used as published previously [52]. For the second nested PCR step, if needed, a modified primer pair amplifying the region of interest (amplicon length 134 bp), FCoV-UCD1-S.3027f and FCoV-UCD1-S.3160r, and the Phusion Hot Start II High-Fidelity DNA Polymerase (Thermo Fisher Scientific) were used as described [52]. Gel electrophoresis was performed with 1.5% agarose gels containing 0.1 mM GelRed (Biotium, Hayward, ON, Canada). Following the addition of Orange G loading dye (Bioconcept, Allschwil, Switzerland) in a 1:5 ratio to the amplified DNA, samples were loaded on the gels and run at 100 V. A 1-kilobase-pair DNA ladder (Fermentas, St. Leon-Rot, Germany), or alternatively, a Gene Ruler DNA Ladder Mix (Thermo Fisher Scientific), was used for molecular size comparisons. Appropriate bands were cut out with sterile razor blades and weighed. PCR amplicons were purified with the MinElute or QIAquick Gel extraction Kit (Qiagen) according to the manufacturer's instructions and sequenced directly. Selected nested PCR amplicons were cloned into the pCR®II-TOPO plasmid with the TOPO TA Cloning Kit for Sequencing (Invitrogen, Thermo Fisher Scientific) using TOP 10 competent cells according to the manufacturer's instructions, after adding A-overhangs using the Taq DNA Polymerase (Sigma-Aldrich). The reaction mix for A-tailing contained 10 µL of PCR product, 1.2 µL Taq Buffer (10×), 0.24 µL dATP (10 mM), 0.1 µL Taq DNA Polymerase (5 U/µL), and 0.46 µL RNase-free water and was incubated at 72 °C for 15 min. Transformed TOP 10 cells were grown overnight at 37 °C on Luria broth plates containing ampicillin. From each cloned RT-PCR amplicon, 10 colonies were picked and cultured overnight at 37 °C in Luria-Bertani liquid medium containing ampicillin. Cultures were centrifuged at 6800× *g* for 3 min and the pellets were used for further manipulation. Plasmids were isolated from the TOP 10 cell pellets with the QIAprep Miniprep Kit (Qiagen) according to the manufacturer's instructions. Concentrations of the eluted DNA were determined using a Nanodrop 2000c (NanoDrop products, Wilmington, NC, USA). Sanger sequencing was performed using M13 forward and M13 reverse primers on 1.2 µg plasmid DNA. Sequencing was performed in a commercial lab (Microsynth, Balgach, Switzerland). Nucleotide sequences were manually edited, assembled, aligned and compared to sequences from Genbank using Geneious Prime® (https://www.geneious.com (accessed on 16 May 2022); Biomatters Ltd., Auckland, New Zealand). Evolutionary analyses were conducted using the Molecular Evolutionary Genetics Analysis software package version X (MEGA X) [53]. Nucleotide sequences were aligned using the ClustalW algorithm [54]. Bootstrap phylogenetic trees were constructed using the Minimum Evolution method [55] and Jukes–Cantor model. A bootstrap analysis was performed to test the stability of the trees with 1000 replicates [56]. Sequences are shown as a FASTA file in the Supplementary Material.

2.6. Statistics

Frequencies of treated FIP cats presenting with effusions, shedding viral RNA in feces, and being RT-qPCR-positive in blood at different time points of the study, or of treated FIP cats and companion cats having high anti-FCoV antibody titers (≥1 > 1:6400) were compared using Fisher's exact test (p_{Fisher}). FCoV RNA loads in fecal swabs and voided

fecal samples were compared using the non-parametric Mann–Whitney U–test. Viral RNA copy number differences in animals between selected treatment days were compared using the non-parametric Wilcoxon signed-rank test (p_W). The correlation of viral loads in the three compartments (feces, blood, and effusions) and antibody titers was calculated using the nonparametric Spearman rank correlation (p_S; r = correlation coefficient). A *p*-value < 0.05 was considered to be statistically significant in all cases. Statistical analysis was performed using the GraphPad Prism 9.3.1 software (GraphPad software, LLC, San Diego, CA, USA).

3. Results

3.1. Treatment Study

Clinical outcomes of successful treatment in cats T1–18 have been described in detail previously [43]. Briefly, all cats survived, demonstrating a full clinical recovery within 84 days after treatment initiation [43]. In all cats, clinical (Karnofsky score, body temperature, and body weight) and laboratory parameters (hematocrit, lymphocyte count, bilirubin, total protein, albumin, globulin, and serum amyloid A concentrations) improved constantly and significantly within the first few days of treatment [43]. The number of cats with effusion had decreased significantly by day 14 after treatment initiation, in comparison to day 0 (p_{Fisher} = 0.0045; Figure 1B, Table 2). On day 83, effusion was no longer present in any of the cats.

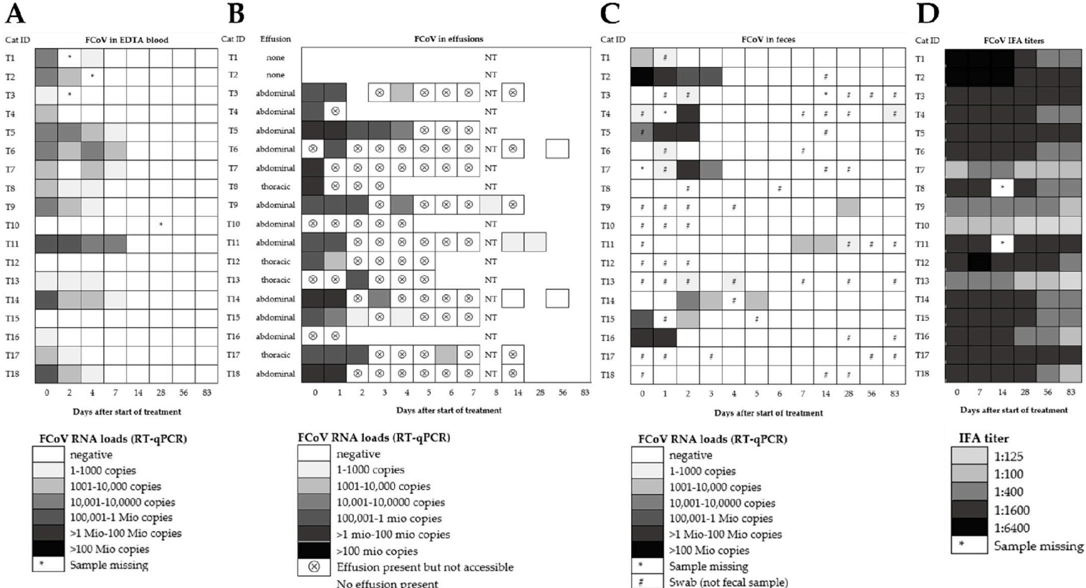


Figure 1. Feline coronavirus (FCoV) viral RNA loads in different compartments and anti-FCoV titers of treated FIP cats (adapted from [43]). (**A**) EDTA anti-coagulated blood; (**B**) Effusions; (**C**) Voided fecal samples or fecal swabs; and (**D**) Antibody titers. FCoV RNA loads were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR) (**A**–**C**). Antibody titers were determined by indirect immunofluorescence assay (IFA). NT, not tested.

3.2. Fecal FCoV RNA Shedding and Viral Loads in Treated FIP Cats

In five of the eighteen treated FIP cats (28%), fecal shedding could neither be detected prior to treatment (day 0) nor at any timepoint throughout the entire study (cats T8, T10, T12, T17, T18; Figure 1C). Only 6/18 cats (33%) tested RT-qPCR-positive in feces on day 0. However, when samples from the first three days of the study were included in the analysis (day 0 to day 2), about 2/3 of the treated FIP cats (11/18; 61%) were positive for FCoV RNA in feces. Fecal virus shedding had stopped in all of these 11 initially shedding cats by day

6, but one cat tested positive later again on day 83 (cat T4). Moreover, two cats (cats T11 and T9) that initially had not shed any viral RNA became RT-qPCR-positive on days 7, 14, and 28 (T11), and on day 28 (T9), respectively. The number of cats shedding FCoV RNA in feces had significantly decreased by day 4 when compared to day 0 ($p_{Fisher} = 0.0408$). Interestingly, in seven cats, viral loads initially increased during treatment (T3, T4, T5, T6, T7, T13, T14), while in three cats (T1, T2, T16), the loads decreased. Moreover, in one cat (T15), the RNA load first decreased and then rebounded before RNA shedding ceased (Figure 2).

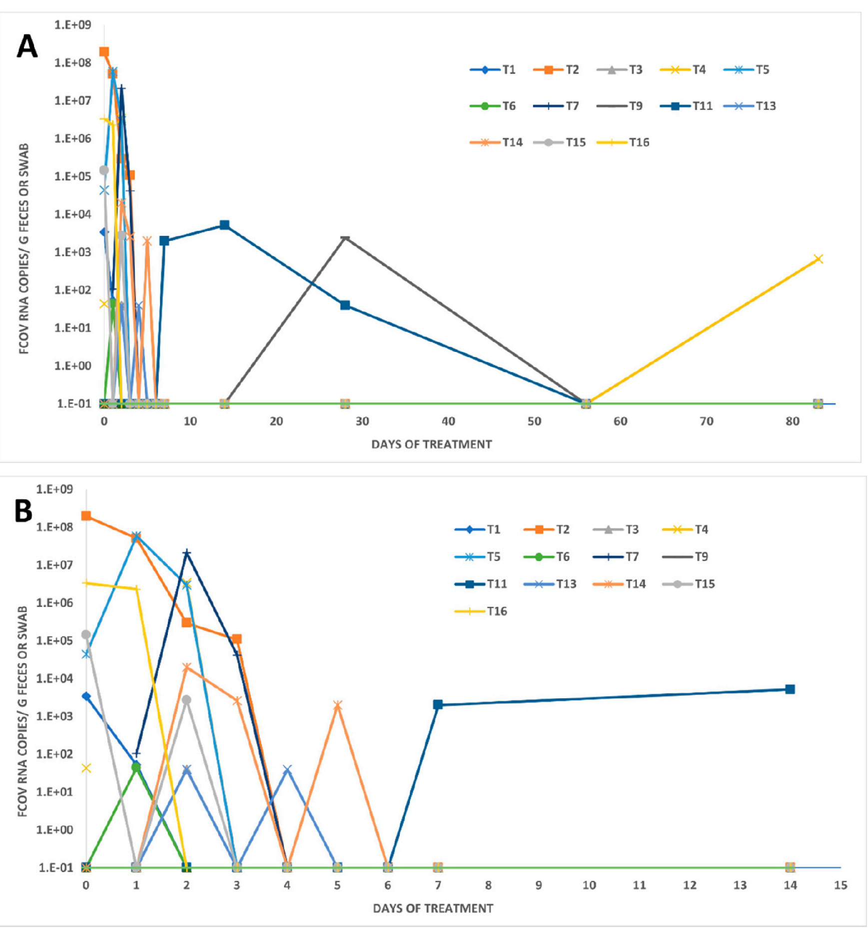


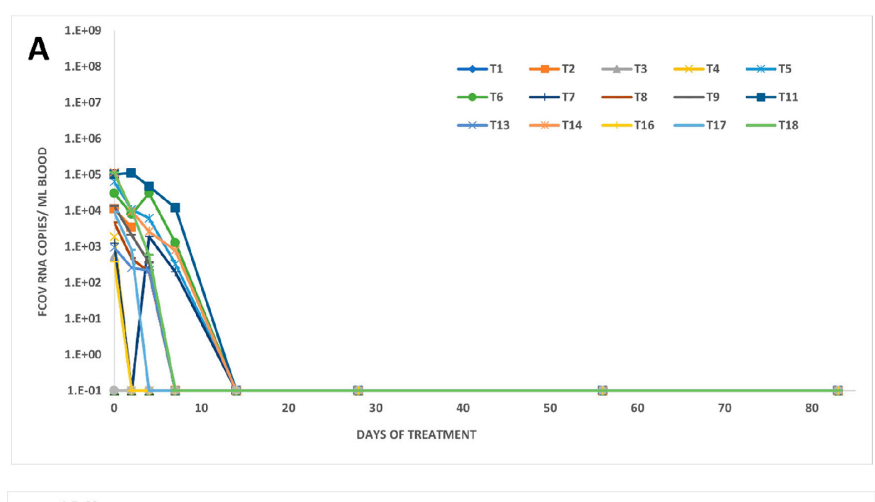
Figure 2. Viral RNA loads in voided fecal samples and fecal swabs of FIP treated cats (T1–T18). Fecal samples were collected from the 18 treated FIP cats (**A**): on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 56, and 83; (**B**): on days 0, 1, 2, 3, 4, 5, 6, 7 and 14. Both voided fecal samples and fecal swabs were collected. Fecal swabs were taken if no voided feces were available. RNA loads were measured by feline coronavirus (FCoV) quantitative reverse transcription polymerase chain reaction (RT-qPCR). Values are given in copy numbers per g feces or swab.

Viral RNA loads in voided fecal samples ranged from almost 196 million copies/g feces on day 0 in cat T2 to 2000 copies/g feces in cat T11 (on day 7) and cat T14 (on day 5). In fecal swabs, loads were lower than in voided fecal samples; loads in swabs were as high as 44,000 copies per swab on day 0 (cat T5) to as low as 40 copies per swab on days 2 (cats T3 and T13) and 4 (cat T13). The influence of the different sample collection methods (swabs versus voided fecal samples) was assessed by comparing the resulting FCoV RNA

loads per PCR reaction; thereby, the RNA loads in fecal swabs were significantly lower than those in voided fecal samples (p_{MWU} = 0.0069).

3.3. FCoV RNA Loads in Other Body Compartments of Treated FIP Cats

Remarkably, in most of the 18 treated FIP cats (15/18; 83%), viral RNA was detected in blood before the start of the treatment (day 0), but by day 7, the number of cats positive for viral RNA in blood had significantly decreased (p_{Fisher} = 0.0020). Blood viral RNA loads in 14 of these 15 cats decreased even earlier than viral loads in feces, by day 2 after treatment initiation (Figures 1A and 3). Blood viral RNA loads ranged from almost 120,000 to about 400 copies per mL blood on day 0. Loads significantly decreased from day 0 to day 2 (p_W = 0.0061; values in 2/16 cats missing and thus two cats excluded; n = 16 cats) and from day 0 to day 7 (p_W < 0.0001; n = 18 cats). In two of the positive cats with an initial decrease (cats T6 and T7), the blood viral RNA load rebounded transiently on day 4 to the same level as on day 0, but decreased again thereafter (Figure 3). The last viral RNA-positive samples were found on day 7. By day 14, no viral RNA was detected in any of the cats (Figure 1A), indicating that all cats had cleared viral RNAemia by that time.



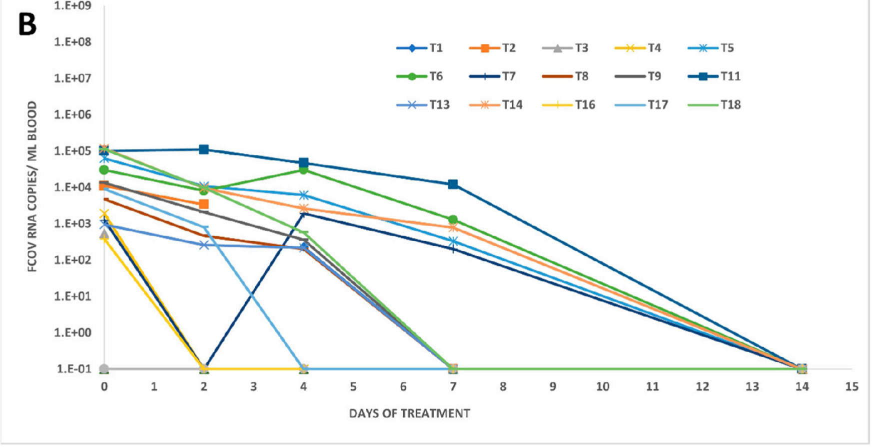
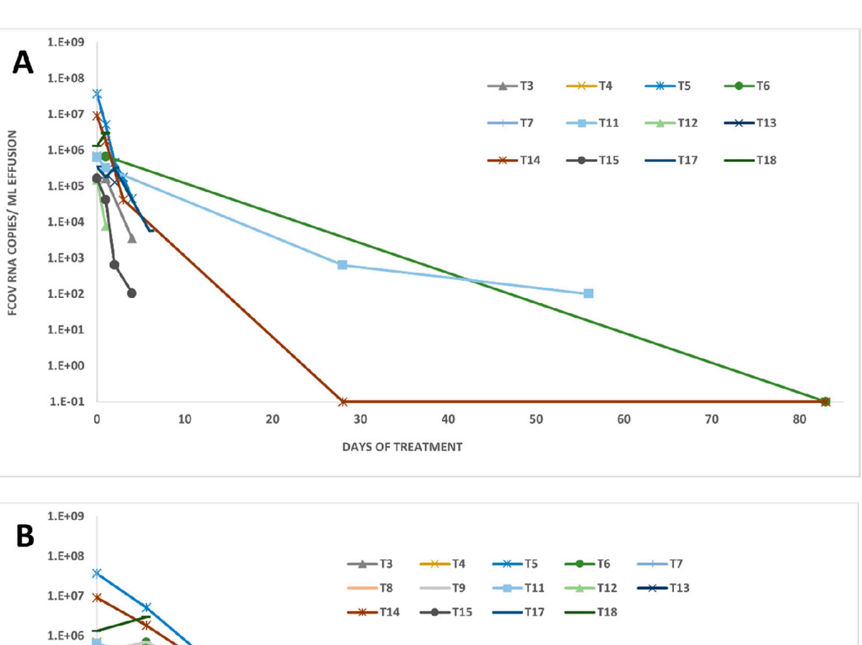


Figure 3. Viral RNA loads in blood samples of treated FIP cats. EDTA blood was collected (**A**): on days 0, 2, 4, 7, 14, 28, 56, and 83 and (**B**): on days 0, 2, 4, 7, and 14 for the determination of viral RNA loads. Loads were measured by feline coronavirus (FCoV) quantitative reverse transcription polymerase chain reaction (RT-qPCR) in cats. Values are given in copy numbers per mL blood.

Sixteen of the eighteen treated FIP cats had effusion at treatment initiation (day 0), and all accessible effusions (n = 12) tested RT-qPCR-positive at that time (Figure 1B). Viral RNA load in effusion decreased rapidly in most cats, while only two cats (T9 and T18) had increased loads one day after treatment initiation (Figures 1B and 4). After the initial increase, viral RNA loads in the effusion of cat T9 decreased from day 1 (684,114 copies/mL) to the last effusion sampling on day 8 (691 copies/mL). The effusion of cat T18 was only accessible on day 0 and day 1. Therefore, it remains unknown if the viral RNA load would have decreased over time in this cat.



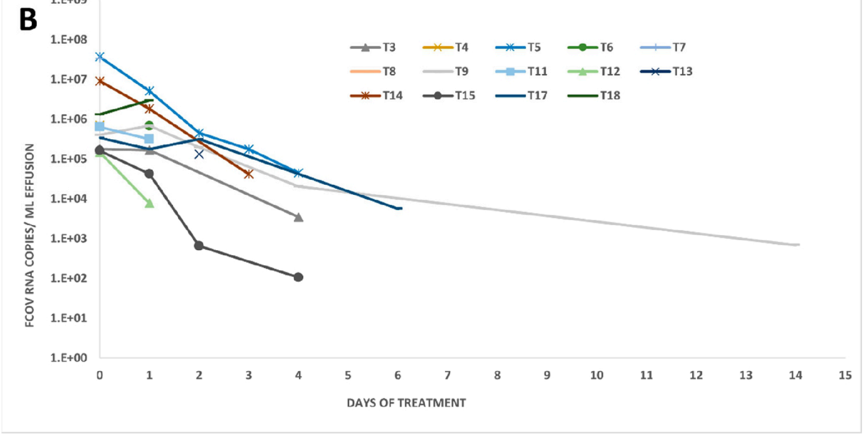


Figure 4. Viral RNA loads in accessible effusions of treated FIP cats. Present and accessible thoracic and abdominal effusions were collected (**A**): on days 0, 2, 4, 7, 14, 28, 56, and 83 and (**B**): on days 0, 2, 4, 7, and 14 for viral RNA load determination. Loads were measured by feline coronavirus (FCoV) quantitative reverse transcription polymerase chain reaction (RT-qPCR) in cats with accessible effusions (n = 16). Values are given in copy numbers per mL effusion. When no value is depicted, either effusions were not (or no longer) accessible or no effusion was present.

Generally, viral RNA was detectable in all but three accessible effusion samples (from cat T6 on day 56 and cat T14 on days 14 and 56), and two of the three had a volume smaller than the 200 µL usually used for TNA extraction. To increase the sensitivity of detection, these three samples were retested by RT-qPCR with 10 replicates, yielding negative results.

There was a good correlation between viral RNA loads in blood and effusion ($p_S = 1.3 \times 10^{-8}$; r = 0.856; 95% CI 0.6990–0.9340). No correlation was found between viral RNA loads in feces and effusion or blood, respectively.

3.4. Anti-FCoV Antibody Titers in Treated FIP Cats

All treated FIP cats were antibody-positive at the beginning of the study (day 0), most of them with high antibody titers (14/18 with titers $\geq$ 1:1600). Anti-FCoV antibody titers declined in 14/18 cats, in some cats starting as early as 28 days after treatment initiation (Figure 1D). In four cats (T3, T5, T11, T17), titers remained unchanged throughout the observation period. In only one cat (T8), a slight titer increase was observed after one week of treatment (Figure 1D). No correlation was found between fecal RNA loads and antibody titers ($p_S = 0.06$; $r = 0.187$; 95% CI 0.0120–0.3710). A very weak correlation between blood RNA loads and antibody titers was found ($p_S = 0.037$; $r = 0.204$; 95% CI 0.0071 to 0.3856).

3.5. Spike Gene Mutations in Fecal Samples, Blood, and Effusions in Treated FIP Cats

Only samples with a cycle threshold (Ct) below 35 (corresponding to a viral RNA load of approximately 1850 copies per mL in blood and effusion samples or 20,200 copies per g in fecal samples) were considered for sequencing (Table 2).

Table 2. Sequencing results of the viral spike gene including positions 1058 and 1060 in fecal samples, blood, and effusion of treated FIP cats.

	Effusions					**Blood**				**Fecal Samples**										
Cat Number	**d0**	**d1**	**d2**	**d3**	**d4**	**d0**	**d2**	**d4**	**d7**	**d0**	**d1**	**d2**	**d3**	**d4**	**d5**	**d7**	**d14**	**d28**	**d56**	**d83**
T1						nb		x	n	x	x	n	n	n	n	n	n	n	n	n
T2						nb	nb		n	**MS**	**MS**	nb	nb	n	n	n	n	n	n	n
T3	nb	**LS**			nb	x		n	n	n	n	x	n	n	n	n		n	n	n
T4	**LS**					x	n	n	n	x		**MS**	n	n	n	n	n	n	n	x
T5	**LS**	**LS**	**LS**	nb	nb	nb	nb	nb	x	x	**MS**	**MS**	n	n	n	n	n	n	n	n
T6		nb				nb	nb	nb	x	n	x	n	n	n	n	n	n	n	n	n
T7	**LS**					x	n	nb	x		x	**MS**	**MS**	n	n	n	n	n	n	n
T8	**LS**					nb	x	x	n	n	n	n	n	n	n	n	n	n	n	n
T9	nb	nb	nb		nb	nb	nb	x	n	n	n	n	n	n	n	n	n	x	n	n
T10						n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
T11	**FS** *	**FS** *				nb	nb	nb	nb	n	n	n	n	n	n	x	x	x	n	n
T12	**MA** *	nb				n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
T13			nb			x	x	x	n	n	n	x	n	x	n	n	n	n	n	n
T14	**MA** *	**MA** *		nb		**MA** *	nb	nb	x	n	n	nb	x	n	x	n	n	n	n	n
T15	**LS**	nb	x		x	n	n	n	n	**MS**	n	x	n	n	n	n	n	n	n	n
T16						x	n	n	n	**MS**	**MS**	n	n	n	n	n	n	n	n	n
T17	nb	nb	nb			nb	x	n	n	n	n	n	n	n	n	n	n	n	n	n
T18	**LS**	**LS**				nb	nb	x	n	n	n	n	n	n	n	n	n	n	n	n

Bold: sequenced samples. LS: leucine at position 1058 and serine at position 1060; MS: methionine at position 1058 and serine at position 1060; FS: phenylalanine at position 1058 and serine at position 1060; MA: leucine at position 1058 and alanine at position 1060; nb: sample with no band detected after nested RT-PCR; x: FCoV RT-qPCR-positive but not sequenced due to low viral RNA load; n: FCoV RT-qPCR-negative sample; gray shading: no sample available. * Confirmed by cloning of the PCR amplicon and sequencing of several clones.

Of the 30 RT-qPCR-positive fecal samples of treated FIP cats (from days 0 to 83), 18 samples qualified for sequencing (Ct < 35), of which 10 could be sequenced successfully. In all 10 sequenced fecal samples, collected between days 0 and 3 of the study from six treated FIP cats, the amino acid sequence corresponded to the FCoV wild-type, with a methionine at position 1058 and a serine at position 1060 of the S protein (MS).

Of the 40 RT-qPCR-positive blood samples of treated FIP cats (from days 0 to 7), 23 qualified for sequencing (Ct < 35), but only one sample from cat T14 collected on day 0 was sequenced successfully. The spike protein in this sample had a methionine at position 1058 but an alanine instead of a serine at position 1060 (MA).

Of the 33 RT-qPCR-positive effusion samples of treated FIP cats (from days 0 to 4), 31 qualified for sequencing (Ct < 35); of these, 15 samples, collected on days 0 to 2 from 10 treated FIP cats were sequenced successfully. In seven out of these ten cats (T3, T4, T5, T7, T8, T15, T18), the amino acid leucin instead of a methionine was found at position 1058 of the S protein (LS; Table 2). In two of the cats (T12 and T14), an alanine was found instead of a serine at position 1060 (MA), and in one cat (T11), an atypical mutation at position 1058 was detected, in that the methionine was replaced by the amino acid phenylalanine

(FS; Table 2). In cat T14, in which the viral spike gene from both effusion and blood could be sequenced, the same mutation was found (MA) in both compartments.

Sequencing results were confirmed in cats T11 (effusion from days 0 and 1), T12 (effusion from day 0), and T14 (effusion from days 0 and 1, and blood from day 0) by cloning of the PCR product and subsequent sequencing. All sequences could be confirmed without exception using at least nine clones in the effusion samples of cats T11 (days 0 and 1), T12 (day 0), and T14 (day 0); three clones in the effusion sample of cat T14 (day 1); and six clones in the blood sample of cat T14 (day 0).

3.6. Fecal FCoV Shedding, Spike Gene Mutations, and Anti-FCoV Antibody Titers in Companion Cats

All companion cats were clinically healthy at the time of sample collection. Eight of twelve (67%) companion cats were positive for viral RNA in feces (Table 3). The percentage of cats shedding viral RNA in feces was not significantly different between companion cats (67%; samples collected at various time points) and treated FIP cats at the start of the treatment (61%; days 0 to 2; Figure 1C). FCoV viral RNA loads in companion cats ranged from almost 75 million copies/g feces (C16) on day 56 to 160,000 copies/g feces (C15 on day 56) in voided fecal samples and from 347,000 copies/fecal swab (C11B on day 83) to about 2000 copies/fecal swab (C11A on day 83).

Table 3. Fecal viral RNA loads and anti-FCoV antibody titers in companion cats.

Companion Cat Number	Corresponding Treated FIP Cat	Day of Sample Collection in Companion Cats	Fecal Samples Collected	Fecal RNA Loads (Copies per g Feces or per Swab)	Sequencing Result	Anti-FCoV Antibody Titer
C1	T1	145	Swab	n	n	1:1600
C2	T2	143	Feces	66,337,219	**MS**	1:100
C4	T4 [1]	135	Swab	27,545	**MS**	1:400
C5	T5	83	Feces	12,990,572	**MS**	1:1600
C11A	T11 [2]	83	Swab	1906	nb	1:100
C11B	T11 [2]	83	Swab	346,600	nb	1:400
C11C	T11 [2]	83	Swab	178,345	nb	1:400
C12	T12	83	Feces	n	n	1:100
C14	T14	56	Swab	n	n	1:25 *
C15	T15	56	Feces	159,536	nb	1:400
C16	T16	56	Feces	74,714,117	**MS**	1:100
C18	T18	56	Swab	n	n	1:100

For better comparison, fecal swab samples are highlighted in gray. Bold: sequenced samples. [1] T4 showed reoccurrence of fecal shedding on day 83. [2] T11 was one of two cats that showed new appearance of fecal shedding during the study (day 7). FIP: Feline Infectious peritonitis; FCoV: Feline Coronavirus; g: gram; MS: methionine at position 1058 and serine at position 1060; nb: sample with no band detected after nested RT-PCR; n: FCoV RT-qPCR-negative sample. * Borderline titer.

All companion cats were antibody-positive (one with a borderline antibody titer; Table 3). The antibody titers of the companion cats (in samples collected at various time points) ranged from 1:25 to 1:1600. Significantly fewer companion cats had titers of $\geq$1:1600 (2/11) compared to the treated FIP cats on day 0 (17/18 cats; p_{Fisher} = 0.0054).

Four of eight positive fecal samples from companion cats were successfully sequenced. In all samples, the amino acid sequence corresponded to the wild-type, with a methionine at position 1058 and a serine at position 1060 of the S protein (MS; Table 3).

3.7. Phylogenetic Analysis of Sequences of Treated FIP Cats and Companion Cats

A phylogenetic analysis of the sequenced 143 bp of the spike gene showed that sequences isolated from each individual animal clustered together (Figure 5). Fecal samples of the companion cats clustered together with the corresponding treated FIP cats.

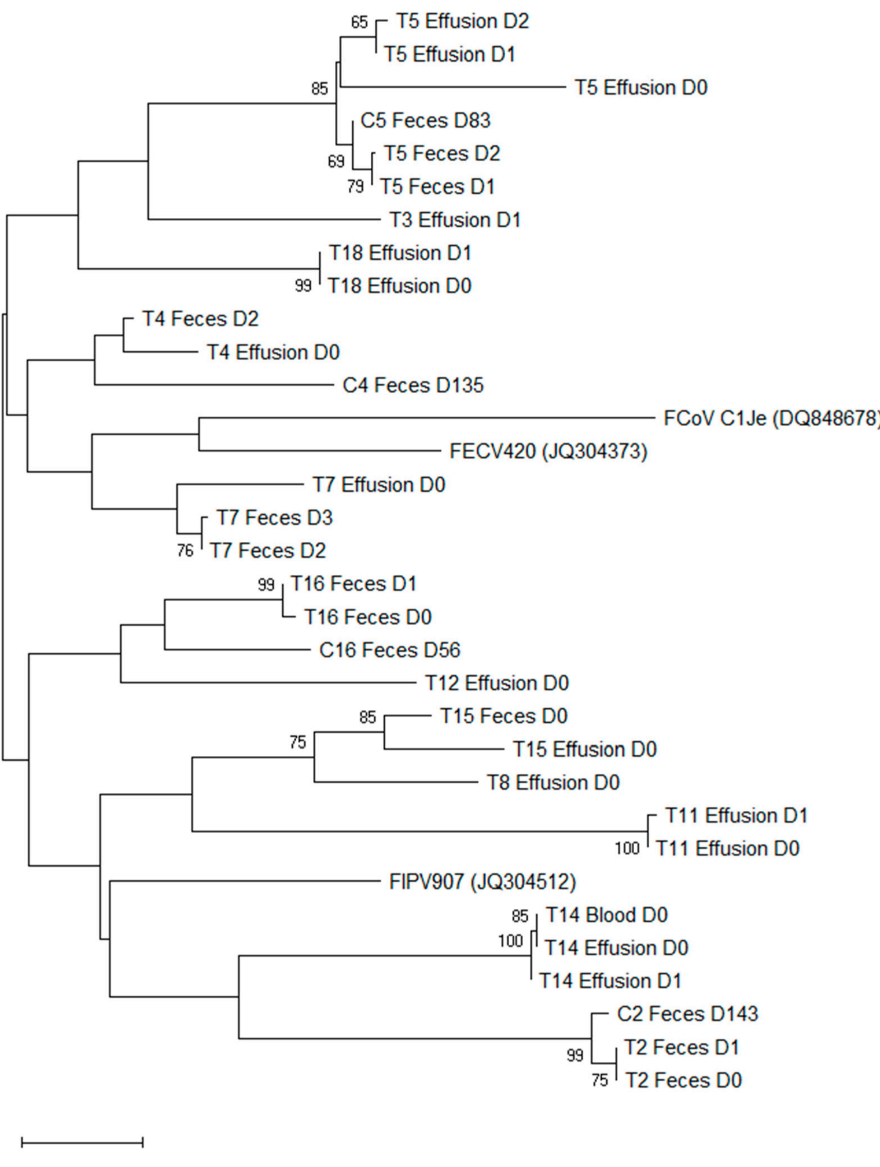


Figure 5. Bootstrap phylogenetic tree of spike (S) gene sequences. The evolutionary history was inferred using the Minimum Evolution (ME) method [55]. The optimal tree is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (values below 60 are not displayed) [56]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes–Cantor method and are in the units of the number of base substitutions per site. The ME tree was searched using the Close Neighbor Interchange (CNI) algorithm [57] at a search level of one. The Neighbor Joining algorithm [58]

was used to generate the initial tree. This analysis involved 86 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). In total, there were 155 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [53]. Each sequenced FCoV strain is defined by the cat ID (T: treated FIP cat; C: companion cat), the compartment from which it has been isolated (feces, blood, effusion), and day of treatment (D#). Three prototype FCoV sequences from Genbank have been used in the tree: an enteric FCoV pathotype (FECV420, Accession no.: JQ304373) and an FIP virus pathotype (FIPV907, Accession no.: JQ304512) from the study [46], and an FIP virus pathotype isolated from the jejunum of an FIP cat (FCoV C1Je, Accession no.: DQ848678).

4. Discussion

This prospective controlled treatment trial in field cats with a confirmed or highly suspected diagnosis of FIP clearly demonstrated that oral treatment with the nucleoside analog GS-441524, contained in the multicomponent drug Xraphconn®, decreased viral RNA loads not only in blood and effusion (where accessible) but also in feces within a short period of time after treatment initiation, and no cats were viremic after day 14 of treatment [43]. These findings demonstrate a highly effective response to the treatment. In addition, antibody titers decreased during the therapy. The current study renders more granular insights into RNA loads and the presence of spike gene mutations in different compartments of treated FIP cats, adding new data on fecal RNA shedding in these cats, and, in addition, collected information on fecal RNA shedding and antibody titers of cats cohabiting with the treated FIP cats.

The majority of the treated FIP cats in the present study (72%) shed viral RNA at some time point throughout the study, particularly during the early days of treatment; only five cats never tested RT-qPCR-positive in fecal samples. Interestingly, on day 0, only 8/18 cats shed FCoV although all had FIP. This observation is not surprising, as in a previous study, no fecal shedding could be detected in the majority of cats with FIP [59]. Nonetheless, in another study, FIP cats were more likely to shed viral RNA in feces compared to cats without FIP [60]. Another previous study found some evidence that an intact FCoV ORF3c is required for intestinal replication [61].

Fecal viral RNA loads decreased significantly during early treatment, and most of the cats had stopped shedding FCoV RNA as early as day 4 of the treatment. Elimination of virus shedding through treatment with the same compound (containing GS-441524) has been described earlier in a study in breeding and rescue catteries with FCoV infection (that did not have FIP) that aimed to reduce the infection pressure in multi-cat households by stopping fecal FCoV shedding [45]. In this study, the authors could prove that a dose of 4 mg/kg q24 h for four days was optimal to stop fecal FCoV RNA shedding in 95% of naturally FCoV-infected, healthy persistent shedders from five households. These results could be reproduced in the present study, where animals were treated with a slightly higher dose (5 mg/kg, with the exception of T1 and T2 that suffered from neurological and/or ocular signs and therefore received a dose of 10 mg/kg) and stopped FCoV shedding by day 4 of treatment. In the study of Addie et al., 2020, in some animals, FCoV shedding restarted upon re-infection or relapse as soon as three days after treatment stopped. In one cat (T4) from the present study, reoccurrence of shedding was detected 83 days after initiation of the treatment and 80 days after shedding had initially ceased in this cat. Moreover, in two cats (T9 and T11), no shedding was detected initially but then was detected later on during the treatment period. Two of these three cats (T4 and T9) started shedding after they had been discharged from the veterinary hospital. These two cats had companion animals in the same household. In the case of cat T4, there was one companion cat (C4), which was sampled at a later time point (day 135), in which the fecal swab tested RT-qPCR-positive. Cat T9 had three companion cats, but no samples were available from those cats. Nonetheless, it is likely that cats T4 and T9 were both re-infected at home by their feline companions during the treatment period and the positive fecal samples in the treated FIP cats most probably represented a superinfection with an intestinal FCoV

from their companion cats. The absence of viremia in the treated cats at the time point of positive testing of their fecal samples supports this assumption. Moreover, clustering of the S gene sequence of viruses from T4 and the companion cat C4 was observed; this further supports the likelihood of a re-infection of the treated FIP cat by its companion cat in the same household. The same could be valid for cat T9, which had two companion cats in the same household. The third cat that started re-shedding (T11) was still in the hospital at that time (day 7). Therefore, it seems reasonable to assume that in this cat, the virus most likely originated from a sanctuary compartment within the cat. A superinfection is rather unlikely (although not completely excluded), since the cat was kept separately from other animals in the hospital. Further insight could be gained by the sequencing of the virus shed by cat T11 and its companion cat; unfortunately, the fecal samples from cat C11 could not be sequenced due to a low viral load.

Intermittent fecal FCoV shedding has been frequently described [8,24]. The fact that a cat during treatment with the nucleoside analog GS-441524 and re-exposure to FCoV could start (re-)shedding the virus demonstrates that treatment does not prevent infection of enterocytes. The concentration of the antiviral in the enterocytes is probably not high enough to prevent re-infection. On the other hand, enterocytes have a very short lifespan [62], which could explain the intermittent shedding (disappearance of cells infected with low pathogenic FCoV once the mutated virus has reached the monocyte/macrophage compartments, and re-infection of new enterocytes). Thus, intestinal FCoV infections in cats should not be treated with GS-441524 or other antiviral compounds, as this poses the risk of mutations and development of resistant virus strains, since up to 90% of cats in multi-cat households are infected with and shed FCoV in their feces [3–6], and re-infections occur continuously and cannot be prevented. Even if a multi-cat environment is cleared from FCoV, it is likely that FCoV will be reintroduced into the household within a short time [45]. Furthermore, a resistant viral strain could persist in an animal. If this animal later develops FIP the antiviral drug would not be efficient anymore. Additionally, resistant FCoV strains could be shed and transmitted to other cats.

A remarkably high percentage of cats with FIP in this study (83%) were viral RNA-positive in blood before treatment with relatively high viral RNA loads. FCoV viremia can last from about 7–21 days following experimental infection and then decline so that in cats with FIP, often no viral RNA is found in the blood [23,37]. On the other hand, a study in shelter cats detected FCoV RNA in the blood of healthy and sick cats without FIP [63]. In many other studies, detection of viral RNA in blood was a rather rare event in cats with confirmed FIP [64–67], making the sensitivity of FCoV RT-qPCR from blood for diagnosis of FIP very low. In addition, the specificity of FCoV RT-qPCR from blood was questioned by the fact that healthy FCoV-infected animals were also sometimes viral RNA-positive in blood [19,63]. Thus, the notion that viremia will likely be over by the time clinical signs of FIP appear [23,37] might have to be reconsidered in view of the current data. However, systemically infected healthy animals without FIP that were RT-qPCR-positive in blood had lower FCoV viral RNA loads than cats with FIP [19,21,30]. Consequently, a positive RT-qPCR from blood alone cannot be recommended for the diagnosis of FIP, but positive results with a high FCoV load can be used to support a diagnosis, and based on the data of the present study, FCoV RT-qPCR from blood could be a valuable diagnostic tool, especially in cats in which no effusion is present.

In 12 out of the 18 treated FIP cats, effusion was present and accessible. In all 12 effusion samples collected on day 0, high viral RNA loads were detected. This result alone supports the utility of the FCoV RNA detection in effusion for the diagnosis of FIP. Previous studies could also amplify FCoV RNA from most (72–100%) of the effusion samples collected from cats with FIP and none from cats without FIP [64,66,68,69]. Few exceptions have been published in which effusion samples from cats with other underlying diseases had positive results for FCoV RNA. Therefore, high FCoV RNA loads in effusion samples, particularly those that also show cytological and biochemical features suggestive of FIP, are highly supportive of FIP. The viral RNA loads in accessible abdominal or thoracic effusions

decreased in all cats over the study period. In most cats, the effusion disappeared or was not accessible anymore before negative RT-qPCR results could be achieved. However, looking at the blood and fecal viral RNA loads, it is assumed that viral RNA was also cleared from the thoracic or abdominal compartment. Therefore, GS-441524 treatment was beneficial in reducing viral RNA loads in effusions, blood, and feces and cured abdominal and/or thoracic effusions in FIP-diseased cats. A good correlation of FCoV RNA loads between blood and effusions was found in the present study, indicating, as expected, exchange between two compartments.

Most cats in the present study with FIP had remarkably high antibody titers, which decreased in most but not all cats over time. It has been known for a long time that specific antibodies are responsible for enhanced FCoV uptake and replication in macrophages by Fc receptors, contributing to clinical manifestations through an Arthus-type reaction [70–72]. Therefore, by reducing the viral loads and thus antibody titers, treatment could also contribute to reducing this immunological reaction and, consequently, the clinical signs in sick cats. A correlation of anti-FCoV antibody titers with the amount and frequency of fecal viral shedding has been previously described [4,33,34]. This was not the case in this study. There were animals with high antibody titers and high viral loads in feces (T2, T4, T5, T14, T15, T16) but more animals with high antibody titers and low to no RNA fecal loads (T1, T3, T6, T8, T11, T12, T17, T18). Conversely, a cat (T7) with relatively low antibody titers shed a high amount of FCoV RNA in feces. Three cats (T9, T10, and T13) had both low antibody titers and low amounts or no viral RNA shedding. These results should lead to reconsideration of the value of measuring the antibody titers for evaluating FCoV fecal shedding; although in many cases, high antibody titers correlate with the likelihood and frequency of FCoV shedding and fecal viral loads, and chronic shedders have higher antibody titers and shed more virus [4], but this is not always the case. Therefore, antibody measurement cannot replace fecal RT-qPCR.

Decreasing antibody titers might be a sign of decreasing viral loads in the cats under therapy, although in the current study only a weak correlation was found between viral RNA loads and antibody titers. Antibody titers are expected to decrease slower than viral loads, since the half life of antibodies can be several weeks, while viral loads are immediately influenced by an effective antiviral therapy. Another factor that might contribute to a slower or no decrease in antibody titers might be continuous antigen stimulation by reinfection with FCoV shedding companion cats in the same household. Of the five cats with high antibody titers on day 83, three cats (T2, T5, T11) had at least one companion cat in the household and all of them tested positive in voided feces or fecal swabs collected on either day 83 or day 143.

The spike protein mediates entry of FCoV into host cells through receptor binding and membrane fusion [73,74]. A full genomic sequencing approach identified a mutation in a nucleotide position that was associated with an amino acid change in the putative fusion peptide of the FCoV S protein [46]. The majority of FCoV isolates from cats with FIP were found to carry this mutation [46,75]. However, according to other studies, the amino acid changes in the spike protein were supposed to be a hallmark of monocyte/macrophage tropism acquisition allowing systemic spread of the virus [60,76]. Nonetheless, not all tissue-derived viral sequences carried this mutation in healthy infected cats [52]. Sequencing of the region of the spike gene encoding for the S protein region containing the mutations M1058L and S1060A [46] revealed a clear tropism of the different strains. In fecal samples of both treated and companion cats of the present study, only the wild-type form with the amino acids methionine (M) and serine (S) was found. On the contrary, in sequenced effusion and blood samples, only the mutated form was detected. These results, together with the results of another study from our group using the same molecular methods in which FCoV RNA detected in various tissues and body fluids of cats without FIP did not contain the M1058L and S1060A S protein mutations [77], support the former hypothesis [46] that these mutations are hallmarks of FIP. Therefore, sequencing of the spike gene would certainly help to diagnose FIP. In most of the systemic samples, the combination of the amino acids

leucine (L) and serine (S) was present, whereas, in the samples of cats T12 and T14, the variant with the amino acids methionine (M) and alanine (A) was present. Interestingly, in one animal (T11), mutations in the genomic sequence led to a not yet described amino acid change with phenylalanine (M1058F). This indicates that the previously described mutations in the spike gene [46] are not the only mutations that can be involved in the development of FIP. Many RT-qPCR-positive samples could not be sequenced in the present study. In general, the lower the viral load, the more difficult it is to obtain a PCR band even when using the nested RT-PCR protocol. A further reason that could prevent amplification of the target sequence is possible sequence variation hampering the primer binding. It was already noticed in other studies that this sequence analysis method only detects mutations in type I FCoVs and not type II FCoVs [60,75]. It would have been interesting to assess viral evolution and antiviral effects on it with a next-generation sequencing approach.

Phylogenetic analysis of the S gene sequences showed a clear clustering of samples coming from the same household, irrespective of the compartment from which they were isolated (feces, blood, or effusions) and of the pathotype (low pathogenic FCoVs or FIP-causing virus). Although the interpretation of this phylogenetic analysis is limited by the short length of the amplicons (about 143 bp) and by the fact that not all samples could be sequenced due to the low viral loads, these results confirm once more the validity of internal mutation theory over the theory of pathogenic strains. The internal mutation theory assumes that FIP causing viruses arise de novo from mutations of low pathogenic FCoV in infected animals, as demonstrated by the phylogenetic clustering of FIP-causing viruses and low pathogenic FCoVs according to geographic distribution rather than clustering according to pathotype [12,52,78,79].

One limitation of the study is that in some cases, voided fecal samples were available, whereas in others, especially after hospital release, fecal swabs were collected. Viral RNA loads per PCR reaction in swabs of treated FIP cats were statistically significantly lower than in voided samples. The collection of voided samples was sometimes impossible since in multi-cat households, it is often difficult to assign collected voided samples to an individual cat with certainty. On the other hand, in cases of swab collection, the risk of an insufficient amount of sample material collected is high. This condition could also have contributed to the absence of correlation between viral RNA loads in feces with those in blood, effusions, and antibody titers, possibly due to an insufficient amount of sample material in swabs. Therefore, if possible, it would be best for future studies to collect both voided and swab samples, but if only one is possible, it should preferably be a voided fecal sample. One could think that a further study limitation was the lack of a control group with untreated FIP cats. However, no control group was chosen purposely. All cats with FIP lacking appropriate treatment either die or have to be euthanized within a few days of diagnosis; the median survival time of untreated cats is only eight to nine days [80,81]. Therefore, for animal welfare and ethical reasons, a control group of FIP cats without treatment was not included in the study.

5. Conclusions

This study in cats with FIP demonstrated that oral treatment with GS-441524 effectively decreased viral RNA loads in feces, blood, and effusion of treated animals. Nonetheless reinfection through FCoV-shedding companion cats is likely to occur.

Viral RNA loads in blood correlated with viral RNA loads in effusion but neither one correlated with RNA loads in feces. In addition, no correlation was found between fecal RNA loads and antibody titers in this study, probably due to the fast antiviral effect of GS-441524 on fecal viral shedding but not on antibody titers.

We identified in one animal a mutation in the genomic sequence that led to a not yet described amino acid change with phenylalanine (M1058F), indicating that further mutations may be involved in the development of FIP. Phylogenetic analysis of S gene sequences showed that FCoV strains cluster according to the household and not the pathotype.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/v14051069/s1, Table S1: Nucleotide sequences of the study in FASTA format.

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V. PUBLIKATION 4: ORIGINAL-PUBLIKATION III

Clinical follow-up and postmortem findings in a cat that was cured of feline infectious peritonitis with an oral antiviral drug containing GS-441524

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Case Report

Clinical Follow-Up and Postmortem Findings in a Cat That Was Cured of Feline Infectious Peritonitis with an Oral Antiviral Drug Containing GS-441524

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Abstract: This is the first report on a clinical follow-up and postmortem examination of a cat that had been cured of feline infectious peritonitis (FIP) with ocular manifestation by successful treatment with an oral multicomponent drug containing GS-441524. The cat was 6 months old when clinical signs (recurrent fever, lethargy, lack of appetite, and fulminant anterior uveitis) appeared. FIP was diagnosed by ocular tissue immunohistochemistry after enucleation of the affected eye. The cat was a participant in a FIP treatment study, which was published recently. However, 240 days after leaving the clinic healthy, and 164 days after the end of the 84 days of treatment, the cured cat died in a road traffic accident. Upon full postmortem examination, including histopathology and immunohistochemistry, there were no residual FIP lesions observed apart from a generalized lymphadenopathy due to massive lymphoid hyperplasia. Neither feline coronavirus (FCoV) RNA nor FCoV antigen were identified by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and immunohistochemistry, respectively, in any tissues or body fluids, including feces. These results prove that oral treatment with GS-441524 leads to the cure of FIP-associated changes and the elimination of FCoV from all tissues.

Keywords: FCoV; FIP; Mutian; Xraphconn®; antiviral chemotherapy; feline coronavirus; therapy; treatment; necropsy

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1. Introduction

Feline infectious peritonitis (FIP) caused by the feline coronavirus (FCoV) is an infectious disease that occurs in felids worldwide. Infection with wildtype FCoV initially only causes a harmless intestinal infection. Mutation of the virus within the host, however, can lead to the disease FIP, which once clinically apparent is always fatal within a short period of time if left untreated [1]. Hitherto identified mutations mainly result in changes in coronaviral spike proteins, which enable the virus to replicate effectively within macrophages and to spread within the cat [2–4]. Subsequent activation of the immune system leads to extensive cytokine release, and thereby exaggerated multisystemic inflammatory lesions. The average survival time without effective treatment is only 8 days after diagnosis [1], and most cats have to be euthanized early due to their severe condition. However, recent studies have demonstrated the efficacy of antiviral compounds containing the nucleoside

analog GS-441524 in cats with FIP [5–7]. Although successful clinical recovery from FIP has previously been reported [7], this case report is the first description of the complete recovery in a cat whose tissues could be examined after a fatal road traffic accident via necropsy, including histopathology as well as FCoV immunohistochemistry (IHC) and quantitative reverse transcription polymerase chain reaction (RT-qPCR).

In the first controlled study (performed by the same study group) using an oral multicomponent compound called Xraphconn®, provided by Mutian Life Sciences Limited, containing GS-441524, 18 cats with naturally occurring and confirmed FIP were treated daily over 84 days [7]. All 18 cats recovered with dramatic resolution of all clinical and laboratory parameters, disappearance of effusion, and complete improvement of neurological signs, if present. Quantitative assessment revealed a large reduction in viral loads (across all measured compartments) within the first few days of treatment. Treatment with Xraphconn® containing GS-441524 was highly effective against FIP, without causing clinically relevant adverse effects [7,8].

One cat participating in the abovementioned study [7] died in an unobserved road traffic accident 164 days after the end of treatment. The aim of the present case report was (1) to describe the clinical course during and after treatment, (2) to screen for pathological sequelae of FIP in a cat treated with an oral antiviral drug by examining tissue samples via necropsy and histopathology, and (3) to search for FCoV antigen and viral RNA by IHC and RT-qPCR, respectively.

2. Case Description

2.1. Signalment and History

A male, neutered, 6-month-old European Shorthair cat was initially presented to a local veterinarian in February 2021. Three of the eight litter mates had died of FIP. One of the siblings that also suffered from confirmed FIP (ocular and neurological manifestation) was another study participant, and was also cured [7]. According to the owner, the cat developed clinical signs of recurrent fever, lethargy, and lack of appetite at the end of January 2021. The cat tested negative for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV). On admission to the local veterinarian at the beginning of February 2021, the cat showed signs of anisocoria, with a relatively miotic, round, and poorly responsive pupil in the left eye (OS). The OS further revealed rubeosis iridis, with marked thickening and bulging of the iris and fibrin precipitates within the anterior eye segment. The intraocular pressure at this time was within normal limits (OS, 11 mmHg; right eye (OD), 16 mmHg). Blood work at the initial examination revealed regenerative anemia (hematocrit, 21.2%; reference range (RR), 29.7–44.5%) and a reticulocyte count of 50.2×10^9/L; all other hematology parameters were unremarkable. The altered hematologic parameters were consistent with FIP. However, the cat did not show signs of neutrophilia or lymphopenia, which can occur in cats with FIP [9]. In the follow-up examination (10 days later), the left globe appeared larger than the right globe. Fibrin deposition within the anterior segment had increased, and was accompanied by a severe hyphema. Fundoscopy revealed partial retinal detachment. The intraocular pressure had increased to 23 mmHg OS (OD 13 mmHg). The OS was enucleated after a third examination 5 days later, due to continuous deterioration and evidence of high-grade anterior uveitis with an intraocular pressure of 48 mmHg (OD 16 mmHg).

The enucleated OS was subjected to histopathological examination. Marked pyogranulomatous uveitis and optic neuritis with retinal detachment were identified. FCoV IHC (as described in 2.5) revealed multiple intralesional immunostaining-positive macrophages, confirming, in combination with the ophthalmologic examination, an ocular manifestation of FIP. Additionally, FIP was confirmed by a positive RT-qPCR result of the ocular tissue (as described in 2.6 and shown in Figure 7).

2.2. *Improvement of Clinical Signs, Ultrasonographic Findings, and Laboratory Abnormalities during Treatment*

Before starting treatment (day 0), a complete physical examination, including determination of the Karnofsky score (see Table 1) [10,11], as well as hematology, serum biochemistry, and a detailed abdominal ultrasound, was performed. The Karnofsky score modified for cats was used to evaluate the general condition and well-being of the cats. The score ranges from 0% (dead), to 100%, which corresponds to a cat with healthy normal general condition [10,11].

Table 1. Karnofsky's score adapted from Hartmann and Kuffer [10,11].

0%	Dead
20%	severely diseased
40%	major changes in the general condition
60%	medium changes in the general condition
80%	minor changes in the general condition
100%	completely normal general condition

In the clinic, the cat presented with reduced general condition, pale mucous membranes, dehydration, a body temperature of 39.1 °C, and a body condition score of 3/9 (Figure 1A). The cat had a Karnofsky score of 70% on day 0. At presentation, and the start of the study, the cat had a body weight of 1.8 kg, measured using a baby scale (AE Adam MTB 20 baby scale, Felde, Germany). Physical examination and determination of the Karnofsky score were performed daily during hospitalization in the clinic (day 0 to day 7) and at all rechecks on days 14, 28, 56, 83, and 168.

Figure 1. Pictures of the cat (**A**) on day 0 (day of first presentation in the clinic) and (**B**) on day 168, 12 weeks after the end of treatment.

The cat was orally treated by daily administration of the multicomponent drug Xraphconn® (Mutian Life Sciences Limited, Nantong, China) containing the nucleoside analog GS-441524, for 84 days. Due to the ocular manifestation, a dose of supposedly

10 mg/kg (according to the manufacturer) was chosen, with the drug being administered according to manufacturer's instructions [7].

During hospitalization, the cat received supportive fluid therapy comprising Ringer's lactate with potassium supplementation at 20 mval/L to control dehydration at individual dosage, calculated by rehydration and maintenance needs. On the first day of treatment (day 0), the cat developed a fever (40.5 °C), whereupon it received a single injection of metamizole (30 mg/kg) intravenously (IV).

From the second day of treatment onward, the cat's appetite improved, and it started to gain weight. The cat was discharged from the clinic on day 7 with a body weight of 2.1 kg. At home, the weight continued to increase steadily, and the cat doubled the initial weight on day 56. Twelve weeks after the end of treatment (day 168), the cat had reached a weight of 4.0 kg (Figures 1B and 2A and Table 2). The Karnofsky score increased to 80% on day 1, and reached 100% on day 7 (Figure 2B). Body temperature decreased to 38.5 °C on day 1, and remained normal for the rest of the study period (Table 2).

Table 2. Physical examination and laboratory parameters of a 6-month-old cat with feline infectious peritonitis. Columns are colored according to the different study sections: orange, hospitalization in the clinic (day 0–7); blue, recheck visits during treatment (day 14, 28, 56, 83); green, follow-up period after the end of treatment. Values marked in bold are outside the reference ranges.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 28	Day 56	Day 83	Day 168
Clinical parameters													
Effusion [1]	+/−	−	−	−	−	−	−	−	−	−	−	−	−
Body weight (kg)	1.81	1.90	1.93	1.94	1.93	1.98	2.00	2.06	2.21	2.47	3.02	3.20	3.89
Karnofsky score (%)	70	80	80	95	95	95	100	100	100	100	100	100	100
Appetite [2]	+	+	++	++	++	+++	+++	+++	++++	++++	++++	++++	++++
Temperature (°C)	**40.5**	38.5	38.7	38.1	37.8	37.8	38.4	38	38.3	37.7	38.4	38.9	38.3
Laboratory parameters													
Hematocrit (L/L)	**0.176**		**0.207**		**0.188**			**0.252**	**0.289**	**0.328**	0.382	0.334	0.479
Mean corpuscular volume (fL)	**38.0**		**39.3**		**39.8**			43.8	43.6	40.8	43.0	**39.3**	51.6
Mean corpuscular hemoglobin (fmol/L)	**0.799**		0.816		0.805			0.852	0.905	0.872	0.866	0.872	**1.250**
Mean corpuscular hemoglobin concentration (mmol/L)	21.0		20.8		20.2			19.4	20.8	21.3	20.2	**22.2**	**24.2**
Reticulocytes (10^9/L)	18.1		50.1		133.6			78.2	20.6	20.9	31.1	41.6	115.1
WBC [3] **(10^9/L)**	10.49		**13.61**		**19.03**			**12.41**	9.33	**13.10**	11.00	**11.38**	**5.39**
Granulocytes (10^9/L): Neutrophils	8.71		7.28		10.78			6.87	5.54	5.97	4.71	3.56	3.57
Granulocytes (10^9/L): Eosinophils	0.01		0.16		0.28			0.24	0.50	0.47	0.37	0.59	0.03
Granulocytes (10^9/L): Basophils	0		0.01		0.01			0.01	0.01	0.01	0.01	0.01	0.01
Monocytes (10^9/L)	**0.64**		**0.91**		**0.93**			0.47	0.38	0.23	0.14	0.26	0.22
Lymphocytes (10^9/L)	1.13		**5.25**		**7.03**			**4.82**	2.90	**6.42**	**5.77**	**6.96**	1.56
Thrombocytes (10^9/L)	**101**		277		452			519	265	345	396	478	205
Liver enzyme activity (IU/L): ALT [4]	36		Np [5]		44			40	30	42	60	61	54
Liver enzyme activity (IU/L): AP [6]	14		np		24			37	63	**106**	80	77	56
Urea (mmol/L)	**4.5**		np		5.0			5.0	6.7	7.4	7.6	6.5	8.5
Creatinine (µmol/L)	62		np		53			50	59	81	88	110	107
Symmetric dimethylarginine (µg/dL)	17.0		13.0		14.0			14.0	11.0	12.0	15.0	14.0	10.0
Bilirubin (µmol/L)	**5.2**		np		2.1			2.5	1.5	0.6	0.8	1.1	0.4
Total protein (g/L)	**109.1**		np		**111.2**			**109.4**	91.5	78.6	67.7	71.0	71.4
Albumin (g/L)	**25.1**		np		26.9			26.4	27.6	34.1	37.5	40.6	42.8
Globulin (g/L)	**84.0**		np		**84.3**			**83.0**	**63.9**	44.5	30.2	30.4	28.6
Albumin/globulin ratio	**0.30**		np		**0.32**			**0.32**	**0.43**	0.77	1.24	1.34	1.50
Serum amyloid A (mg/L)	0.5		**6.3**		**5.3**			**4.2**	**19.6**	**5.8**	1.0	**5.1**	0
Viral loads													
FCoV [7] **RNA feces (copies/g)**	3437	53	0	0	0	0	0	0	0	0	0	0	0
FCoV RNA blood (copies/mL)	11,473				229			0	0	0	0	0	0
Antibody titers													
FCoV antibody titer (IFA [8]**)**	1:6400							1:6400	1:6400	1:600	1:400	1:400	1:400

[1] Effusion was graded from +++ (high-grade) to–(no effusion); [2] Appetite was graded from + (reduced appetite) to ++++ (normal appetite); [3] WBC, white blood cells; [4] ALT, alanine aminotransferase; [5] np, not performed; [6] AP, alkaline phosphatase; [7] FCoV, feline coronavirus; [8] IFA, indirect immunofluorescence assay.

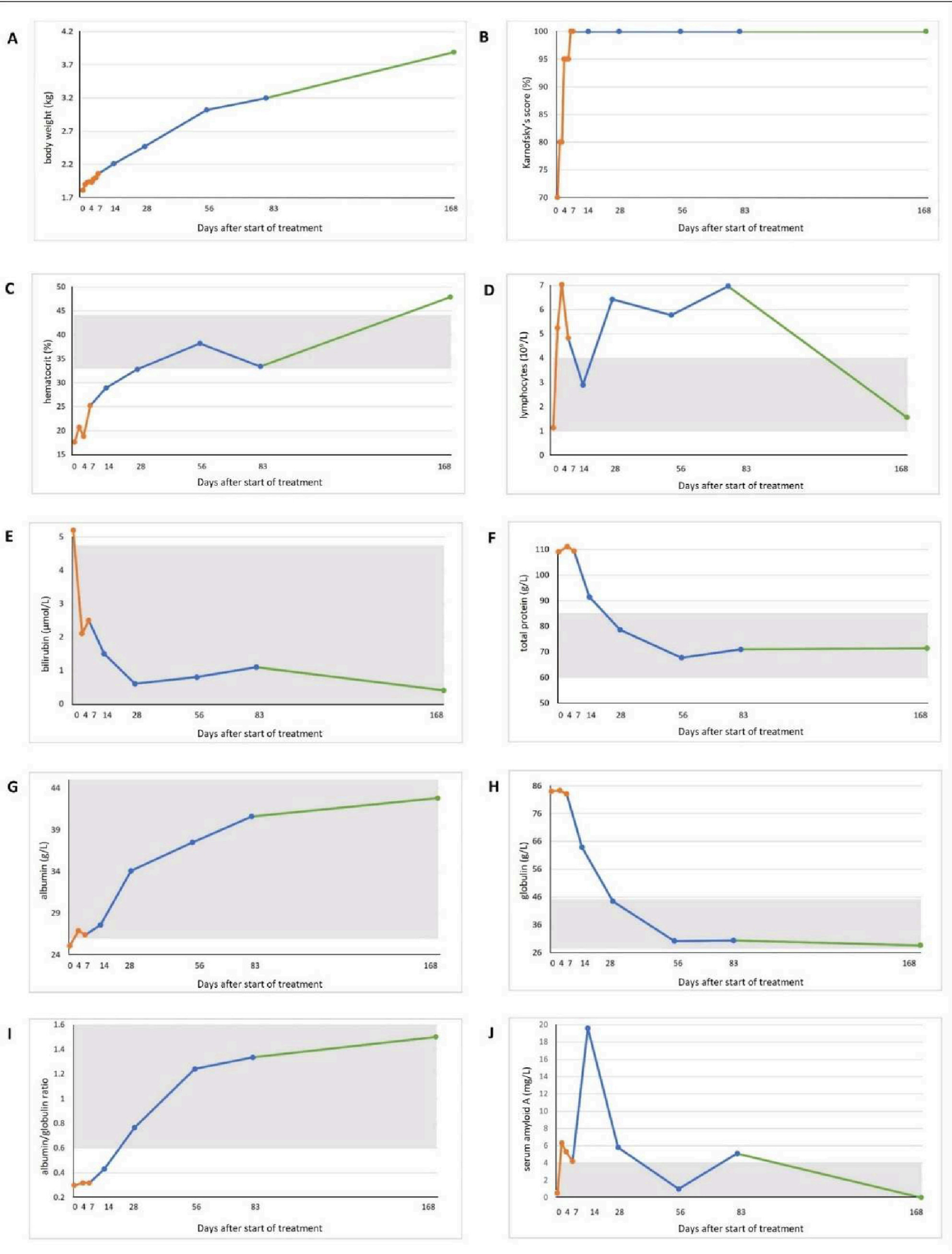

Figure 2. Timeline visualizing improvement of clinical and laboratory parameters throughout the study course. Lines are colored according to the different study sections: orange, hospitalization (day 0–7); blue, recheck visits during treatment (day 14, 28, 56, 83); green, follow-up period at the end of treatment. Grey shading marks the reference ranges, if present. (**A**) Body weight. (**B**) Karnofsky score modified for cats [10]. (**C**) Hematocrit. (**D**) Lymphocyte count. (**E**) Bilirubin concentration. (**F**) Total protein concentration. (**G**) Albumin concentration. (**H**) Globulin concentration. (**I**) Albumin/globulin ratio. (**J**) Serum amyloid A concentration.

Abdominal ultrasound was performed using the Logiq E9 ultrasound machine (GE Healthcare) and an 8-MHz microconvex probe, with the cat in dorsal recumbency after clipping the fur. Upon presentation (day 0), the most notable finding was bilateral renomegaly. Regarding longitudinal measurements, the left and right kidney were 4.7 cm and 4.6 cm in size, respectively (Figure 3A,B), with a hypoechoic subcapsular rim on both sides. The surface of the left kidney was irregular, and the cortical parenchyma of both kidneys appeared hyperechoic and mottled. There was poor corticomedullary definition and a small amount of anechoic fluid in the retroperitoneal space. Intestinal lymph nodes were mildly enlarged with a homogeneous texture.

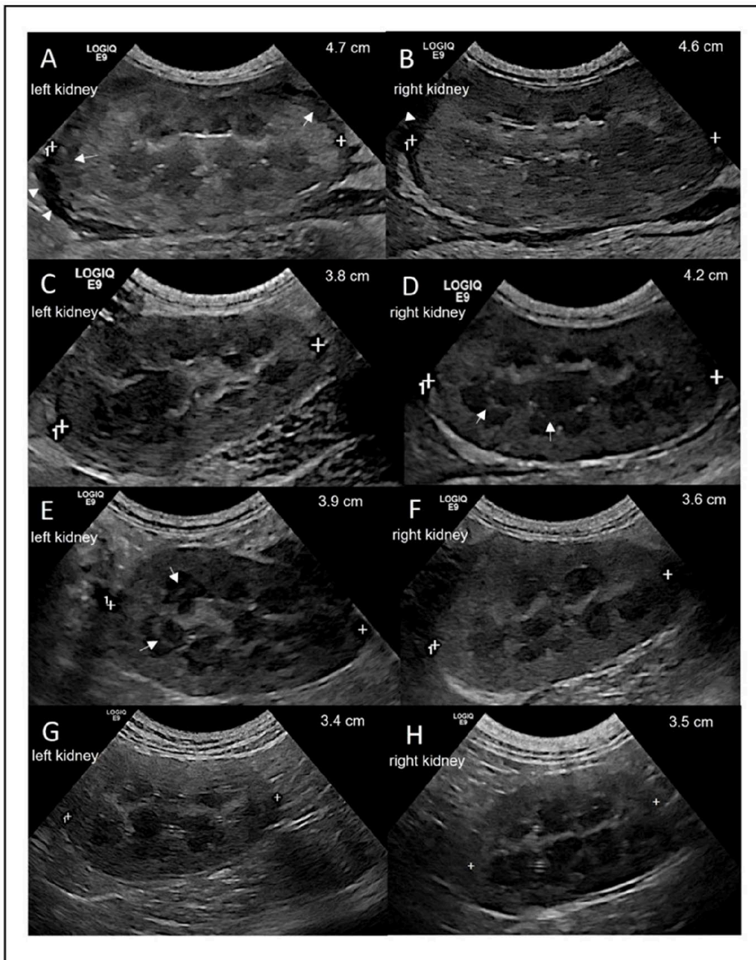

Figure 3. Ultrasonographic longitudinal view of the left and right kidney on days 0, 7, 14, and 168. (**A**,**B**) Day 0 with arrow heads showing free fluid in the retroperitoneal area and arrows showing hypoechoic subcapsular rim. (**C**,**D**) Day 7 with arrows showing the medullary rim sign. (**E**,**F**) Day 14 with arrows showing the medullary rim sign. (**G**,**H**) Day 168 with normal size, structure, texture, and echogenicity.

On day 7, the lengths of the left and right kidneys had decreased to 3.8 and 4.2 cm, respectively (Figure 3C,D). The cortex of both kidneys had a homogenous texture, and both kidneys had a distinct corticomedullary definition. The retroperitoneal fluid was no longer visible. A poorly defined medullary rim sign was observed in the right kidney on day 7 (Figure 3D) and in the left kidney on day 14 (Figure 3E). Both kidneys were considered

normal in size, structure, texture, and echogenicity on day 56. The intestinal lymph nodes remained mildly enlarged throughout the study period.

Hematology was performed on days 0, 2, 4, 7, 14, 28, 56, 83, and 168 using an automatic analyzer (Cell-Dyn 3500, Abott Laboratories, Chicago, IL, USA). Differential blood count was additionally performed manually on blood smears exposed to Haema Quick Staining/Diff-Quick staining (LT-SYS®, Eberhard Lehmann GmbH, Berlin, Germany) if hematology parameters were abnormal. On day 0, the cat presented with severe non-regenerative, hypochromic, and microcytic anemia, and moderate thrombocytopenia. On day 2, hematocrit and reticulocyte counts increased, indicating early regeneration. On day 7, the cat was discharged from the clinic with a hematocrit of 0.252 L/L. On day 56, anemia had resolved (Table 2 and Figure 2C). By day 2, the lymphocytes, which were initially within the lower RR (day 0), had transitioned into mild lymphocytosis. Thrombocyte count was within the RR. Throughout the rest of the treatment period, lymphocyte counts indicated mild lymphocytosis, but 12 weeks after the end of treatment, the lymphocyte count was within the RR (Table 2 and Figure 2D). All other hematology parameters were within the RR throughout the entire study period (Table 2).

Serum biochemistry parameters were measured on days 0, 4, 7, 14, 28, 56, 83, and 168 using an automatic analyzer (Hitachi 911, Roche, Grenzach-Wyhlen, Germany). Symmetric dimethylarginine (SDMA) concentration was analyzed at IDEXX Diavet AG (Bäch, Switzerland) using a high-throughput immunoassay, and serum amyloid A (SAA) concentration was determined using a latex agglutination turbidimetric immunoassay reaction (LZ Test SAA, Eiken Chemical Co., Ltd., Tokyo, Japan) on a cobas® c 501 clinical chemistry analyzer (Roche Diagnostics AG, Rotkreuz, Switzerland). On day 0, the cat showed signs of mild hyperbilirubinemia, mild hyperproteinemia, and mild hypoalbuminemia (Table 2 and Figure 2E–G). SAA was low (Table 2 and Figure 2J) and SDMA was in the upper RR (Table 2). On day 4, hyperbilirubinemia was no longer observed (until the end of the observation period) (Figure 2E). Furthermore, the alkaline phosphatase activity was mildly elevated on day 28, and urea concentration was mildly decreased on day 0. Total protein concentration continued to increase (both globulin and albumin concentrations) until day 7. From there on, until the end of therapy, globulin concentration decreased until it was finally within the RR on day 28. Albumin concentration continued to increase until the end of treatment (Figure 2F–H). SDMA concentration was within the RR at all times during treatment. SAA concentration increased to a maximum value on day 14, but was within the RR on day 168 (Figure 2J). All other parameters were within the RR.

2.3. Changes in Viral Loads and Anti-FCoV Antibody Titers

The courses of the viral load in blood (on days 0, 4, 7, 14, 28, 56, 83, and 168) and feces (on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 56, 83, and 168) were analyzed by RT-qPCR as previously described [7,8]. Fecal samples were collected using voided samples (on days 0, 2, 3, 4, 5, 6, 7, 14, 28, 56, and 83) or fecal swabs (on days 1 and 168). The viral RNA load in blood before treatment was 11,473 copies/mL blood. On day 4, only 229 FCoV RNA copies/mL blood were detectable. From day 7 onward, no FCoV RNA was detectable in the blood. In feces, excretion of 3437 viral RNA copies/g feces was detectable on day 0. On day 1, only 53 FCoV RNA copies/fecal swab could be detected by RT-qPCR. From day 2, until the end of treatment, viral RNA was no longer detectable in feces (Figure 4A).

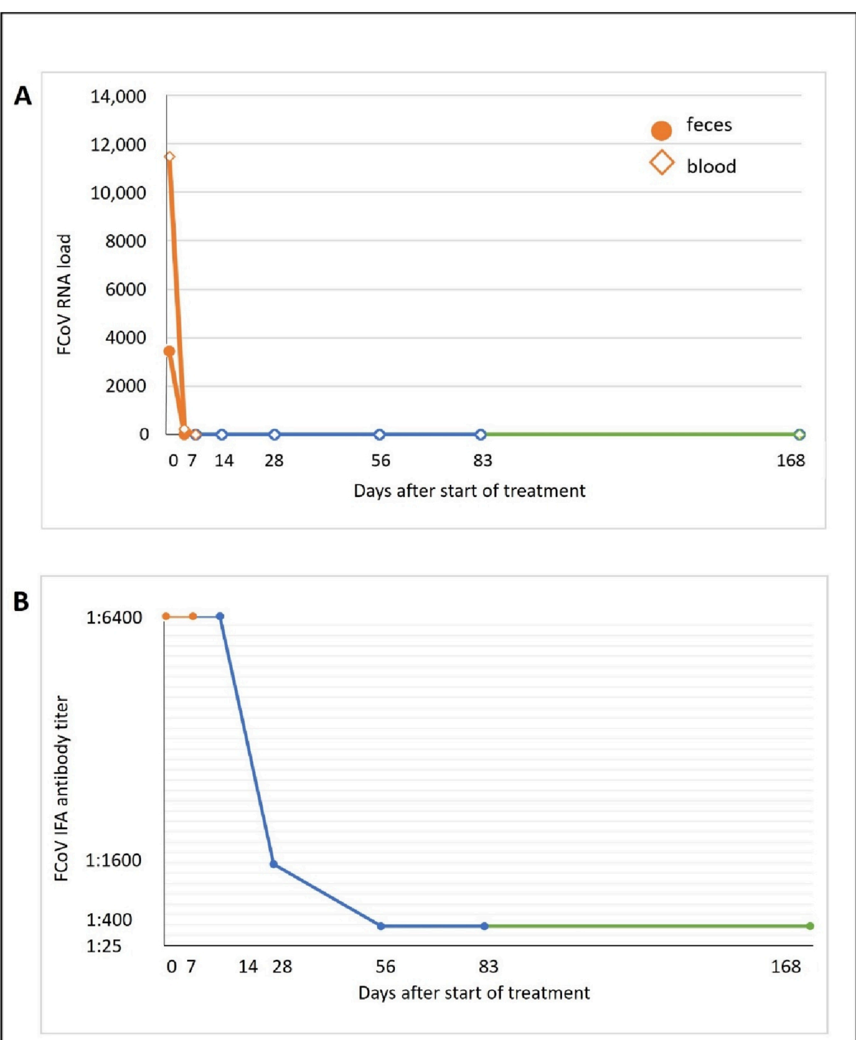

Figure 4. Feline coronavirus (FCoV) viral RNA loads in blood and fecal samples and serum anti-FCoV antibody titer. Lines are colored according to the different study sections: orange, hospitalization in the clinic (days 0–7); blue, recheck visits during treatment (days 14, 28, 56, 83); green, follow-up period at the end of treatment. (**A**) FCoV RNA loads in ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood (presented in copy numbers/mL) and feces (presented in copy numbers/g). FCoV RNA loads were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR). Dot, feces; rhombus, blood. (**B**). Serum anti-FCoV antibody titer. Antibody titer was determined via indirect immunofluorescence assay (IFA).

Anti-FCoV antibody titers in serum (on days 0, 7, 14, 28, 56, 83, and 168) were determined by indirect immunofluorescence assay (IFA) as previously described [7,8,12–14]. The cat exhibited very high anti-FCoV antibody titer levels at the beginning (1:6400 from day 0 until day 14) of the treatment period. From day 56 on, the antibody titer levels decreased to 1:400 (Figure 4B).

2.4. Necropsy and Histopathology

A total of 164 days after completion of antiviral treatment, the cat went missing without preceding clinical signs. When the cat was found dead next to the road by the

owners, it was immediately submitted to necropsy. A full postmortem examination was performed within 24 h after death. Upon dissection and gross examination, paired samples were taken from all visceral organs and tissues to be (1) snap-frozen for molecular analysis and (2) transferred into 10% neutral-buffered formalin for histopathology and IHC. Fixed samples were trimmed and underwent automated tissue processing, paraffin embedding, and sectioning at 3 µm slice thickness. Sections were routinely stained with hematoxylin–eosin for histopathological evaluation. Further sections of liver and intestine underwent Giemsa and Gram staining.

Upon external inspection and superficial dissection, the carcass had undergone rigor mortis. The body showed prototypic lesions associated with road traffic accidents, including superficial abrasions, subcutaneous and intramuscular hematomas of the head and neck area, and frayed claws. Death occurred due to forced ventral hyperflexion of the head and cervical spine, leading to the luxation of the atlanto-occipital joint and complete spinal cord tear at the medullospinal junction. The other parts of the head, including the OD, were unremarkable. Upon dissection of the body cavities, there were no indications of FIP. In particular, there were no effusions or serosal and subserosal changes. However, generalized lymphadenomegaly of both the internal and peripheral lymph nodes was observed, which was most extensive in the mesenteric lymph nodes, as well as swelling of the tonsils. The spleen only showed mild splenomegaly. Respiratory tract and cardiovascular system, as well as the gastrointestinal tract, liver, and pancreas, were unremarkable. Both kidneys were normal in size; cortex and medulla were clearly delineated and highly unremarkable. No retroperitoneal fluids were evident, and the lower urinary tract was normal.

Histopathology confirmed the peracute medullospinal injury and excluded pre-existent central nervous system (CNS) changes. The enlargement of the lymphatic tissues histologically corresponded to a severe generalized follicular lymphoid hyperplasia with clearly delineated germinal centers, mantle and marginal zones, and distinct periarteriolar lymphocyte sheaths (Figure 5A,B,D). Histological examination of the other organs also confirmed the absence of FIP-associated changes. The lungs instead presented with a moderate multifocal alveolar emphysema (Figure 6A). Liver sections showed mild oligofocal lymphocytic infiltrates within portal areas and limiting plates, consistent with mild chronic portal and periportal hepatitis. Moreover, there were occasional, randomly distributed and variably sized foci of lytic hepatocellular necroses associated with individual degenerate polymorphonuclear neutrophils and lymphohistiocytic infiltrates (Figure 6B, dashed line). The distribution was typical for the hematogenous spread of bacteria from the guts via the portal vein after passing the mucosal barrier. Accordingly, there were some foci within better-preserved areas of the intestinal mucosa, with an overall increase in the densities of lymphocytes and plasma cells within the propria, accompanied by degenerate polymorphonuclear neutrophils. Hematoxylin–eosin sections, and those stained via Giemsa and Gram staining techniques, exhibited rather diffuse bacterial overgrowths, with mixed morphology ranging from cocci to elongated rods. During necropsy, the OD was removed, and was found to lack any changes indicative of FIP, in contrast to the OS, which had been enucleated prior to antiviral treatment, and showed a fibrotic scar in the area of the ciliary body (Figure 7).

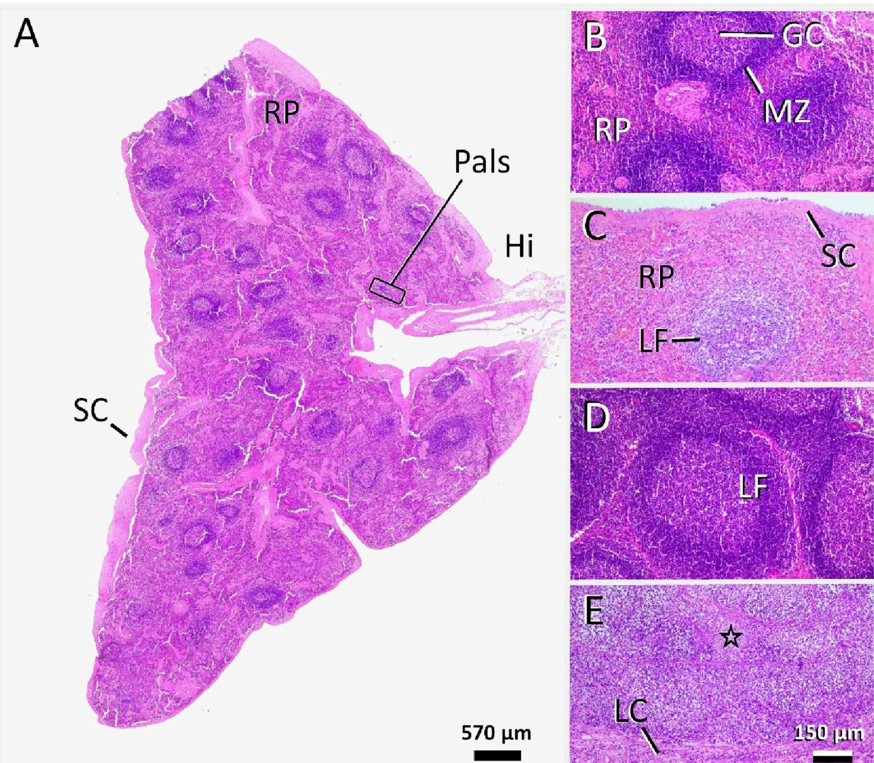

Figure 5. Lymphatic tissue changes in the cat (**A**,**B**,**D**) compared to a cat with active feline infectious peri-tonitis (FIP) (**C**,**E**). Follicular lymphoid hyperplasia was evident in all lymphatic tissues, including the spleen (**A**,**B**), lymph nodes (**D**), and Peyer's patches and tonsils (not shown). Note the large lymphoid follicles (**A**,**B**; **D**: LF) with prominence of germinal centers (**A**,**D**; **B**: GC) and clearly de-lineated mantle zones (**A**,**D**; **B**: MZ). In the spleen (**A**,**B**), red pulp (**A**,**B**: RP) and periarteriolar lymphoid sheaths (**A**: Pals) were discernible, corresponding to splenic activation type 1 [15]. Le-sions, typically associated with splenic (**C**) and lymph node (**E**) involvement in FIP, including ef-facement of follicular zones and delineation, as well as polymorphonuclear and macrophageal in-filtrate and fibrin deposition (**E**: asterisk), were not seen. Markers: GC, germinal center; Hi, hilus (spleen); LC, lymph node capsule; LF, lymph follicle; MZ, mantle zone; Pals: periarteriolar lym-phoid sheath; RP, red pulp (spleen); SC, splenic capsule. Stain (**A**–**E**): hematoxylin–eosin.

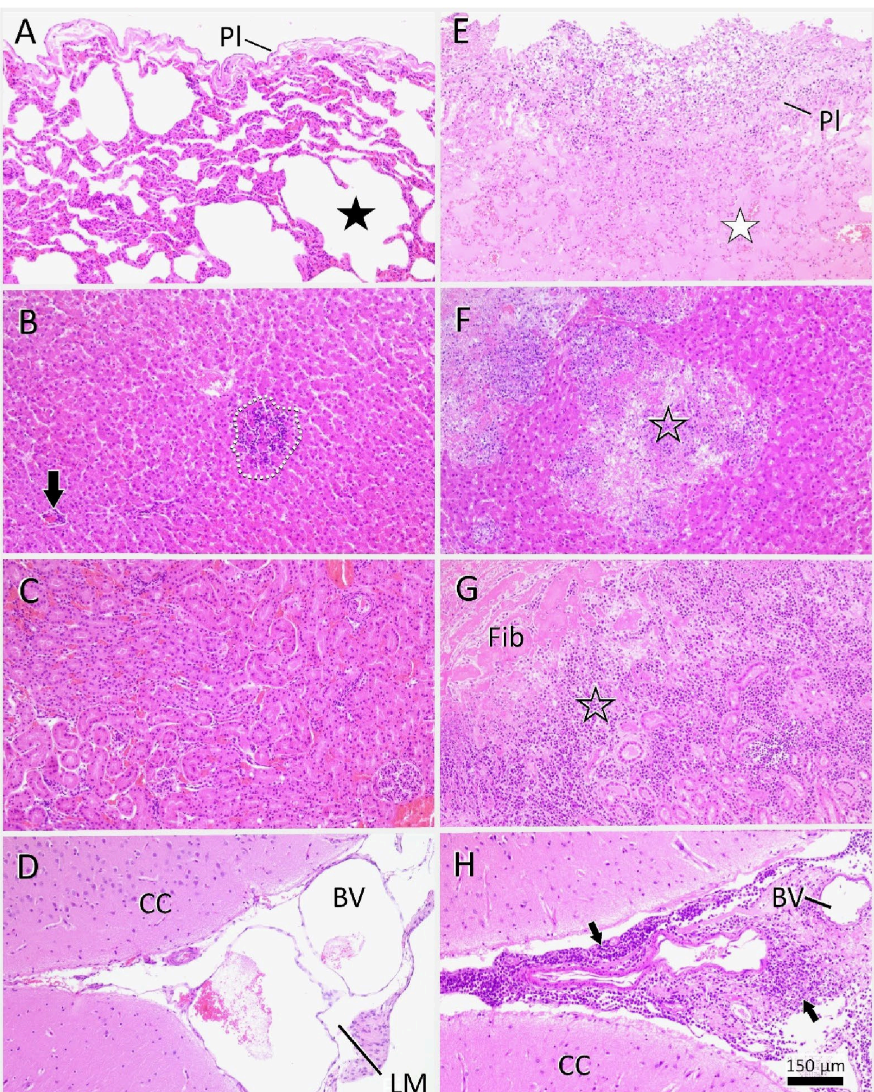

Figure 6. Histology of lungs, liver, kidney, and brain of the cat (**A**–**D**) as opposed to cats with local feline infectious peritonitis (FIP)-associated changes (**E**–**H**). **Lungs**: Note, that the fatally injured cat pre-sented with an alveolar emphysema (**A**: black asterisk), while FIP-typical pleural changes (**E**: Pl) and proteinaceous alveolar oedema (**E**: white asterisk) were not observed. **Liver**: The cat showed further signs of mild lymphocytic periportal hepatitis (**B**: black arrow), and multifocal necrotizing hepatitis (**B**: dashed line). Once again, fibrinoid and pyogranulomatous liver changes in cats with FIP (**F**: empty asterisk) were not evident. **Kidneys**: In the kidneys a mild congestion was seen (**C**). In FIP (**G**), fibrin (**G**: Fib) and pyogranulomatous changes (**G**: empty asterisk) can extend from the capsule into the depth of renal parenchyma with effacement of cortical architecture. **Brain**: Apart from the peracute medullospinal tear, the central nervous system (CNS) condition was unre-markable (**D**), including FIP predilection sites of the leptomeninges (**D**: LM)and brain ventricles (not shown). In CNS manifestation of FIP (**H**), there were mostly ill-defined fibrinonecrotic and pyogranulomatous changes in the meninges intermingling with extensive lymphoid infiltrates (**H**: arrows). Markers: BV, blood vessels; CC, cerebral cortex; LM, leptomeninx; Pl, pleura. Stain (**A**–**H**): hematoxylin–eosin. Scale bar applies to all photomicrographs.

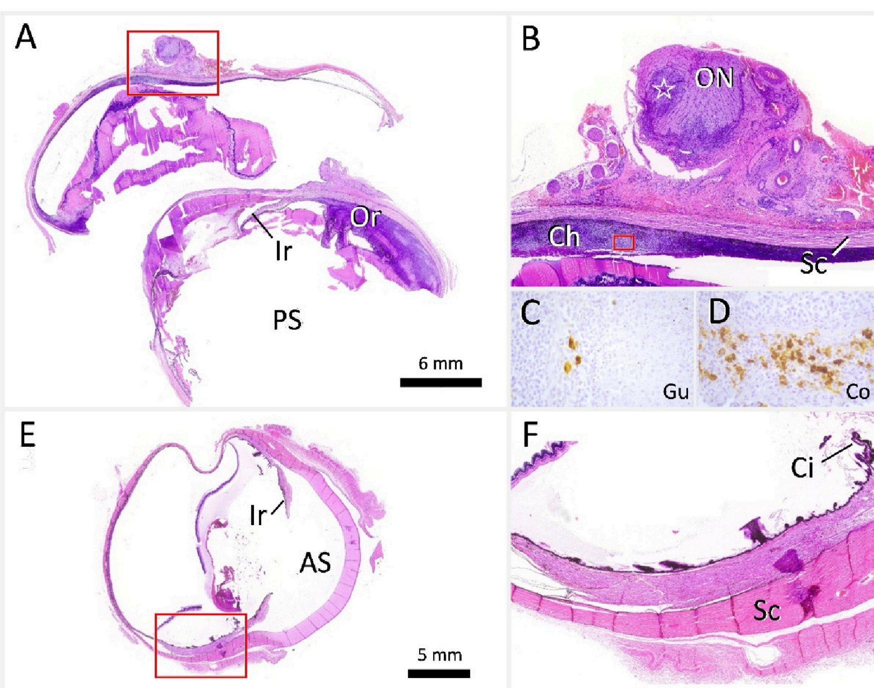

Figure 7. Comparison of the left eye (**A**–**C**), enucleated before antiviral treatment, and the right eye (**E**,**F**), harvested during necropsy. The feline infectious peritonitis (FIP)-affected left eye showed extensive pyogranulomatous and lymphocytic inflammation affecting mainly the optic nerve (**A**; **B**: ON, asterisk) and choroid (**A**; **B**: Ch), thereby extending from the posterior pole to the ora serrata (**A**: Or). Fibrin precipitates, proteinaceous fluid, and free-floating cells were abundantly present in both anterior and posterior segments (**A**: PS). On immunohistochemistry of Gusti´s left eye (**C**: Gu), there were scattered brown macrophages immunopositive for feline coronavirus antiges. The signal was similar to that of polymerase chain reaction (PCR)-confirmed control tissue (**D**: Co). No such changes were observed in the right eye (**E**,**F**) from necropsy. Markers: AS, anterior segment; Ch, choroid; Ci, ciliary body; Ir, iris; ON, optic nerve; Or, ora serrata; PS, posterior segment; Sc, sclera. Stain (**A**,**B**,**E**,**F**): hematoxylin–eosin. Stain (**C**,**D**): diaminobenzidine tetrahydrochloride (brown), hematoxylin counterstain. Red frames: localisers of higher magnification pictures: (**A**,**B**; **B**,**C**; **E**,**F**).

2.5. IHC for FCoV Antigen

Tissue sections from all areas sampled (including tonsils, mandibular lymph nodes, mesenteric lymph nodes, spleen, mesenterium, stomach, duodenum, jejunum, cecum, colon, rectum, kidneys, liver, pancreas, brain, spinal cord, OD) underwent immunohistochemical investigation, and tested negative for FCoV antigen expression (Figure 6 and Table 3). IHC was performed using FIPV3-70 monoclonal antibodies (Linaris Biologische Produkte GmbH, Dossenheim, Germany), on formalin-fixed, paraffin-embedded tissue sections as described previously [16]. Negative controls were included in every IHC staining process. These were two brain sections from a cat with confirmed FIP affecting the central nervous system, into which the antibody was substituted using phosphate-buffered saline (PBS), and by an irrelevant mouse monoclonal antibody (Bo-18). Additionally, and to ensure adequate performance of the antibody, a positive tissue control (tissue from a cat with confirmed FIP) was included in every IHC run. Samples were considered positive for FIP in IHC if typical histopathological lesions were present (e.g., pyogranulomatous and fibrinonecrotic tissue lesions at predilection sites with the exclusion of other pathogens), and FCoV antigen was detected within macrophages in these lesions. Samples were considered negative for FIP in

IHC if no histopathological lesions suggestive of FIP were detected, and if FCoV antigens were absent in all tissue samples, including lymph nodes (Table 3).

Table 3. Results of immunohistochemistry (IHC) for feline coronavirus (FCoV) antigen and quantitative reverse transcriptase PCR (RT-qPCR) in different tissues compared to control tissues.

Tissue	IHC for FCoV Antigen	FCoV RT-qPCR [1] (Viral Load)	18S rRNA RT-qPCR [1] (RNA Quality Control) CT [2]-Value
mandibular lymph node	negative	negative	15.09
jejunum	negative	negative	15.86
duodenum	negative	negative	14.08
spleen	negative	negative	14.18
colon	negative	negative	16.02
mesenterial lymph node	negative	negative	15.57
kidney	negative	negative	20.62
caecum	negative	negative	14.28
rectum	negative	negative	15.21
liver	negative	negative	20.54
brain	negative	negative	20.82

[1] RNA diluted 1:5; [2] cycle threshold values.

Additional sections from the spleen, lymph nodes, and liver were stained for lymphocyte markers CD3, CD20, and CD79a. In lymphatic tissues, cell phenotypes segregated with the distribution of physiological T cell and B cell areas. Liver infiltrates mainly consisted of T cells, with only a few B lymphocytes.

2.6. Tissue FCoV RT-qPCR

From each sampled organ (mandibular lymph nodes, mesenteric lymph nodes, jejunum, duodenum, cecum, colon, rectum, spleen, kidneys, liver, brain, and OD), 30 mg of frozen tissue was transferred to a soft tissue homogenizing tube of the Precellys Lysing Kit CK14 (Labgene Scientific SA, Châtel-St-Denis, Switzerland), and 600 µL of buffer RLT of the RNeasy Mini Kit (Qiagen AG, Hombrechtikon, Switzerland) containing 1% beta-mercaptoethanol (GBiosciences, St. Louis, MO, USA) was added. Tissues were homogenized twice for 1 min at 5000 Hertz on the Precellys 24 tissue homogenizer (Labgene Scientific SA, Châtel-Saint-Denis, Switzerland), followed by a centrifugation at 17,601 x g for 3 min. RNA was extracted from the 600 µL supernatant using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA was eluted in 30 µL RNase/DNase-free water and stored at −80 °C until further use. All samples were tested undiluted and diluted (1:5) for FCoV RNA by RT-qPCR as described previously [17]. Additionally, all RNA samples from tissues were tested undiluted and diluted (1:5) for the presence of the 18S rRNA housekeeping gene to test for sufficient TNA and absence of potential PCR inhibition. The master mix consisted of 1X Ag-Path RT-PCR buffer (AgPath-IDTM One-step RT-PCR Kit; Applied Biosystems, Rotkreuz, Switzerland), 1.0 µL Array Script reverse transcriptase and AmpliTaq Gold DNA polymerase (AgPath-IDTM One-step RT-PCR Kit; Applied Biosystems), 1X 18s rRNA Dye Mix (VIC/MGB) EUK (Applied Biosystems), and nuclease-free H_2O was added to a final volume of 20 µL. All FCoVRT-qPCRs were run with 5 µL of TNA in a final volume of 25 µL. Positive and negative controls were run in parallel using a ABI 7500 Fast instrument (Applied Biosystems). An FCoV RNA standard curve was run in parallel as a positive control and to determine the viral RNA copy number [8]. As negative controls, an extraction control (PBS) and DNase/RNase-free water were used. In the 18S rRNA RT-qPCR all tissue tested positive, showing no inhibition at a 1:5 dilution.

The lower the cycle threshold (CT) value, the higher the viral load. The cycling conditions were the same as for the FCoV RT-qPCR.

All examined tissue samples, including the OD, were negative for FCoV RNA (Table 3). In comparison, the OS, enucleated before treatment, tested positive for FCoV, with a cycle threshold (CT) value of 25.38.

3. Discussion

This case report describes a cat that participated in a clinical trial investigating the efficacy of an oral antiviral drug to treat FIP. Without treatment, virtually all cats suffering from FIP die, making FIP one of the most lethal diagnoses in feline medicine. The survival rate of the 18 cats in this study, however, was 100% [7]. The cat described here was included in the study as it fulfilled the inclusion criteria of (1) a diagnosis of FIP established by IHC, (2) negative test results for FIV and FeLV infection, and (3) absence of other severe diseases. After initial presentation with anterior uveitis, recurrent fever, apathy, and lack of appetite, the cat showed a very swift response to treatment, with rapid improvement of clinical and laboratory parameters leading to full, relapse-free recovery. The cat was treated with 10 mg/kg GS-441524 according to the manufacturer, but it has to be considered that recent additional analysis of the provided drug suggested that a tablet of the multicomponent drug Xraphconn® contains more GS-441524 than officially stated by the manufacturer (data not shown, personal communication J. Horak).

As the analyses show, it is difficult to rely on statements about an unapproved drug. Different illegal antiviral drugs are manufactured under non-standardized conditions. Cats receiving GS-441524 from their owners are treated at variable doses due to the variety of manufactures that produce the drug under uncontrolled conditions. The cats in the cited study were treated with a significantly higher dose than assumed, and only mild side effects occurred [7]. Whether the cat would have been virus-free with a lower dose requires further research.

The main clinical abnormality of FIP in the cat presented in this case report was anterior uveitis, manifesting as anisocoria, aqueous flare, and an optic neuritis with retinal detachment, which is consistent with the typical clinical signs observed in cats with ocular FIP [18]. FIP is the most commonly identified cause of uveitis in young cats. According to a study of the North Carolina Veterinary School, 19 of 120 cats (15.8%) with uveitis had FIP [19]. A study from the UK showed a similar distribution pattern, with FIP being the cause of uveitis in 15/92 cats (16.3%) [18]. Confirmation of FIP as the cause of uveitis is often difficult. Clinical signs are not pathognomonic, and aqueous humor analysis, including cytology and screening for infectious diseases is often unspecific [20–22]. Detection of FCoV antigens within macrophages via immunostaining of ocular tissue can confirm the diagnosis, although false-positive results are possible using immunocytochemistry on aqueous humor [23]. In the cat of the present case report, the OS was enucleated to confirm FIP. Histopathology of the OS revealed marked pyogranulomatous uveitis, pyogranulomatous neuritis of the optic nerve, and retinal detachment. In IHC, multiple macrophages were positive for FCoV antigen. In contrast, the OD had shown no abnormalities on ophthalmic examinations, although at necropsy, a fibrotic scar could be seen on the OD in the area of the ciliary body. It is conceivable that the virus infiltrated not only the OS, but also the contralateral eye, and that these "microlesions" were too minor to be clinically conspicuous and recognized by ophthalmic examination. In both IHC for FCoV antigen and tissue-based FCoV detection using RT-qPCR, FIP could no longer be detected in the enucleated eye postmortem, suggesting cure by treatment with Xraphconn®. Thus, the scarring could be an indication of previous lesions caused by FIP after healing.

A case report from the US described treatment of four cats with FIP and neurological and/or ocular signs [24]. The cats were treated with GS-441524 via subcutaneous injections at a dose of 5–10 mg/kg, applied once daily for at least 12 weeks. All of the four cats responded to treatment initially, including remission of ocular signs. Serial ophthalmic examinations revealed healing of ocular changes presenting as chorioretinal scars, similar

to the changes in the cat described in the present case report. However, one cat of the case series of [24] experienced two relapses, and was ultimately euthanized after two courses of treatment. Postmortem examination revealed lymphocytic, histiocytic uveitis, and choroiditis, and viral antigens could be detected in various tissues, including the eye, by IHC. This could either be caused by viral persistence or recurrent FCoV infection and mutation. This cat received GS-441524 at a lower dose (5 mg/kg) than the cat described in this current case report. It is known that drug levels of GS-441524 in aqueous humor are lower than in serum [5]; thus, it is likely that the higher dose used in the present study was more effective [24]. This was corroborated by the fact that results from both IHC and RT-qPCR conducted on ocular and other tissues were negative for FCoV antigen and RNA, respectively, in the cat in the present report. Further studies are needed to investigate whether an intermediate dose of GS-441524 might be adequate to permanently stop viral replication in cats with ocular FIP.

The medullary rim signs, which was visible in both kidneys, also disappeared towards the end of treatment. Medullary rim signs can have various causes, but has been described in association with FIP. In a retrospective study including 243 cats showing medullary rim signs, 15 of these cats were finally diagnosed with FIP [25]. Therefore, treatment with GS-441524 likely also cured FIP-associated changes in the cat's kidneys.

No residual FIP lesions were present in the cat 164 days after the end of treatment with the multicomponent drug Xraphconn®, apart from a generalized lymphadenopathy due to massive lymphoid hyperplasia. The involvement of mesenteric lymph nodes could partially resemble a consequence to the presumed mild suppurative bacterial enteritis. Gastrointestinal infection and the subsequent portogenic involvement of the liver, on the other hand, cannot explain lymphoid hyperplasia at distant sites such as the tonsils, and mandibular and superficial cervical lymph nodes. The presence of lymphadenopathy could be an incidental unrelated finding, possibly caused by a recent (re)infection with FCoV, or could be an indication of a "long FIP syndrome". It is possible that some cats have a genetic predisposition of developing FIP. According to current theory on FIP pathogenesis, FIP occurs within cats that are genetically predisposed to being unable to control viral replication effectively, resulting in uncontrolled virus replication and increased opportunity for mutations. These mutations lead to a switch in pathogenicity of the virus, resulting in a variant that is able to efficiently replicate in macrophages. It is the ability to replicate in macrophages in an uncontrolled fashion that distinguishes FIP-causing variants from low-pathogenic feline coronavirus isolates [26,27]. To further characterize the predisposition to developing FIP, single-nucleotide polymorphisms (SNP) in the feline IFN-γ gene were previously investigated, and certain genotypes were described as FIP-susceptible factors [28,29]. It was also demonstrated that cats with FIP have lower IFN-γ production than cats infected with FCoV without FIP [30–33]. In the present cat, there were other known cases of FIP in the cat's family history, which suggests a genetic predisposition of this family. However, FCoV infection neither as relapse nor as newly acquired was found by IHC or RT-PCR that would explain the lymphadenopathy. Lymphadenopathy could be considered as a sign of an exaggerated genetically conditioned reaction of the immune system. Also, persistence of lymphadenopathy after elimination of the FCoV could be discussed as a "long FIP syndrome". In human medicine, cases have been described in which viral RNA persisted after clinical resolution of an acute infection, such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the long COVID syndrome [34]; however, what is detected by RT-PCR is mostly fragmented RNA strains [35]. In the present case report, RT-PCR did not test positive in any tissue of the cats, and thus, no fragmented persisting RNA was found. Nonetheless, lymphadenopathy might be an aftereffect of FIP, without the presence of virus. Further studies are needed to prove whether these changes are due to a long-term adverse effect of treatment or potentially associated with a "long FIP syndrome".

The initially seen fluid in the retroperitoneal space was not present in the postmortem examination. In FIP, lesions are induced by immune complexes deposited at the wall of

blood vessels, subsequently activating the complement cascade, and damaging vascular tissues [36]. In addition to the spleen, the omentum and the mesenteric lymph nodes are tissues with high viral loads [37]. However, no virus was presented in the lymphoid tissue of the cat analyzed here. Moreover, it remains to be determined whether the preceding FIP might have paved the way for intestinal infection e.g., by affecting local or systemic immunocompetence.

FCoV was no longer detected in any tissue of the cat. In addition, viral load decreased in the blood within a short period of time after treatment initiation, and fecal shedding stopped by day 2. These results indicate that the cat was 100% cured of FIP. The antibody titer decreased during treatment, although it did not become negative. Possibly, the titer would have continued to decrease after infection was cleared, as described previously in FCoV-infected cats without FIP. Anti-FCoV antibodies can sometimes be measured after the clearance of (harmless) FCoV infection for several months, and this is not a sign of viral persistence [38].

FIP relapses after treatment with GS-441524 have been described in a few cases [5]. In these cases, the question arises whether a reinfection with FCoV and a new mutation took place, or whether the virus could not be completely eliminated in these cats and was still present in individual tissues. The presently described cat was 100% free of the virus, making relapses after appropriate treatment very unlikely. Unfortunately, the drug used for the treatment of FIP in this cat is currently not legally available for veterinary use in most countries, forcing well-meaning owners to self-diagnose and treat their cats based on judgment of non-veterinary lay people and social media groups. Thus, there is an urgent need for respective official bodies and industry to work towards a swift licensing process of the drug, so that it can be legally used by veterinary experts to offer supervised treatment to cats suffering from FIP. The limitation of this case report is that only one cat was examined via necropsy. The other study cats are still alive at the time of publication.

4. Conclusions

This is the first report describing clinical and laboratory as well as postmortem findings in a cat cured of FIP that subsequently died accidentally. GS-441524 was highly effective in this cat, and neither signs of FIP nor FCoV RNA or antigens could be detected postmortem. GS-441524 is currently the most effective treatment of FIP, and should be licensed for veterinary use as soon as possible.

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VI. Publikation 5: Original-Publikation IV

Long-term follow-up of cats in complete remission after treatment of feline infectious peritonitis with oral GS-441524

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Original Article

Long-term follow-up of cats in complete remission after treatment of feline infectious peritonitis with oral GS-441524

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Abstract

Objectives Feline infectious peritonitis (FIP), a common disease in cats caused by feline coronavirus (FCoV), is usually fatal once clinical signs appear. Successful treatment of FIP with oral GS-441524 for 84 days was demonstrated recently by this research group. The aim of this study was to evaluate the long-term outcome in these cats.

Methods A total of 18 successfully treated cats were followed for up to 1 year after treatment initiation (9 months after completion of the antiviral treatment). Follow-up examinations were performed at 12-week intervals, including physical examination, haematology, serum biochemistry, abdominal and thoracic ultrasound, FCoV RNA loads in blood and faeces by reverse transcriptase quantitative PCR and anti-FCoV antibody titres by indirect immunofluorescent assay.

Results Follow-up data were available from 18 cats in week 24, from 15 cats in week 36 and from 14 cats in week 48 (after the start of treatment). Laboratory parameters remained stable after the end of the treatment with undetectable blood viral loads (in all but one cat on one occasion). Recurrence of faecal FcoV shedding was detected in five cats. In four cats, an intermediate short-term rise in anti-FcoV antibody titres was detected. In total, 12 cats showed abdominal lymphadenomegaly during the follow-up period; four of them constantly during the treatment and follow-up period. Two cats developed mild neurological signs, compatible with feline hyperesthesia syndrome in weeks 36 and 48, respectively; however, FcoV RNA remained undetectable in blood and faeces, and no increase of anti-FcoV antibody titres was observed in these two cats.

Conclusions and relevance Treatment with GS-441524 proved to be effective against FIP in both the short term as well as the long term, with no confirmed relapse in all study participants during the 1-year follow-up period. Whether delayed neurological signs could be a long-term adverse effect of the treatment or associated with a 'long FIP syndrome' needs to be further evaluated.

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Keywords: Feline coronavirus; feline infectious peritonitis; nucleoside analogue; Mutian; Xraphconn®; antiviral chemotherapy; GS-441524; therapy; long-term observation; survival

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Introduction

Feline infectious peritonitis (FIP) is caused by feline coronavirus (FcoV) that occurs in two different biotypes (lowly pathogenic FcoV and highly pathogenic FIP-associated FcoV).[1–4] Lowly pathogenic FcoV is endemic in cats and the prevalence of infection is up to 90%, especially in multi-cat environments, but only 4–10% of FcoV-infected cats develop FIP,[5,6] a fatal immune-mediated disease characterised by granulomatous vasculitis and perivasculitis.[7] Most of the cats with FIP die if no effective antiviral treatment is available.[3,8]

However, since 2016, antiviral drugs that inhibit the replication of FcoV have been developed. The protease inhibitor GC376, which inhibits the viral 3C- or 3C-like protease,[9,10] which is necessary for the replication of FcoV, was shown to be effective in cats with experimentally induced FIP[9] and in field cats with FIP,[10] although not all cats could achieve complete remission. A second antiviral drug, the nucleoside analogue GS-441524, the active form of the prodrug remdesivir,[11] showed promising results in recently published studies (Table 1). In the first controlled prospective oral treatment study, 18 cats with confirmed FIP were successfully treated with the oral antiviral drug Xraphconn®, containing GS-441524, for 12 weeks.[12]

Since no data describing the detailed long-term outcome in cats with FIP treated with antiviral drugs are available so far, the aim of this study was to prospectively evaluate the outcome in the first year after the start of therapy with oral GS-441524 in these abovementioned 18 successfully treated cats,[12] evaluating clinical outcome, laboratory and virological parameters.

Materials and methods

Study population and protocol

A total of 18 cats that successfully completed a 12-week FIP treatment with the oral multicomponent drug Xraphconn® (Mutian Life Sciences Limited) containing GS-441524, q24h PO, entered long-term monitoring. All cats included in the treatment study had confirmed FIP (diagnosis according to the diagnostic tool of the Advisory Board on Cat Diseases).[13] The cats had to have clinical and clinicopathological abnormalities typical for FIP.[17–19] In addition, direct detection of FCoV had to be positive either by immunohistochemistry from affected organs or by detection of mutated strains of FCoV with commercial reverse transcriptase (RT)-quantitative PCR (qPCR) from effusion, blood or an organ fine-needle aspirate. Mutation PCR used targeted FCoV spike gene mutations leading to spike protein substitutions M1058L and S1060A. In the compound Xraphconn®, the active ingredient GS-441524 was identified with mass spectrometry and nuclear magnetic resonance (referred to as MT-0901 in the package insert). Other ingredients mentioned were Radix scrophulariae, Platycodon grandiflorum, Phyllostachys pubescens, Forsythia suspensa and Anemarrhena asphodeloides. The tablets were film-coated and could be divided to obtain the exact dosage. Beside the main ingredients, ingredients of the coating were hydroxypropyl cellulose, PEG 6000, crystalline cellulose and titanium oxide (for package insert, see Supplementary Material 1). Two different tablets in concentrations of 2.5 mg and 10 mg, according to the manufacturer, were available. The dosage of Xraphconn® tablets was adjusted daily to the current weight of the cats and always administered at the same time each day on an empty stomach. Half an hour after tablet administration, the cats were offered food. From the time the cats were treated at home, the owners received personalised instructions, including videos, on how to administer the tablets. The tablets could be administered with the help of a tablet feeder or wrapped in a little bit of food. Owners were instructed to call in at any time in case of problems. However, this happened only once, whereupon the owner immediately came to the clinic, where he was shown again how to administer the tablet. Cats with neurological and/or ocular signs (n = 2; cats 1 and 2) received a presumed dose of 10 mg/kg, and cats without these signs (n = 16) received 5 mg/kg (according to the manufacturer's instructions).[12] Further analysis of one tablet of the provided drug, however, suggested that one tablet of Xraphconn® of the batch used for the study contained more than double the amount of the GS-441524 than officially stated by the manufacturer (a 200 mg tablet investigated by liquid chromatography mass spectrometry contained 22.15 mg GS-441524 and not 10 mg as presumed; J Horak, unpublished data). Therefore, cats with neurological/ocular signs possibly received 22.15 mg/kg and cats without these signs possibly received 11.06 mg/kg. Initially (day 0), the enrolled cats showed laboratory changes that are considered typical for FIP (hyperproteinaemia: 6/18, 33.3%; hyperglobulinaemia: 13/18, 72.2%; hyperbilirubinemia: 12/18, 66.7%; hypoalbuminemia: 11/18, 61.1%; low albumin-globulin ratio: 16/18, 88.9%; anaemia: 12/18, 66.2%; and lymphopenia: 4/18, 22.2%). Initially, 16/18 cats had abdominal and/or thoracic effusion and two cats had neurological and/or ocular signs. The median age was 7.7 months (range 4.7–56.5 months). The general condition as well as the clinical and laboratory parameters had improved rapidly (significant difference were found between days 1–28 and day 0 for each parameter). The amount of effusion

Table 1 Overview of retrospective and prospective, experimental and field studies with reported relapses of feline infectious peritonitis after treatment with different drugs containing the active ingredient GS-441524

Study	Study design	Number of cats	FIP diagnosis	Dosage of GS-441524	Duration of treatment	Adverse effects	Relapse	Final outcome
Murphy et al, 2018[20]	Prospective experimental	10	Experimentally infected	2–5 mg/kg SC q24h	2 weeks	Local irritations at the injection sites	2/10* 4–6 weeks after finishing treatment	10/10 in remission (survival >8 months)
Pedersen et al, 2019[36]	Prospective field	31	Diagnosis based on clinical and laboratory changes, effusion analysis, in addition in some cats RT-qPCR, IHC	2 mg/kg SC q24h	12 weeks	Local irritations at the injection sites 1/31 cats: increase of urea and SDMA concentrations	8/26† within 3–84 days after finishing treatment	25/26 in remission (at the time of publication)
Dickinson et al, 2020[37]	Case series	4	No definitive diagnosis	5–10 mg/kg SC q24h	12 weeks	–‡	1/4 cats euthanised after 216 days (after two treatments with GS-44152)	3/4 in remission (survival time 354–528 days)
Jones et al, 2021[38]	Retrospective survey	393	No definitive diagnosis	2–16 mg/kg SC q24h	12 weeks	Local irritations at the injection sites	50/393§ after finishing treatment	380/393 in remission at time of survey
Yin et al, 2021[39]	Retrospective	127	No definitive diagnosis	2–4 mg/kg q24h** (some cats in addition GC376 6–8 mg/kg, q24h)	4–>8 weeks	–‡	1/30 (euthanised)	29/30 in remission (no time points mentioned)
Katayama and Uemura, 2023[40]	Prospective field	324	Diagnosis based on clinical and laboratory changes, qPCR detection of FCoV in blood and effusion	130–200 mg/kg SC q24h or PO (100 mg formulation contained 5 mg of GS-441524)	12 weeks	–‡	11/324 (second treatment cycle 200 mg/kg, q24h, for 42 days)	290/324 survived

*Relapse: 4–6 weeks after the end of treatment, followed by a successful second treatment cycle

†Relapse: 8/31 relapse (Ø 23 days after end of treatment), followed by a second treatment cycle: 5/8 cats with 4 mg/kg SC q24h, the cats responded and were in remission at the time of publication; 3/8 cats with 2 mg/kg SC q24h: 1/3 cats relapsed again and was euthanised, 2/3 responded well, but relapsed for the second time, followed by a successful third treatment cycle

‡No adverse effects were mentioned in this study

§Of the relapsed cats, 2.7% received a second treatment cycle

**Route of administration was not indicated

FCoV = feline coronavirus; FIP = feline infectious peritonitis; IHC = immunohistochemistry; RT-qPCR = reverse transcriptase-quantitative PCR; SDMA = symmetric dimethylarginine

and viral loads in the effusion decreased steadily and all cats were clinically and neurologically unremarkable at completion of treatment.[12]

After the 12 weeks, all cats (18/18) had completed treatment successfully, which was defined by unremarkable clinical and neurological examinations, haematology and serum biochemistry parameters mostly in normal ranges, no signs of effusion, ocular and central nervous system disease, and no detectable viral RNA in blood.[12] Haematology was performed using an automatic analyser (Cell-Dyn 3500; Abott Laboratories). A differential blood count was additionally performed manually on blood smears stained with Haema Quick Staining/Diff-Quick staining (LT-SYS®; Eberhard Lehmann GmbH) when haematology parameters were abnormal. Serum biochemistry parameters were measured using an automatic analyser (Hitachi 911; Roche). The concentration of symmetric dimethylarginine (SDMA) was analysed at IDEXX Diavet AG (Bäch) using a high-throughput immunoassay, and the concentration of serum amyloid A (SAA) was determined using a latex agglutination turbidimetric immunoassay reaction (LZ Test SAA; Eiken Chemical Co, Ltd) on a cobas® c 501 clinical chemistry analyser (Roche Diagnostics AG). The general condition and well-being of the cats were evaluated using Karnofsky's score (modified for cats by Hartmann and Kuffer,[14] a classification scale from 0% [dead] to 100% [normal health condition]). The reference range for Karnofsky's score was defined as 90–100%.

Follow-up examinations were performed in weeks 24, 36 and 48 after the start of treatment (168, 252 and 336 days after the start of treatment [weeks after start of treatment will be given henceforth]), which equals 12, 24 and 36 weeks after the completion of treatment. All examined parameters are summarised in Table 2.

This study complied with the German guidelines for prospective studies and was approved by the Government of Upper Bavaria (reference number 55.2-2532.Vet_02-20-52) and by the ethical committee of the Centre for Clinical Veterinary Medicine of LMU Munich (reference number 288-11-10-2021). Owners of the cats gave their written informed consent to participate in the study.

FCoV viral loads and anti-FCoV antibody titres

Viral loads in blood and faeces were analysed by real-time TaqMan RT-qPCR as previously described,[12,15] and anti-FCoV antibody titres in serum were determined by indirect immunofluorescence assay (IFA) as previously described[12,15] at all three follow-up examinations (weeks 24, 36 and 48).

Statistical analysis

Data were analysed using the statistical language R version 4.0.3 (R Core Team, 2020). For every single animal (served as random effects in subsequent models), repeated measurements were performed on several days; therefore, all variables were evaluated with generalised linear mixed-effects models. After model fitting, all contrasts (differences) between individual days were assessed using estimated marginal means, with a Bonferroni P value correction for multiple comparisons ($P \leqslant 0.05$).

Results

Clinical signs, laboratory values and diagnostic imaging

In week 24, 18/18 cats, in week 36, 15/18 cats and in week 48, 14/18 cats were examined. Three cats were lost to follow-up since two cats (cats 3 and 9) were not presented due to missing owners' compliance and one cat had died in a road traffic accident in week 35 (cat 1). On post-mortem examination, the latter cat showed no residual FIP lesions; only a generalised lymphadenomegaly was noted. Moreover, neither FCoV RNA nor FCoV-antigen was identified by RT-qPCR and by immunohistochemistry, respectively, in any tissues or body fluids, including faeces of this cat.[16] One cat (cat 10) showed new onset of neurological signs resembling feline hyperesthesia syndrome (FHS) in week 36 and was therefore treated with a second 84-day treatment of GS-441524 by its owners without prior consultation with the study group and was not presented for the last follow-up examination (Table 3).

All cats had achieved a modified Karnofsky score[14] of 100% at the end of the 12-week treatment period and remained healthy, with clinical and laboratory parameters mostly within reference intervals throughout the follow-up period (Table 4, Figure 1), with the exception of two cats with signs compatible with FHS (described in detail below). The physical parameters (such as body weight, appetite and general condition), which normalised during and towards the end of treatment, also remained consistently normal during the follow-up period. At the start of the treatment, 16 cats had abdominal lymphadenomegaly on ultrasound examination, which in 4/16 cats persisted during the entire treatment and follow-up period. During weeks 24–48, a total of 12 cats showed abdominal lymphadenomegaly (Table 4).

Changes in viral loads and anti-FCoV antibody titres

In blood, no FcoV RNA could be detected in any cat during the follow-up period, with one exception (cat 8). This cat was FCoV RNA-positive in blood before treatment, become negative from day 7 onwards but was positive again at the first re-check in week 24 (200 copies/ml, CT value 38.0), but tested negative again at further re-checks (Figure 2a).

In faeces, five other cats (cats 2, 6, 7, 11 and 16) were RT-qPCR-positive at least once during the follow-up period; 2/5 cats (cats 2 and 16) were positive at all three re-checks (with decreasing copy numbers per g faeces/per swab); 3/5 cats were positive only in week 48 (Figure 2b).

Anti-FCoV antibodies in serum were still present in all 18 cats at the first re-check (week 24), in 14/15 cats at the

Table 2 Overview of the clinical and the laboratory parameters evaluated during the follow-up period at the individual time points

Parameter		Follow-up examinations		
Days after start of treatment		Follow-up 1 (168 days)	Follow-up 2 (252 days)	Follow-up 3 (336 days)
Weeks after start of treatment		24	36	48
Weeks after end of treatment		12	24	36
Physical examination	Including measurement of body weight (measured by a baby scale [AE Adam MTB 20 baby scale; Felde]) and determination of Karnofsky's score, modified for cats[14]	+*	+	+
Neurological examination†	Including evaluation of posture, gait, cranial nerves, postural reactions, spinal reflexes, palpation of the spine	+	+	+
Ultrasound	Abdominal and thoracic‡	+	+	+
Haematology	As previously described[12]	+	+	+
Clinical chemistry	Including SDMA and SAA as previously described[12]	+	+	+
FCoV RNA load in blood	Analysed by RT-qPCR as previously described[12]	+	+	+
FCoV RNA load in faeces	Analysed by RT-qPCR as previously described[12]	+	+	+
Anti-FCoV antibody titre	Determined by indirect immunofluorescence assay as previously described[12]	+	+	+

*+ examinations performed
†If neurological abnormalities were seen.
‡Abdominal ultrasound was performed in all cats; thoracic ultrasound was performed only in cats in which thoracic effusion or changes in the thorax were present before or during treatment
FCoV = feline coronavirus; SAA = serum amyloid A; SDMA = symmetric dimethylarginine

Table 3 Overview of the study population available for the follow-up examinations (numbers show the total number of cats available at the corresponding follow-up examination)

Study population	Follow-up 1	Follow-up 2	Follow-up 3
Number of cats	18	15	14
Death (not related to FIP)	0	1	1
Lack of owner compliance	0	2	3

FIP = feline infectious peritonitis

second and in 13/14 cats at the third re-check (Table 4). Four cats (cats 3, 8, 16 and 18; all of them had companion cats at home) had a short single time-point increase of anti-FCoV antibody titres (Figure 2c).

Signs compatible with feline hyperesthesia syndrome

Two cats (cats 8 and 10) that had not shown neurological abnormalities before or during the treatment developed new-onset mild neurological signs compatible with FHS during follow-ups. These signs were characterised by attacks of excessive licking and twitching of the skin in the lumbar region.

Cat 8 developed signs compatible with FHS in week 47, shortly before the third follow-up re-check. The episodes were characterised by frequent, uncontrolled twitching of the ears, repetitive skin twitches in the lumbar area and tail chasing, occurring several times a day (video, see Supplementary Material 2). Usually, the cat would retreat and hide after such an episode. However, the cat did not appear dazed or disturbed during or after the episodes. In week 48, clinical and laboratory examinations were unremarkable, and no FCoV RNA could be detected in blood and faeces. The anti-FCoV antibody titre increased from 1:100 (week 24) to 1:400 (week 36) and had decreased again at the last follow-up re-check (1:100) (week 48). Two weeks later (week 50) and a few days after the cat had been neutered, mild diarrhoea was reported. At that time (when the cat was readmitted), FCoV faecal shedding (CT value 32.6) was detected. Ultrasound examination at that time showed abdominal lymphadenomegaly and no evidence of free abdominal fluid. Lymph node cytology

Table 4 Selected clinical and laboratory parameters and anti-FCoV antibody titres and viral loads of the cats

Parameter	Treatment				Follow-up		
	Day 0	Day 7	Day 14	Day 83	1 (week 24)	2 (week 36)	3 (week 48)
Clinical parameters							
Karnofsky's score (RI: 90–100)	70 (40–100)	100 (80–100)	100 (–)*	100 (–)	100 (–)	100 (80–100)	100 (–)
Number of cats with values below RI/total cats	17/18	1/18	0/18	0/18	0/18	1/15	0/14
Temperature (°C) (RI: 38.0–39.3)	39.0 (37.5–40.5)	38.4 (37.8–39.5)	38.7 (37.3–39.5)	38.9 (37.9–39.4)	38.8 (37.4–39.4)	38.7 (37.2–39.4)	38.3 (37.2–39.1)
Number of cats with values above RI/total cats	6/18	1/18	1/18	1/18	2/18	1/15	0/14
Body weight (kg)	2.8 (1.8–6.3)	2.8 (1.7–4.4)	2.9 (2.0–4.5)	3.8 (2.7–5.8)	4.2 (2.7–6.3)	5.0 (2.9–6.9)	4.9 (3.0–6.5)
Number of cats with abdominal lymphadenomegaly/total cats	16/18	12/18	17/18	14/18	10/18	7/15	6/14
Number of cats with signs compatible with FHS/total cats	0/18	0/18	0/18	0/18	0/18	1/15	1/14 †
Laboratory parameters							
Haematocrit (l/l) (RI: 33–44)	**30.2** (16.7–44.9)	**27.7** (18.5–43.8)	**30.1** (21.4–48.4)	38.9 (32.5–47.7)	43.1 (35.3–54.2)	**44.1** (32.6–48.7)	41.9 (37.6–51.2)
Number of cats with values below RI/total cats	12/18	16/18	12/18	1/18	0/18	1/15	0/14
Lymphocytes (×10⁹/l) (RI: 1–4)	1.8 (0.42–16.4)	**4.4** (1.62–15.0)	3.2 (1.4–8.2)	**4.5** (2.0–40.7)	3.3 (1.5–6.5)	3.6 (1.3–5.1)	3.2 (1.6–6.0)
Number of cats with values below RI/total cats	4/18	0/18	0/18	0/18	0/18	0/15	0/14
Number of cats with values above RI/total cats	5/18	11/18	6/18	10/18	8/18	5/15	4/14
Bilirubin (µmol/l) (RI: 0–4.74)	**9.7** (0.0–72.9)	3.1 (0.2–11.7)	1.4 (0.0–5.8)	0.5 (0.0–1.1)	0.5 (0.0–1.0)	0.6 (0.1–1.1)	0.7 (0.2–1.2)
Total protein (g/l) (RI: 60–85)	75.9 (59.0–114.4)	**88.9** (54.9–116.9)	78.8 (64.3–104.7)	68.3 (59.9–85.0)	72.2 (62.0–77.6)	73.8 (64.9–80.5)	72.9 (63.8–81.8)
Albumin (g/l) (RI: 26–56)	**23.2** (12.2–38.7)	27.1 (20.8–35.5)	29.7 (19.2–33.4)	35.7 (31.3–40.6)	40.5 (35.2–47.3)	40.7 (38.3–43.3)	40.0 (35.6–44.9)
Globulin (g/l) (RI: <45)	**56.5** (32.0–88.1)	**59.8** (27.8–88.0)	**52.0** (45.1–71.3)	31.4 (25.7–51.6)	32.8 (17.5–42.4)	32.4 (26.6–40.6)	32.5 (21.7–44.5)
Albumin/globulin ratio (RI: >0.6)	**0.4** (0.2–1.0)	**0.5** (0.2–1.0)	**0.5** (0.4–0.6)	1.2 (0.7–1.4)	1.3 (0.8–2.5)	1.3 (1.0–1.5)	1.3 (0.8–1.9)
SAA (mg/l) (RI: 0–3.9)	**146.7** (0.5–244.7)	**6.2** (0.0–52.0)	**4.3** (2.0–100.2)	**5.1** (0.0–9.9)	2.1 (0.0–4.0)	3.0 (0.0–11.7)	2.9 (1.0–16.2)
Number of cats with SAA values above RI/total cats	16/18	13/18	11/18	11/18	1/18	3/15	3/14

(Continued)

Table 4 (Continued)

Parameter	Treatment				Follow-up		
	Day 0	Day 7	Day 14	Day 83	1 (week 24)	2 (week 36)	3 (week 48)
Anti-FCoV antibody titres							
Anti-FCoV antibody titre (IFA)	1600 (100–6400)	1600 (100–6400)	1600 (100–6400)	400 (25–1600)	400 (25–6400)	400 (0–1600)	100 (0–1600)
Number of cats with anti-FCoV antibodies/total cats	18/18	18/18	18/18	18/18	18/18	14/15	13/14
Viral loads							
FCoV RNA faeces (copies/g)	0 ($0–19.6 \times 10^7$)	0 ($0–2.0 \times 10^3$)	0 ($0–5.2 \times 10^3$)	0 (0–671)	0 ($0–25.8 \times 10^7$)	0 ($0–2.2 \times 10^6$)	0 ($0–14.0 \times 10^4$)
Number of cats with FCoV RNA in faeces[‡]/total cats	11/18[§]	1/18	1/18	1/18	2/18	2/15	5/14
FCoV RNA blood (copies/ml)	6.866 ($0–11.8 \times 10^4$)	0 ($0–11.9 \times 10^2$)	0 (–)**	0 (–)	0 (0–200)	0 (–)	0 (–)
Number of cats with FCoV RNA in blood[‡]/total cats	15/18	5/18	0/18	0/18	1/18	0/15	0/14
FCoV RNA in accessible[††] effusion (copies/ml)	67.2×10^4 (15.0×10^4–37.3×10^6)	–[‡‡]	319 (0–637)	–	–	0	–
Number of cats with effusion/total cats	16/18	10/18	6/18	0/18	2/18	4/15	0/14
Number of cats with accessible[††] effusion/total cats	12/16	0/10	2/6	0/18	0/2	1/4	0/0
Number of cats with FCoV RNA in accessible[††] effusion[‡]/total cats	12/12	0/0	2/2	0/0	0/0	0/1	0/0

Values are given as n or median (range). Columns are coloured according to the different study sections: orange = hospitalisation in the clinic (days 0 and 7); blue = re-checks during the treatment (days 14 and 83); green = follow-up period after the end of treatment (weeks 24, 36 and 48). Values marked in bold are outside the reference intervals. For the parameters for which the values were outside the reference interval during the follow-up period, the number of cats with values below and/or above the interval is shown

*No range because all values were the same (100%) at this time point

†Cat 10 developed signs compatible with FHS in week 36 and was therefore treated with a second 84-day treatment of GS-441524 by its owners without prior consultation with the study group and was not presented for the last follow-up examination

‡Number of cats that were viral RNA-positive (≥1 copy per ml of blood/effusion and per g of faeces/ per swab) at the individual time points

§Within the first 3 days, overall excretion could be detected in the faeces of 11 cats

**No range because all values were viral RNA-negative in blood (0 copy per ml of blood) at this time point.

††'Accessible' means that in these cases, effusion could be seen on ultrasound, and it was possible to obtain a sample; in the other cases, a small amount of effusion could be seen on ultrasound examination, but sampling was not possible

‡‡Some cats showed effusion on ultrasound examination but it was not possible to obtain any sample, therefore no sample for FcoV RNA measurement was available.

FCoV – feline coronavirus; FHS – feline hyperesthesia syndrome; IFA = indirect immunofluorescence assay; RI – reference interval; SAA – serum amyloid A

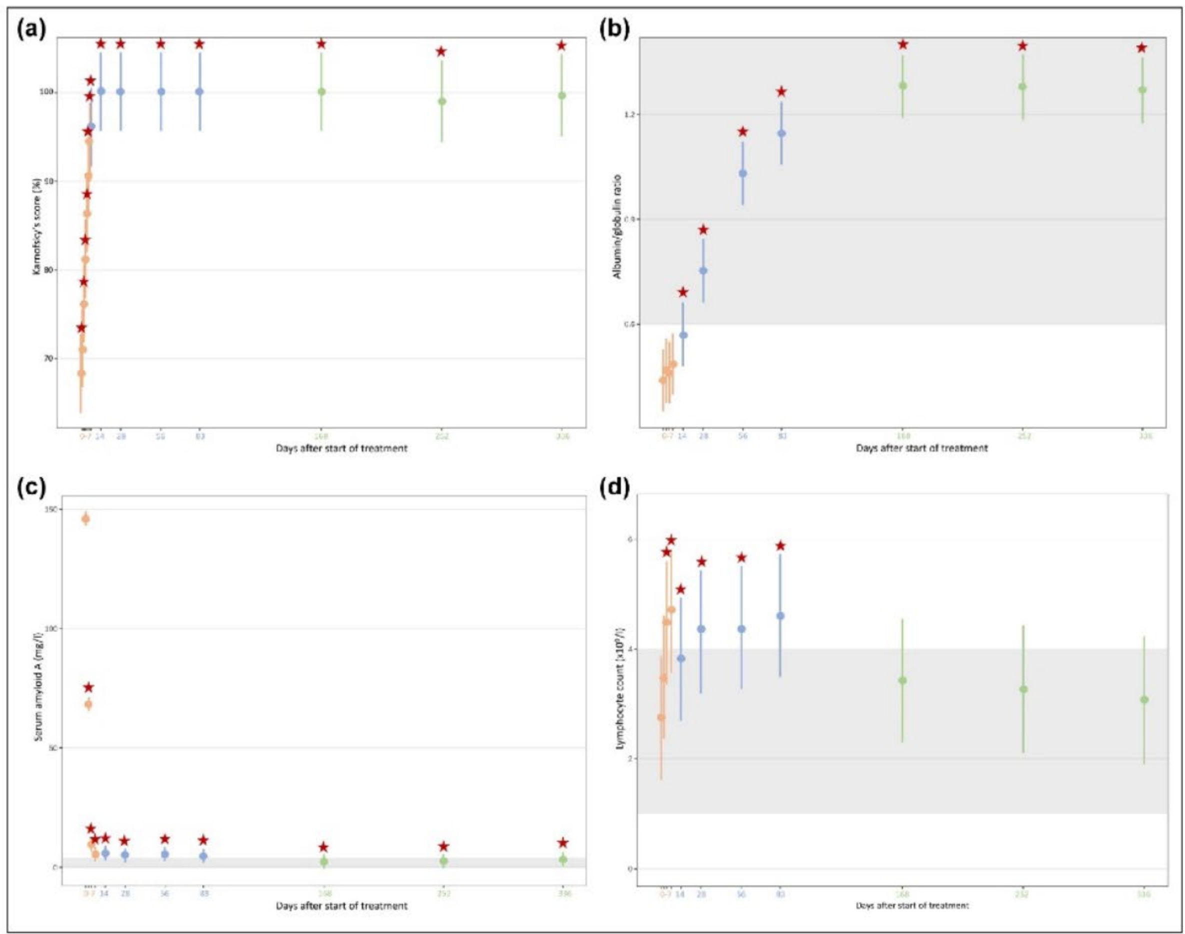

Figure 1 Timeline showing the improvement of selected clinical and laboratory parameters before, during and after the treatment in predictive values and 95% confidence intervals. Values and days are coloured according to the different study sections. Orange = treatment and hospitalisation in the clinic (days 0–7); blue = treatment at home period with re-checks (days 14, 28, 56 and 83); green = follow-up period after the end of treatment (days 168 [week 24], 252 [week 36] and 336 [week 48]). The grey area marks the reference interval of each parameter. (a) Karnofsky's score (%) modified for cats by Hartmann and Kuffer (1998) (from 0%, indicating 'dead', to 100%, indicating 'completely normal general condition').[14] (b) Albumin/globulin ratio. (c) Serum amyloid A (mg/l). (d) Lymphocyte count ($\times 10^9$/l). Red asterisks mark significant differences ($P \leqslant 0.05$) between parameters on the different treatment days compared to day 0 (before treatment), measured with linear-mixed robust linear-mixed effects models

revealed reactive lymphocytes with isolated neutrophils and plasma cells consistent with reactive hyperplasia. In the lymph node, FCoV RNA could be detected at a very low load (CT value 40). No FCoV RNA was detected in blood. Physical, dermatologic, ocular and neurological examinations were unremarkable. Palpation of the spine and tail identified no sites of pain and no lesions. Laboratory examinations including *Toxoplasma gondii* antibody IgG IFA and *Toxoplasma gondii* antibody IgM IFA were unremarkable. Further diagnostic work-up with MRI, cerebrospinal fluid (CSF) analysis and electroencephalography (EEG) was offered but declined by the owners. To exclude ectoparasite infestation as a possible cause of signs compatible with FHS, the cat was treated once with a spot-on (esafoxolane, eprinomectine, praziquantel); however, this did not lead to improvement and episodes continued to occur. The frequency and severity of episodes improved significantly after 6 months without any other medications and up to the day of submission, episodes occur only very rarely.

Cat 10 developed episodes with twitching skin on the back and restlessness several times a day in week 35. The owners reported additional signs of difficulties to jump on objects, increased body temperature (40°C) and anorexia for 2 days. The same evening, the cat was treated with supportive treatment at another hospital (a single dose of metamizole, amoxicillin/clavulanic acid and fluid therapy for 2 days). The owners reported that they had started a second course of GS-441524 treatment using a substance from an unknown source the evening of the

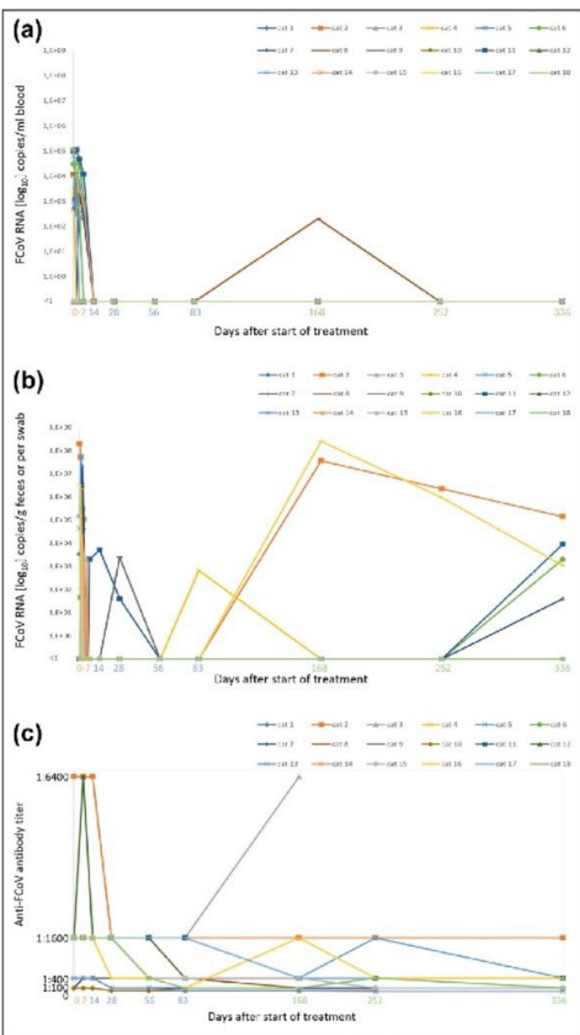

Figure 2 Viral RNA loads of each cat in (a) blood (copies per ml) and (b) faeces (copies per g or per swab) before, during and after antiviral treatment. Blood samples were collected on days 0, 2, 4 and 7 during hospitalisation in the clinic, at the re-checks during the treatment (days 14, 28, 56 and 83) and the follow-up period after the end of treatment (days 168 [week 24], 252 [week 36] and 336 [week 48]). Faecal samples were collected on days 0–7, days 14, 28, 56 and 83 and days 168 (week 24), 252 (week 36) and 336 (week 48). RNA loads were measured by feline coronavirus (FCoV) reverse transcriptase-quantitative PCR. (c) The anti-FCoV antibody titres of the 18 cats before, during and after antiviral treatment. The samples were collected on days 0 and 7 during hospitalisation in the clinic, at the re-checks during the treatment (days 14, 28, 56 and 83) and the follow-up period after the end of treatment (days 168 [week 24], 252 [week 36] and 336 [week 48]); anti-FcoV antibody titre was determined by indirect immunofluorescence assay (IFA)

day of discharge. The next day, the cat was presented to the authors' research group for the second follow-up (week 36). No further signs of reluctance to jump were reported and appetite and body temperature were normal. Physical and neurological examinations (including mental status, posture and gait, cranial nerves, postural reactions, spinal reflexes, perineal reflex) at that time were unremarkable. A thorough palpation of the spine failed to disclose any sites of pain. Haematology and serum biochemistry revealed no abnormalities. Ultrasound examination showed abdominal lymphadenomegaly and a very small amount of free fluid (RT-qPCR-negative for FCoV RNA). No viral RNA was detected in blood and faeces, and anti-FCoV antibody titres were unchanged (1:25, since week 4 after the start of treatment). Further diagnostic work-up with MRI, CSF analysis and EEG were offered but declined by the owners. Subsequently, the owners reported that signs resembling FHS (episodes of twitching skin on the back and restlessness several times a day) persisted throughout the second 12-week treatment course, and only disappeared a few weeks to months later. On the day of submission of the manuscript, the cat remained clinically healthy without any further episodes.

Discussion

This study demonstrates the successful oral treatment of cats with FIP with a multicomponent drug containing GS-441524 over 12 weeks resulting in sustained remission. No cat experienced a relapse of the initial clinical and laboratory signs indicative of FIP[17–19] over a 1-year follow-up period since the initiation of treatment. In a previous study by Murphy et al on 10 experimentally infected cats treated with GS-441524 (2–5 mg/kg SC q24h) for 2 weeks, 2/10 cats had a relapse 4–6 weeks after treatment.[20] The reason for these relapses might be a lower drug exposure (lower daily doses used and shorter treatment duration) compared to the present study (with 5–10 mg/kg PO q24h, as stated by the manufacturer) for 84 days. Additional analyses of the drug used in the study indicate that one tablet of the multicomponent drug Xraphconn® contained more GS-441524 than officially stated by the manufacturer (J Horak, unpublished data). Although all clinicopathological parameters typically altered in cats with FIP (increased total protein, globulin and bilirubin concentrations, decreased albumin concentration, albumin/globulin ratio, haematocrit and lymphocyte count)[17–19] improved significantly within a few days of treatment in the experimental study,[20] the 2-week treatment might have been too short for relapse-free FIP treatment after all. Since the optimal duration of treatment is unknown, the question arises whether 12 weeks are necessary to achieve long-term remission or whether a shorter duration could also be sufficient. The concentration of the active substance GS-441524 in one tablet was determined, which showed that the concentration is different from that stated by the manufacturer. This raises the suspicion that all tablets might contain different amounts of GS-441524. As not every tablet could be evaluated, it

can be suspected that each tablet might contain a different amount of GS-441524 due to the unlicensed and uncontrolled production. However, it is important to know the exact concentration of the active ingredient given to a cat as adverse effects of under- or overdosing are not known.

Within the follow-up period, all cats constantly showed a normal general condition (including cats 8 and 10 despite them developing FHS) and a constant or still increasing body weight. A mild to moderate lymphocytosis was seen in 14/18 (77.7%) cats during the treatment period.[12] During the follow-up period, a very mild lymphocytosis was still present in some cats. Since the severity of initial lymphopenia is considered a negative prognostic factor in FIP,[21] lymphocytosis during treatment might indeed be a positive prognostic factor for recovery. In general, lymphocytosis in cats can be caused by excitement (stress after transport to the clinic and all examinations performed) to which cats are very sensitive. It remains of particular interest to further determine the immunological processes and cellular immune response against FCoV before, during and after treatment. Haematocrit was very mildly decreased in only one cat at the second follow-up. SAA was minimally elevated in some cats, but as this parameter is a non-specific inflammatory marker, it might have been slightly elevated for many reasons. During treatment, there was not only a normalisation of clinicopathological parameters, but also a rapid elimination of the viral RNA in blood, effusion (if still present at all) and faeces. At the end of treatment (week 12), all cats were FCoV RNA-negative in blood and remained negative during the follow-up period, with one exception. One cat (cat 8) had temporarily a very low FCoV RNA blood load of 200 copies/ml in week 24. Intestinal reinfection with FCoV and translocation into blood would be likely as the anti-FCoV antibody titre in this cat has risen to week 36 (1:100 in week 24 and 1:400 in week 36), even though FCoV could not be detected in faeces in either week 24 or week 36. Alternatively, the virus was still present in another compartment (eg, CSF) during the entire treatment period and translocated into blood.

Five cats (cats 2, 6, 7, 11 and 16) became enterally reinfected in the follow-up period as indicated by faecal FCoV shedding, compatible with the fact that all of them had companion cats at home. While three cats showed re-shedding only in week 48, two cats (cats 2 and 16) continued to shed during the entire follow-up period. No relapse of clinical signs compatible with FIP has occurred in the five cats. Only 1/5 re-shedding cats (cat 16) showed a rise of anti-FCoV antibody titres during the follow-up period. Already during the treatment period, re-shedding could be seen in one cat (cat 4) and new onset of shedding in two cats (cats 9 and 11). Reinfections caused by their partner cats are most likely, as sequencing of FCoV RNA from faeces was shown in four cases[15]—8/12 partner cats were positive for FCoV in faeces, and in 4/8 of these samples, phylogenetic analysis showed common clusters with the corresponding FIP cats[15]; therefore, in the five cats shedding during follow-ups, the most likely source of reinfection were the partner cats. Thus, antiviral drugs should not be used just to stop faecal FCoV excretion, as reinfections occur continuously, even though a recently published study by Addie et al reported a complete stop of FCoV shedding in 29 healthy cats treated with oral GS-441524 (2.0–4.0 mg/kg PO q24h for 4–7 days, in 4/29 after a second round of treatment). In 18/29 cats, the known duration of absence of FCoV in faeces after the completion of treatment was in the range of 1–157 days. However, it must be noted that not all cats in that study underwent follow-up testing of FCoV shedding; 11/29 cats were not re-tested after the treatment. Therefore, it is not known whether these cats might have become reinfected later.[22] In addition, viral resistance can occur, as demonstrated in an *in vitro* study. FCoV is indeed able to mutate and has already developed resistances to the antiviral protease inhibitor GC376[23]; it is, in the opinion of the authors, contraindicated and not recommended to administer GS-441524 in cats not suffering from FIP. Only cats with proven FIP after a thorough diagnostic approach should receive this antiviral treatment. The main focus in a multi-cat household should be to reduce the risk of FCoV infection and transmission (eg, by thorough hygiene, a sufficient number of litter trays and, if possible, outdoor access).[17]

A total of 12 cats showed abdominal lymphadenomegaly during the follow-up period (at any time point). This could be interpreted as a sign of an exaggerated, genetically determined reaction of the immune system comparable with a prolonged healing process or a persistent stimulus (eg, of the presence of residual virus, as it is possible that FCoV was still present in certain compartments). Severe generalised follicular lymphoid hyperplasia was also found in the cured cat that died in a traffic accident.[16] The high proportion of cats with abdominal lymphadenomegaly could be a result of a recent (re)infection with FCoV, but this is not very likely as many different cats showed this change without any other signs of reinfection. Alternatively, lymphadenomegaly might be associated with long-term sequela of the FIP itself or possibly be a long-term adverse effect of antiviral treatment. Further clinical trials with cytological examination of the enlarged lymph nodes, especially in long-term observational trials, would be interesting to understand more about the persistence of abdominal lymphadenomegaly.

The two cats (cats 8 and 10) that developed signs compatible with the FHS after successful treatment of FIP with GS-441524 are the first published cases of such a syndrome after FIP treatment. However, in a round table discussion at the congress of the International Society for Companion Animal Infectious Diseases (ISCAID) (September 2022 in Glasgow, UK), other researchers reported a few similar observations. FHS is a poorly

understood syndrome, first described in 1980,[24] and comprises episodes of twitching and rolling of the skin of the back and signs of discomfort, which mostly appear spontaneously, sometimes triggered by touching of the lumbar area. Various medical conditions have been reported to cause signs indicative of FHS including dermatologic, orthopaedic, neurological or behavioural diseases.[24–28] In many cases, the underlying cause cannot be detected.[29] In the two cats from the present study, a neurological cause was considered the most likely as clinical, dermatological and orthopaedic examinations were unremarkable, and no spinal or tail pain was identified. Yet, meningitis cannot be excluded without further examinations. It is striking that these two cats initially had no neurological abnormalities but developed signs compatible with FHS after successful treatment. Since FCoV RNA was not detected in blood, and laboratory parameters and neurological examinations were unremarkable, these signs were not considered as a relapse of FIP. This was further supported by the subsequent good clinical condition (besides the signs compatible with FHS) in both cats.

Cat 8 started faecal re-shedding of FCoV and showed an increase in anti-FCoV antibody titres from 1:100 to 1:400 (2 weeks after the third follow-up; week 50) a few weeks after the onset of signs compatible with FHS. However, the increase in anti-FCoV antibody titre likely only indicated reinfection with FCoV, but not a relapse of FIP.

In cat 10, the owner reported short-term (2 days) clinical signs that could have been suggestive of a relapse of FIP (increased body temperature, reluctance to jump, spinal hyperesthesia) at approximately the same time as the onset of signs resembling FHS, but these other signs disappeared after 2 days. At the second follow-up, neither these nor other neurological signs indicative of multifocal neurological disease were verified.[30,31] This cat was started on another 84-day treatment of presumptive GS-441524 SC by the owner, but the owner reported that the signs resembling FHS persisted throughout the second treatment and only later resolved spontaneously.

It is possible that mild neurological signs could also be a type of 'long FIP syndrome'. In a study on children with COVID-19 and paediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 (PIMS-TS), 4/27 children who had been previously healthy without any SARS-CoV-2-related signs or other comorbidities developed new-onset neurological signs. The neurological situation improved in all children, with 2/4 children showing a complete recovery after 11 and 18 days, respectively.[32] In the present study, no virus could be detected in the analyses allowed by the owners, which raises the hypothesis that long-term immunological effects triggered by the virus might also contribute to the development of neurological manifestations resembling FHS.

Since neurological signs compatible with FHS appeared only a few weeks to months after the treatment, this could also be a long-term adverse effect (eg, neuropathy) of GS-441524 treatment. In the treatment of cats with GS-441524, there are no long-term data on potential adverse effects to date. In addition, in human medicine, relatively little is known from human trials on remdesivir, which is the prodrug of GS-441524, except for the occurrence of delirium in two patients in an open-label study of COVID-19 disease[33] and one possible report of neurological complications in a phase 1 trial of Ebola virus disease.[34] However, experience from previous viral pandemics suggests that the immunological response to the viruses themselves has the potential to cause neuropsychiatric manifestations, including encephalopathies and psychoses.[35] Further studies with controlled drug concentrations are needed. This is especially important since in the currently available unlicensed preparations, additives and their possible effects are not known. However, these compounds are still widely used, as GS-441524 is not licensed and the production of and treatment with drugs containing GS-441524 is not allowed in most countries (with the exception of the United Kingdom and Australia).

The main limitations of the study were that complete follow-up examinations were not available in all cats and that the actual amount of the active ingredient GS-441524 each cat received was unknown. Furthermore, the owners of the two cats with signs compatible with FHS opted against further diagnostic work-up with EEG, MRI and/or CSF. Another limitation of this study was that cytology was not performed in all cats with abdominal lymphadenomegaly, which could have provided more information on the cause of the biology of the persistence of enlarged abdominal lymph nodes.

Conclusions

This is the first long-term (1 year) follow-up study of cats with a confirmed diagnosis of FIP treated with GS-441524 resulting in sustained remission. Thus, treatment with GS-441524 was effective against FIP in both the short-term and long-term because all cats are still alive on the day of submission of the present manuscript (except the one cat that died in a road traffic accident), which is more than 2 years after the diagnosis, and physical, neurological and laboratory examinations remained unremarkable throughout the observation period. Two cats of this cohort developed neurological signs resembling FHS but these neurological signs did not progress and appeared to resolve spontaneously several months later. Whether mild neurological signs compatible with FHS and/or abdominal lymphadenomegaly are associated with a 'long FIP syndrome' are an adverse effect of treatment, or reflect the presence of residual virus in these compartments, or whether signs resembling FHS are unrelated to the treatment of FIP requires further investigation (summarised in Box 1).

Box 1 Key findings and questions raised by the present long-term follow-up study

KEY FINDINGS and **QUESTIONS** raised by the present long-term follow-up study

- Treatment with oral GS-441524 proved to be effective against FIP not only short-, but also long-term over 1 year, without any relapse in the study participants
- Persistence of abdominal lymphadenomegaly can be observed long-term in cats with sustained remission. Reasons for this might be a. . .
  - . . .prolonged healing process? (exaggerated, genetically determined reaction of the immune system)
  - . . .persistent stimulus? (residual virus in abdominal lymph nodes)
  - . . .long-term adverse effect of antiviral treatment?
- Delayed new-onset of neurological signs compatible with feline hyperesthesia syndrome occurred after treatment with oral GS-441524. Reasons for this might be a. . .
  - . . .'long FIP syndrome'?
  - . . .long-term adverse effect of antiviral treatment?
- New-onset faecal FcoV shedding can occur, most likely due to a reinfection from companion cats

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Informed consent Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken (either prospective or retrospective studies). Written informed consent was obtained from the owners described in this work for the procedure(s) undertaken. For any animals or humans individually identifiable within this publication, informed consent (either verbal or written) for their use in the publication was obtained from the people involved.

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VII. DISKUSSION

FIP ist eine tödliche Infektionskrankheit bei Hauskatzen (CAVE et al., 2002). Bis vor kurzem konnten Katzen nur symptomatisch behandelt werden; umso wichtiger ist es, eine wirksame kausale Therapie gegen FIP zu finden (**Übersichtsartikel**). Frühere Therapieversuche gingen über die symptomatische Therapie (z. B. Glukokortikoide, Propentofyllin), immunmodulatorische Ansätze (z. B. Interferone, Polyprenyl-Immunstimulanz) bis hin zur antiviralen Therapie mit einem Protease-Inhibitor (GC376) und einem Nukleosid-Analogon (GS-441524) (WEISS et al., 1990; ISHIDA et al., 2004; RITZ et al., 2007; LEGENDRE & BARTGES, 2009; FISCHER et al., 2011; KIM et al., 2016; LEGENDRE et al., 2017; PEDERSEN et al., 2018). Die symptomatische Therapie führte nur zu einer kurzzeitigen Verbesserung der klinischen Symptome, aber nicht zur Heilung. Auch die immunmodulatorische Therapie erwies sich als nicht sehr erfolgsversprechend (WEISS et al., 1990; RITZ et al., 2007; LEGENDRE & BARTGES, 2009; LEGENDRE et al., 2017). Die antiviralen Medikamente GC376 und GS-441524 waren jedoch in mehreren Studien *in vitro* und *in vivo*, sowohl experimentell, als auch im Feld, wirksam und konnten das Leben von an FIP erkrankten Katzen verlängern, wobei die Wirksamkeit von GS-441524 im Vergleich zu GC376 deutlich besser war (KIM et al., 2016; PEDERSEN et al., 2018). Beide antiviralen Wirkstoffe sind nicht zugelassen und können von Tierärzten in Deutschland nicht angewendet werden. Nicht-lizenziertes GS-441524 wird daher von Katzenbesitzern über den Schwarzmarkt aus nicht kontrollierten Produktionen bestellt und in Eigenregie ohne tierärztliche Betreuung angewendet.

Bisher gab es keine kontrollierte prospektive Studie zum oralen Einsatz von GS-441524. In der ersten prospektiven, kontrollierten Therapiestudie zur Behandlung der FIP (**Publikation 1**) konnte die Effektivität des antiviralen Wirkstoffes GS-441524 (Xraphconn®, Mutian, China) bei oraler Verabreichung an Katzen mit FIP erstmals bewiesen werden. Die Wirksamkeit von GS-441524 wurde in früheren Studien ausschließlich bei subkutaner Verabreichung nachgewiesen (MURPHY et al., 2018; PEDERSEN et al., 2019). In diesen Studien traten jedoch bei mehr als der Hälfte der behandelten Katzen lokale Hautreaktionen auf (MURPHY et al., 2018; PEDERSEN et al., 2019). Solche Entzündungsreaktionen sind nicht nur schmerzhaft, sie könnten auch zur Entstehung feliner Injektionsstellen-assoziierter

Sarkome (FISS) führen (HARTMANN et al., 2015). Darüber hinaus ist es für Besitzer oft schwierig, die eigene Katze (ohne tierärztliche Hilfe aufgrund der Nicht-Zulassung des Medikamentes) selbst zu spritzen, insbesondere, da die Injektionen, die den Katzen über einen langen Zeitraum (84 Tage) täglich gespritzt werden müssen, mit Schmerzen verbunden sind. Aus diesen Gründen ist eine orale Verabreichung des Medikamentes von großem Vorteil. GS-441524 wird illegal in China hergestellt und ist als Injektionslösung und in Tablettenform (seit 2020) auf dem Schwarzmarkt erhältlich (ADDIE et al., 2020a). Eine orale Gabe ermöglicht eine stress- und schmerzfreie Applikation. Nichtsdestotrotz verwendeten laut den Ergebnissen einer retrospektiven Studie die Mehrheit der Besitzer (71,8 %; 282/393) Injektionen, um die eigene an FIP erkrankte Katze zu therapieren. Nur 8,1 % (32/393) der Besitzer verwendeten Tabletten; 20,1 % der Besitzer kombinierten Injektionen und Tabletten (JONES et al., 2021). Ein Grund, weshalb Besitzer häufiger Injektionen verwendeten, könnte die Unwissenheit der Besitzer und/oder eine nicht ausreichende Datenlage gewesen sein, da es bis zu diesem Zeitpunkt nur einen veröffentlichten Fallbericht zur oralen Therapie mit GS-441524 bei einer Katze mit neurologischer FIP gab (ADDIE et al., 2020a). Tabletten sind im Vergleich zu Injektionslösungen teurer; dies könnte ein weiterer Grund für die häufigere Verwendung von Injektionen statt Tabletten sein. Die Studie der **Publikation 1** untersuchte erstmals die Wirksamkeit dieser oralen Therapie an einer größeren Gruppe von Katzen mit FIP unter kontrollierten Bedingungen.

Alle eingeschlossenen Katzen befanden sich zum Zeitpunkt der Veröffentlichung von **Publikation 1** in vollständiger und rückfallfreier Remission (18/18, 100 %). Katzen ohne neurologische und/oder okuläre Symptome erhielten 5 mg/kg Körpergewicht (KG), Katzen mit neurologischen und/oder okulären Symptomen 10 mg/kg KG der aktiven antiviralen Substanz über einen Zeitraum von 84 Tagen 1 x täglich. Die Dosierungen bezogen sich dabei auf die Angaben des Herstellers (persönliche Kommunikation und Packungsbeilage). Analysen im späteren Verlauf (**Publikation 3**) konnten zeigen, dass die Tabletten mehr als das Doppelte an GS-441524 enthielten, als angenommen. In einer ähnlichen Studie, die die Wirksamkeit von GS-441524 bei subkutaner Verabreichung (2 – 4 mg/kg, q24h, SC, für 84 Tage) untersuchte, wurden 31 Katzen mit FIP eingeschlossen. Davon verstarben 5/31 Katzen zu Beginn der Therapie; 26/31 (83,9 %) Katzen schlossen einen kompletten Therapiezyklus von 12 Wochen ab. 18/26 (69,2 %) Katzen befanden

sich in anhaltender Remission; 8/26 (30,8 %) Katzen hatten einen Rückfall und mussten erneut therapiert werden (PEDERSEN et al., 2019). Die Erfolgsrate war in der vorliegenden Studie somit deutlich höher. Dies könnte mit einer höheren Dosierung von GS-441524 in Zusammenhang stehen. So erhielten Katzen der vorliegenden Studie mehr als die doppelte (den später vorgenommenen Analysen zufolge möglicherweise mehr als die vierfache) Dosis. Außerdem wurde in der vorliegenden Studie die Dosis täglich an das aktuelle Gewicht der Katzen angepasst, um bei Gewichtszunahmen Unterdosierungen zu vermeiden. Ein zweiter denkbarer Grund für die höhere Wirksamkeit der oralen Therapie in der vorliegenden Studie könnte sein, dass tatsächlich alle eingeschlossenen Katzen nachweislich FIP hatten. Dies war möglicherweise bei den früheren Studien nicht immer der Fall. In der vorliegenden Studie wurde die Diagnose FIP gestellt, wenn (1) FCoV direkt nachgewiesen wurde mittels positiven immunhistochemischen Nachweis von FCoV-Antigen in Makrophagen veränderter Organe (FELTEN & HARTMANN, 2019; ABCD, 2021) und/oder einer positiven kommerziellen RT-PCR (Reverse-Transkriptase-Polymerase-Kettenreaktion) mit Nachweis der FCoV-Spike (S)-Genmutationen (M1058L und S1060A) im Erguss, Blut oder in Feinnadelaspiraten eines veränderten Organs. Zusätzlich mussten Katzen für die Diagnosestellung (2) FIP-typische klinische und labordiagnostische Veränderungen (ABCD, 2021; ABCD, 2022) aufweisen. Dank dieser mehrstufigen Diagnosestellung, empfohlen von Experten des European Advisory Board On Cat Diseases (ABCD), wurden nur Katzen mit dem antiviralen Medikament behandelt, die tatsächlich an FIP erkrankt waren. Katzen, die nicht an FIP, sondern an anderen Erkrankungen litten, wurden nicht in die Therapie-Studie eingeschlossen, so, wie dies möglicherweise in früheren Studien der Fall war. Darüber hinaus könnte die intensive Betreuung und unterstützende symptomatische Therapie der Katzen wesentlich zu der hohen Erfolgsquote der vorliegenden Studie beigetragen haben. So erhielten Katzen neben einer intravenös angepassten Flüssigkeitstherapie bei Bedarf Antipyretika, Analgetika, Appetitanreger, Antiemetika und/oder Antibiotika sowie intensive Betreuung, mit deren Hilfe Komplikationen oder andere Erkrankungen schnell diagnostiziert und behandelt werden konnten. Eine Katze entwickelte beispielsweise während des stationären Aufenthaltes einen Pyothorax, welcher ohne tierärztliche Betreuung nicht direkt diagnostiziert und dementsprechend auch nicht therapiert worden wäre. Neben der FIP können auch nicht FIP-assoziierte Probleme auftreten, wie z. B. eine Nierenmineralisierung bei

einer der Studien-Katzen, die ebenfalls unbemerkt geblieben wäre, wenn die Besitzer ihre Katze ohne tierärztliche Aufsicht behandelt hätten und zu erheblichen Problemen (z. B. Obstruktion der harnableitenden Wege) geführt hätte. Dies zeigt deutlich, wie wichtig eine an die Probleme und Bedürfnisse der einzelnen Katzen angepasste veterinärmedizinische Behandlung ist, die jedoch eine legale Zulassung von GS-441524 zwingend voraussetzt.

Das chinesische Präparat Xraphconn®, mit dem die Katzen in der vorliegenden Studie therapiert wurden, stammte aus einer nicht kontrollierten Produktion. Deshalb und aufgrund widersprüchlicher Angaben des Herstellers zur aktiven Substanz wurden strukturchemische Analysen mittels Massenspektrometrie und Kernspinresonanzspektroskopie durchgeführt. Diese Analysen identifizierten GS-441524 als (einziges) Adenin-C-Nukleosid-Ribose-Analogon (**Publikation 1, Abbildung 7 und 8**). Im Weiteren enthält Xraphconn® (neben GS-441524) noch weitere Inhaltsstoffe (laut Packungsbeilagen: pflanzliche Inhaltstoffe), die theoretisch durch synergistische Effekte ebenfalls zur hohen Wirksamkeit der antiviralen Therapie hätten beitragen können. In einer kürzlich veröffentlichten Studie wurde die Kombination von GS-441524 mit Itraconazol, einem Antimykotikum und Inhibitor der Cholesterinsynthese, in Zellkultur auf eine potentiell synergistische antivirale Wirkung untersucht. Itraconazol konnte die antivirale Wirkung von GS-441524 und damit die Hemmung der Replikation von FCoV verstärken (DOKI et al., 2022). Ein ähnlicher Effekt wäre auch durch die in Xraphconn® enthaltenen pflanzlichen Inhaltstoffe denkbar.

In der Studie der **Publikation 1** wurde bei 15/18 Katzen (83,3 %) der an FIP erkrankten Katzen bei Studieneinschluss FCoV-RNA im Blut nachgewiesen. Bei all diesen Katzen konnte die Virämie spätestens bis Tag 14 nach Beginn der Therapie gestoppt werden. Dies deutet auf einen exzellenten antiviralen Effekt des Medikamentes hin. Auch decken sich die *in-vivo*-Ergebnisse mit der Wirksamkeit des Medikamentes *in vitro*. In Zellkultur konnte durch GS-441524 ein rascher Rückgang der Viruslast in mit FCoV-infizierten Crandell-Rees-Feline-Kidney-(CRFK-) Zellen und somit eine ausgezeichnete Wirksamkeit gezeigt werden (**Publikation 1, Abbildung 2**).

Die Tatsache, dass bei 83,3 % der an FIP erkrankten Katzen bei Studieneinschluss FCoV-RNA (382 – 118.052 Kopien/ml) im Vollblut nachgewiesen werden konnte, war überraschend. In bisherigen Studien wurde FCoV im Blut von Katzen mit

bestätigter FIP nicht (zuverlässig) nachgewiesen (PEDERSEN et al., 2015; FELTEN et al., 2017a). Auf der anderen Seite kann FCoV-RNA auch bei gesunden mit FCoV-infizierten Katzen im Blut gefunden werden (MELI et al., 2004; FISH et al., 2018). Aus diesen Gründen wird eine FCoV-RT-PCR aus Blut für die Diagnose von FIP allein nicht empfohlen. Ein positiver FCoV-Nachweis im Blut kann aber zur Unterstützung der Diagnose in Kombination mit FIP-typischen Veränderungen labordiagnostischer Parameter und klinischer Präsentation verwendet werden.

Bei 12/18 (66,7 %) behandelten Katzen in der Studie zu **Publikation 1** war zu Studienbeginn ein Erguss vorhanden und punktierbar. In allen 12 Ergussproben, die an Tag 0 (vor Start der Therapie) entnommen wurden, konnte eine hohe Viruslast (150.139 – 37.295.159 Kopien/ml) nachgewiesen werden (**Publikation 1, Abbildung 5; Publikation 2, Abbildung 4**). Dieses Ergebnis bestätigt den Nutzen des FCoV-RNA-Nachweises im Erguss für die Diagnose von FIP. In früheren Studien wurde FCoV-RNA in 72 – 100 % der Ergussproben von Katzen mit FIP amplifiziert; Ergussproben von Katzen ohne FIP lieferten keine positiven Ergebnisse (DOENGES et al., 2017; FELTEN et al., 2017b; LONGSTAFF et al., 2017; STRANIERI et al., 2018). Der Nachweis von FCoV-RNA in Ergussproben, insbesondere in solchen, die auch zytologisch und biochemisch auf FIP hindeuten, ist somit ein starkes Indiz für FIP. Die Menge an Erguss (thorakal/abdominal) nahm bei allen Katzen während des Studienzeitraumes ab; bereits ab Tag 2 konnte ein signifikanter Unterschied zu Tag 0 in Bezug auf die Ergussmenge gesehen werden. Ab Tag 28 der Therapie hatte keine Katze mehr Erguss. Im Weiteren konnte eine deutliche Abnahme der FCoV-Viruslast im Erguss festgestellt werden. Solange Erguss in punktierbarer Menge vorhanden war, konnte auch (noch) FCoV-RNA nachgewiesen werden. Vergleichsuntersuchungen zur Viruslast und deren Abnahme in Ergussproben gibt es bisher nicht.

Katzen der vorliegenden Studie zeigten FIP-typische labordiagnostische Abweichungen (Erhöhung der Gesamtprotein- (6/18; 33,3 %), Globulin- (13/18; 72,2 %) und Bilirubinkonzentrationen (12/18; 66,7 %); Erniedrigung der Albumin-Konzentration (11/18; 61,1 %), des Albumin-Globulin-Verhältnisses (16/18; 88,9 %), des Hämatokrits (12/18; 66,7 %) und der Lymphozytenzahl (4/18; 22,2 %)) (HARTMANN et al., 2003; RIEMER et al., 2016), die sich unter der Behandlung mit GS-441524 innerhalb kürzester Zeit normalisierten. Je nach Parameter war bereits ab Tag 2 – 28 eine signifikante Verbesserung im Vergleich

zu Tag 0 zu erkennen (**Publikation 1, Abbildung 3 und 4**). Die Körperinnentemperatur war bei einigen Katzen (6/18; 33,3 %) erhöht, sank aber bereits in den ersten Tagen des stationären Aufenthaltes ab. Während der Kontrollbesuche ab Tag 14 (zu diesen Zeitpunkten waren die Katzen bereits wieder zu Hause) war die Körperinnentemperatur wieder etwas höher als in den ersten Tagen des stationären Aufenthaltes; dies könnte durch den Transport-Stress erklärt werden. Serum Amyloid A (SAA), ein wichtiges akute-Phase-Protein bei Katzen, welches bei FIP stark erhöht sein kann und auf eine schwere Entzündungsreaktion hinweist (SASAKI et al., 2003; GIORDANO et al., 2004; TECLES et al., 2015; HAZUCHOVA et al., 2017), war bei 16/18 (88,9 %) Katzen zum Zeitpunkt der Diagnosestellung in der Tat sehr hoch, sank jedoch bei allen Katzen innerhalb kürzester Zeit (bereits ab Tag 2) signifikant ab. Der schnelle Rückgang der SAA-Konzentrationen als Reaktion auf die Behandlung spiegelt die exzellente Wirksamkeit von GS-441524 und Abnahme der Hyperinflammation wider (**Publikation 1, Abbildung 3 und 4**).

In der Studie zu **Publikation 2** wurde die FCoV-RNA-Ausscheidung der behandelten FIP-Katzen (**Publikation 1**) im Kot untersucht. Interessanterweise schieden an Tag 0 nur 6/18 (33,3 %) Katzen FCoV-RNA mit dem Kot aus. Dies deckt sich mit der Studie von Chang und Kollegen. In dieser Studie schieden 6/17 (35,3 %) der Katzen FCoV-RNA mit dem Kot aus, obwohl alle Katzen an FIP erkrankt waren (CHANG et al., 2010). In einer anderen Studie hingegen schieden 64,6 % der Katzen mit FIP FCoV-RNA mit dem Kot aus (BARKER et al., 2017). In der vorliegenden Studie waren 13/18 (72,2 %) Katzen zu unterschiedlichen Studienzeitpunkten positiv in der RT-PCR im Kot. Ab Tag 4 der Behandlung war nur noch eine Katze FCoV-positiv. Dies bestätigt den antiviralen Effekt der Therapie. In einer Studie, die zum Ziel hatte, den Infektionsdruck in Mehrkatzenhaushalten (durch die Eliminierung der fäkalen FCoV-RNA-Ausscheidung) zu senken, konnte die FCoV-RNA-Ausscheidung bei 21/22 (95,5 %) der natürlich FCoV-infizierten gesunden Dauerausscheider aus 5 Haushalten durch die Behandlung mit Xraphconn® (4 mg/kg, q24h, für 7 Tage) gestoppt werden. Allerdings schied eine Katze innerhalb von 9 Tagen erneut FCoV-RNA mit dem Kot aus. Ein großer Nachteil dieser Studie war, dass nicht bei allen 22 Katzen Follow-up-Kotuntersuchungen durchgeführt wurden, sodass weitere Reinfektionen (im späteren Verlauf) möglicherweise unbemerkt blieben (ADDIE

et al., 2020b). Auch bei einer Katze (Katze 4) der Studie zu **Publikation 2** konnte 83 Tage nach Beginn der Behandlung und 80 Tage nach FCoV-RNA-Ausscheidungsstopp eine erneute Ausscheidung festgestellt werden. Eine Katze (Katze 9) schied erstmals nach Ende des stationären Aufenthaltes FCoV-RNA mit dem Kot aus. Katze 4 und Katze 9 lebten mit Partnerkatzen zusammen. Kot der Partnerkatze von Katze 4, der an Tag 135 beprobt wurde, war FCoV-RNA-positiv. Katze 9 hatte 3 Partnerkatzen, von denen jedoch keine Kotproben zur Analyse verfügbar waren. Es ist wahrscheinlich, dass sich beide Katzen (Katze 4 und Katze 9) während des Behandlungszeitraumes bei ihren Partnerkatzen reinfizierten. Eine Sequenzierung des FCoV konnte tatsächlich auch eine Übereinstimmung der S-Gen-Sequenz von Katze 4 und ihrer Partnerkatze beweisen (**Publikation 2, Abbildung 5**). Dies unterstützt die Annahme einer Reinfektion mit FCoV durch die im selben Haushalt lebende Partnerkatze. Eine weitere Katze (Katze 11) schied erstmals an Tag 7 (also noch während des stationären Aufenthaltes) FCoV-RNA mit dem Kot aus. Obwohl Katze 11 in der Klinik von anderen Katzen getrennt gehalten wurde, ist eine Reinfektion (z. B. über Personal) möglich. Bis zu 90 % der Katzen in Mehrkatzenhaushalten sind mit FCoV infiziert und scheiden das Virus über den Kot aus (PEDERSEN, 2009; DRECHSLER et al., 2011; FELTEN et al., 2020; KLEIN-RICHERS et al., 2020). Reinfektionen treten kontinuierlich auf und können in Mehrkatzenhaushalten nicht verhindert werden. Auch wenn ein Mehrkatzenhaushalt vorübergehend FCoV-frei ist, ist es wahrscheinlich, dass FCoV innerhalb kurzer Zeit wieder in den Haushalt eingeschleppt wird (ADDIE et al., 2020b). Intestinale FCoV-Infektionen bei Katzen ohne FIP sollten daher nicht mit GS-441524 oder anderen antiviralen Präparaten behandelt werden. Eine solche Behandlung birgt das Risiko der Entwicklung resistenter Virusstämme. Resistente Virusstämme könnten in der Katze persistieren und (irgendwann) FIP hervorrufen; antivirale Medikamente wären dann nicht mehr wirksam. Zudem könnten resistente FCoV-Stämme ausgeschieden und auf andere Katzen übertragen werden. Die wirksame antivirale Therapie muss unbedingt auf Katzen mit FIP beschränkt werden.

Die meisten Katzen hatten hohe anti-FCoV-Antikörpertiter (14/18; 77,8 %; ≥ 1:1600) (**Publikation 1, Abbildung 5; Publikation 2, Abbildung 1)**. Bei insgesamt 14/18 (77,8 %) Katzen fielen diese ab, bei 5/18 (27,8 %) Katzen bereits 28 Tage nach Beginn der Behandlung. Bei 4/18 (22,2 %) Katzen blieb der anti-

FCoV-Antikörpertiter während des gesamten Beobachtungszeitraumes unverändert. Anti-FCoV-Antikörper sind mitunter für eine vermehrte FCoV-Aufnahme und -Replikation in Makrophagen durch fragment-crystalline-Rezeptoren (Fc-Rezeptoren) verantwortlich; außerdem können sie durch eine Arthus-ähnliche Reaktion zu klinischen Symptomen beitragen (JACOBSE-GEELS et al., 1982; PEDERSEN, 1987; OLSEN et al., 1992). Die Senkung der Viruslast mittels antiviraler Therapie scheint demnach auch die immunologische Reaktion der Katzen zu verringern.

Eine Korrelation zwischen dem anti-FCoV-Antikörpertiter und der fäkalen FCoV-Virusausscheidung wurde in vergangenen Studien beschrieben (HARTMANN, 2005; FELTEN et al., 2020). Diese Korrelation konnte aber in der Studie zu **Publikation 2** nicht nachgewiesen werden. 6/18 (33,3 %) Katzen hatten hohe anti-FCoV-Antikörpertiter und eine hohe FCoV-Viruslast im Kot; 8/18 (44,4 %) Katzen hatten einen hohen anti-FCoV-Antikörpertiter und eine niedrige FCoV-Viruslast oder gar kein FCoV im Kot. Umgekehrt schied 1/18 (5,6 %) Katzen mit relativ niedrigen anti-FCoV-Antikörpertitern eine hohe Menge an FCoV-RNA mit dem Kot aus. 3/18 (16,7 %) Katzen hatten sowohl niedrige anti-FCoV-Antikörpertiter als auch nur geringe Mengen oder keine virale FCoV-RNA im Kot. Die Ergebnisse der vorliegenden Untersuchung sollten genutzt werden, um den Stellenwert der Bestimmung von anti-FCoV-Antikörpern für die Bewertung der FCoV-Ausscheidung im Kot zu überdenken; wenngleich hohe anti-FCoV-Antikörpertiter mit der FCoV-Ausscheidung und der fäkalen Viruslast korrelieren können und chronische Ausscheider oft höhere anti-FCoV-Antikörpertiter haben und mehr Virus ausscheiden als intermittierende Ausscheider (FELTEN et al., 2020), ist dies nicht immer der Fall. Eine anti-FCoV-Antikörpermessung kann die fäkale RT-qPCR (quantitative Reverse-Transkriptase-Polymerase-Kettenreaktion) daher nicht ersetzen. In der aktuellen Studie konnte nur eine schwache Korrelation zwischen viraler RNA-Last und anti-FCoV-Antikörpertitern festgestellt werden; sinkende anti-FCoV-Antikörpertiter könnten ein Zeichen für eine sinkende Viruslast bei den behandelten Katzen sein. Anti-FCoV-Antikörpertiter nehmen langsamer ab als die Viruslast, da die Halbwertszeit von anti-FCoV-Antikörpern mehrere Wochen beträgt, während die Viruslast durch eine wirksame antivirale Therapie direkt abnimmt. Ein weiterer Faktor, der dazu beitragen könnte, dass anti-FCoV-Antikörper langsamer oder gar nicht abfallen, könnte eine kontinuierliche

Antigenstimulation durch Reinfektion über FCoV-ausscheidenden Hauskatzen im selben Haushalt sein. Von den 5 Katzen mit hohen anti-FCoV-Antikörpertitern an Tag 83 hatten 3 Katzen (Katze 2, 5 und 11) mindestens eine Partnerkatze; alle Partnerkatzen waren im Kot (Tag 83 oder Tag 143) FCoV-RNA-positiv.

In der Studie zu **Publikation 2** wurde eine Sanger-Sequenzierung der S-Gen-Region, die für die S-Protein-Region kodiert, durchgeführt. Die *in-vivo*-Mutationstheorie besagt, dass aufgrund von Mutationen in verschiedenen Genen, wie z. B. des S-Gens des FCoV, das hochpathogene FIP-verursachende Virus entsteht (POLAND et al., 1996; VENNEMA et al., 1998). Punktmutationen im S-Gen treten häufig an 2 Nukleotidpositionen (23531 und 23537) auf, die zu einer Aminosäuren-Substitution an Position 1058 (Leucin statt Methionin; M1058L) oder Position 1060 (Alanin statt Serin; S1060A) führen. Diese Mutationen werden häufig bei FIP nachgewiesen (CHANG et al., 2012). In den Kotproben der behandelten Katzen und denen der jeweiligen Partnerkatzen wurde nur der FCoV-Wildtyp mit den Aminosäuren Methionin und Serin gefunden. Im Gegensatz dazu wurde in sequenzierten Erguss- und Blutproben nur die mutierte Form nachgewiesen. Eine vorherige Studie, die dieselben molekularen Methoden anwendete, zeigte, dass bei Katzen ohne FIP (21/87; 24,1 %) zwar FCoV-RNA in verschiedenen Geweben und Körperflüssigkeiten nachweisbar war, jedoch kein mutiertes FCoV (JÄHNE et al., 2022). Diese Ergebnisse sowie die Ergebnisse der vorliegenden Studie (**Publikation 2**) stützen die Hypothese, dass die Mutationen an den Nukleotidpositionen 23531 und 23537 FIP-typisch sind. Eine Sequenzierung des Spike-Gens könnte demnach zur Diagnose FIP beitragen. In der Mehrheit der Proben (Blut und Erguss) wurden die Aminosäuren Leucin und Serin und in den Proben von Katze 12 und 14 die Aminosäuren Methionin und Alanin nachgewiesen. Bei Katze 11 konnte sogar eine noch nicht beschriebene Mutation in der genomischen Sequenz nachgewiesen werden, die zu einer Aminosäuren-Substitution mit Phenylalanin (M1058F) führte. Dies deutet darauf hin, dass die zuvor beschriebenen Mutationen im S-Gen (CHANG et al., 2012) nicht die einzigen Mutationen sind, die an der Entstehung der FIP beteiligt sind. Einige RT-qPCR-positive Proben konnten in der vorliegenden Studie nicht sequenziert werden, vermutlich, weil es bei niedriger FCoV-Viruslast schwierig war, eine PCR-Bande zu erhalten. Ein weiterer Grund, der die Amplifikation der Zielsequenz verhindern könnte, wären Sequenzvariationen, die die Primer-Bindung behindern. In früheren

Studien wurde bereits festgestellt, dass die Sanger-Sequenzierung nur Mutationen in FCoV vom Serotyp I und nicht vom Serotyp II nachweisen kann (BARKER et al., 2017; DECARO et al., 2021). Studien, die die virale Evolution mit einem Next-Generation-Sequencing-Ansatz bewerten, wären wünschenswert.

Die phylogenetische Analyse der Sequenzen des S-Gens in der vorliegenden Studie zeigte eine eindeutige Übereinstimmung zwischen Katzen, die aus demselben Haushalt stammten, unabhängig vom verwendeten Probenmaterial (Blut, Erguss und Kot) und unabhängig vom Pathotyp (niedrig pathogenes FCoV oder FIP-verursachendes FCoV). Obwohl die Interpretation dieser phylogenetischen Analyse durch die kurze Länge der Amplikons (circa 143 bp) beeinträchtigt war, bestätigen diese Ergebnisse die Theorie der internen Mutation. Kernaussage der internen Mutationstheorie ist, dass das FIP-verursachende Virus *de novo* aus Mutationen des harmlosen FCoV in infizierten Katzen entsteht. Dies wurde mittels phylogenetischer Clustering des FIP-verursachenden FCoV und FCoV nach geografischer Verteilung (und nicht nach Pathotyp) belegt (VENNEMA et al., 1998; BROWN et al., 2009; PEDERSEN et al., 2009; LUTZ et al., 2020).

Die genaue Konzentration von GS-441524 in Xraphconn® war bei der in der Studie zu **Publikation 1** verwendeten antiviralen Therapie nicht bekannt. Eine 200 mg Xraphconn®-Tablette sollte laut Herstellerangaben 10 mg der aktiven Substanz enthalten. Eine durchgeführte Analyse (**Publikation 3**) deutet jedoch darauf hin, dass eine Tablette mehr als das Doppelte an GS-441524 enthielt, als vom Hersteller offiziell angegeben. Wie die Analysen zeigten, ist es schwierig, sich auf Herstellerangaben eines nicht zugelassenen hergestellten Medikaments zu verlassen. Bei der Behandlung mit einem illegalen Präparat muss also damit gerechnet werden, dass unterschiedliche Dosen verabreicht werden, und/oder die enthaltenen Wirkstoffkonzentrationen variieren können. Auch trotz höherer Dosierung des antiviralen Medikamentes in der vorliegenden Studie wurde die Behandlung jedoch gut vertragen. Es traten keine schwerwiegenden Nebenwirkungen auf.

In früheren Studien, in denen GS-441524 verabreicht wurde, standen Nebenwirkungen der Therapie hauptsächlich in Zusammenhang mit den subkutanen Injektionen (z. B. Schmerzreaktionen, oberflächliche Hautläsionen, Hautulzerationen). Die häufigste Nebenwirkung, die in der vorliegenden Studie beobachtet wurde, war dagegen eine Lymphozytose, die bei 14/18 (77,8 %) Katzen

auftrat, die meist leicht bis mäßig war. Eine Studie bei Katzen mit experimentell induzierter FIP zeigte ebenfalls einen deutlichen Anstieg der Lymphozyten-Anzahl während der GS-441524-Behandlung (MURPHY et al., 2018). Eine Lymphozytose bei Katzen kann durch Stress oder durch Antigenstimulation verursacht werden. Der Altersmedian der Studienkatzen lag bei 7,7 Monaten, und obwohl jüngere Katzen oftmals eine höhere Lymphozyten-Anzahl (bei 7 Monate alten Katzen: $5{,}3 \pm 1{,}2 \times 10^9$/l) aufweisen (VON DEHN, 2014), war die Lymphozytose der Studienkatzen zu ausgeprägt (Tag 7: $1{,}62 - 14{,}97 \times 10^9$/l, Median $4{,}39 \times 10^9$/l), um nur durch das junge Alter erklärt zu werden. Eine Katze entwickelte an Tag 83 sogar eine massive Lymphozytose ($40{,}7 \times 10^9$/l), ähnlich wie bei einer lymphatischen Leukämie oder einem Lymphom (GEISEN & HARTMANN, 2021). Ein Lymphom wurde aber durch Ausschluss von Malignität und Klonalität mittels PARR- (Polymerase-Kettenreaktion für Antigen-Rezeptor-Gen-Rearrangement) Analyse ausgeschlossen. Die erhöhte Anzahl an Lymphozyten deutete daher auch bei dieser Katze auf eine reaktive Lymphozytose hin. Die Lymphozytose erinnert an ein in der Humanmedizin beschriebenes inflammatorisches Immunrekonstitutionssyndrom bei HIV- (humanes Immundefizienz-Virus) infizierten Patienten als Folge der antiviralen HIV-Therapie (TAPPUNI, 2011). Eine Lymphopenie, ein häufiges Merkmal (PALTRINIERI et al., 2003; RIEMER et al., 2016) und negativer prognostischer Faktor (RIEMER et al., 2016) bei Katzen mit FIP, wird durch eine zunehmende Apoptose von B- und T-Zellen, die durch die Expression von Tumornekrosefaktor-α ausgelöst wird, verursacht (HAAGMANS et al., 1996; KIPAR et al., 2001; DEAN et al., 2003; TAKANO et al., 2007a; TAKANO et al., 2007b). Infolgedessen könnte es nach Abklingen der Zytokin-Wirkung zu einer überschießenden Immunantwort kommen. Darüber hinaus wurde bei FCoV-infizierten Katzen ohne FIP eine Hyperplasie von B- und T-Zellen (KIPAR et al., 1999; KIPAR et al., 2001; KIPAR et al., 2006) und eine erhöhte Anzahl zirkulierender T-Zellen (PALTRINIERI et al., 2003) nachgewiesen. Es wäre daher von besonderem Interesse, diese immunologischen Merkmale und die zelluläre Immunantwort gegen FCoV vor und nach der Behandlung an FIP erkrankter Katzen weiter zu charakterisieren.

Als weitere Nebenwirkung der Xraphconn®-Behandlung wurde in der Studie zu **Publikation 1** eine Eosinophilie (11/18; 61,1 %) festgestellt. Bei 10/11 (90,9 %) Katzen mit Eosinophilie wurden Kotuntersuchungen durchgeführt, um einen

Endoparasitenbefall auszuschließen. Bei 2 dieser Katzen konnte eine Infektion mit *Giardia spp.* nachgewiesen werden; die Infektion wurde (während der antiviralen Therapie) mit Fenbendazol behandelt. Interessanterweise wurde ein Anstieg der Eosinophilen-Anzahl auch in der Humanmedizin bei Patienten mit COVID-19 (coronavirus disease 2019) während der Genesung nachgewiesen. Die Eosinophilie (sogenannte „Morgenröte der Genesung") wird bei Patienten mit COVID-19 als positiver prognostischer Marker gewertet (GLEICH, 2013; KROEGEL & SCHREIBER, 2018; FRAISSÉ et al., 2020; MATEOS GONZÁLEZ et al., 2021). Anzeichen einer (behandlungsbedingten) allergischen Überempfindlichkeitsreaktion, verbunden mit der Eosinophilie, wurden bei Katzen in der vorliegenden Studie nicht beobachtet.

1/18 (5,6 %) Katzen entwickelte am letzten Tag der Therapie eine leichte Heinz-Innenkörperchen-Anämie. Die Bildung von Heinz-Innenkörperchen ist das Ergebnis einer oxidativen Schädigung des Hämoglobins und der Erythrozyten. Die Bildung kann zur Hämolyse führen (REINHART et al., 1986). Bei Katzen wurde eine vermehrte Bildung von Heinz-Innenkörperchen bereits im Zusammenhang mit der Verabreichung bestimmter Arzneimittel und Substanzen, wie Propofol (BAETGE et al., 2020), Acetaminophen (WEBB et al., 2003) oder Propylenglykol (CHRISTOPHER et al., 1990), nachgewiesen; ein Zusammenhang zwischen der wiederholten Verabreichung von GS-441524 und der Bildung von Heinz-Innenkörperchen bei dieser Katze ist ebenfalls denkbar. Weitere Studien sollten die Quantifizierung der Heinz-Körper-Innenkörperchen während der antiviralen Behandlung einschließen, um einen möglichen kausalen Zusammenhang zu klären.

Bei 11/18 (61,1 %) Katzen wurde zwischen Tag 2 und Tag 83 ein Anstieg der Leberenzymaktivitäten festgestellt, der jedoch meist nur leicht bis mäßig ausgeprägt war. Da GS-441524 in der Leber metabolisiert wird, könnte dieser Metabolismus möglicherweise zu einer erhöhten Stoffwechselrate mit erhöhten Leberenzymaktivitäten führen. Erhöhungen der Leberenzymaktivität wurden in einer zuvor unveröffentlichten Studie als unerwünschte Wirkungen bei der Behandlung mit GS-441524 berichtet (ADDIE et al., 2020b). Ein Anstieg der Aktivität der Alanin-Aminotransferase (ALT) wurde bei 7/18 (38,9 %) Katzen beobachtet; davon wiesen 2 Katzen eine hochgradige Erhöhung der ALT (> 350 IU/L) an Tag 56 der Therapie auf, die dann mit Silymarin, einem pflanzlichen Wirkstoff zur Unterstützung der Leberfunktion, behandelt wurde. Am

letzten Tag der Therapie (Tag 83) war bei beiden Katzen die ALT-Konzentration wieder im Referenzbereich. Ein leichter bis mäßiger Anstieg der Aktivität der alkalischen Phosphatase (AP) wurde bei 8/18 (44,4 %) Katzen festgestellt. Am letzten Tag der Therapie (Tag 83) war jedoch bei der Mehrheit der Katzen (16/18; 88,9 %) die AP-Konzentration im Referenzbereich.

In der vorliegenden Studie gab es keine Hinweise für eine Nierentoxizität. Die Konzentrationen des symmetrischen Dimethylarginins (SDMA), ein sehr empfindlicher Parameter für die Nierenfunktion, blieben während der gesamten Behandlung im Referenzbereich. Eine Katze (mit Thorax- und Abdominalerguss) hatte an Tag 0 vor der Behandlung eine SDMA-Konzentration von 18 µmol/l (Referenzbereich 0 – 18 µmol/l), höchstwahrscheinlich infolge einer Beeinträchtigung der glomerulären Filtrationsrate durch die FIP. Nach Beginn der antiviralen Behandlung war die SDMA-Konzentration wieder im Referenzbereich. Eine weitere Katze hatte eine renale Azotämie und erhöhte SDMA-Konzentration, die bereits bei Studienaufnahme vorhanden waren. Daher lag auch bei dieser Katze der Verdacht nahe, dass die Nierenveränderungen durch FIP verursacht worden waren. Im weiteren Verlauf der Studie entwickelte diese Katze eine einseitige Nierenmineralisierung und eine leichte Pyelektasie, die nach Abschluss der Behandlung zu einer Harnleiterobstruktion führte, höchstwahrscheinlich verursacht durch einen Ureterolithen. Die Katze zeigte keine offensichtlichen klinischen Anzeichen einer Nierenerkrankung. Da die Azotämie bereits vor Beginn der Therapie auftrat, ist es unwahrscheinlich, dass die Nierenveränderungen eine Nebenwirkung des Studienmedikamentes (Xraphconn®) waren, sondern vielmehr die Folge einer fortschreitenden Nierenerkrankung, die nicht mit der Behandlung assoziiert war. In einer früheren Studie (PEDERSEN et al., 2019) zeigte eine mit GS-441524 behandelte Katze einen progressiven Anstieg der Harnstoff-Konzentration und einen plötzlichen Anstieg der SDMA-Konzentration während wiederholter Behandlungszyklen, so dass vorsorglich beschlossen wurde, die Behandlung abzubrechen. Allerdings wurde in dieser Studie keine Messung des spezifischen Gewichts des Urins vorgenommen, so dass das Vorliegen einer renalen Azotämie nicht bestätigt werden konnte. Die Harnstoff- und SDMA-Konzentrationen dieser Katze sanken nach Behandlungsende wieder in die Referenzbereiche (PEDERSEN et al., 2019). Auch in einem Fallbericht über eine Katze mit FIP, die ebenfalls mit Xraphconn® behandelt wurde, wurde während der

Behandlung eine erhöhte SDMA-Konzentration festgestellt, die sich nach Absetzen der Behandlung normalisierte (ADDIE et al., 2020a). In der vorliegenden Studie konnten jedoch keine Anzeichen einer Nephrotoxizität durch das Studienmedikament festgestellt werden.

Ein Abfall des Hämatokrits, der Konzentration des Gesamtproteins und des Albumins während der ersten Tage des stationären Aufenthaltes der Katzen der vorliegenden Untersuchungen (**Publikation 1**) sind vermutlich auf die häufigen Blutentnahmen zurückzuführen; ebenso könnte dafür ein Verdünnungseffekt aufgrund der unterstützenden Flüssigkeitstherapie im Rahmen der symptomatischen Therapie ursächlich gewesen sein. Dieser Effekt war nach Ende der Infusionstherapie und bei größerem Zeitabstand zwischen den Blutentnahmen reversibel.

Alle Katzen der Studie zu **Publikation 1** erhielten eine einheitliche Dosis des Studienmedikamentes. Wie bereits genannt, erhielten Katzen ohne neurologische und/oder okuläre Symptome 5 mg/kg KG, Katzen mit neurologischen und/oder okulären Symptomen 10 mg/kg KG. Eine Dosisanpassung des antiviralen Medikamentes auf Grundlage von weiterbestehenden veränderten Laborparametern oder eine standardisierte zusätzliche symptomatische Therapie, wie sie zum Teil in sozialen Medien empfohlen wird, war in der Studie zu **Publikation 1** weder notwendig noch angezeigt.

Die Wirksamkeit von GS-441524 konnte nicht nur durch den Verlauf der klinischen und labordiagnostischen Parameter und die Abnahme der Viruslast im Blut, Erguss und Kot bewiesen werden, sondern auch durch postmortale Gewebeanalysen einer Katze bestätigt werden (**Publikation 3**). Eine der Katzen („Gusti“) aus **Publikation 1** starb 164 Tage nach Ende der (erfolgreichen) antiviralen Behandlung bei einem Autounfall und wurde zur Obduktion freigegeben. Die Katze hatte bei Erstvorstellung eine durch FIP ausgelöste Uveitis gezeigt, die sich in Anisokorie, Tyndall-Phänomen und Optikusneuritis mit Netzhautablösung manifestierte, entsprechend den typischen klinischen Anzeichen, die bei Katzen mit okulärer FIP beobachtet werden (WEGG et al., 2021). FIP ist die häufigste Ursache einer Uveitis bei jungen Katzen. Laut einer Studie der North Carolina Veterinary School hatten 19/120 Katzen (15,8 %) mit Uveitis FIP (JINKS et al., 2016). In einer Studie aus dem Vereinigten Königreich hatten 15/92 Katzen (16,3 %) mit Uveitis FIP (WEGG et al., 2021). Die Diagnosestellung FIP bei Uveitis ist jedoch oftmals

schwierig. Klinische Anzeichen bei diesen Katzen sind oft nicht pathognomonisch, und selbst die Analyse des Kammerwassers, einschließlich Zytologie und Screening auf Infektionskrankheiten, verläuft oft unspezifisch (LAPPIN et al., 1995; LAPPIN, 2000; LINN-PEARL et al., 2015). Der Nachweis von FCoV-Antigenen in Makrophagen durch Immunfärbung von Augengewebe kann die Diagnose bestätigen, wenngleich falsch-positive Ergebnisse bei der Immunzytochemie des Kammerwassers möglich sind (FELTEN et al., 2018). Bei der Katze aus **Publikation 3** wurde das linke betroffene Auge enukleiert, um FIP zu bestätigen. Die Histopathologie des linken Auges zeigte eine ausgeprägte pyogranulomatöse Uveitis, eine pyogranulomatöse Neuritis des Sehnervs und eine Netzhautablösung. Bei der Immunhistochemie (IHC) waren mehrere Makrophagen positiv für FCoV-Antigen. Im Gegensatz dazu war die ophthalmologische Untersuchung des rechten Auges vor Therapie unauffällig. In der späteren Sektion konnte eine fibrotische Narbe im Bereich des Ziliarkörpers des rechten Auges nachgewiesen werden. Daher ist denkbar, dass das Virus nicht nur das linke Auge, sondern auch das rechte Auge infiltriert hatte, vorhandene "Mikroläsionen" jedoch zu klein waren, um in der ophthalmologischen Untersuchung aufzufallen. Mittels postmortaler Analysen (IHC, gewebebasierter FCoV-Nachweis mittels RT-qPCR) konnte FIP im rechten Auge nicht mehr nachgewiesen werden; die Narbenbildung könnte aber ein Hinweis auf frühere, durch FIP verursachte Läsionen sein, was auf eine Heilung durch die Behandlung mit Xraphconn® schließen lässt. In einer Fallstudie aus den USA wurden 4 Katzen mit FIP und neurologischen und/oder okulären Anzeichen beschrieben, die mit GS-441524 (5 – 10 mg/kg, SC, q24h) über mindestens 84 Tage behandelt wurden. Alle 4 Katzen sprachen zunächst auf die Behandlung an. Serielle ophthalmologische Untersuchungen zeigten eine Abheilung der okulären Veränderungen, die sich als chorioretinale Narben darstellten, ähnlich zu den Veränderungen bei der in **Publikation 3** beschriebenen Katze (DICKINSON et al., 2020). Eine Katze aus der amerikanischen Fallserie erlitt jedoch Rückfälle und wurde schließlich (nach 2 Behandlungszyklen) euthanasiert. Die postmortale Untersuchung ergab eine beidseitige lymphozytäre und histiozytäre Uveitis sowie eine Chorioiditis; mittels IHC konnten in den Augen und in weiteren Geweben virale Antigene nachgewiesen werden. Diese Veränderungen könnten entweder auf eine virale Persistenz oder eine wiederkehrende FCoV-Infektion und erneute Mutation zurückzuführen sein (DICKINSON et al., 2020). Die Katze erhielt GS-441524 in einer niedrigeren Dosis

(5 mg/kg) (DICKINSON et al., 2020) als die in **Publikation 3** beschriebene Katze. Die Wirkstoffkonzentration von GS-441524 ist in Kammerwasser niedriger als in Serum (MURPHY et al., 2018); es ist daher wahrscheinlich, dass die in der vorliegenden Studie verwendete höhere Dosis wirksamer war. So waren die IHC- als auch die RT-qPCR-Untersuchungen aus allen Geweben bei der Katze aus **Publikation 3** negativ für FCoV-Antigen und -RNA. Es war also nirgendwo in der Katze mehr Virus nachweisbar. Weitere Studien sind nötig, um zu untersuchen, welche Dosis von GS-441524 ausreichen könnte, um die Virusreplikation bei Katzen mit okulärer FIP effektiv zu stoppen.

Die bei der Katze von **Publikation 3** zu Therapiebeginn aufgetretenen „medullary rim signs“ beider Nieren sowie Aszites im Bereich des Retroperitonealraumes im Bereich der Nieren verschwanden ebenfalls unter Therapie. „Medullary rim signs“ können verschiedene Ursachen haben und wurden auch in Verbindung mit FIP beschrieben. In einer retrospektiven Studie mit 243 Katzen, die „medullary rim signs“ aufwiesen, wurden bei 15/243 (6,2 %) Katzen letztendlich FIP diagnostiziert (FERREIRA et al., 2020). Es ist daher denkbar, dass die Behandlung mit Xraphconn® auch die FIP-assoziierten Veränderungen in den Nieren der Katze verschwinden ließ. 84 Tage nach Beendigung der Behandlung mit Xraphconn® konnten bei der Katze in der klinischen Untersuchung, Bildgebung und Labor keine FIP-typischen Läsionen mehr nachgewiesen werden, abgesehen von einer generalisierten Lymphadenopathie aufgrund einer massiven lymphatischen Hyperplasie. Die vergrößerten mesenterialen Lymphknoten könnten eine Folge einer gastrointestinalen Infektion (postmortale histologische Untersuchung des Darmes: Lymphozyten und Plasmazellen in der Propria, begleitet von degenerierten polymorphkernigen Neutrophilen) sein, die jedoch die Lymphknotenhyperplasie in den Tonsillen sowie den mandibulären und superfizialen zervikalen Lymphknoten nicht erklären kann. Das Vorhandensein der Lymphadenopathie könnte auch ein Zufallsbefund sein, der möglicherweise durch eine kürzlich erfolgte Reinfektion mit FCoV verursacht wurde. Es ist möglich, dass einige Katzen eine genetische Veranlagung für die Entwicklung von FIP haben. Nach der derzeitigen Theorie zur Pathogenese tritt FIP bei Katzen auf, die genetisch veranlagt sind, die Virusreplikation nicht wirksam kontrollieren zu können. Dies führt zu einer vermehrten und unkontrollierten Virusreplikation, verbunden mit einer erhöhten Wahrscheinlichkeit auftretender Mutationen. Mutierte Viren können sich effizient

in Makrophagen replizieren. Die Fähigkeit, sich in Makrophagen unkontrolliert zu vermehren, unterscheidet die FIP-verursachenden Varianten von wenig pathogenen FCoV-Isolaten (PEDERSEN et al., 2009; SHIRATO et al., 2018). Zur weiteren Charakterisierung der Prädisposition, um die Anfälligkeit von Katzen für das Entstehen von FIP zu untersuchen, wurden Einzelnukleotid-Polymorphismen im felinen Interferon (IFN)-γ-Gen untersucht, und bestimmte Genotypen letztendlich als FIP-anfällige Faktoren beschrieben (HSIEH & CHUEH, 2014; BARKER et al., 2020). Es wurde nachgewiesen, dass Katzen mit FIP eine geringere IFN-γ-Produktion aufweisen als Katzen, die mit FCoV infiziert, aber nicht an FIP erkrankt sind (GUNN-MOORE et al., 1998; KISS et al., 2004; GELAIN et al., 2006; GIORDANO & PALTRINIERI, 2009). Bei der Katze der **Publikation 3** gab es in der Familienanamnese weitere bekannte Fälle von FIP. Dies lässt möglicherweise auf eine genetische Prädisposition schließen. Es wurde jedoch weder eine FCoV-Infektion als Rezidiv noch als Reinfektion mittels IHC oder RT-PCR in den postmortalen Analysen nachgewiesen. Die Lymphadenopathie könnte letztendlich auch als Zeichen einer überschießenden, genetisch bedingten Reaktion des Immunsystems gewertet werden oder ein "long-FIP-Syndrom" darstellen. In der Humanmedizin wurden Fälle beschrieben, in denen virale RNA nach Abklingen klinischer Symptome persistierte, wie z. B. nach SARS-CoV-2-Infektionen beim sogenannten “long-COVID-Syndrom“ (GRIFFIN, 2022); allerdings handelt es sich bei den mittels RT-PCR nachgewiesenen Viren meist um fragmentierte RNA-Stämme (RAMAKRISHNAN et al., 2021). Im vorliegenden Fallbericht der **Publikation 3** fiel die RT-PCR in keinem Gewebe der Katze positiv aus, so dass keine persistierende RNA gefunden wurde. Nichtsdestotrotz könnte die Lymphadenopathie eine Folgeerscheinung der FIP sein, ohne dass noch Virus vorhanden ist. Weitere Studien wären wichtig, um nachzuweisen, ob die Lymphadenopathie auf eine langfristige negative Auswirkung der Behandlung zurückzuführen ist oder möglicherweise mit einem "long-FIP-Syndrom" zusammenhängt.

Aszites war bei der postmortalen Untersuchung nicht mehr vorhanden. Bei FIP werden Gefäßläsionen durch Immunkomplexe hervorgerufen, die sich an den Wänden der Blutgefäße anheften, die Komplementkaskade aktivieren und das Gefäßgewebe schädigen (KIPAR et al., 1998). Gewebe mit hoher FCoV-Viruslast sind neben der Milz, das Bauchnetz und die mesenterialen Lymphknoten

(PEDERSEN et al., 2015). FCoV wurde jedoch in keinem Gewebe der Katze mehr nachgewiesen. Die FCoV-Viruslast im Blut sank innerhalb kurzer Zeit nach Beginn der Behandlung ab; auch die FCoV-RNA-Ausscheidung im Kot stoppte bereits am 2. Tag nach Therapiestart. Diese Ergebnisse zeigen, dass die Katze zu 100 % von der FIP geheilt war. Der anti-FCoV-Antikörpertiter nahm während der Behandlung ab, wurde jedoch bis Tag 168 nicht negativ. Möglicherweise wäre der anti-FCoV-Antikörpertiter nach Abklingen der Infektion weiter gesunken. Anti-FCoV-Antikörper können nach dem Abklingen einer (harmlosen) FCoV-Infektion noch mehrere Monate lang persistieren; dies ist kein Zeichen für eine Viruspersistenz (ADDIE & JARRETT, 2001). FIP-Rezidive nach Behandlung mit GS-441524 wurden in einigen wenigen Fällen beschrieben (MURPHY et al., 2018). In diesen Fällen stellt sich die Frage, ob eine Reinfektion mit FCoV und eine neue Mutation stattgefunden hat oder ob das Virus bei diesen Katzen nicht vollständig eliminiert werden konnte und in einzelnen Geweben noch vorhanden war. Die hier beschriebene Katze „Gusti“ war zu 100 % FCoV-frei, so dass ein Rückfall nach Therapie sehr unwahrscheinlich ist.

Die Katzen der **Publikation 1** wurden zur Langzeitbeobachtung im Rahmen von regelmäßigen Kontrolluntersuchungen nach Ende der Therapie in 12-wöchigen Abständen (1. Kontrolluntersuchung: Woche 24, 2. Kontrolluntersuchung: Woche 36, 3. Kontrolluntersuchung: Woche 48) begleitet (**Publikation 4**). Die Katzen zeigten im weiteren Verlauf keine Auffälligkeiten in den labordiagnostischen und klinischen Parametern. Während des Beobachtungszeitraumes von einem Jahr trat bei keiner der Katzen ein FIP-Rückfall auf. Rückfälle wurden in früheren Studien beschrieben. In einer Studie von Murphy et al. bei 10 experimentell infizierten Katzen, die 2 Wochen lang mit GS-441524 (2 – 5 mg/kg, q24h, SC) behandelt wurden, zeigten 2/10 (20 %) der Katzen 4 – 6 Wochen nach Behandlungsende einen Rückfall und mussten in einem 2. Therapiezyklus erneut therapiert werden (MURPHY et al., 2018). Gründe für Rückfälle in der Studie von Murphy et al. könnten eine zu niedrig gewählte Dosis des Medikamentes gepaart mit einer zu kurzen Behandlungsdauer sein.

Am Ende der Therapie waren alle Katzen im Blut FCoV-RNA-negativ und blieben es über den gesamten Beobachtungszeitraum. Nur eine Katze (Katze 8) wurde in Woche 24 vorübergehend FCoV-RNA-positiv im Blut mit einer niedrigen Viruslast von 200 Kopien/ml (cycle-threshold (CT)-Wert: 38,0). Bei den weiteren

Kontrolluntersuchungen war die Katze wieder FCoV-RNA-negativ im Blut. Der anti-FCoV-Antikörpertiter stieg bei dieser Katze (Katze 8) von 1:100 in Woche 24 auf 1:400 in Woche 36 an, obwohl zu keinem Zeitpunkt FCoV-RNA im Kot nachgewiesen werden konnte. Eine intestinale Reinfektion mit FCoV ist daher unwahrscheinlich. Eher ist eine systemische Translokation von FCoV anzunehmen; möglicherweise war das Virus während des gesamten Behandlungszeitraumes noch in einem anderen Kompartiment (z. B. Liquor) vorhanden und ging dann ins Blut über.

5 Katzen (5/17; 29,41 %; Katze 2, 6, 7, 11, 16) reinfizierten sich während der Langzeitbeobachtung erneut mit FCoV; dies zeigte sich mit einer erneuten FCoV-RNA-Ausscheidung über den Kot. Jede der 5 Katzen hatte Partnerkatzen oder Freigang. Nur bei 1/5 (20 %) der wieder ausscheidenden Katzen (Katze 16) stieg der anti-FCoV-Antikörpertiter während der Langzeitbeobachtung an. Partnerkatzen sind also die wahrscheinlichste Quelle der Reinfektion. Antivirale Medikamente sollten also nicht eingesetzt werden, um die fäkale FCoV-RNA-Ausscheidung zu stoppen, da Reinfektionen kontinuierlich auftreten.

12 Katzen (12/17; 70,59 %) zeigten während der Langzeitbeobachtung eine abdominale Lymphadenopathie. Die Lymphadenopathie könnte als Folge einer möglichen kürzlich erfolgten Reinfektion mit FCoV auftreten. Dies ist eher unwahrscheinlich, da die Katzen keine weiteren Anzeichen einer Reinfektion aufwiesen. Auch eine überschießende genetische bedingte Reaktion des Immunsystems, ausgelöst durch einen persistierenden Stimulus oder eine noch andauernde Heilung wären als Erklärung für die persistierende Lymphadenopathie vorstellbar. Zudem kann die persistierende Lymphadenopathie eine Langzeitfolge der FIP selbst oder eine Langzeit-Nebenwirkung der antiviralen Behandlung sein. Weitere klinische Studien über einen längeren Zeitraum sind erforderlich, um die Ursachen weiter zu erforschen.

2 Katzen (Katzen 8, 10) entwickelten nach erfolgreicher Behandlung mit GS-441524 milde neurologische Symptome, die einem felinen Hyperästhesiesyndrom (FHS) ähnelten. Katze 10 entwickelte in Woche 35 mehrmals tägliche Episoden von Zuckungen der Haut im Rückenbereich und unruhigem Verhalten. Katze 8 entwickelte in Woche 47, also kurz vor der Kontrolluntersuchung in Woche 48, mehrmals tägliche Episoden von extremen Ohrenspiel, Schwanzjagen, Zuckungen der Haut im Rückenbereich. FHS ist ein Syndrom, das erstmals 1980 (TUTTLE,

1980) beschrieben wurde und Episoden von Zuckungen der Haut sowie Anzeichen von Unbehagen umfasst, die meist spontan auftreten oder durch Berührung ausgelöst werden. Ursächlich hierfür könnten neben dermatologischen (hypersensitive Dermatitis), orthopädischen (Trauma), neurologischen (Erkrankungen des Gehirnes oder Rückenmarkes, z. B. Erkrankungen der Bandscheiben, Neoplasien oder Meningomyelitis) auch verhaltensbedingte (zwanghaftes Verhalten) Erkrankungen sein (TUTTLE, 1980; CIRIBASSI, 2009; MARIONI-HENRY, 2010; PAKOZDY et al., 2014; AMENGUAL BATLE et al., 2019). Des Weiteren werden schmerzhafte Neuropathien oder fokale epileptische Anfälle diskutiert (GÓMEZ ÁLVAREZ & SOLER ARIAS, 2021). In vielen Fällen kann die zugrundeliegende Ursache nicht festgestellt werden. Die Ultraschalluntersuchung bei Katze 10 ergab einen vergrößerten abdominalen Lymphknoten und eine sehr geringe Menge freier Flüssigkeit (RT-qPCR-negativ für FCoV-RNA). Im Blut und Kot wurde keine FCoV-RNA nachgewiesen; auch der anti-FCoV-Antikörpertiter war unverändert (1:25, seit Woche 4 nach Beginn der Behandlung). Die Besitzer befürchteten einen Rückfall und die Katze wurde eigenständig von den Besitzern erneut 84 Tage mit GS-441524 therapiert. Die neurologischen Symptome hielten während des 2. Therapiezyklus an und klangen erst später spontan ab. Bei Katze 8 stieg vor Entwicklung der neurologischen Symptome der anti-FCoV-Antikörpertiter von 1:100 (Woche 24) auf 1:400 (Woche 36) an und sank daraufhin wieder auf 1:100 (Woche 48) ab. 2 Wochen später (Woche 50) und einige Tage, nachdem die Katze kastriert wurde, zeigte die Katze milden Durchfall. Zu diesem Zeitpunkt wurde der Kot positiv auf FCoV-RNA (CT-Wert: 32,6) getestet und die Ultraschalluntersuchung zeigte ebenfalls einen vergrößerten abdominalen Lymphknoten, jedoch keinen Hinweis auf freie abdominale Flüssigkeit. Der Lymphknoten wurde punktiert und die Lymphknotenzytologie (reaktive Lymphozyten mit vereinzelten Neutrophilen und Plasmazellen) sprach für eine reaktive Hyperplasie. In dem Lymphknoten konnte FCoV-RNA in sehr geringer Menge nachgewiesen werden (CT-Wert: 40,0). Im Blut wurde keine FCoV-RNA nachgewiesen. Zudem wurde die Katze negativ auf *Toxoplasma-gondii*-Antikörper (Immunglobuline (Ig) G und IgM) mittels Immunfloureszenz-Testes (IFT) getestet. Um einen Ektoparasitenbefall als mögliche Ursache für die Anzeichen von FHS auszuschließen, wurde Katze 8 einmalig mit einem Spot-on (Esafoxolaner, Eprinomectin, Praziquantel) behandelt; dies führte jedoch nicht zu einer Besserung und die Symptome, die dem FHS

ähnelten, traten weiterhin auf. Die Häufigkeit und der Schweregrad der Episoden verbesserten sich nach 6 Monaten deutlich ohne weitere Medikamente. Bis zum Tag der Einreichung der **Publikation 4** traten die Episoden bei beiden Katzen nur noch sehr selten auf. Bei beiden Katzen der vorliegenden Studie war die klinische, dermatologische und neurologische Untersuchung unauffällig. Auch zeigten die Katzen keine Schmerzhaftigkeit entlang der Wirbelsäule. Aufgrund der unauffälligen Laborparameter beider Katzen sowie der virologischen Untersuchungen wurde ein Rückfall als unwahrscheinlich angesehen.

Im Zusammenhang mit der COVID-19-Pandemie wurden Kinderärzte weltweit mit einer neuen Krankheit bei Kindern konfrontiert, einem Syndrom, das mit einer SARS-CoV-2-Infektion assoziiert ist und als multisystemisches Entzündungssyndrom bei Kindern (MIS-C) bezeichnet wird. Vergleicht man die klinischen Merkmale von FIP und MIS-C, so lassen sich bemerkenswerte Parallelen feststellen (RIPHAGEN et al., 2020; ALBERER & VON BOTH, 2021). In einer Studie bei Kindern mit MIS-C wurden ebenfalls neu auftretende neurologische Symptome beschrieben. 4/27 (14,81 %) der Kinder, die zuvor gesund waren und keine anderen Komorbiditäten aufwiesen, entwickelten neurologische Symptome (einschließlich Kopfschmerzen, Symptome der Lokalisation im Hirnstamm und Kleinhirn, Muskelschwäche und verminderte Reflexe). Die bei 2 Kindern durchgeführte Liquor-Untersuchung zeigte keine Anzeichen einer Infektion. Die neurologischen Symptome verbesserten sich bei allen Kindern, 2/4 (50 %) der Kinder zeigten eine vollständige Genesung. Ähnlich wie in der vorliegenden Studie der **Publikation 4** konnte bei den Kindern auch kein Virus nachgewiesen werden (ABDEL-MANNAN et al., 2020). Möglicherweise könnte es sich also auch bei den leichten neurologischen Symptomen der Katzen um eine Art "long-FIP-Syndrom" handeln, ausgelöst durch immunologische Langzeiteffekte des FCoV. Da die neurologischen Symptome erst einige Wochen bis Monate nach der Behandlung auftraten, wäre auch eine späte unerwünschte Nebenwirkung (z. B. Neuropathie) der GS-441524-Behandlung möglich. Weitere Studien mit kontrolliert produzierten Medikamenten sind daher erforderlich.

Es stellt sich die Frage, ob Remdesivir, dass für die Behandlung von Menschen mit schwerer COVID-19 zugelassen ist, für die Behandlung von FIP bei Katzen nützlich sein könnte. Remdesivir wurde von Tierärzten in Australien zur Behandlung von Katzen mit FIP eingesetzt, obwohl noch keine veröffentlichten

Studien vorliegen. Remdesivir ist ein Prodrug von GS-441524 und wird mit begrenztem Erfolg bei Patienten mit akuter COVID-19-Erkrankung eingesetzt (AGOSTINI et al., 2018; YAN & MULLER, 2020). Remdesivir muss als Injektion verabreicht werden, da es keine akzeptable orale Bioverfügbarkeit aufweist. Im Gegensatz dazu erwies sich die orale Aufnahme von GS-441524 bei Mäusen als wirksam gegen COVID-19 (AGOSTINI et al., 2018; XIE & WANG, 2021). Ein weiterer Vorteil von GS-441524 gegenüber Remdesivir scheint die geringere Lebertoxizität zu sein, die eine Dosiseskalation ermöglicht, um eine wirksame Behandlung von systemischen Coronavirus-Infektionen zu gewährleisten (YAN et al., 2021); GS-441524 wird in Leber- und Lungengewebe direkt in das aktive Nukleosid-Triphosphat umgewandelt. Die Ergebnisse der vorliegenden Studien könnten daher auch für die Humanmedizin wichtig sein.

Eine Limitation der vorliegenden Therapiestudie (**Publikation 1**) bestand darin, dass keine unbehandelte Kontrollgruppe in die Untersuchung einbezogen wurde. Es ist jedoch aus ethischen Gründen nicht vertretbar, Katzen mit FIP unbehandelt zu lassen, da diese ohne antivirale Behandlung innerhalb weniger Tage sterben (RITZ et al., 2007; FISCHER et al., 2011). Eine weitere Limitation ist, dass die potenzielle Wirksamkeit der zusätzlichen pflanzlichen Komponenten in den Xraphconn® Tabletten (außer GS-441524) nicht bekannt ist. So ist denkbar, dass neben GS-441524 noch weitere Inhaltsstoffe für den Therapieerfolg verantwortlich sind. Dies wäre ein interessanter zusätzlicher Ansatz für Untersuchungen in der Zukunft. Aufgrund der oralen Verabreichung der Behandlung konnten Katzen in einem moribunden Zustand nicht in die Studie aufgenommen werden. Ein oral verabreichtes Medikament hätte in einem solchen Zustand aufgrund der niedrigen Stoffwechselrate und der Tatsache, dass orale Medikamente in einem inaktiven Verdauungstrakt kaum absorbiert werden, keine Wirkung gehabt. 2 Katzen im moribunden Zustand konnten nicht in die Studie aufgenommen werden und mussten aus ethischen Gründen in Agonie euthanasiert werden. Eine weitere Limitation ist die geringe Patientenzahl der aktuellen Studie. Mit einer höheren Anzahl an Studienteilnehmern wäre vielleicht auch eine Erhebung von aussagekräftigen prognostischen Markern möglich.

Eine Limitation der **Publikation 2** ist, dass während des stationären Aufenthaltes Kotproben zur Analyse zur Verfügung standen, wohingegen bei den

Kontrolluntersuchungen in einigen Fällen lediglich Kottupfer untersucht werden konnten, da einige Besitzer mit Mehrkatzenhaushalten Kotproben nicht eindeutig der behandelten Katze zuordnen konnten. Dabei fiel auf, dass die virale FCoV-RNA-Last in Kottupfern signifikant niedriger war als in den gesammelten, abgesetzten Kotproben. Eine geringere Menge Probenmaterial (an Kottupfern) könnte auch dazu beigetragen haben, dass keine Korrelation zwischen der FCoV-Viruslast im Kot und den anti-FCoV-Antikörpertitern bestand. Allerdings wurde kein direkter Vergleich der FCoV-RNA-Last in Kotproben *versus* Kottupfern von ein- und derselben Katze vorgenommen.

Hauptlimitation der **Publikation 4** war, dass nicht alle therapierten Katzen zu den Kontrolluntersuchungen aufgrund von mangelnder Besitzer-Compliance vorgestellt werden konnten. Zudem verstarb eine der 18 Katzen 164 Tage nach Ende der Therapie (**Publikation 3**). Auch die Ablehnung der Besitzer von weiteren diagnostischen Maßnahmen zur Abklärung des FHS, wie Magnetresonanztomographie (MRT), Liquor-Untersuchung und Elektroenzephalographie (EEG), stellen eine Limitation der **Publikation 4** dar.

Zusammenfassend lässt sich sagen, dass die vorliegenden Ergebnisse (**Publikation 1, 2, 3 und 4**) eindeutig zeigen konnten, dass durch die Verabreichung von GS-441524 bei Katzen mit FIP eine Remission ohne gravierende Nebenwirkungen erreicht werden kann. Auch in der Langzeitbeobachtung blieben die Katzen in vollständiger Remission (für insgesamt ein Jahr nach Behandlungsbeginn) ohne Rückfall. Somit war die Behandlung mit GS-441524 sowohl kurz- als auch langfristig wirksam gegen FIP. Leider ist das Medikament derzeit in vielen Ländern nicht legal für den tierärztlichen Gebrauch erhältlich. Dies stellt Besitzer und Tierärzte vor ein ethisches Dilemma. Besitzer sind gezwungen, ihre Katzen auf der Grundlage der Meinung nicht-tierärztlicher Laien und Gruppen in sozialen Medien selbst zu diagnostizieren und zu behandeln. Die illegal angebotenen Medikamente werden in nicht kontrollierten Produktionen hergestellt, und es besteht keinerlei Garantie für die Zusammensetzung (Reinheit und Konzentration) des Wirkstoffes.

Es ist daher dringend erforderlich, dass die zuständigen Behörden und die Industrie auf eine rasche Zulassung des Medikamentes hinarbeiten, damit es von Tierärzten legal verwendet werden kann, um Katzen, die an FIP leiden, unter tierärztlicher Aufsicht zu behandeln. So wäre auch die notwendige intensivmedizinische Betreuung in den ersten Tagen der Behandlung gesichert. Die regelmäßigen

Tierarztbesuche sind wichtig für die Kontrolle des Therapieerfolges, zur Detektion potentieller Nebenwirkungen und um eine unterstützende symptomatische Behandlung an die Probleme und Bedürfnisse der einzelnen Katzen anzupassen. Die Behandlung von Katzen mit FIP sollte daher nicht allein den Besitzern überlassen werden. GS-441524 ist derzeit die einzige nachgewiesene wirksame Behandlung von FIP und sollte sobald wie möglich für die Tiermedizin zugelassen werden.

VIII. ZUSAMMENFASSUNG

Die feline infektiöse Peritonitis (FIP), verursacht durch das feline Coronavirus (FCoV), ist eine häufige Erkrankung bei Katzen, die unbehandelt tödlich verläuft. Ziel der **Publikation 1** dieser Doktorarbeit war es, die Wirksamkeit und Toxizität des oralen antiviralen Medikamentes Xraphconn® (Mutian, China) *in vitro* und *in vivo* bei Katzen mit FIP in Bezug auf die Überlebensrate, die Entwicklung klinischer und labordiagnostischer Parameter, den Verlauf der Viruslasten, der anti-FCoV-Antikörper sowie der Nebenwirkungen zu untersuchen. Mittels Massenspektrometrie und Kernspinresonanzspektroskopie wurde die antiviral wirksame Substanz des Studienmedikamentes GS-441524 identifiziert. Xraphconn® zeigte in der Zellkultur eine exzellente antivirale Aktivität. Durch die Therapie mit dem oral verfügbaren GS-441524 befanden sich 18 Katzen zum Zeitpunkt der **Publikation 1** in anhaltender Remission. Die hohe Wirksamkeit des Medikamentes konnte durch die schnelle Verbesserung der klinischen und labordiagnostischen Parameter und anhand der Abnahme der Viruslasten im Blut, Erguss und Kot bewiesen werden. Die klinischen und labordiagnostischen Parameter verbesserten sich rapide (je nach Parameter signifikanter Unterschied zu Tag 0 ab Tag 2 – 28). Es traten keine schwerwiegenden Nebenwirkungen auf.

Publikation 2 untersuchte neben dem Verlauf der Viruslasten im Blut, Erguss und Kot und des FCoV-Antikörpertiters auch das Vorhandensein von Spike-Gen-Mutationen. Zusätzlich wurden die Partnerkatzen der behandelten Katzen auf Vorhandensein von FCoV-RNA-Ausscheidung mit dem Kot sowie von Spike-Gen-Mutationen und anti-FCoV-Antikörpertiter untersucht. Die orale Behandlung mit GS-441524 führte bei Katzen mit FIP zu einer signifikanten Abnahme der viralen RNA-Last im Blut, im Erguss und im Kot. Dennoch kann es zu einer erneuten FCoV-Ausscheidung kommen, wenn Katzen durch ihre Partnerkatzen erneut mit FCoV in Berührung kommen. Spezifische Spike-Gen-Mutationen, die mit der systemischen FCoV-Ausbreitung in Zusammenhang stehen, wurden im Blut und Erguss der behandelten FIP-Katzen gefunden, jedoch nicht im Kot der behandelten Katzen oder der Partnerkatzen. Eine neue Mutation des FCoV konnte identifiziert werden, die zu einer noch nicht beschriebenen Aminosäure-Substitution führte. Dies deutet darauf hin, dass weitere Mutationen an der Entwicklung von FIP beteiligt sein könnten.

Auch die postmortalen Befunde einer genesenen Katze (**Publikation 3**), die zu 100 % geheilt und virusfrei war, unterstreichen die hervorragende Wirkung des Wirkstoffes. Die verwendeten Tabletten enthielten jedoch eine höhere Konzentration von GS-441524 als vom Hersteller offiziell angegeben (**Publikation 3**). Die durchgeführten Analysen zeigten also, dass es schwierig ist, sich auf die Herstellerangaben eines nicht zugelassenen Medikaments zu verlassen. Bei der Behandlung mit einem illegalen Präparat muss also damit gerechnet werden, dass unterschiedlich hohe Dosen verabreicht werden und die enthaltenen Wirkstoffkonzentrationen variieren können.

Die Katzen der **Publikation 1** wurden über einen Zeitraum von einem Jahr zur Langzeitbeobachtung begleitet, um eventuelle Rückfälle nach erfolgreicher Therapie oder Nebenwirkungen frühzeitig erkennen zu können (**Publikation 4**). Eine Katze starb bei einem Verkehrsunfall 164 Tage nach Therapieende (**Publikation 3**). Die übrigen 17 Katzen zeigten keine Auffälligkeiten in den labordiagnostischen und klinischen Parameter. Bei 17/18 Katzen konnte kein FCoV im Blut nachgewiesen werden; nur eine Katze war mit einer sehr niedrigen Viruslast zu einem Studienzeitpunkt RT-qPCR-positiv. 5 Katzen zeigten eine erneute fäkale FCoV-Ausscheidung. 2 Katzen entwickelten sehr milde neurologische Symptome (vereinbar mit Symptomen eines felinen Hyperästhesie-Syndrom). Diese beiden Katzen waren im Blut und Kot zu jeder Zeit FCoV-negativ. Auch konnte kein anti-FCoV-Antikörperanstieg gesehen werden. 12 Katzen wiesen während der Langzeitbeobachtungen eine abdominale Lymphadenopathie auf.

Leider ist das Medikament GS-441524 derzeit in vielen Ländern nicht legal für den tierärztlichen Gebrauch erhältlich. Dies stellt Besitzer und Tierärzte vor ein ethisches Dilemma. Die Besitzer sind gezwungen, ihre Katzen auf der Grundlage von Meinungen nicht-tierärztlicher Laien und Gruppen in sozialen Medien selbst zu diagnostizieren und zu behandeln. Die illegal angebotenen Medikamente werden in nicht kontrollierten Produktionen hergestellt und es besteht keinerlei Garantie für die Zusammensetzung (Reinheit und Konzentration) des Wirkstoffes.

GS-441524 stellt eine hervorragende Option für die orale Behandlung von FIP dar. Es sollte auch als potenziell wirksame Behandlungsoption für andere schwere Coronavirus-assoziierte Krankheiten bei verschiedenen Tierarten erforscht werden.

IX. SUMMARY

Feline infectious peritonitis (FIP) caused by feline coronavirus (FCoV), is a common disease in cats and fatal if untreated. The aim of **publication 1** of this doctoral thesis was to investigate the efficacy and toxicity of the oral antiviral drug Xraphconn® (Mutian, China) *in vitro* and *in vivo* in cats with FIP concerning survival rate, development of clinical and laboratory diagnostic parameters, progression of viral loads, anti-FCoV antibodies, and side effects. Mass spectrometry and nuclear magnetic resonance were used to identify the active antiviral substance of the study drug, which was identified as GS-441524. Xraphconn® showed excellent antiviral activity in cell culture. As a result of treatment with orally available GS-441524, 18 cats were in sustained remission at the time of **publication 1**. The strong efficacy of the drug was proven by the rapid improvement of clinical and laboratory parameters and by the decrease in viral loads in blood, effusion and feces. The clinical and laboratory parameters improved rapidly (significant difference to day 0 from day 2 to 28, depending on the parameter). No serious adverse events occurred.

Publication 2 investigated the course of viral loads in blood, effusion, feces and anti-FCoV antibody titers as well as the presence of spike gene mutations. In addition, the companion cats of the treated cats were examined for the presence of FCoV RNA excretion with feces, spike gene mutations and anti-FCoV antibody titers. Oral treatment with GS-441524 effectively reduced viral RNA loads in blood, effusion and feces in cats with FIP. However, reinfection occurred if cats are re-exposed to FCoV through their companion cats. Specific spike gene mutations associated with systemic FCoV spread were detected in blood and effusion of treated cats, but not in feces of treated or companion cats. A novel mutation of FCoV was identified that resulted in an amino acid substitution not yet described. This suggests that other mutations than those already described can be involved in the development of FIP.

The postmortem findings of a recovered cat (**publication 3**), which was 100% cured and virus-free, also underlies the excellent efficacy of the compound. The tablets used contained a higher concentration of GS-441524 than officially stated by the manufacturer (**publication 3**). The analyses showed that it is difficult to rely on the

manufacturer's claims of an unapproved manufactured drug. Thus, when treating with an illegal preparation, different doses will be administered, and the drug concentrations contained will vary.

The cats of **publication 1** were followed for a period of one year as a long-term observation in order to detect any relapses after successful treatment or side effects at an early stage (**publication 4**). One cat died in a traffic accident 164 days after the end of treatment (**publication 3**). The remaining 17 cats showed no abnormalities in laboratory and clinical parameters. FCoV-RNA was not detected in blood in 17/18 cats; only one cat was RT-qPCR positive with a very low viral load at one time point. Five cats showed recurrent fecal FCoV shedding. However, 2 cats developed very mild neurologic signs (compatible with symptoms of feline hyperesthesia syndrome). These 2 cats were FCoV-negative in blood and feces. Also, no anti-FCoV antibody titers increase could be seen. 12 cats exhibited abdominal lymphadenopathy during the long-term follow-up.

Unfortunately, GS-441524 is not currently legally available for veterinary use in most countries. This poses an ethical dilemma to owners and veterinarians. Owners are forced to self-diagnose and treat their cats based on the opinions of non-professional people and social media groups. The illegally available medications are produced without control and there is no guarantee of the composition (purity and concentration of the active ingredient).

GS-441524 is an excellent option for the oral treatment of FIP. It should also be further explored as a potentially effective treatment option for other severe coronavirus-associated diseases in various animal species.

X. LITERATURVERZEICHNIS

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