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Unlicensed antiviral products used for the at-home treatment of feline infectious peritonitis contain GS-441524 at significantly different amounts than advertised

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OBJECTIVE
To analyze the content of unlicensed GS-441524-like products being used as a largely successful at-home treatment for cats suspected to have FIP. The remdesivir content and pH were also measured.

SAMPLE
127 injectable and oral samples from 30 of the most popular brands of black market producers.

METHODS
Unlicensed GS-441524-like products were procured through donations and tested for GS-441524 and remdesivir content by liquid chromatography with tandem mass spectrometry. A pH meter measured the pH of injectable samples.

RESULTS
Of the 87 injectable formulations, 95% contained more (on average 39% more) GS-441524 than expected based on the producer's marketed concentrations. The average pH (1.30 pH) was well below the physiologic pH conditions recommended for SC injections. The oral formulations were more variable, with 43% containing more GS-441524 (on average 75% more) than expected and 58% containing less (on average 39% less) than the expected content. There was minimal variability in GS-441524 content between replicate samples in the injectables formulations (measured by coefficient of variation). One injectable and 2 oral samples additionally contained remdesivir.

CLINICAL RELEVANCE
All unlicensed products used for the at-home treatment of FIP that we tested contain GS-441524. The injectables generally contain significantly more drug than advertised at a below-physiologic pH. Unlicensed oral products vary more widely in drug content and suffer from unconventional dosing and labeling. These data should highlight the need for regulation of these products and the development of legal pathways to procure GS-441524.

Keywords: antiviral, feline infectious peritonitis, GS-441524, unlicensed, LC-MS/MS

Feline coronavirus (FCoV) is endemic within the domestic cat population and occurs as 2 biotypes. The first, feline enteric coronavirus (FECV), usually results in nonspecific, nonfatal signs. By some mechanism not fully described in the literature, FCoV can turn into a much deadlier disease, FIP, with the highest incidence occurring in cats ages 4 to 16 months.[1] A definitive diagnosis can often be challenging in the absence of reliable antemortem tests, and there are no FDA-approved therapies available. The disease is almost invariably fatal when left untreated.[2]

Over the past 5 years, research into antiviral therapies has highlighted some promising outcomes for cats diagnosed with FIP. One such antiviral, GS-441524, demonstrated therapeutic efficacy in cats with experimentally induced and naturally occurring FIP.[3–7,8,9] GS-441524 is the active metabolite of remdesivir, which was authorized by the FDA for emergency use in human COVID-19 patients in 2020.[10] There are currently no legal means of prescribing GS-441524

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by a veterinarian in the US or in most other countries worldwide. The existence of a lifesaving therapy but no legal means of acquiring it has created an unprecedented demand for the GS-441524 compound by owners in the US. The accumulation of online resources as well as social media support communities (such as "FIP Warriors" and others) has facilitated thousands of owners in acquiring and treating their pets with little to no supervision from veterinarians.[7] Until now, the true contents and pH of these formulations, compared to what the unregulated manufacturing label describes, have not been analyzed. The ambiguity surrounding the true contents of these products is one of many issues prohibiting veterinary involvement with FIP therapy. The authors recognize that the term *unlicensed* may not apply perfectly to this scenario because licensing of biologics through the USDA does not apply to pharmaceuticals. A term for the products resulting from this unprecedented form of citizen's veterinary medicine does not currently exist in the literature. However, the term *unlicensed* has been found in multiple places in the literature regarding "black market" antivirals for FIP and is therefore used in this report.[7,11] We hypothesized that the unlicensed products do contain GS-441524, and at greater amounts than as labeled. We further hypothesized that the average pH of injectable formulations would be low (< 2.0 pH), given previous descriptions of the solubility of this compound.[3,5]

Despite this lack of oversight, at-home treatment with unlicensed GS-441524 products has been found to be an effective treatment for cats diagnosed with FIP based on owner-reported data.[7] We hope that the results of this study allow owners to make more informed decisions about treatment plans as well as encourage ethical and legal drug development of therapeutics for cats with FIP. Identifying which formulations are safe and effective for owners to purchase and administer to their cats diagnosed with FIP is of the utmost importance. An understanding of the contents and pH of these unregulated compounds will empower owners and veterinarians with options regarding treatment and will help further characterize this unique public health phenomenon.

Methods

Disclaimer

This report evaluates products that are not legally available in the US and are only available in the US on the black market. This drug is neither approved by the FDA nor commercially available through legal entities in the US.

Sample collection

Samples of commonly used injectable and oral GS-441524 formulations from various black market producers were solicited from the FIP treatment community using social media. All samples were donated between October and December of 2022 to The Ohio State University College of Veterinary Medicine. No samples were purchased directly from producers. Following collection, samples were sent to the University of California-Davis School of Medicine Molecular Pharmacology Shared Resource for an analysis of the GS-441524 and remdesivir content in each sample. The pH of injectable formulations was also measured.

Actual GS-441524 content via liquid chromatography with tandem mass spectrometry

Chemicals—Remdesivir (> 99%; AdipoGen Life Sciences Inc), GS-441524 (> 99%; MedChemExpress), and deuterated remdesivir-D5 (> 99%; Acanthus Research Inc) were ordered as control compounds. Methanol and acetonitrile (ACN) of analytical grade (Thermo-Fisher Scientific Inc) and ammonium formate and formic acid (Sigma-Aldrich) were purchased.

Analysis via liquid chromatography with tandem mass spectrometry—An ultrafast liquid chromatography system (Prominence; Shimadzu Corp) coupled with a mass spectrometer (4000 QTRAP; AB Sciex) was used for quantification. For the analysis of injectable samples, the samples were diluted 20,000 times by ACN/water solution (1:1, v/v), then centrifuged at 15,700 X *g* for 5 minutes at 4 °C. Five-µL supernatant was directly injected for liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. For the analysis of tablets and capsules, the tablet or the powder of the capsule was dissolved with a designated amount of ACN/water solvent (1:1, v/v) to achieve the concentration of 1.0 mg/mL by shaking incubated at 100 rpm for 1.5 hours at room temperature. Then 1 mL of dissolved solution was centrifuged at 15,700 X *g* for 10 minutes at 4 °C. The supernatant was collected and filtered with a 0.22-µL filter. After the filtrate was diluted 1,000 times by ACN/water solution (1:1, v/v), 5 µL was injected for LC-MS/MS analysis. The mobile phase was composed of 10mM ammonium formate in water with 0.1% formic acid (solution A) and acetonitrile with 0.1% formic acid (solution B). The flow rate was set at 0.3 mL/min. The separation of GS-441524 and remdesivir was conducted on a reverse-phase C18 column (AQUASIL [4.6 X 50 mm ID, 3.0-µm particle size]; Thermo) maintained at 40 °C by an optimal gradient elution: 0 to 1.5 minutes, 3% to 80% solution B; 1.5 to 2.8 minutes, solution B maintained at 80%; 2.8 to 3.0 minutes, 80% to 3% solution B, with a total run time of 5 minutes. The MS/MS analysis was operated under positive mode with optimized parameters: curtain gas, 10 psi; nebulizer gas, 20 psi; auxiliary gas, 20 psi; ion spray voltage, 5,500 V; and temperature, 500 °C. The optimal multiple reaction monitoring transitions were m:z (mass to charge ratio) $[M + H]^+$ 292.3→202.1 for GS-441524, m:z $[M + H]^+$ 603.4→402.3 for remdesivir, and m:z $[M + H]^+$ 608.6→407.2 for the internal standard. The retention times of GS-441524 and remdesivir were 2.78 and 3.47 minutes, respectively. The calibration curves were linear over the range of 100 to 4,000 ng/mL for GS-441524 and 10 to 400 ng/mL for remdesivir, with the mean correlation coefficients greater than

0.99. Precisions evaluated at 150, 500, and 3,000 ng/mL for GS-441524 and 15, 50, and 300 ng/mL for remdesivir, respectively, were lower than 2.7%, and accuracy was in the 90.0%-to-106.7% range.

Expected GS-441524 content

The content of GS-441524 (and remdesivir) determined by LC-MS/MS was compared to the expected value for each sample. The expected concentration of injectables (mg/mL) and expected strength of oral formulations (mg/pill) were determined via the label found directly on the sample or the producer's website, where possible. In the cases where this information was not available, members of the FIP community who donated the samples were consulted as to how the samples were advertised or described by the producer. The latter method was only necessary for the injectable formulations that were donated in unmarked vials from the producer without a website to reference.

The oral GS-441524 samples included both tablets and capsules. Because of the various unconventional labeling methods for these oral formulations, some calculations were necessary to express the appropriate expected strength in terms of milligrams per pill. It is known within the FIP treatment community that producers report the strength of the pills based on what is thought to be bioavailable to the cat.[12] Therefore, the strength of the pill expressed on the label reflects the producer's estimation of the equivalent SC dose. This is presumably done to make the process of switching between injectable and oral formulations easier for lay owners. Ten oral samples reported the amount of GS-441524 content in milligrams per pill (pills from Brava, Ocean, Rose, and Valor **[Table 1]**). The authors assumed these values were only 50% of what was truly contained in the pill and adjusted the expected amount (for example, if labeled "Brava 5mg," the pill would be expected to contain 10 mg/pill of GS-441524). Six samples were labeled "*actual* mg/pill," which was assumed by the authors to reflect the true amount, so no further calculations were required. In 19 samples, only dosage instructions were provided without including the strength of the pill (mg/pill). For example, the only

Table 1—The oral formulations tested in this study are listed. The product description includes information found on the label, website, or elsewhere regarding content of the pill or the listed directions. The various methods of labeling and identifying these pills are highlighted in this column by the variety of descriptions present. Based on the product description, a presumed GS-441524 (GS) content was extrapolated based on treatment instructions, the recommended 5-mg/kg/d dosage, and the approximately 50% bioavailability of GS. The actual content was determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Products	Product description	N	Presumed GS content (mg/pill)	Actual GS (mg/pill)	Difference (%)
Aura	12 h/1 kg	1	5	5.1	2.8
Aura	24 h/1 kg	1	10	11.2	11.5
Aura	12 h/3 kg	1	15	12.9	–14.3
Aura	24 h/2 kg	1	20	14.2	–29.3
Brava	5 mg	1	10	3.9	–60.6
Brava	10 mg	1	20	11.4	–43.0
Capella	1kg	2	10	14.9 ± 3.9	48.5
Capella	2 kg	2	20	12.0 ± 4.3	–39.8
Capella	5 kg	1	50	14.2	–71.7
CureFIP	2.5–4 kg	1	75	13.7	–81.8
Lucky	1 kg	2	10	14.7 ± 3.6	46.8
Lucky	2 kg	2	20	17.1 ± 3.1	–14.4
Lucky	4 kg	1	40	15.0	–62.5
Meadow*	12 mg	1	12	20.5	70.8
Meadow*	24 mg	2	24	16.5 ± 0.2[a]	–31.4
Meadow*	48 mg	2	48	25.8 ± 7.9	–46.3
Mutian	100 mg	2	10	19.9 ± 3.3	99.0
Mutian	200 mg	1	20	38.1	90.5
Mutian Xraphconn	50 mg	1	5	18.0	260.5
Mutian Xraphconn	100 mg	1	10	42.6	326.0
Ocean	7.5 mg	1	15	6.2	–58.5
Ocean	15 mg	1	30	12.8	–57.4
Panda**	1 kg	1	10	18.9	89.0
Panda**	2 kg	1	20	20.8	4.0
Phoenix Blue	1 kg	2	10	11.1 ± 0.1[b]	11.0
Phoenix Red	1 kg	1	10	10.5	4.5
Rose	2.5mg	1	5	3.7	–27.0
Rose	10 mg	1	20	14.6	–27.0
Rose	11.6 mg	1	23.2	17.6	–24.3
Rose	20 mg	1	40	28.3	–29.3
Rose	30 mg	1	60	48.9	–18.5
Valor	8 mg	1	16	11.6	–27.3

Values from samples with replicates are listed as an average with an SD and coefficient of variation.
N = Number of replicates.
*Also known as Harmony. **Also known as Maxpaw.
[a,b]Values are significantly ($P < .05$) different.

information on the label or website instructed "give 1 pill/1 kilogram/ 24 hours" or simply "1kg." To provide a comparison for the results, an estimated strength was calculated based on the dosage instructions provided and the current average recommended dose of 5 mg/kg/d of GS-441524 for treatment of FIP in cats without ocular or neurologic involvement.[4,7,13] However, dosage regimes are known to vary widely.[7] Once the strength of the pill in milligrams was determined, it was further adjusted to reflect 50% bioavailability as was done with the other samples (eg, a pill labeled "1 kg" would contain 5 mg based on the current recommended therapeutic dose, then be adjusted to reflect a bioavailability of 50%, so would be presumed to contain 10 mg by the authors).

Finally, the active ingredient in Xraphconn and Mutian tablets (both made by the company Mutian) is listed as MT-0901, but previous studies have determined the active ingredient to actually be GS-441524.[5] It is believed the 50-, 100-, and 200-mg Xraphconn tablets are intended to have a dose equivalent to 2.5 mg, 5 mg, and 10 mg of GS-441524.[8,12] The 5 samples were then adjusted to reflect 50% bioavailability.

The method of reporting the expected GS-441524 concentration in the injectable samples was generally more straightforward, as they were almost all marketed in milligrams per milliliter by the producer. It was necessary to estimate the expected strength for only one of the injectable samples (OM) by converting the dosage instructions in a similar manner as was done with the oral formulations.

pH

The pH values of the injectable samples were directly measured by a pH meter (pH 700, Oaklon). The calibration of the pH meter adopted the 1-point calibration method of standard buffer solution (pH 4.01).

Statistics

Excel, version 1808 (Microsoft Corp), was used to calculate the percent difference between the expected and determined GS-441524 content in each sample as well as to calculate mean and SD of all parameters. To assess variability of GS-441524 content between replicate samples, the coefficient of variation (CV) was calculated to express SD as a percent of the mean. A 2-sample paired *t* test was used to determine the significance between the expected and determined concentrations/strengths. The mean and SD of the percent difference from the expected concentration/strength as well as the pH were also determined in products with replicate samples.

Results

Sample collection

In this study, we collected 127 samples (87 injectable and 40 tablets/capsules) that included 30 different products sold under various names (*brands*), all of which were tested for GS-441524 and remdesivir by LC-MS/MS. Because samples were procured through donations, the number of replicates for each product was limited to availability and ranged from 2 to 9 (Tables 1 and **2**). The location where the samples were manufactured was reported by the donor if known. Eighty-eight samples (69%) were manufactured in China, 18 (14%) in the US, 13 (10%) in Canada, 5 (4%) in Malaysia, and 1 (1%) in Poland; for 2 samples (2%), the location was unknown. Samples were procured from donors across the world. The brands listed in this study as Meadow, Panda, and Freecat are also known as Harmony, Maxpaw, and Bliss respectively. Additionally, Mutian and Xraphconn products are created by the same producer, named Mutian. There is awareness in the FIP therapy community that other brands may have a common producer, and the branding names are changed frequently.

GS-441524 and remdesivir content

The content of GS-441524 reported by the producer was compared to the amount determined by LC-MS/MS. The content of remdesivir in each sample was also determined. All 127 samples contained GS-441524, and 3 samples (Mutian M3 injectable formulation, Panda 1-kg oral formulation, and Panda 2-kg oral formulation) additionally contained remdesivir.

GS-441524 actual and expected content in 87 injectable samples is presented in Table 2 (for a complete list of samples, refer to **Supplementary Tables S1 and S2**). Of these 87 samples, 83 (95%) contained more GS-441524 than the reported concentration and were on average 39 ± 17% (range, 2% to 111%) higher. Four (5%) injectable samples contained less drug than the expected concentration and were on average 18 ± 8.4% (range, 7% to 26%) lower. All 4 samples were made by different manufacturers (CureFIP, Mutian, Petronium, and Freecat). According to current Good Manufacturing Process regulations by the FDA, products must contain 95% to 105% of the active pharmaceutical ingredient at the time of manufacturing. Only 2 total of 87 injectable samples were within the acceptable range. Including all 87 injectable samples, a paired sample *t* test demonstrated that the actual concentration was significantly higher than the expected concentration ($P < .01$). We performed individual statistical comparisons where replicate samples of the same product were available. Of the 25 samples with replicates, in 9 (Aura 20 [$P = .17$], Bliss [$P = .034$], Lucky 15 [$P = .001$], Lucky 20 [$P = .002$], Oscar [$P = .001$], Panda 15 [$P = .008$], Phoenix 15 [$P < .001$], Seka [$P = .008$], and Valor [$P < .001$]) the actual concentration was significantly different from the expected concentration.

GS-441524 actual versus expected content in 40 oral samples is presented in Table 1. Of these 40 samples, 17 (43%) contained more GS-441524 than expected and were on average 75 ± 91.3% (range, 2.8% to 326%) higher ($P < .001$). Twenty-three (58%) samples contained a level of GS-441524 below the expected strength and were on average 39 ± 20% (range, 3.5% to 81.8%) lower ($P = .002$). Assuming an acceptable content range is 95% to 105% of the expected content, 3 of 40 oral samples were within the acceptable range. The 5 oral products manufactured by Mutian (Mutian 100 [n = 2], Mutian 200 [1], Xraphconn 50 [1], Xraphconn 100 [1]) ranged from

Table 2—The claimed and actual amounts of GS-441524 (GS) in the injectable formulations are listed. The claimed amount is the amount of GS the authors believe is in the formulation based on the label or research. The actual amount of GS was determined by LC-MS/MS.

Products	N	Claimed GS content (mg/mL)	Actual GS (mg/mL)	Difference (%)	CV % (GS)	pH	CV % (pH)
Aino	2	16.9	30.0 ± 8.1	77.2	—	1.08 ± 0.23	—
Aura 15	2	15	22.3 ± 2.7	48.6	—	1.43 ± 0.04	—
Aura 17	2	17	23.4 ±1.1	37.6	—	1.4 ± 0.08	—
Aura 20	2	20	27.3 ± 0.3[a]	36.5	—	1.3 ± 0.02	—
Azul	1	17	27.2	60.0	—	1.29	—
Bliss	2	15	27.2 ± 0.9[b]	81.0	—	1.2 ± 0	—
Brava	2	15	18.1 ± 0.4	20.5	—	1.3 ± 0.11	—
Capella	2	15	24.8 ± 1.6	65.0	—	1.2 ± 0.03	—
CureFIP 15	1	15	20.3	35.2	—	1.37	—
CureFIP 20	2	20	25.1 ± 1.1	25.5	—	1.4 ± 0.08	—
CureFIP 30	2	30	29.1 ± 8.6	–3.0	—	1.1 ± 0.02	—
Fenh	2	20	25.8 ± 2.5	28.8	—	1.3 ± 0.14	—
Fina	1	17	22.9	34.7	—	1.40	—
Freecat	3	15	15.9 ± 4.5	5.8	28.2	1.3 ± 0.18	13.8
Karma	1	18	24.6	36.7	—	1.33	—
Kittycare 16.8	2	16.8	20.2 ± 1.7	20.3	—	1.4 ± 0.04	—
Kittycare 20	1	20	20.4	2.0	—	1.32	—
Lucky 15	4	15	22.2 ± 1.1[c]	47.8	4.8	1.4 ± 0.11	7.8
Lucky 20	4	20	28.0 ± 1.6[d]	40.1	5.6	1.3 ± 0.08	6.3
Meadow* 17.5	2	17.5	26.0 ± 0.9	48.3	—	1.4 ± 0.18	—
Meadow* 20	2	20	30.6 ± 2.6	52.8	—	1.3 ± 0.21	—
Mutian II	1	17	14.3	–15.8	—	1.21	—
Mutian M3	1	0	0.42	—	—	2.59	—
Mutian Xraphconn II	2	17	21.7 ± 1.1	27.4	—	1.5 ± 0.19	—
Ocean 15	1	15	19.9	32.7	—	1.08	—
Ocean 18	1	18	22.6	25.6	—	0.93	—
OM**	1	12	15.4	28.3	—	1.26	—
Oscar	6	15	20.1 ± 1.6[e]	34.0	8.1	1.3 ± 0.04	3.1
Panda*** 15	2	15	23.3 ± 0.1	55.3	—	1.2 ± 0.40	—
Panda*** 17	1	17	23.5	38.2	—	1.42	—
Patronium	1	15	14	-6.9	—	1.64	—
Phoenix 15	9	15	20.5 ± 0.7[f]	36.9	3.5	1.3 ± 0.06	4.6
Phoenix 20	1	20	26.6	33.0	—	1.13	—
Proline	3	15	18.4 ± 2.9	22.4	15.8	1.5 ± 0.23	15.4
Rainbow	2	20	26.4 ± 2.1	31.8	—	1.3 ± 0.20	—
Rose 20	2	20	26.8 ± 2.8	33.8	—	1.1 ± 0.01	—
SAK	1	17	23.2	36.5	—	1.19	—
Seka	4	15	21.9 ± 2.2	46.1	10.0	1.2 ± 0.01	1.0
Sion	1	15	21.8	45.3	—	1.19	—
Star	1	15	21.3	42.0	—	1.32	—
Trusted	1	15	22.2	48.0	—	0.89	—
Valor	3	17.5	23.8 ± 0.4[g]	36.0	1.5	1.5 ± 0.12	8.0

Replicate samples (when available) are presented as an average with an SD and a CV. The pH and the CV are listed. The CV is a measure of variability between replicate samples and was calculated when 3 or more samples were available. Meadow is also known as Harmony and Panda is also known as Maxpaw.

— = Not available. CV = Coefficient of variation. N = Number of replicates.

*Also known as Harmony. **The manufacturer, OM, did not advertise the amount of GS in the product. A presumed concentration was calculated based on treatment instructions (1 syringe for cats < 5 lb) and the recommended 5-mg/kg/d dose of GS. ***Also known as Maxpaw.

[a–g]Values are significantly different ($P < .05$).

90.5% to 326% higher than the expected content. Of note, the producers of CureFIP 2.4 to 4 kg (n = 1), Meadow 12 mg (1), Meadow 24 mg (2), and Meadow 48 mg (2) provided an "actual" amount of GS-441524 on the label to indicate no bioavailability calculations had been made; therefore, the expected values were not adjusted in any way. The amount of GS-441524 content was greatly lower than the value advertised in CureFIP 2.4 to 4 kg (81.8% less), Meadow 24 mg (31.4% less), and Meadow 48 mg (46.3% less). Meadow 12 mg had 70.8% more GS-441524 than expected. We performed individual *t* tests on the 8 samples with replicates. The actual content of Meadow 24 was significantly lower ($P = .014$) and that of Phoenix Blue ($P = .029$) was significantly higher than the presumed amount.

Variability among replicate samples of the same product

There were replicate samples in 25 of the injectable and 8 of the oral formulations. The number of available replicate samples varied significantly (for some products only 2 replicates were available, while others had 9). The variability of GS-441524 content between 3 or more replicate samples of the same unlicensed formulation was analyzed by calculation of

the CV (Table 1). The CV was used to express SD as a percent of the mean. The CV of injectable formulations ranged between 1.5% and 28.2% (n = 8). The authors defined the acceptable variability between samples as a CV of under 10%. This would indicate the amount of GS-441524 between replicate samples varies to an acceptable degree. Six out of the 8 injectable products had a CV under 10%. The Phoenix 15 samples benefited from a robust sample size (n = 9) and were found to have very consistent GS-441524 content between samples (CV, 3.5%).

Remdesivir content

The amount of remdesivir in each sample was also determined by LC-MS/MS because deuterated remdesivir was included as an internal control for the technique. Three products in this study were found to contain remdesivir. Mutian M3 had 10.88 mg/mL of remdesivir and only 0.4 mg/mL of GS-441524. This was the only product advertised to contain remdesivir (per the donor; this could not be verified on the producer's label or website) and was not expected to contain any GS-441524. Remdesivir was also detected in the samples of Panda 1 kg (5.91 mg) and Panda 2 kg (0.749 mg), though not reported by the producer. The Panda 1-kg sample contained almost 30% as much remdesivir (5.91 mg/pill) as GS-441524 (18.9 mg/pill).

pH of injectable formulations

The pH of injectable GS-441524 samples was on average 1.30 ± 0.15 (range, 0.89 to 2.59 pH). The CureFIP website marketed their injectable products at a pH of 1.9 to 2.2, and the pH of all 5 samples was between 1.07 and 1.46 pH. Meadow and Rose marketed their injectable products as having a pH of 1.8, and the actual pH was determined to be 1.3 pH (Meadow 17.5), 1.11 pH (Meadow 20), and 1.28 pH (Rose 20). The CV ranged between 1% and 15.4%, indicating minimal variability in pH between replicate samples. Samples manufactured by Bliss (n = 2) were sourced from different donors and were found to have the same pH (1.15). The largest variability in pH between replicate samples was found in the 2 Panda 15 samples (0.94 vs 1.5 pH). The highest pH among the samples in this study (2.59) was found in Mutian M3 (this was the sample found to contain mostly remdesivir).

Discussion

In this study we analyzed several commonly used formulations (ie, brands) of unlicensed GS-441524 being sold over the internet to treat FIP for their actual drug contents. We hypothesized that the GS-441524 formulations would be marketed at a concentration lower than their actual GS-441524 content. All 127 samples analyzed in this study contained some amount of GS-441524, and 3 also contained remdesivir. LC-MS/MS determined the actual amount of GS-441524 to be higher than the expected concentrations in 95% of the tested injectable samples. We speculate that the producers often include excess drug in their injectable products intentionally to gain a competitive advantage in this market (ie, potentially making their product appear more effective than others), and/or to compensate for poor purity, poor stability, or lot-to-lot variability. Research into the use of these unlicensed compounds by our group and others must be interpreted in light of the differences between labeled and actual contents of these products.

A little more than half of the oral formulations were below the expected GS-441524 content. As a result of this variability, a pairwise comparison of expected to actual drug content of oral formulations was not significant. A nontraditional method of reporting the amount of GS-441524 content in the oral formulations posed significant challenges and forced our group to make assumptions when attempting to determine the amount of drug expected to be in the pills. Presumably, in an effort to make the conversion from injectable to oral treatments easier for lay owners, producers report the amount of GS-441524 that is orally bioavailable to the cat (or the approximate SC dose equivalent), instead of the amount of drug actually contained in the pill. Therefore, black market producers are thought to include significantly more GS-441524 in oral formulations relative to the labeled strength.[12] Further studies describing the bioavailability of oral GS-441524 in felines may be useful for this field; the 50% adjustment made in this study reflects extrapolations from other peer-reviewed and non–peer-reviewed GS-441524 pharmacokinetic studies.[12–14] Further, the convention for many of these products is to label on a "per kilogram of cat" basis, rather than listing the actual milligram content of drug contained within the tablet or capsule. For example, a label stating "12 h/1 kg" would instruct the owner to give 1 pill/1 kg of cat (eg, 3 pills for a 3-kg cat), every 12 hours. This required us to assume a standard average recommended dosage of 5 mg/kg to calculate the expected amount of drug in the pills.[4,5,7,8,9,13] Both of these factors (adjusting for bioavailability and the unconventional labeling system) complicated our analysis of the expected versus actual amount of GS-441524 in the oral formulations and represented a limitation of this study.

Due to ease of administration, decreased morbidity in the patient (eg, injection site reactions and pain upon administration), and decreasing cost of pills over time, oral formulations of GS-441524 are becoming more common.[7,12] The findings of this study suggested that the actual amount of GS-441524 can be quite low relative to the expected amount in some brands, although a more robust sample size is necessary to identify trends within specific brands. The most compelling evidence of this trend can be found in the 6 samples that list an "actual GS-441524" content on the label in addition to the "per kilogram of cat" designation. First, the specification of an actual amount suggests the unconventional method of taking bioavailability into account within drug labeling is in fact a commonly used practice by black market producers (supporting this study's adjustment of the expected content). Second, of the 6 samples, 5

had less GS-441524 than advertised as the "actual" amount on the label. This small subset of samples reflects the overall trend displayed by the rest of the oral samples. Thus, there is a risk of insufficient GS-441524 content in unlicensed oral samples.

Analysis of the injectable formulations did not suffer from this same limitation because bioavailability is not an issue and the convention for almost all black market producers is to report the drug content of those products in milligrams per milliliter (with the exception of the brand OM, where a similar calculation was done to that of the oral products, as described above). While unconventional and confusing package labeling make it difficult to extrapolate from the percent differences calculated in this study for the oral formulations, the actual amount of GS-441524 found by LC-MS/MS can be used to better inform treatment decisions. Availability of reliable, ethical, and approved injectable and oral GS-441524 products would best serve cat owners and the veterinary community.

The samples in this study containing less GS-441524 than expected raise some concerns for treatment efficacy when used and the potential for the development of resistance. In this limited set of samples, products contained up to 66% less than the expected content of GS-441524. Resistance may be more common in animals with prolonged treatment at a lower-than-appropriate dose.[3,11] Despite the relatively high degree of variability in the GS-441524 content of these unlicensed products, they are nevertheless reported to be an effective treatment for FIP patients.[5,7] In vitro toxicity studies have shown GS-441524 to have a large safety margin.[5] Crandell-Rees feline kidney cells display no signs of toxicity when treated with GS-441524 concentrations as high as 100μM,[4] although rigorous safety studies and adverse event reporting of GS-441524 have not been performed.

Of note, there may be a legal pathway in the US to produce GS-441524 for use in FIP. The FDA recently released Guidance for Industry No. 256, which states interested parties can nominate bulk drug substances for compounding in non–food-producing animals, provided there is a justified therapeutic deficiency. If GS-441524 was nominated to be included on this list, compounders could produce this drug ethically and possibly decrease or eliminate the illegal black market trade. The authors encourage further pursuit of this avenue in light of this study's findings.[15]

The variability of GS-441524 content between products with more than 1 sample was measured by calculating a CV. The average CV (8.9%) reflected a low degree of variability between the injectable samples. While the GS-441524 content in the Phoenix 15 samples (n = 9) had 37% more GS-441524 than expected, the CV suggested minimal variability in this producer's samples (3.5%).

Remdesivir (GS-5734) is the monophosphoramidate prodrug of GS-441524.[16] One unlicensed product in this study set (Mutian M3) is thought, by the FIP treatment social media community, to contain remdesivir. However, LC-MS/MS detected remdesivir in 3 of the tested samples. In addition to Mutian M3, both oral products from the brand Panda (also known as Maxpaw) contained some amount of remdesivir. While one sample contained a very small amount of the compound, the other contained a third as much remdesivir as GS-441524. Additionally, the Mutian M3 sample, which was expected to only contain remdesivir, also contained a small amount of GS-441524.

While we do not have any details on the manufacturing process utilized by Panda or Mutian, it is possible that it leads to some unintentional formation of remdesivir as a byproduct. It may also represent contamination by other products produced in the same setting, or even intentional substitution. Contamination of GS-441524 products with remdesivir may not pose a risk to the patient's health, as remdesivir has also been used to treat cats with FIP.[17,18] In fact, remdesivir can legally be prescribed by veterinarians in Australia, the UK, and New Zealand for the treatment of FIP through the compounding pharmacy BOVA. GS-441524 can also be prescribed through BOVA.[19] Therefore, the 2 samples in this study that additionally contained remdesivir (Panda 1 kg and Panda 2 kg) would most likely have little effect on the cat. Still, the finding of remdesivir in some products highlights uncertainty surrounding the quality and purity of unlicensed GS-441524 from overseas producers. In contrast, FDA regulation of manufactured pharmaceuticals includes a robust assessment of the active and inactive pharmaceutical ingredient identity, strength, quality, and purity and includes stability studies, potential toxicities, environmental impact, and microbial testing. The lack of any regulation of these products and proper safety protocols highlights serious concerns for animal welfare. The scope of analysis in this study was limited to only the quantity of the active pharmaceutical ingredient due to the limitations of the targeted LC-MS/MS.

Due to the unlicensed nature of this industry, packages misrepresenting the contents and intended use of these GS-441524 products are common (Supplementary Tables S1 and S2). Products are shipped in boxes labeled as facial masks, serums, or cat nutrients, possibly to curb suspicion during shipment **(Supplementary Figures S1 and S2)**.[12] Additionally, some products are marketed vaguely as FIP cures with active ingredients other than GS-441524. For example, the brand OM markets their products as containing 50 mg of sea sponge extract, and Mutian products are advertised to also contain vitamin B12. Mutian lists its active ingredient as MT-0901 and is described by the company as an adenosine nucleoside analogue.[8] While the LC-MS/MS technique used here was unable to determine whether sea sponge extract, B12, or any other adenosine nucleoside analogues were present in the samples, all 127 samples did contain GS-441524.

The pH of the injectable GS-441524 samples spanned a relatively narrow range (0.89 to 1.69 pH), with an average pH (1.30) well below a physiologic pH. Of the producers that advertised their injectable products at a certain pH (8 samples in this study), all determined pH values were lower than the marketed value. While there is no published FDA guidance on

the recommended pH of animal drugs used SC, the range usually is close to physiological conditions to minimize pain and tissue damage (per communication with FDA Center for Veterinary Medicine Compliance). While the low pH most likely causes tissue pathology, there have been no rigorous studies to confirm the adverse effects associated with injections, as would have been performed during the FDA approval process. Previous experimental studies (using GS-441524 sourced from pharmaceutical companies) followed a dilution protocol of 5% ethanol, 30% propylene glycol, 45% polyethylene glycol 400, and 20% water, adjusting the pH of the solution with HCl to 1.5 pH in one study and 3 to 4 pH in another.[3,6] The producer's protocol for dilution of unlicensed injectable GS-441524 samples was only reported by the CureFIP brand (which followed the same dilution as previously mentioned). GS-441524 has low aqueous solubility at a pH of 5 to 7.4 and high solubility at a pH < 1.[20] Due to the low pH necessary for the dilution of GS-441524, pain upon injection and injection site reaction have been documented.[3,6,7] Mutian M3 (mostly containing remdesivir) was determined to have the highest pH (2.59 pH). Remdesivir has been found to be slightly less painful upon injection, and this study suggests this could possibly be due to the higher pH in solution.[21] Investigation into diluents that can allow a pH that more closely matches physiologic levels may decrease patient morbidity. Of the replicate injectable samples tested, the average CV fell below 10%, indicating a small amount of variability in pH between replicate products.

This study investigated the GS-441524 content via LC-MS/MS of 127 samples comprising 30 different unlicensed brands of GS-441524 marketed to treat FIP. The actual GS-441524 content was, on average, significantly higher than what was marketed for the injectable formulations and had minimal variability in the amount of drug between replicate samples of the same product. The average pH was more acidic than claimed by the producers and well below the physiologic pH conditions recommended for SC injections. The amount of GS-441524 in the oral formulations varied more widely above and below the expected content with a high degree of variability between replicate samples. The risk of relapse or development of drug resistance due to insufficient drug dosing is of concern with any unlicensed formulation, and owners should be vigilant for signs of decline while switching to oral forms of GS-441524. Despite these findings, the benefits of the oral form over the injectable may outweigh these risks at this time. It is important for owners, veterinarians, and researchers alike to be aware of the highly unconventional methods used to label the oral formulations of GS-441524, as described here. Further work is necessary to understand whether some of the unlicensed formulations may contain compounds other than GS-441524 and remdesivir. The results of this study will inform ongoing research into the use of these unlicensed products and encourage the creation of legal avenues for procurement of GS-441524 to safely treat feline patients.

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Disclosures

Nicole Jacque, in her role as an administrator for FIP Global CATS, is involved in the distribution and sales of several products listed in this report.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.