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PEQUEÑOS ANIMALES

Nº 3

- Polyprenyl Immunostimulant Treatment of Cats with Presumptive Non-Effusive Feline Infectious Peritonitis In a Field Study

  Tratamiento inmunosimulante de poliprenil de gatos con peritonitis infecciosa felina presuntiva no efusiva en un estudio de campo

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SUMARIO

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Tratamiento inmunosimulante de poliprenil de gatos con peritonitis infecciosa felina presuntiva no efusiva en un estudio de campo

Palabras clave: peritonitis infecciosa felina, poliprenil inmunoestimulante, aumento de la supervivencia, enfermedad crónica, coronavirus felino, estudio de campo

Keywords: *feline infectious peritonitis, Polyprenyl Immunostimulant, increased survival, chronic disease, feline coronavirus, field study*

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La peritonitis infecciosa felina (FIP) es una enfermedad mortal sin tratamiento clínicamente efectivo. Este estudio de campo evaluó el tratamiento con poliprenil inmunoestimulante (PI) en gatos con la forma no efusiva de FIP. Debido a que la supresión inmunitaria es un componente importante en la patología de la FIP, planteamos la hipótesis de que el tratamiento con un estimulante del sistema inmunitario aumentaría los tiempos de supervivencia de los gatos con FIP seco. Sesenta gatos, diagnosticados con FIP seca por veterinarios de atención primaria y especialistas y que cumplían con los criterios de aceptación, fueron tratados con PI sin la selección intencional de casos menos graves.

Feline infectious peritonitis (FIP) is a fatal disease with no clinically effective treatment. This field study evaluated treatment with Polyprenyl Immunostimulant (PI) in cats with the non-effusive form of FIP. Because immune suppression is a major component in the pathology of FIP, we hypothesized that treatment with an immune system stimulant would increase survival times of cats with dry FIP. Sixty cats, diagnosed with dry FIP by primary care and specialist veterinarians and meeting the acceptance criteria, were treated with PI without intentional selection of less severe cases. The survival time from the start of PI treatment in cats diagnosed with dry FIP showed that of the 60 cats with dry FIP treated with PI, 8 survived over 200 days, and 4 of 60 survived over 300 days. A literature search identified 59 cats with non-effusive or dry FIP; no cat with only dry FIP lived longer than 200 days. Veterinarians of cats treated with PI that survived over 30 days reported improvements in clinical signs and behavior. The survival times in our study were significantly longer in cats who were not treated with corticosteroids concurrently with PI. While not a cure, PI shows promise in the treatment of dry form FIP, but a controlled study will be needed to verify the benefit.

Introduction

Feline infectious peritonitis (FIP) is considered to be one of the most devastating diseases of domestic cats with an incidence of 2–12% (1) in multi-cat environments. FIP has long been considered fatal (2–4) and a leading cause of mortality in young cats. No clinically effective treatments exist for FIP (5). FIP has an effusive form with abdominal and thoracic fluid accumulations; a median survival time of 9 days was noted in 21 cats with effusive FIP (6). Dry (non-effusive) FIP is often characterized by pyogranulomatous infiltrates in the liver, kidneys, lymph nodes, eyes, and central nervous system. The dry form of FIP has longer survival times within a range of 1–200 days noted in 59 cats (6–13). Two cats with mixed dry and wet forms FIP treated with combinations of corticosteroids, human alpha interferon, and nelfinavir survived 181 and 477 days (7).

Mutation of the enteric coronavirus that induces a tropism for macrophages initiates the disease process (3, 14). Cell-mediated immunosuppression due to a decrease in CD4+ lymphocytes is commonly seen in cats with FIP (14). Deficiencies in cell-mediated immunity promote an exuberant production of antibodies to the coronavirus, which results in deposition of immune complexes. With immunosuppression being

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a major component of pathophysiology, treatment with an immune stimulant is a rational approach.

Polyprenyl Immunostimulant (PI) is a veterinary biologic licensed by the U. S. Department of Agriculture for the reduction of the severity of signs of feline herpesvirus and is safe in cats over 8 weeks of age. It was used in our pilot study to treat cats with the dry form of FIP and produced promising results (15). It upregulates Th-1 type pathway via toll-like receptors (16) and may thus be of benefit in the diseases involving suppression of cellular immunity. In this field study, we tried to determine if PI treatment increases survival time and quality of life in cats diagnosed with dry FIP.

Materials and Methods

General Study Design

The field study had a single treatment arm, without a placebo control group, and was limited to cats with non-effusive or dry FIP. Only cats in the United States and Canada were accepted. Cats were diagnosed and treated by their primary care veterinarians in conjunction with, in many cases, veterinary specialists. The veterinarians' usual laboratories performed diagnostic tests. The study measured survival times from the start of PI treatment to death or euthanasia in terminal condition. The survival data from this study were compared to the historic data from a number of published articles. The study included cats of all signalments with clinical signs that represented the clinical spectrum of dry form FIP and were accepted and treated regardless of the severity of the disease or current treatments. Addition of appetite stimulants, antiemetics, antibiotics, vitamins, or special diets was not prohibited in our study, but the protocol advised against the use of corticosteroids because they cause immunosuppression. This study was carried out in accordance with the recommendations of the University of Tennessee Office of Laboratory Animal Care. The protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee Protocol #1946.

Case Recruitment

Preliminary findings of a prior pilot study were published in 2009 (15). The Veterinary Information Network site published a note of this trial in February 2010. Following the publication of the article and the note, practicing veterinarians with suspected or confirmed FIP cases contacted the Principal Investigator (AML) via e-mail or phone. All cats diagnosed with dry FIP by their veterinarian were considered and assigned a number (**Figure 1**). The initial diagnostics were done by veterinarians and reviewed and assessed for acceptance by AML based on sufficient data to support the diagnosis. In eight instances, AML accepted the cats into the study without all laboratory diagnostics if the diagnosis was made by invasive techniques [immunohistochemistry (IHC), histopathology of biopsied material, or cytology of aspirates].

The initial veterinarian diagnosis was supported using the diagnostic approach proposed by Addie *et al*. (2) as reflected by our data collection form (**Table 1**), which included questions about patient age, history, environment, and observations such as pyrexia, weight loss, lethargy, anorexia, presence of abdominal lesions (masses, enlarged mesenteric lymph nodes). Required laboratory tests included complete blood count, biochemistry, and antibody titers to feline coronavirus (FCoV) and to pathogens that may mimic FIP. Surgical biopsy or aspiration of suspected lesions was encouraged. Necropsy was offered at the University of Tennessee free of charge or veterinarians could provide results of necropsy done by their providers.

The accepted cats received PI at 3 mg/kg orally three times per week and were clinically assessed by their veterinarians initially and then on follow-up examinations (monthly was recommended), which collected the data for

the analysis as shown in **Table 2**. Submission of the initial and follow-up laboratory test results was required. Refills of PI were shipped to collaborating veterinarians after the results of the follow-up evaluations were received. Monitoring of the progress of the cats accepted into the study was continued until death or euthanasia.

Veterinarians were advised to taper corticosteroid treatments if started before the study, but they were allowed to continue corticosteroid therapy at the lowest effective dose to maintain appetite and well-being.

Quality of life assessment was done using responses on the questionnaires and communications by the primary care veterinarians and owners. In many cases, more extensive comments were recorded in the cat's medical records, and all records were analyzed for the comments. We considered communications by veterinarians and owners regarding restoration of routines, activity levels, appetite improvement, etc. as indicators of the quality of life. **Table 3** shows the questionnaire used to provide the data collected in the study.

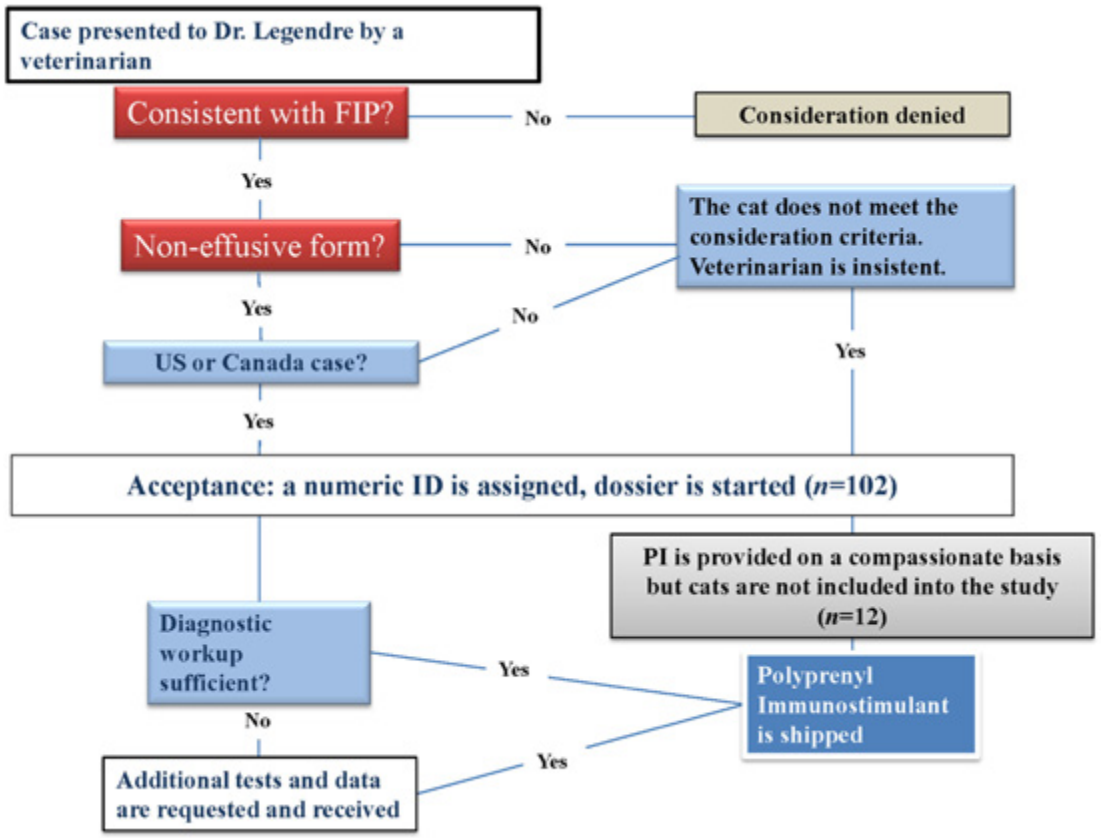

Figure 1. *Study decision-making tree at a glance.*

ID #	Date Polyprenyl Immunostimulant (PI) shipped		
Breed	Multi cat?	Origin	
Age now	Age at diagnosis		
Type of feline infectious peritonitis	Changes?	Histopathology?	
Date of Diagnosis	Date of PI start	Feline coronavirus (FCoV) titer	Necropsy

	Baseline	Follow-up								
Date		30 days	60 days	90 days	120 days	150 days	180 days	210 days		
Weight										
FCoV titer										
Total protein										
Globulins										
Albumin										
HCT										
WBC										
Neutrophils										
Lymphocytes										
ALT										
Temperature										
Uveitis										
Neuro										
Diarrhea										
Abdominal mass										
Steroids										

Survival, days (check)										
Date of death										

Table 1. *Data collection form.*

Tabla de contenido:

Table 2. *Data analysis form.*

Case #/Name		
Breed		
Male/female		
Neutered		
Age		
Weight		Did not start Polyprenyl Immunostimulant (PI) (check)

Clinical signs	**1 or 0**
Ocular	
Neuro	
Decreased appetite	
Fever 102.5+	
Lethargy/depression	
Abdominal lesions	
Weight loss	

1 = yes
0 = no

Weight loss	1 or 0 compared to start
@ 30 days	
@ 60 days	
@ 90 days	
@ 120 days	
@ 150 days	

Weight gain	1 or 0 compared to start
@ 30 days	
@ 60 days	
@ 90 days	
@ 120 days	
@ 150 days	

Weight, kg	
@ 30 days	
@ 60 days	
@ 90 days	
@ 120 days	
@ 150 days	
Status/survival	
@ 30 days	
@ 60 days	
@ 90 days	
@ 120 days	
@ 150 days	
>150 days	
Other	
Lost to follow-up	

Improvement (subjective owner/vet assessment)	
@ 30 days	
@ 60 days	
@ 90 days	
@ 120 days	
@ 150 days	
>150 days	

Diagnosis	**1 or 0**
Abdominal imaging	
Surgery	
Histopathology	
Cytology	
Elevated globulins	
Elevated total protein	
Decreased albumin	
Feline coronavirus titer	
1:400	
1:800	
1:1,600+	
Elevated WBC	
Decreased HCT	
Elevated neutrophils	
Decreased neutrophils	
Elevated lymphocytes	
Decreased lymphocytes	
Palpable/visible abdominal mass	
Enlarged mesenteric lymph nodes	
Colon involvement	

Treatment	
PI	
Steroids at start of PI	
Steroids at 1 month after start of PI	
Steroids at 2 months after start of PI	
Other	
Other	

Survived after PI start	days

Tabla de contenido:

Physical exam findings				
Date of exam			Cat's name	
			Owner's name	
Initial exam or recheck exam (mark)				
Date of first dose of Polyprenyl Immunostimulant (PI)				
Current dosing schedule				
Please answer the following questions based on the current examination. If you check YES, give additional details including locations, duration, and severity as necessary				
			Comments	
Weight loss	Yes	No	Current weight:	
Weakness	Yes	No		
Appetite	Yes	No		
Vomiting	Yes	No		
Diarrhea	Yes	No		
Fever	Yes	No		
Lameness	Yes	No		
Neurologic signs	Yes	No		
Paraplegia	Yes	No		
Ocular signs	Yes	No		
Other	Yes	No		
Initial history/history since last exam				
PI Study **Concomitant medications**				
Start date	End date	Drug	Dosage	Route

The questionnaires were submitted with results of laboratory tests.

Table 3. *Questionnaire for collection of patient information from the initial and follow-up examinations.*

Statistical Analysis of Study Results

Skewness and kurtosis statistics found non-normal distributions for all temporal variables associated with survival. Therefore, non-parametric statistics were employed to yield inferences based on the respective research questions. Between-subjects comparisons for age groups and disease groups were conducted using Kruskal–Wallis and Mann–Whitney U-tests. In addition to means and SD, medians and interquartile ranges were reported to give context to non-parametric statistical findings. Kaplan–Meier survival curves were used to display the cumulative survival of cats across time. t-tests were employed for the comparison of mean and SD. An alpha value of 0.05 assumed statistical significance, and all analyses were conducted using SPSS Version 21 (Armonk, NY, USA: IBM Corp.).

Results

Consideration and Acceptance

Consideration began March 1, 2010, and ended May 6, 2011. A total of 102 cats were considered for the study. All cats were diagnosed by their primary care veterinarians. There were 60 cats that met the qualification criteria and were therefore accepted into the study, and 23 of those had been referred to specialists, including veterinary ophthalmologists (12), internists (6), neurologists (3), ophthalmologist and a neurologist jointly (1), and a veterinary cardiologist (1). The remaining considered cats were disqualified before or during the study for the following reasons: insufficient diagnostic information (10), died before PI arrived (10), wet form FIP (10; PI was provided on a compassionate basis), treatment was stopped after one to two doses (2), overseas cases (2; PI was provided on a compassionate basis), incorrect diagnosis (2; PI was provided to one of the two cats on a compassionate basis), cats that were lost to follow-up because the veterinarian never provided any information after PI was shipped (5) and one cat enrolled in error, i.e., started the treatment prior to March 1, 2010 (1). The accepted cats were evaluated and treated by veterinarians throughout the USA (58), and in Canada (2) in their practices. Fewer than 32%

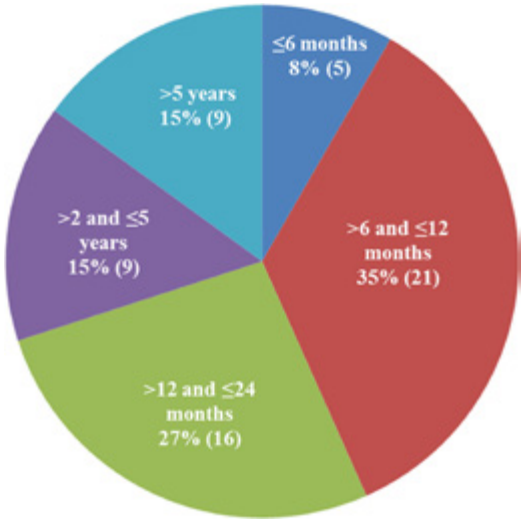

Figure 2. *Age distribution of the qualified patients. The majority of cats diagnosed with dry feline infectious peritonitis were under 2 years of age (70%).*

(19 of 60) of the cats were diagnosed at 10 days or less before the start of PI treatment. The remaining 41 cats were diagnosed with dry FIP 11 or more days before the beginning of treatment with PI. The mean time span between the diagnosis and the treatment was 22.97 ± 21.60 days. The cat that died after the administration of the first dose was diagnosed 161 days before the start of PI treatment.

Diagnostics

Signalment

There were 25 female, 1 hermaphrodite, and 34 male cats; 38 were non-purebred and 22 were purebred (5 Ragdoll, 4 Siberian, 3 Bengal, 3 Maine Coon, 2 Siamese, 2 Sphynx, 1 Tonkinese, 1 Manx, and 1 Birman). Data on the household were provided for 30 cats; 22 of the 30 were from multi-cat households and 8 from single-cat households. The age distribution of the 60 cats is shown in **Figure 2**. Seventy percent of the cats were under 24 months old, and 43% were under a year of age.

Clinical Signs

Fifty-nine of the 60 cats accepted into the study had clinical signs of FIP listed on the algorithm proposed by Addie *et al*. (2). The presence of clinical signs caused the cats' owners to bring the cat to their primary veterinarian for examination. During this initial examination, the clinical signs were documented in medical records. **Figures 3A,B** show the clinical signs and their distribution in the cats accepted into this study. Fifty-four of 59 cats showed two or more clinical signs (**Figure 3B**) with five cats having one sign. Of the five cats with one sign, 2/5 were neurologic, 1/5 ocular, 1/5 was evaluated because of persistent vomiting (an abdominal mass in the ileocolic region was discovered at the examination), and no data of a comprehensive initial examination were provided for one whose abdominal mass was discovered at physical examination.

One cat was included in the study that had no clinical signs when seen for neutering. This 8-month-old, male Siberian cat had a 10.7 g/dL serum total protein on a pre-anesthesia screening. The albumin was 2.1 g/dL, and the globulin was 8.6 with an A/G ratio of 0.24. The serum electrophoresis was interpreted as polyclonal gammopathy. The cat was anemic with a hematocrit of 23%. The coronavirus antibody titer was positive at 1:1,600, and the feline leukemia antigen was negative and the feline immunodeficiency antibody titer was negative. Thoracic radiographs showed an increase in cranial mediastinal density, and enlarged mesenteric lymph nodes were seen on abdominal ultrasound. Lymph node aspirates showed an increase in neutrophils on aspirate cytology.

Fifty-nine of the 60 cats on the study were categorized into one of five subforms of dry FIP based on the initial clinical signs, physical examination findings by primary veterinarians (and specialists where applicable) and diagnostic workup. One cat had no clinical signs. The subform categories were distributed as follows: mixed (18/59), gastrointestinal (16/59), non-localized (11/59), ocular (9/59), neurologic (5/59).

At the initial presentation, the cats in the gastrointestinal category had anorexia (15/16), diarrhea (4), and/or vomiting (3), which were the primary

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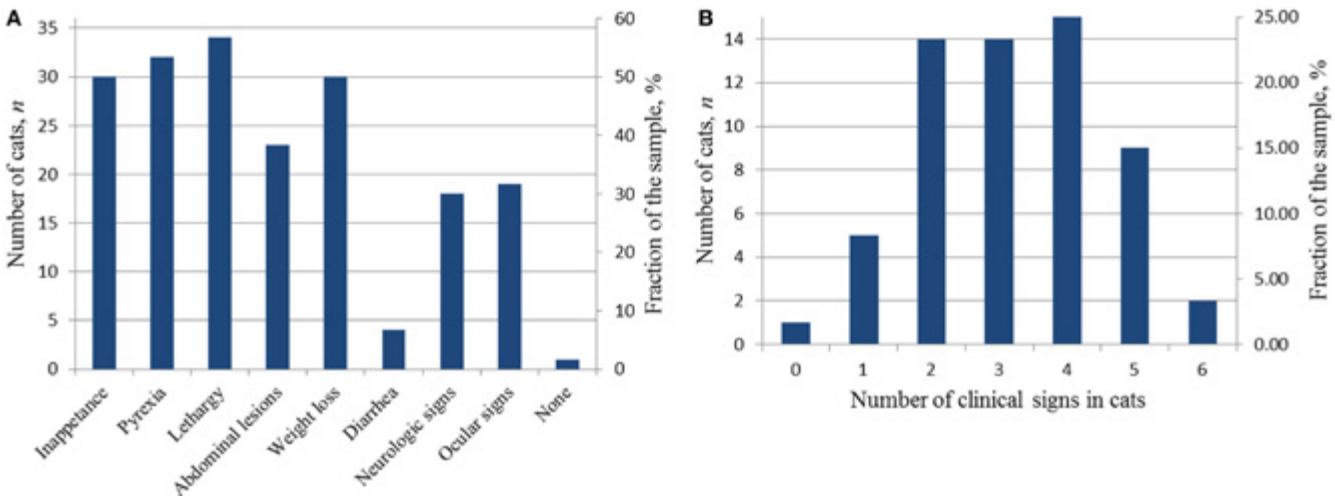

Figure 3. *Diagnostic clinical signs in the cats accepted into the study at the time of the initial presentation. (A) Distribution of clinical signs in the study sample; (B) distribution of the number of clinical signs in individual cats.*

reasons for the veterinary visit. Abdominal masses were found in 23/59 cats in the study, and the cats were categorized into either the gastrointestinal or mixed subform depending on whether they had additional clinical signs more often associated with another subform.

Ocular changes were reported in 17 cats and included anterior uveitis (17/17), retinal detachment (2/17), and keratic precipitates (4/17). One cat had a corneal ulcer. In 10/17 cats, ocular signs were the reason for the initial veterinary visit, and the cats were assigned to the ocular subform. The other 7 cats with ocular signs also had neurologic, abdominal, or non-localized signs and were classified as having mixed form. Neurologic signs, such as seizures, ataxia, and disorientation, were the main reason for the veterinary evaluation in 5/59 cats, and they were categorized as neurologic subform. The "non-localized" category (11/59) included cats with persistent fever uncontrollable-with-antibiotics, lethargy, anorexia, and/or weight loss. The mixed subform cats had simultaneous signs from two or more subcategories, such as ocular combined with neurologic signs (e.g., uveitis and seizures).

Hematology, Serology, Differential Testing

Results of hematology tests from blood drawn during the initial examination are shown in **Table 4**. The blood testing data were unavailable for nine cats initially diagnosed based on histology and cytology findings consistent with FIP which were accepted as diagnostic. Two cats had serology and hematology tests done after the acceptance and the start of the treatment, and those later data are not included on the table. In some cases the testing did not include items of interest, thus the data sets are not complete.

Jaundice was observed in one cat whereas hyperbilirubinemia was noted in 8/50 cats. Anemia (HCT < 29%) was observed in 28 of 49 cats. Increased WBC counts were noted in 20/50, neutrophilia was observed in 24/44, and lymphopenia in 16/48 cats.

Hyperglobulinemia and/or an albumin/globulin (A/G) ratio ≤0.6 were noted in 48 of the 50 (96%) cats; two cats had A/G ratio equal to 0.8. The mean and SD of the value in the whole group was 0.37 ± 0.14, and the spread is shown in **Table 4**.

The antibody titers were tested by IFA in 49 cats and, on the scale proposed by

Measurement	Reference interval	Data sets received, *n*	Mean	WNL, *n* (%)	Below normal, *n* (%)	Above normal, *n* (%)
Albumin, g/dL	2.3–3.9	51	2.5 ± 0.5	32/51 (62.7)	19/51 (37.3)	0
Total protein, g/dL	5.9–8.5	51	9.8 ± 1.5	12/51 (23.5)	0	39/51 (76.5)
Globulins, g/dL	3.0–6.6	51	7.3 ± 1.6	18/51 (35.3)	0	33/51 (64.7)
A/G ratio	0.4–0.8	50[a]	0.37 ± 0.14	See the breakdown below:		
	<0.8			2/50 (4.0)[b]	48/50 (96.0)	0
	<0.6				36/50 (72.0)	N/A
	<0.4				32/50 (64.0)	N/A
Total bilirubin, mg/dL	0.0–0.4	50	0.5 ± 1.1	42/50 (84.0)	N/A	8/50 (16.0)
WBC, 10^3/µL	4.2–15.6	51	15.6 ± 10.6	27/51 (52.9)	1/51 (2.0)	23/51 (45.1)
HCT, %	29–45	49	29.4 ± 6.9	20/49 (40.8)	28/49 (57.1)	1/49 (2.0)
Neutrophils	Varies	44	N/A	19/44 (43.2)	1/44 (2.3)	24/44 (54.5)
Lymphocytes	Varies	48	N/A	32/48 (66.7)	16/48 (33.3)	0

Seven animals were accepted based on the results of histopathology and cytology, and hematology results for two cats were received after the start of the treatment.
[a]In one cat, concentrations of individual fractions were provided in lieu of the total globulin level and showed markedly elevated serum globulins and gammopathy. In another cat, no albumin levels were provided.
[b]A/G ratio was 0.8 in two cats; no cat had A/G ratio above 0.8.
WNL, within normal limits.

Table 4. *Hematology test results at the first presentation considered in the feline infectious peritonitis diagnosis.*

Addie *et al*. (17), were ranked from high positive (400–1,280; n = 13) to very high positive (>1,280, n = 36); 10 of the 49 had titers > 12,800. In the two cats with low positive titers (100), the diagnosis was confirmed by immunostaining of biopsied lesions for the FCoV antigen. The 7b ELISA was used in three cats. The 3c PCR test done in three cats and showed negative results in two; these two cats were retested by IFA with positive results and also had the diagnosis by cytology.

We collected data sets on differential testing on 50 cats; the tests included FeLV antigen (45), feline immunodeficiency virus (FIV) (45), and toxoplasma (18). Except in one cat who was FIV positive, all other test results were negative. No serologic data were available for 10 cats. In the group with probable diagnosis with four missing data sets, one cat was diagnosed by neurologist based on the results of spinal tap and MRI, and another one had uveitis as a 3.3 lb 7-month Maine Coon kitten. In the group with the diagnosis confirmed by histologic, cytologic, and immunochemical methods, of the six cats without differential data sets, four were diagnosed by IHC and two by histology on the biopsied tissues.

Specialized Laboratory Testing

Specialized laboratory testing methods used to confirm the dry FIP diagnosis are summarized in **Table 5**. Histology and cytology were performed in 36 cats and were conclusive in 34, they were further validated by immunostaining for FCoV antigen in 13/36 cats. One cat with pyogranulomatous mesenteric lymphadenopathy on lymph node aspirate also had elevated FCoV transcripts in the RT-PCR test on the same sample.

Three cats with neurologic disease had the diagnosis confirmed by CSF tap, and two of those had MRI results consistent with the FIP diagnosis. For one cat with ocular form ocular centesis followed by quantitative PCR confirmed the presence of high titers of FCoV subgenomic mRNA of the M gene.

Necropsy was done of 15/60 cats. In 3/15, the necropsy was done of cats whose diagnosis was previously confirmed antemortem by histology or cytology. Histopathological analysis of the necropsied tissues was conclusive for FIP for 14/15 cats and inconclusive for the 965-day survivor.

Concurrent Treatments Used in the Study

Of the 60 cats accepted into the field study, 13 received PI as the only treatment; the other 45 cats received treatments before the enrollment and/or concurrently with the PI including one or more of appetite stimulants, antiemetics, antibiotics, corticosteroids, vitamins, and/or special diets. There were no data on concurrent treatments for two cats.

Sixty-two percent of the cats (36/60) were prescribed corticosteroids orally at the time of the initial diagnosis (27/36),

Test type	Other tests on the same cat	Total tests, *n*		Total histology, cytology, necropsy, *n*	Total CSF tap, *n*	Total ocular centesis, *n*	Total imaging, *n*
		By test	Total				
Cytology on fine needle aspirate		1[a]	24	36			
Histology	Only	8					
	+IHC	12					
	+Necropsy	2					
	+IHC+necropsy	1					
Necropsy	Only	12					
CSF tap cytology	Only	1			3		
	+MRI	2					
Q-PCR (m-gene mRNA) on aqueous humor		1				1	
Thoracic and abdominal imaging (X-rays and ultrasound)	Only	3					6
	+Histology or cytology or necropsy	3[c]					
Consistent with FIP				34/36	3/3	1/1	3/3[c]
Inconclusive				1/25[a]	0	0	0
Inconsistent with FIP				1/25[b]	0	0	0

[a]*Low cellularity.*
[b]*Necropsy results inconsistent with FIP; details are provided in the text.*
[c]*The count is included into histology, cytology, necropsy total (n = 36).*

Table 5. *Specialized tests used in support of the diagnosis.*

topically (ocular, 7/36), or both (2/36). In 4/36 cats; the corticosteroid treatment was stopped before or shortly after beginning the treatment with PI. Statistics on the use of corticosteroids are presented in **Table 6**. During the study, 31 cats received corticosteroids concurrently with PI (7 ocular topical and 25 systemic or systemic with ocular topical), and 27 cats were treated with PI without concurrent corticosteroids.

The four cats whose corticosteroid treatment was stopped in compliance with the requested test protocol before or at the start of PI treatment all had clinical signs consistent with FIP: weight loss (4/4), ataxia (1/4), abdominal masses (3/4), pyrexia (2/4), lethargy (2/4), anorexia (2/4) with the number of clinical signs from 2 to 5. Their laboratory tests also supported the diagnosis.

Two veterinarians started corticosteroid treatment when the cats deteriorated, one at 9 and at one 24 days before death; these cases were end-of-life care and were not counted as corticosteroid receivers. One cat was given one dose of nelfinavir the day before dying by her veterinarian owner.

Duration of Survival Post-Diagnosis and Clinical Progress

Duration of survival was determined as the time from the start of the PI treatment to death or euthanasia. Of the 60

Form	Treatment	*n*	Survival, days mean ± SD	Survival range, days
Ocular	PI	3	99.33 ± 109.77	32–226
	PI + ToCS	2	185.50 ± 159.10	73–298
	PI + SyCS	4	67.75 ± 80.19	6–184
Neurologic	PI + SyCS	5	38.80 ± 38.21	5–100
Gastrointestinal	PI	11[a]	252.45 ± 533.25	15–1,829
	PI + SyCS	5	53.20 ± 75.60	7–185
Non-localized	PI	6[b]	268.83 ± 363.00	4–965
	PI + ToCS	1[c]	60	60
	PI + SyCS	4	11.00 ± 13.47	7–31
Mixed	PI	8[b]	77.38 ± 94.87	1–131
	PI + ToCS	6	32.67 ± 28.49	4–39
	PI + SyCS	4	17.25 ± 16.46	6–77
No signs	PI	1	148	148

PI, treated with Polyprenyl Immunostimulant, no concurrent corticosteroid treatment; PI + SyCS, treated with Polyprenyl Immunostimulant and systemic corticosteroids concurrently (includes 1 combined systemic and topical ocular corticosteroid treatment); PI + ToCS, treated with Polyprenyl Immunostimulant and topical ocular corticosteroids concurrently.
[a]*Includes two cats whose corticosteroid treatment was stopped at the start of PI treatment.*
[b]*Each number includes one cat whose corticosteroid treatment was stopped at the start of PI treatment.*
[c]*The cat initially diagnosed with non-localized form started ocular topical corticosteroid treatment after uveitis developed.*

Table 6. *Survival time by the subform of the disease. No statistically significant differences were observed between any groups.*

cats treated with PI, 16 survived for over 100 days, 8 cats survived for over 200 days, 4 cats survived for over 300 days (one additional cat survived for 298 days and is not counted here), 2 for over 900 days, and 1 cat for 1,829 days.

The survival times of the cats in the three groups, i.e., (1) treated with oral corticosteroids concurrently with PI, (2) treated with topical ocular corticosteroids concurrently with PI; and (3) treated with PI without concurrent use of corticosteroids were significantly different from each other (p = 0.03,

Kruskal–Wallis test). **Table 7** lists all statistical data, and the survival curves are presented in **Figure 4**. For cats who did not receive corticosteroids the median survival time was 73.5 days (n = 27). For the cats that received corticosteroids by any route concurrently with PI, the median survival time was 21.5 days (n = 31). The difference in survival times between the groups (corticosteroid receiver versus non-receiver) was significant (p = 0.003, Mann–Whitney U-test). There was no significant difference in the survival times of the cats treated with corticosteroids systemically or topically (p = 0.57, Mann–Whitney U-test). The four cats whose initial corticosteroid treatment was stopped before the treatment with PI survived for 79, 91, 279, and 1,829 days. No data on concurrent treatments were available for two cats diagnosed by histology (1) and histology with IHC (1).

For the cats receiving PI alone and whose diagnosis was not confirmed by cytology, histology (ante- or post-mortem) survival times were 261.1 ± 329.7 days (4–965 days, median 148 days, n = 7); the cats who were treated with PI and corticosteroids concurrently with PI survived 52.8 ± 74.2 days (3–298 days, median 22 days, n = 19). The difference in the survival between the cats treated or untreated with corticosteroids concurrently with PI was significant (p = 0.03).

Treatment	*n*	Survival statistics, days				*p*-value (Mann–Whitney *U*-test)
		Range	Median	Mean	SD	
All study cats (*n* = 60)						
No concurrent corticosteroids	27	3–1,829	73.5	201.4	378.6	0.003
Concurrent corticosteroids:	31	3–298	21.5	47.5	49.3	
systemic	24	3–185	16	40.5	71.4	0.57
topical	7	4–298	30	51.2	103.3	
No data on concurrent treatments	2	1–15	N/A	8	9.9	N/A
Diagnosis confirmed by specialized tests on biopsied tissues (*n* = 34)						
No concurrent corticosteroids	20	3–1,829	63	180.5	400.0	0.03
Concurrent corticosteroids	12	4–185	20.5	38.9	51.3	
No data on concurrent treatments	2	1–15	N/A	8	9.9	N/A
Diagnosed without confirmation on biopsied tissues (*n* = 26)						
Necropsy/cytology inconclusive, no concurrent corticosteroids (*n* = 2)	7	4–965	148	261.1	329.7	0.04
No concurrent corticosteroids (*n* = 5)						
Concurrent corticosteroids	19	3–298	22	52.8	74.2	

Table 7. *Survival of cats treated with or without corticosteroids concurrently with Polyprenyl Immunostimulant by the method of the diagnosis.*

Cats whose diagnosis was confirmed by any type of analysis of biopsied or necropsied samples and treated with PI without concurrent corticosteroids survived 180.5 ± 400.0 days (3–1,829 days, median 63 days, n = 20); the cats treated with PI and corticosteroids concurrently survived 38.9 ± 51.3 days (4–185 days, median 20.5 days, n = 12). Two cats with inconclusive results of cytology (1) and necropsy (1) were accounted for as unconfirmed by those methods. The difference in the survival between the cats treated or untreated with corticosteroids concurrently with PI was significant (p = 0.04). A subgroup in which the diagnosis was also confirmed by IHC included eight cats treated with PI only (survived 8–1,829 days) and four cats treated with PI concurrently with corticosteroids (survived 7–185 days). No concurrent treatment data were available for one cat who survived one day. The size of those subgroups was insufficient to render statistical power to the analysis.

There was no significant difference in survival times between groups treated with PI only and diagnosed without biopsy-based tests (n = 20) versus those diagnosed with biopsy-based tests (n = 7; p = 0.27, Mann–Whitney U-test). Similarly, there was no difference in survival times between groups treated with corticosteroids concurrently with PI, which were diagnosed with tissue

biopsies (n = 12) or without it (n = 19; p = 0.93 Mann–Whitney U-test).

No significant difference was found between the survival times for the cats receiving oral (n = 24) or topical ocular corticosteroids (n = 7, p = 0.57, Mann–Whitney U-test); and the median survival times were 16 and 30 days, respectively. No significant differences were noted between survival times of cats with different subforms of the disease regardless of the use of steroids (**Table 6**). Survival times of cats belonging to age groups under 6, 7–12, 13–24 months, and over 25 months did not differ significantly (p = 0.90, Kruskal–Wallis test).

After the beginning of treatment, the non-effusive form progressed to effusive in six cats (10%), and five of those died or were euthanized within 2 weeks thereafter. One cat (#31) whose initial corticosteroid treatment was stopped at the beginning of PI trial developed palpable abdominal masses and effusion after 3 months of the treatment, which resolved by the next monthly visit. After 6 months on the treatment, a small mass was palpated and a small amount of fluid in mid-cranial abdomen was identified per the veterinarian's records. The mass remained unchanged for the next two monthly visits, and the cat developed a distended belly with palpable fluid by the eighth month of the PI treatment. The amount of the fluid decreased by the next monthly check. Between 1 and 8 months on the treatment, the cat was doing clinically well, gained weight, returned to normal routines, played, and had an appetite. The cat started declining in the ninth month of the PI treatment, lost weight, and there was an increased amount of abdominal fluid, and the cat died naturally after 279 days from the start of the treatment.

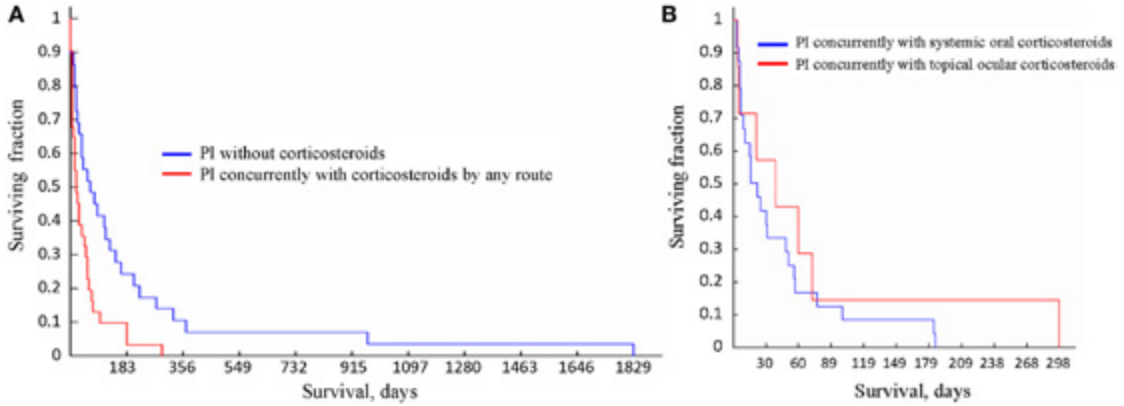

Figure 4. *Survival curves for the cats receiving different treatments. (A) Survival of the cats treated with Polyprenyl Immunostimulant (PI) without concurrent corticosteroids (blue) was significantly longer (p = 0.003, Mann–Whitney U-test) than of the cats treated PI with corticosteroid administered concurrently by any route (red). (B) Survival of the cats treated with PI with concurrent corticosteroid administration topically (red) or orally (blue) did not differ significantly (p = 0.57, Mann–Whitney U-test).*

	Cat #2 (A)	Cat #52 (B)	Cat #78 (C)	Cat #105 (D)
Age at Dx	11 years	12 months	6 months	3 years
Sex	FS	FS	MN	MN
Breed	DMH	Bengal	DSH	DLH
Housing density	2 cats	>3 cats	>20 cats	>3 cats
Household and FIP history	Not known	Previously lost 3 cats to FIP	Rescued by a rescue with FIP outbreak. Foster queen died of FIP	Not known
Keeping condition	Inside	Inside	Inside	Inside
Survival time, days	**375**	**334**	**965**	**1,829**
Initial presentation and diagnostics				
Days between initial visit and the start of PI	36	23	19	31

(*Continued*)

Table 8. *Case summaries for the cats with feline infectious peritonitis (FIP) surviving over 300 days on Polyprenyl Immunostimulant (PI) treatment.*

Table 8. *Continued*

	Cat #2 (A)	Cat #52 (B)	Cat #78 (C)	Cat #105 (D)
Diagnostic signs	Frequent vomiting, persistent diarrhea, weight loss. Not active. Palpable abdominal mass	Weight loss, vomiting, persistent diarrhea with occasional blood streak	URI, weight loss, weakness, possible diarrhea (too many cats to tell), poor appetite	Sudden weight loss, abdominal mass noted on ultrasound
Additional clinical signs	CRF (Dx 1 month after FIP)	None	Submandibular lymphoadenopathy, gingivitis, conjunctivitis	Mild gingivitis at the end of life
Differential	FIV-neg, FeLV-neg, toxoplasma-neg	FIV-neg, FeLV-neg, toxoplasma-neg	FIV-neg, FeLV-neg, toxoplasma-neg	FIV-neg, FeLV-neg, toxoplasma-neg
Albumin, g/dL	2.3	2.6	2.6	2.3
Tp, g/dl	9.3	10.8	8.3	8.8
Globulin, g/dl	7.3	8.2	5.7	6.5
A/G ratio	0.3	0.3	0.5	0.4
Bilirubin, mg/dL	0.1	0.1	0.1	0.4
WBC cells/uL	23,400	22,600[a]	20,300	11,700
HCT, %	30.9	31.0[a]	8.4	33 (N 32–49)
Neutrophilia	YES	YES[a]	YES	NO
Lymphopenia	YES	YES[a]	YES	YES
Monocytosis	YES	NO[a]	NO	NO
Feline coronavirus (FCoV) titer	1:6,400 (ELISA IFA)	1:320 (7B ELISA)	1:1,600 (ELISA IFA)	1:800 (ELISA IFA)
FIP subtype	GI	GI	Non-localized	GI
Specialized laboratory testing and findings	Ultrasound, resection, and anastomosis of ileocecal region. Biopsies had pyogranulomatous reaction, IHC was FCoV antigen-positive	Not done	Not done	Polyclonal gammopathy, FIP mRNA-, ultrasound, FNA, biopsy. Histopathology revealed pyogranulomatous lymphoadenitis and pancreatitis. IHC positive for FCoV antigen
Concurrent medications	Calcitrol 10 mg orally daily, Sucralfate PRN, Metoclopramide PRN	Metoclopramide PRN	PI treatment stopped after about 700 days	PI was tapered to 2× weekly after 3 years and to once weekly after 4 years
Progress on the PI treatment				
Hyperglobulinemia and A/G ratio	No major change	No major change	Changed to WNL after 2 months	Decreased to normal range after 1 month
Anemia	No. Before death only	No. Last test 40 days before death	Hematocrit increased to >30% after 2 months on PI. No data after 700 days	Increased to >35% after 2 months on PI, decreased before death
Diagnostic clinical signs	Resolved	Resolved	Resolved	Resolved
Life quality	Improved and returned to normal	Returned to normal	Improved, stable (records until the end of PI treatment). Cystitis Dx after 600 days on PI	Returned to normal
Cause of death	Euth: weight loss, lethargic, dehydrated, trouble breathing	Euth: inappetance, lethargy, weight loss, fever	Euth *in extremis*	Euth: anorexia, vomiting, severe azotemia indicating kidney failure or severe trauma from infection or toxin/ischemic injury. No abdominal mass on ultrasound before death
Necropsy	Pleural effusion, small mesenteric mass, close to the resection site. Multifocal granulomatous colitis and hepatitis consistent with FIP	Not done	Mild changes consistent with prior hepatic injury and nephritis, cystitis cystica	Not done

[a]*Tested 9 days after the start of the PI treatment.*

CRF, chronic renal failure, chronic renal insufficiency; Euth, euthanasia; FCoV, feline corona virus; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; FNA, fine needle aspirate; IHC, immunohistochemistry; URI, upper respiratory infection; WNL, within normal limits.

Three of the 17 cats with ocular disease survived for over 180 days. Their initial signs included anterior uveitis (3), keratic precipitate (1), discoloration (1), anisocortia (1). In two of those cats the anterior uveitis was significantly improved or resolved after 2 months on PI treatment with no corticosteroids. The uveitis did not improve in the one cat who was receiving topical ocular corticosteroids concurrently with PI, and the eyes were enucleated.

In the 13/22 cats with palpable abdominal masses that survived over 30 days (life span 57–1,829 days), the reduction or resolution of the abdominal masses was noted in 6/13 during the first or second monthly follow-up examinations, three of the six cats received corticosteroids together with PI. One of the six cats (#31, described above), whose initial neurologic signs resolved and palpable masses were no longer reported after 1 month of the treatment, redeveloped a small palpable abdominal mass and abdominal fluid at 6 months into the treatment and 3 months prior to her natural death at 279 days; the corticosteroid treatment of this cat was stopped before PI treatment began. In another cat who received no corticosteroids, the masses were initially resected and did not reappear until 1 month prior to euthanasia at 374 days. The masses remained unchanged on palpation in 4/13 cats; all four cats received no corticosteroid treatment. The findings were confirmed by ultrasound tests in one of those four, two cats died before the follow-up examination, and follow-up data were not available for one cat.

Four cats survived over 300 days and were considered long term survivors. Their records were scrutinized in considerable detail after death. The summary of the data for these cats is presented in **Table 8** and **Figure 5**. All four cats were brought initially to their veterinarians with signs typical of dry FIP, including inappetance (3/4), lethargy (3/4), and weight loss (4/4). One cat had persistent diarrhea and vomiting; in two of the four cats, abdominal masses were detected by their veterinarians. The A/G ratios for all four cats ranged from 0.3 to 0.5. Three of the four cats were anemic, and all had moderate to high coronavirus antibody titers of 1:320 (K-ELISA), 1:800, 1:1,600 and 1:6,400 (all three by IFA). In two of the four cats, histopathology of biopsied tissues had pyogranulomatous inflammation, and the diagnosis was validated by immunostaining for FCoV antigen. Cat 105, the longest survivor (1,829 days), initially received prednisolone to control the weight loss but the corticosteroid therapy was discontinued at the start of PI treatment. Based on reports by owners and veterinarians, all four cats returned to normal behavior by the first checkup visit, about 1 month from the beginning of the treatment, and the weight either stabilized or increased. The clinical signs of vomiting, diarrhea, lethargy, etc. also resolved. Three cats lost weight before death; data were unavailable for one. The A/G ratios increased after 2 months on the PI treatment in the two longest survivors, and reached >0.8, and anemia improved in one and resolved in another one. The borderline anemia, although somewhat improved, was present in the Cats 2 and 52 survivors and became more pronounced before death. The treatment was stopped after about 700 days in Cat 78, and no laboratory test data are available from that time until his death at 965 days when necropsy was performed. The PI treatment frequency was tapered first to twice weekly and then to once weekly in Cat 105. All cats were euthanized in extremis. The necropsies on the Cat 2 showed lesions consistent with FIP. The necropsy on Cat 78 showed lymphoplasmacytic interstitial nephritis and cystitis cystica without lesions of FIP. Toxicosis, azotemia, and ischemia probably associated with renal failure led to the euthanasia of Cat 105. The ultrasound showed no masses on the internal organs and the results were unremarkable; no necropsy was performed.

A number of veterinarians and owners voluntarily provided information on

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the quality of life in communications, and veterinarians commented from the first recheck on after 30 days of PI treatment using the questionnaire. More quality-of-life-related comments were recorded by veterinarians on medical records which were analyzed. We found improved quality-of-life comments for 32 of the 34 cats that survived for more than 30 days. All comments for those 32 cats indicated an improvement in the perceived quality of life ("return to normal," "as before the disease," "appears healthy," got back to normal routines, and/or improvement in appetite, mobility, socialization, and responsiveness) during the preceding period, most commonly every month on the treatment. Weight stabilized or increased in 31/32 patients, while 1/32 continued to lose weight while showing improvement in behavior and appetite.

No toxicity or adverse events due to the administration of PI were reported by the veterinarians or owners.

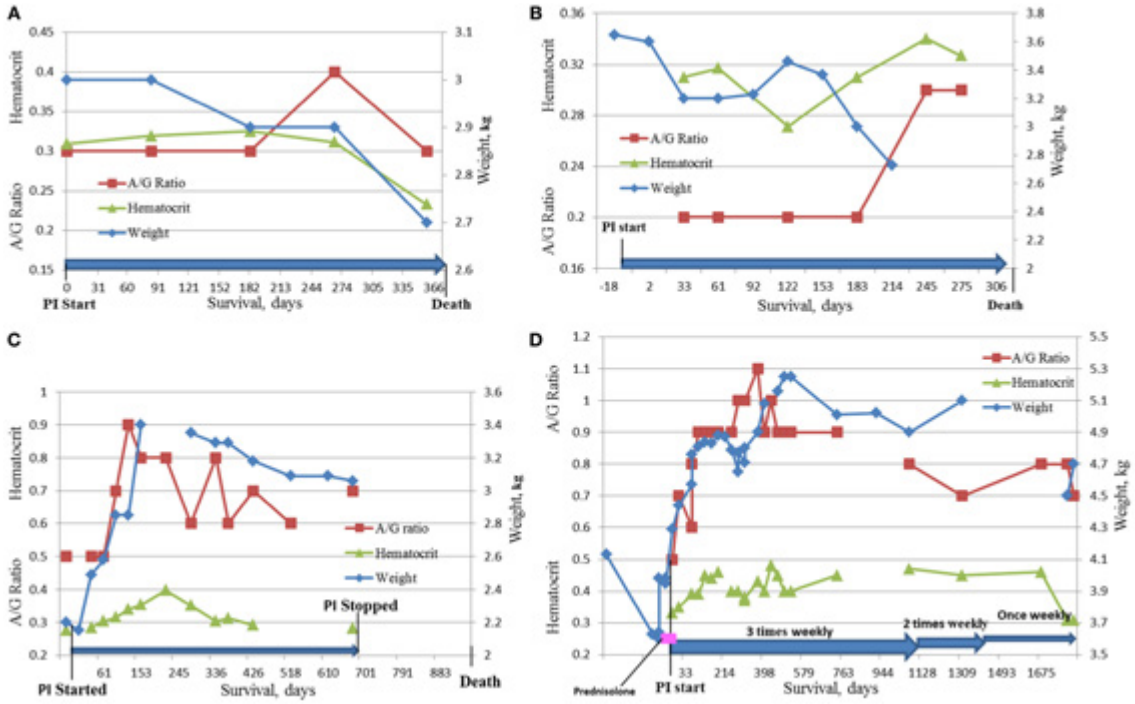

Figure 5. *Dynamics of HCT, A/G ratio, and weight in the four longest survivors on the study. (A) Cat #2, 375 days survival; (B) Cat # 52, 334 days survival; (C) Cat #78, 965 days survival; (D) Cat #105, 1,829 days survival.*

Discussion

We report that of the 60 cats with presumed non-effusive FIP diagnosed using the recognized algorithm (2) and treated with PI 1 cat survived for 1,829 days, 2 cats for over 900 days, 4 cats survived over 300 days, 8 for over 200 days (one of those survived 298 days), and 16 lived over 100 days from the start of treatment. The 31 cats given oral corticosteroids or receiving topical ocular corticosteroids concurrently with PI survived a mean of 47. 5 ± 49.3 days (3–298 days, median 21.5 days), while the 27 cats treated with PI without corticosteroids survived a mean of 201.4 ± 378.6 days (3–1,829 days, median 73.5 days), which is significantly longer (**Table 7**; **Figure 4A**). Currently the most common therapy for FIP is corticosteroid (2, 4).

Of the 35 cats started on corticosteroids at the time of diagnosis, the treatment was stopped in 4 cats before the start on PI, while 31 were continued on corticosteroids concurrently with PI. The four cats whose corticosteroid treatment was stopped survived a mean of 569.50 ± 844.65 days (79–1,829 days, median 91 days). These four cats had multiple signs of the disease, and none of the four cats at the time of diagnosis appeared less severely affected than the other cats in our study.

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In the subgroups of FIP, there was a difference in the likelihood of corticosteroid use, with 31% (5/16) in the GI subform of FIP receiving corticosteroids to 100% (5/5) in neurologic subforms receiving corticosteroids. Both topical and ocular corticosteroids appeared to reduce survival times when given concurrently with PI (**Figure 4B**).

We accepted all cats that met the inclusion criteria and did not intentionally select less severe cases for the study. No assessment of the severity of the disease was done. Our first assumption was that corticosteroids were used in the more severe cases but we could find no justification for that assumption. But the small sample size does not allow ruling out that the shorter survival times of the cats concurrently treated with PI and corticosteroids may indirectly reflect the severity of the disease.

We observed no statistically significant differences between survival of the cats either for the subforms of the disease (ocular, neurologic, gastrointestinal, non-localized, or mixed), or between different age groups (≤6 months, 7–12 month, 13–24 months, and over 25 months) which may be because of a very wide variance in each of the groups. We did not have a sufficient number of cats in each group for the statistical power to validate any conclusions.

The literature offers limited data on survival times of cats with dry form FIP with or without treatments. Cats in the literature with dry form FIP treated with corticosteroids and supportive care had a survival range of 1–200 days (n = 51). One early study (8) examined field records for intestinal, granulomatous manifestation (n = 26), and reported the survival time in this non-effusive FIP form as "up to 9 months" in a cat that was lost to follow-up. More careful retrospective record studies reported the survival time at 7–45 days in cats with histologically confirmed diagnosis (8 effusive, 5 non-effusive, no separate data provided; 9), and 7–60 days (n = 4, 1 of 4 was on IFNα-rHU; 10). Reports of clinical studies in natural infection put survival with dry FIP at 38 days (n = 1; 11), 1–171 days (n = 11; 6), 6–33 days without treatment (n = 4), and 4–42 days in cats previously given an FIP vaccine as a preventative (n = 4; 13).

The literature mentions an individual cat diagnosed with the dry form that survived for 200 days with glucocorticoid and ω-interferon treatment (6) and two cats survived 181 and 477 days with mixed dry/wet form treated with glucocorticoid, human α-interferon, and nelfinavir (7). A recent mention (18) of a natural survival time of over a year without treatment in cats with dry form FIP referred to cats that were treated with PI (15).

The survival times from this study were compared to published data of cats with dry form FIP. The data suggest a lengthening of the survival times in cats treated with PI. The survival times equaled or exceeded the longest survival times reported in the literature for dry FIP, with 8 of 60 cats in our study exceeding the maximum reported number of 200 days.

The diagnosis of dry FIP in our field study was done at different levels of diagnostic certainty, and we compared survival times in the subgroups—diagnosed, or not, by histologic, cytologic, or immunostaining tests and found no statistically significant differences based on the proof of the diagnosis, although there were significant differences between cats treated with corticosteroids concurrently with PI or not. There was no significant difference in the survival times of cats who did not receive steroids regardless of whether they were diagnosed by any biopsy-based method or diagnosed without invasive methods. The survival time was the same regardless of the diagnostic method used.

A comparison of mean and SD values for the survival times of cats with dry FIP showed that the survival time of cats in our study was significantly longer for the group treated with PI without concurrent corticosteroid treatment (201.4 ± 378.6 days, n = 27) than the published 38.4 ± 48.8 days (n = 11; 7); p

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= 0.04. The survival time for the group treated with corticosteroids concurrently with PI was 47.45 ± 49.26 days (n = 31), which does not significantly differ (p = 0.59) from the published value (7). Corticosteroids are the usual treatment for FIP signs; however, they need to be used with caution if PI is used.

Feline infectious peritonitis is considered 100% fatal which, in our study plan, precluded for ethical reasons an experimental design that used an untreated placebo group. For statistical comparison of data, a placebo group is ideal. This study was modeled on human studies that use single-arm trials of anti-cancer drugs with survival as the end-point. In those studies, no placebo or best accepted therapy is used because of the lack of known beneficial treatment and the universally grave prognosis (19). Our study was a field trial dependent on primary care veterinarians to identify candidates for the study and treat those accepted in accordance with the protocol. On ethical grounds and considering the reluctance of cat owners to participate in a placebo-controlled study, we elected the presented study design.

Our decision to exclude cats with wet form FIP was based on the rapid progression of the wet form (median of 9 days; 5) which we assumed correctly to be faster than the expected median time needed for acceptance of a cat into the study (which turned out to be 22.97 ± 21.60 days). Additionally, our prior limited studies of PI in effusive FIP did not appear promising while treatment of dry form FIP was encouraging.

The diagnosis of dry FIP was done at three levels of certainty: (1) highly probable, diagnosis supported by history, clinical signs, and laboratory findings without specialized lab tests; this category included neurologic forms in which biopsies are not possible and ocular forms when ocular centesis was used; (2) with histologic or cytologic confirmation of the diagnosis with or without immunostaining; this group also included cats with confirmation done on necropsied tissues; and its subgroup (3) with the confirmation of the presence of the FCoV antigen by IHC. There was no statistically significant difference in the survival between the cats diagnosed at different certainty levels and treated with PI only. We could not compare survival times between the subgroups treated with corticosteroids due to insufficient sample power.

Making an antemortem diagnosis of dry FIP is notoriously hard in the absence of a single, standardized test and relies on the combination of non-specific "disease-indicators" especially if the owners do not allow invasive procedures. Test data in clinical chemistry, hematology, serology, clinical assessment, histology, etc. have different predictive values and are not pathognomonic (2, 14, 18, 20). In the absence of a single, 100% reliable diagnostic approach, all diagnostic efforts are aimed to increase the "index of suspicion" as it was called by Diaz and Poma (21). The requirements for a "gold standard" vary between research groups with some accepting histopathology and others stressing IHC (14, 20), while the consensus document by Addie *et al*. (2) avoids the definition of the "definitive diagnosis" altogether. In clinical practice, most diagnoses are made by laboratory findings consistent with FIP and excluding other diseases (3, 18).

The primary care veterinarians and veterinary specialists treating these cats were comfortable with the diagnosis of dry form FIP, but we are using the term "presumptive FIP" because the "gold standard" of coronavirus antigen by IHC in lesions consistent with dry form FIP was not achieved with all cats. The cats in this study were diagnosed by their veterinarians based on the assessment of the combination of signalment, clinical signs, and as many tests as necessary to rule out other diseases and support the diagnosis, relying on experience (18), in the mode documented by Rohrbach *et al*. (22). The diagnosis, made by the primary care veterinarian in conjunction with specialists as needed, was reviewed by AML using crite-

ria consistent with the evidence-based algorithm (2). Specialized testing such as histology, cytology, CSF tap, PCR on aqueous humor, IHC, and necropsy was done in 36/60 cats. When the presumptive diagnosis is made, owners are often reluctant to get the confirmation through the invasive procedures.

The diagnosis for each cat on the study was established individually, and we also compared our data to the data published for cats with a confirmed diagnosis of FIP (1, 7, 20, 23). Signalment and housing density were similar to the reported data (23) with the majority of the cats being young, non-pedigreed, and originating from multi-cat households. The age distribution of the FIP patients in our study underscored a well-established age bias for FIP (12, 22). Clinical presentations observed in our sample were similar to the reported for the cats with dry form FIP (20, 23).

Diseases that may be clinically similar to FIP were excluded by specific tests in most cats. Feline leukemia virus (FeLV) antigen and FIV antibody were measured in 50/60 cats and one of those was positive for FIV antibodies. The FIP diagnosis of the only FIV-positive cat was given by a neurologist based on the results of MRI and CSF tap. Antibodies for toxoplasma were measured in 18 cats and all were negative. Ten cats were not tested for FeLV and FIV; six had the diagnosis confirmed by specialized tests on the biopsied tissues (four IHC and two histology), and the other four had a number of strong FIP indicators and considered presumptive.

We analyzed the initial biochemical parameters of diagnostic value for FIP. In young cats, with high serum globulin and low A/G ratios, there are few conditions except FIP that are likely. Plasma cell and B-lymphocyte malignancies can produce a monoclonal gammopathy, but these conditions are rare in young cats. Chronic inflammatory or infectious conditions such as chronic abscesses and pyothorax could increase serum globulin levels but these conditions would likely be identified by diagnostic evaluation. Hyperglobulinemia and/or an albumin/globulin (A/G) ratio ≤0.6 were noted in 48 of the 50 (96%) cats; two cats had A/G ratio equal 0.8. At the initial evaluation of the cats in our study, there was hyperglobulinemia in 64.7% of the cats, which is similar to the value reported in the literature in the combined wet-dry group (7). The albumin/globulin ratio offers the highest positive prediction value for FIP (20). In our group it was 0.37 ± 0.14, which is consistent with the reported values for FIP in general (20) and lower than the mean and SD reported for the initial presentation by Tsai *et al.* (7).

Hyperbilirubinemia occurred in 16% of the cats which is consistent with dry form FIP and is lower than reported in the groups with wet FIP (7, 23). Hyperglobulinemia is an important diagnostic sign because it is rarely associated with diseases other than FIP in young kittens, which is the most often affected group.

The initial hematologic parameters of diagnostic value for FIP were analyzed. Leukocytosis and neutrophilia were found in 45.1 and 54.5% of the cats, respectively; both values are consistent with the published (23). Lymphopenia was present in 33.3% of the study cats consistent with previous studies (23).

Coronavirus antibody titers are somewhat helpful in the diagnosis of FIP (2, 4, 17). Negative serum antibody titers make the diagnosis of FIP unlikely, while high and very high titers are supportive of the diagnosis. However, mid-range to high antibody titers can occur with coronavirus infection without FIP. All tested cats accepted to the study had coronavirus antibody titers with most of the cats having high (400–1,280; n = 13), very high (>1,280, n = 36), or extremely high positive titers (>12,800, n = 10).

The only cat on the study without signs of FIP was diagnosed by the veterinarian after a pre-neuter checkup. The follow-up cytology of the mesenteric lymph node aspirate was inconclusive because the cellularity was insufficient

to definitively demonstrate pyogranulomatous inflammation, although there was no evidence of neoplasia or other infectious organisms. The cat developed effusion and died after 148 days of the treatment with PI and corticosteroids concurrently.

The four cats who survived over 300 days had clinical signs and diagnostic tests consistent with FIP at initial examination. A detailed history and diagnostic information is given in **Table 8** and **Figures 5A–D**. Although cats 52 and 78 did not have histopathology or IHC, their age (12 and 6 months), exposure in multi-cat, FCoV environments, clinical signs, laboratory findings, and high FCoV antibody titers were sufficient to establish a clinical diagnosis. Cats 2 and 105 had their diagnoses confirmed by histology and cytology and IHC. All four showed clinical improvement and returned to normal behavior. Cats 2 and 52 had only modest improvements in laboratory findings despite clinical improvements (**Figures 5A,B)**. The clinical decline in those two cats started 2 weeks before their death and was accompanied by weight loss, anorexia, worsening anemia, reappearance of abdominal masses at the resection site (Cat 2), and pleural effusion in the same cat. Redevelopment of the abdominal mass and progression of the disease is common in the cats where the masses were resected (8).

The two longest survivors, cats 78 and 105, had weight loss at diagnosis but gained weight while on the PI treatment. The weight gain in cat 105 started 1 week before the trial and was attributed to prednisolone (5 mg daily). The prednisolone treatment was tapered and stopped during the first week of the PI treatment and the cat continued gaining weight. The clinical improvements and weight gain in both cats were accompanied by improvements in A/G ratios and hematocrits (**Figures 5B,C**). In both cats, PI treatment was either stopped or its frequency was decreased after over 2 years of survival. Cat 105 died of renal failure. Both cats declined at the end of life and were euthanized in extremis. The lack of pyogranulomatous changes in the biopsied tissues in cat 78 is consistent with the observation that cats experimentally infected with FIPV and who had clinical disease and survived were "free of lesions" post-mortem (14, 24). The necropsy changes of mild hepatic firbrosis and mild chronic lymphoplasmacytic interstitial nephritis are not adequate to account for the demise of the cat. There was no evidence of lesions of FIP. The A/G ratio of 0.5 at the initial diagnosis was returned to normal suggesting resolution of the FIP. No necropsy was performed on cat 105.

We did not use a formalized assessment of the quality of life. We collected information about clinical and behavior changes from progress reports, communications, and veterinarian charts. All progress reports and notes indicated an improved quality of life, returning to normal pre-diagnosis behavior as expressed in comments by both owners and veterinarians, e.g., "Very energetic … doing well," "acts normal," etc. In the medical records, cats were noted as having more energy, being more playful, interacting more with owners, and essentially returning to their pre-FIP behavior. In the 34 cats that lived for 30 + days, clinical improvement, i.e., an improvement in one or more signs such as increased appetite or an abatement of fever, was anecdotally noted after 10–14 days (four to six doses of PI); 10/34 reported weight gain, 5/34 reported weight loss, the weight remained stable for 17 cats, no records were filed for 2 cats. Generally speaking, the cats treated with PI returned to regular routines with occasional "bad days" until a precipitous decline led to death or euthanasia within days.

Our results suggest that PI benefits cats clinically diagnosed with dry FIP by increasing survival times and improving quality of life but a controlled study will be needed to verify the benefit of PI in the treatment of FIP. While not a cure, PI may maintain FIP cats as a chronic condition as opposed to the fast-progressing fatal disease. It may

Tabla de contenido:

be possible to predict and monitor the survival by the normalization of A/G ratio and hematocrit. Survival times with PI treatment are significantly longer when corticosteroids are not used concurrently.

Author Contributions

AL: conception and design of the work, interpretation of the data, revising the manuscript critically for important intellectual content, final approval of the version to be published. TK: post-study acquisition of the data, analysis and interpretation of data for the work, drafting the work and revising it critically for important intellectual content, final approval of the version to be published. GG: data acquisition, entry and organization, final approval of the version to be published. VB: data acquisition, entry and organization, revising the data and the manuscript critically for important intellectual content, final approval of the version to be published. RH: data analysis and interpretation, revising the manuscript critically for important intellectual content, final approval of the version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest Statement

AL, GG, VB, and RH do not have a financial interest in Sass & Sass, Inc. TK is an employee and a minor stakeholder in Sass & Sass, Inc. VB and RH were consultants to Sass & Sass. No financial incentives were provided to owners and veterinarians participating on the study.

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https://axoncomunicacion.net/tratamiento-inmunosimulante-de-poliprenil-de-gatos-con-peritonitis-infecciosa-felina-presuntiva-no-efusiva-en-un-estudio-de-campo/

Tabla de contenido:

La **dilución urinaria** es la **clave en el manejo de las urolitiasis**

La saturación de la orina es el principal factor de riesgo para la formación de urolitos, por tanto, será fundamental disminuir la concentración urinaria y favorecer la diuresis.

Aumentar el nivel de sodio y/o de humedad en la dieta, resulta eficaz para disminuir la sobresaturación relativa de la orina tanto de estruvita como de oxalato cálcico.

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A través de la alimentación se puede influir en la concentración urinaria y conseguir orina insaturada, que es la clave en el manejo de las urolitiasis. También se puede modificar el pH, que influye en la formación de algunos urolitos.

Dilución urinaria

La forma más sencilla de producir orina insaturada es favorecer la diuresis. El aumento del flujo urinario reduce la concentración de sustancias litogénicas e incrementan la frecuencia de la micción, lo que ayuda a eliminar los cristales libres que se formen en las vías urinarias.

Para estimular la diuresis es necesario potenciar el consumo de agua, bien administrando alimentos húmedos, que contienen de un 70 a un 80% de agua, añadiendo agua a la alimentación o incrementando ligeramente el contenido de cloruro sódico de los alimentos secos.

Se ha demostrado que este aumento del cloruro sódico alimentario incrementa tanto la ingesta de agua como la producción de orina y disminuye la sobresaturación urinaria en gatos y perros[1].

Sobresaturación relativa

La sobresaturación relativa (SSR) de la orina es un factor clave en la formación de cristales en el tracto urinario y una herramienta eficaz para valorar el riesgo de formación de cristales y cálculos.

Se trata de un método utilizado de manera generalizada en humanos y que también se ha validado para perros y gatos. Consiste en la evaluación del riesgo de formación de los diversos cristales urinarios determinando la concentración de doce electrolitos diferentes de una muestra urinaria, así como el pH. Los datos son analizados mediante un programa informático que calcula las concentraciones de gran número de complejos interactivos presentes entre los iones de la orina[2,3].

La SSR es específica de cada mineral y puede definir tres zonas de saturación diferentes.

- Zona de baja saturación: significa que la orina está insaturada. Cualquier cristal de estruvita que se añadan en este estado se disolverá. Aunque no es posible la dilución de cálculos de oxalato, tampoco crecerán ni se volverán a formar en animales predispuestos.
- Zona de saturación metaestable: a este nivel de saturación, no se formarán nuevos urolitos de oxalato cálcico o de estruvita espontáneamente, pero los urolitos de estruvita no se disolverán y pueden crecer.
- Zona de sobresaturación: la orina está sobresaturada y supone un ambiente inestable en el que puede producirse la formación espontánea de cristales homogéneos, agregación y crecimiento de estos.

Estos márgenes pueden obtenerse con una alimentación adecuada y se ha demostrado que es posible crear alimentos con efecto preventivo frente a la estruvita y, a la vez, contra el oxalato.

SSR y potencial de cristalización

LA ORINA PRODUCIDA ES	RIESGO DE FORMACIÓN DE ESTRUVITA	VALOR DE SSR DE LA ESTRUVITA	RIESGO DE FORMACIÓN DE OXALATO CÁLCICO (CaOx)	VALOR DE SSR DE CaOx
INSATURADA (ZONA DURANTE EL TRATAMIENTO Y LA PREVENCIÓN)	• No se formarán nuevos urolitos de estruvita • Los urolitos de estruvita se disolverán*	1	No se formarán nuevos urolitos de CaOx Los urolitos de CaOx presentes no crecerán	1
METASTABLE (ZONA DE PREVENCIÓN)	• No se formarán urolitos de estruvita • Cualquier urolito de estruvita presente puede crecer	2,5	No se formarán urolitos de CaOx Cualquier urolito de CaOx presente puede crecer	12
SOBRESATURADA (ZONA DE PELIGRO)	• Se pueden formar nuevos urolitos de estruvita • Los urolitos de estruvita presentes crecerán		Se pueden formar nuevos urolitos de CaOx Los urolitos de CaOx presentes crecerán	

No obstante, el empleo de cloruro sódico para estimular la sed y la diuresis ha suscitado cierta controversia, ante la posibilidad de que afectara a la excreción urinaria de calcio, a la presión arterial y a la función renal.

Sodio y excreción del calcio

En humanos, la ingesta elevada de sal se ha asociado con una mayor excreción de calcio urinario. En perros se hicieron inicialmente las mismas observaciones, lo que llevó a suponer que los alimentos enriquecidos con sodio podrían favorecer la urolitiasis por oxalato cálcico. Sin embargo, aunque la ingesta de sodio aumenta la excreción de calcio, la concentración de calcio no se incrementa debido al aumento concomitante del volumen urinario y lo que se observa es un descenso significativo de la SSR del oxalato cálcico[4].

Sodio y presión arterial

La relación entre el contenido de sal en la alimentación humana y la hipertensión es objeto de intensos debates. Por extrapolación también surgieron dudas en torno al efecto del sodio del alimento sobre la presión arterial del perro y el gato, y se llevaron a cabo distintas investigaciones al respecto.

Estudios realizados en perros sanos demostraron que incrementar la ingesta de NaCl de 8 a 120 _mol/kg no afecta a la presión arterial, lo que sugiere que los perros son insensibles a la sal[5].

Esto significa que, en perros no enfermos, la regulación del contenido corporal de NaCl por el riñón es un mecanismo eficiente y capaz de responder de manera apropiada a cambios en la ingesta de NaCl. Si bien cabría suponer que los perros con ERC podrían ser sensibles a la sal, los estudios experimentales realizados en perros con

azotemia inducida similar en grado a los estadios IRIS II y III indican que no es así[6].

En otro estudio experimental en gatos con azotemia inducida, similar en grado a los estadios IRIS II y III de ERC, la ingesta de sal no tuvo efecto sobre la presión arterial[7].

En otros estudios tampoco se han obtenido pruebas de que el incremento moderado del sodio alimentario (hasta 3,2 g Na/1000 kcal) influya en la presión arterial de los perros y los gatos sanos[8].

En conjunto, los estudios realizados en perros y en gatos sugieren que ni la presión arterial ni la hipertensión sistémica son sensibles a la sal en ninguna de las dos especies.

Modificación del pH urinario

La modificación del pH urinario a través del alimento o por medios clínicos puede resultar muy efectiva para el control de algunos urolitos, aunque no de todos. La acidificación de la orina produce un marcado aumento de la solubilidad de la estruvita y es fundamental para su disolución. Por el contrario, la alcalinización urinaria es importante porque aumenta la solubilidad de los urolitos de urato y cistina. En general, la eficacia de la alimentación aumenta si también reduce la excreción urinaria de los cristaloides que intervienen en la formación del urolito[9].

Otros tipos de cálculos, como los de oxalato cálcico, no son sensibles a la modificación del pH. Además, es aconsejable intentar conseguir un pH urinario que evite la precipitación posterior y potencie la excreción de otros minerales que podrían coprecipitar o actuar como inhibidores.

Conclusión

Aunque existen medidas específicas para el manejo de las urolitiasis, la disminución de la concentración urinaria y la estimulación de la diuresis, son medidas beneficiosas y comunes en el manejo general de las urolitiasis.

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TODOS LOS PROBLEMAS URINARIOS MERECEN SER SOLUCIONADOS

La enfermedad del tracto urinario inferior abarca una gran variedad de afecciones, se manifiesta a través de diversos signos y puede estar causada por múltiples problemas o la comorbilidad de diversas patologías.

Gracias a más de 50 años de ciencia, a una observación meticulosa y a la colaboración con veterinarios, sabemos que una nutrición especializada puede tener un papel fundamental en la recuperación de pacientes con problemas urinarios, así como en la salud general de los animales.

Por eso, disponemos de una amplia gama de soluciones nutricionales a medida para los problemas específicos del sistema urinario, ahora con innovaciones de última generación.

URINARY

Ante **patologías renales,** como la enfermedad renal crónica, es fundamental proporcionar un aporte nutricional adecuado

La enfermedad renal crónica en perros y gatos es una patología más frecuente de lo que nos pensamos. Gemma Baciero, veterinaria del departamento de Comunicación Científica de Royal Canin, señala las causas que pueden provocar la aparición de esta enfermedad y cómo debe abordarse desde un punto de vista nutricional.

Gemma Baciero

Veterinaria, Acre. GTNC AVEPA

Comunicación Científica Royal Canin

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Habéis lanzado una gama denominada Royal Canin Renal y surge siempre la siguiente duda: ¿son tan importantes los problemas renales en nuestras mascotas? ¿No sería suficiente con una dieta para animales mayores?

Dentro de las patologías renales, la enfermedad renal crónica (ERC) es la patología renal más frecuente en el perro y el gato, con una prevalencia global en estas especies entre el 0.5-1.5% y el 1-3%, respectivamente. Esta es una enfermedad irreversible y progresiva, para la que el manejo nutricional con una dieta renal formulada con unos requerimientos específicos, es clave.

¿Qué problemas pueden acarrear los problemas renales en nuestros perros?

Los signos clínicos de la ERC pueden ser muy inespecíficos: poliuria y polidipsia, pérdida de peso, menor actividad, debilidad, náuseas, vómitos.

¿Ocurren estos problemas a cualquier edad o existe una tendencia más acentuada según el animal va envejeciendo?

La ERC es más frecuente en pacientes geriátricos, hasta el 10% de los perros y el 35% de los gatos de edades avanzadas pueden presentar ERC en diversos estadios, pero puede afectar a perros y gatos de cualquier edad.

¿Existe algún tipo de predisposición asociada a la raza del perro?

Si bien se relaciona la ERC con animales de edad avanzada, no se suele asociar a la raza del perro, aunque en algunas fuentes citan ciertas razas, por ejemplo, Doberman, Cocker o Sharpei como razas que pueden presentar problemas renales hereditarios.

¿Una alimentación inadecuada, una incorrecta hidratación puede llevarnos a sufrir esta patología?¿Hay alguna enfermedad, infecciosa, metabólica o de cualquier otro tipo, que pueda desencadenarnos este tipo de problemas?

Hay diferentes causas que pueden provocar una insuficiencia renal aguda que puede desembocar en enfermedad renal crónica. Situaciones de isquemia renal (situaciones de hipotensión, hipovolemia y shock) y la acción de sustancias nefrotóxicas (aminoglicósidos, quimioterápicos, AINEs, metales pesados, etilenglicol entre otros) son las principales causas de insuficiencia renal aguda en el perro y el gato. Otras causas son las glomerulopatías (glomerulonefritis y amiloidosis), hipercalcemia, pielonefritis, leptospirosis, obstrucción urinaria y diabetes mellitus.

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¿Qué diferencia hay entre vuestra presentación en paté y cualquier otra presentación en forma de alimento seco o húmedo?

Todas las dietas Renal estás formuladas para cumplir un perfil nutricional específico, que se caracteriza por el control del aporte de proteína y fósforo entre otros. También es muy importante una muy buena palatabilidad ya que es frecuente que estos pacientes pierdan el apetito. En algunos casos una presentación húmeda, como la de Renal canine paté puede resultar más atractiva para el animal y suponer ser una buena opción para poder lograr una ingesta diaria adecuada.

¿Qué deberían recomendar los veterinarios a los propietarios de perros adultos para tratar de prevenir la insuficiencia renal?

Una buena recomendación es la de hacer chequeos periódicos de la función renal de los animales en su madurez, para poder detectar de forma precoz la enfermedad y así comenzar a tomar las medidas adecuadas lo antes posible.

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Assisting Decision-Making on Age of Neutering for 35 Breeds of Dogs: Associated Joint Disorders, Cancers, and Urinary Incontinence

Ayudar a la toma de decisiones sobre la edad de la castración de 35 razas de perros: trastornos articulares asociados, cánceres e incontinencia urinaria

Palabras clave: displasia del codo, displasia de cadera, desgarro cruzado craneal, linfoma, tumor de mastocitos, hemangiosarcoma, osteosarcoma

Keywords: *elbow dysplasia, hip dysplasia, cranial cruciate tear, lymphoma, mast cell tumor, hemangiosarcoma, osteosarcoma*

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La castración (incluida la esterilización) de perros machos y hembras en el primer año después del nacimiento se ha convertido en una rutina en los Estados Unidos y en gran parte de Europa, pero una investigación reciente revela que para algunas razas de perros, la castración puede estar asociada con un mayor riesgo de trastornos articulares debilitantes y algunos cánceres, lo que complica las decisiones de Los trastornos articulares incluyen displasia de cadera, desgarro o ruptura del ligamento cruzado craneal y displasia del codo.

Neutering (including spaying) of male and female dogs in the first year after birth has become routine in the U.S. and much of Europe, but recent research reveals that for some dog breeds, neutering may be associated with increased risks of debilitating joint disorders and some cancers, complicating pet owners' decisions on neutering. The joint disorders include hip dysplasia, cranial cruciate ligament tear or rupture, and elbow dysplasia. The cancers include lymphoma, mast cell tumor, hemangiosarcoma, and osteosarcoma. In previous studies on the Golden Retriever, Labrador Retriever and German Shepherd Dog, neutering before a year of age was associated with increased risks of one or more joint disorders, 2–4 times that of intact dogs. The increase was particularly seen with dogs neutered by 6 months of age. In female Golden Retrievers, there was an increase in one or more of the cancers followed to about 2–4 times that of intact females with neutering at any age. The goal of the present study was to expand and use the same data collection and analyses to cover an additional 29 breeds, plus three varieties of Poodles. There were major breed differences in vulnerability to neutering, both with regard to joint disorders and cancers. In most cases, the caregiver can choose the age of neutering without increasing the risks of these joint disorders or cancers. Small-dog breeds seemed to have no increased risks of joint disorders associated with neutering, and in only two small breeds (Boston Terrier and Shih Tzu) was there a significant increase in cancers. To assist pet owners and veterinarians in deciding on the age of neutering a specific dog, guidelines that avoid increasing the risks of a dog acquiring these joint disorders or cancers are laid out for neutering ages on a breed-by-breed and sex basis.

Introduction

In the U.S. and much of Europe, the practice of neutering male and spaying female dogs (herein both referred to as neutering) has become routine (1) and is increasingly being performed at, or before, 6 months of age. At the same time, several investigations have revealed that joint disorders and some cancers may increase in association with neutering of males and/or females. For example, in studies that did not focus on specific breeds or ages of neutering, one found that hip dysplasia and cranial cruciate ligament tears or ruptures were significantly more likely in neutered than intact males and females (2). Another study found that neutering was associated with a 3-fold increase in excessive tibial plateau angle (3), which is a risk factor for develop-

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ment of cranial cruciate ligament tears or rupture. Neutering is reported to be a risk factor for canine intervertebral disc herniation in Dachshunds (4). Certain cancers are also known to be more likely in neutered than intact dogs. The occurrence of lymphoma was found to be higher in spayed than intact females (5), as was the occurrence of mast cell tumors (6) and hemangiosarcoma (7). A study of over 40,000 dogs utilizing the Veterinary Medical Database found that neutered males and females were more likely to die of cancer than intact dogs (8). A recent finding was that the absence of estrogen from spaying females was associated with accelerated brain aging (9). Another recent report from the Golden Retriever Lifetime Project is that neutering at <6 months increases the risk of cranial cruciate ligament injury (10). Most of the studies cited above offer no useful clinical information or guidelines with regard to the various diseases that may occur in association with neutering in a specific breed.

In an attempt to address the absence of breed-specific information on joint disorders and cancers associated with neutering, we undertook a project focusing on various specific breeds using data collection and analyses with our extensive veterinary hospital database where the same diagnostic criteria could be applied to all breeds. We started with popular breeds well-represented in the database, initially with the Golden Retriever (11, 12), Labrador Retriever (12) and German Shepherd Dog (13). The joint disorders examined included cranial cruciate ligament tears or rupture (CCL), hip dysplasia (HD) and elbow dysplasia (ED). The cancers examined, which previous studies found could be affected by neutering, were lymphoma/lymphosarcoma (LSA), hemangiosarcoma (HSA), mast cell tumors (MCT), and osteosarcoma (OSA).

In the Labrador Retrievers, Golden Retrievers, and German Shepherd Dogs, there was an increase in the incidence of one or more of the joint disorders with neutering in the first year in males and females to 2–4 times >3–5% incidence in intact dogs. In female Golden Retrievers, neutering at any age was associated with an occurrence of one or more of the cancers followed to 2–4 times higher than the 5 percent incidence in intact females. But in male Golden Retrievers, and in male and female Labrador Retrievers and German Shepherd Dogs, there was no evident increase in cancers above that of the dogs left intact. Preliminary analyses from some small-dog breeds revealed no apparent increased risks of joint disorders with neutering. Thus, the research that had been undertaken revealed a wide range of breed-specific differences in disease vulnerability to neutering.

The purpose of this study was to analyze, in a variety of additional breeds, the increased risks, if any, of the above specified joint disorders and cancers associated with neutering male and female dogs at various ages, so as to increase the information available to pet owners and veterinarians for consideration when making decisions regarding neutering specific dogs. We added 29 new breeds to the study, separating three varieties of Poodles, for a total of 32 breed groups (referred to as breeds); this made a total of 35 breeds with the Goldens, Labs and German Shepherds included. The goal was to use the same veterinary hospital database and diagnostic criteria for the diseases as was used with the published studies on the retrievers and German Shepherds so as to allow for direct comparisons among various breeds. The primary purpose was to offer readers some evidence-based information on breed-specific differences with vulnerability to neutering, including suggested guidelines for neutering ages to avoid increasing long-term health risks of neutering, if any. A secondary, unforeseen, purpose was to document breed-specific differences in the increases in some cancers associated with removal of gonadal hormones, as an area for possible research on genetic aspects of cancer occurrence.

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Methods

Ethics Statement

Hospital records of the Veterinary Medical Teaching Hospital (VMTH) provided the retrospective dataset used. In conformity with the campus policy, faculty of the University of California-Davis, School of Veterinary Medicine, are allowed use of the record system for research purposes. No animal care and use committee approval was required, and strict confidentiality of the owners and their dogs was maintained.

Subjects Breed Categories

In addition to the Golden Retriever, Labrador Retriever, and German Shepherd Dog, the other breeds chosen for this project included those most frequently occurring in the database and those chosen to obtain a sampling of giant breeds or small-dog breeds. The final list of 35 (including three varieties of Poodle) represented in the present study are, alphabetically, the: Australian Cattle Dog, Australian Shepherd, Beagle, Bernese Mountain Dog, Border Collie, Boston Terrier, Boxer, Bulldog, Cavalier King Charles Spaniel, Chihuahua, Cocker Spaniel, Collie, Corgi (Pembroke and Cardigan combined), Dachshund, Doberman Pinscher, English Springer Spaniel, German Shepherd Dog, Golden Retriever, Great Dane, Irish Wolfhound, Jack Russell Terrier, Labrador Retriever, Maltese, Miniature Schnauzer, Pomeranian, Poodle-Miniature, Poodle-Standard, Poodle-Toy, Pug, Rottweiler, Saint Bernard, Shetland Sheepdog, Shih Tzu, West Highland White Terrier, and Yorkshire Terrier.

Study Parameters

The present study examined the occurrence in both sexes of the joint disorders: HD, CCL and ED. Also examined in both sexes were the cancers LSA, HSA, MCT, and OSA, because these had been shown in some multi-breed studies to be increased in risks with neutering. In addition, mammary cancer (MC), pyometra (PYO), and urinary incontinence (UI) were examined in female dogs. Of interest was the possible association of early neutering and the occurrence of intervertebral disc disorders (IDD) in the Corgi and Dachshund, two breeds known to be at risk for these diseases. All of the above diseases were examined with regard to dogs neutered in one of the age periods of: <6 mo., 6–11 mo., 1 year (12 to <24 mo.) or 2–8 years, or left intact. The diseases were tracked until the dogs were last seen at the hospital, or through 11 years of age, if seen past their 12th birthday.

Mammary cancer is a late occurring cancer with the median age of diagnosis being 10.1 years in one study (14). Tracking cancers through 11 years of age would be presumably sufficient to catch most cases of MC if the case record had information extending to that age. However, most case records did not extend to that age. As an additional point of comparison, percentages of MC occurrence were looked at in just females tracked through 8 years of age or beyond, including diagnosed MC cases beyond the 12th birthday cut-off, which was the cut-off used for all other data.

Data Collection and Presentation

The computerized hospital record system of the VMTH provided the dataset. The hospital, with currently over 50,000 cases admitted per year, is a secondary and tertiary facility as well as being a primary care facility. The statistical evaluations, with standardized diagnostic criteria applied to various diseases and taking into account sex and different ages of neutering, required a large database with a computerized record system. The study focused on proportional differences in disease occurrences between the neuter age groups and intact dogs of the same breed and sex.

The study period represented 15 years of data for most breeds. The inclusion criteria were date of birth, age at neutering (if neutered), and age of diagnosis or onset of clinical signs for diseases of interest. As mentioned, age at neutering was designated as <6 mo., 6–11 mo., 1

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year (12 to <24 mo.), and 2–8 years (2 to <9 years). The term "early neutering" is sometimes used below to refer to neutering in the first year, combining cases for both the <6 mo. and 6–11 mo. neuter periods. For MC, PYO, and UI, only females were examined. While UI does occur in males, it is predominantly an issue in females.

For all neutered dogs that developed a disease of interest, records were examined to confirm that the dog was neutered prior to the diagnosis or signs of the disease. If the dog developed signs of the disease prior to neutering, the dog was considered intact for analysis of that disease. However, for any disease that occurred after neutering, the dog was considered neutered for analysis of that disease. For any disease of interest that occurred before 12 months of age, the dog was removed from that disease analysis, but included in analyses of other diseases. Therefore, the number of cases for various diseases varied in the analyses for different disease occurrences.

The age at neutering was sometimes not included in the hospital records, so telephone calls to the referring veterinarians were made to obtain the neutering dates or ages. Nonetheless, there were many neutered dogs where age at neutering was not available from the VMTH records or the referring veterinarian, so these dogs were excluded from the study. Of course, this was not an issue with the sample of intact dogs, so there were proportionately more intact cases in the final dataset for each breed than would be expected in the general population. However, the proportion of dogs with a disease, whether intact or neutered, was not affected by the overrepresentation of intact dogs in the database.

The criteria for disease diagnoses were the same as in previous studies on the retrievers and German Shepherd Dog (11–13). A dog was considered as having a disease of interest if the diagnosis was made at the VMTH, or by a referring veterinarian and later confirmed at the VMTH. For joint disorders (HD, ED, and/or CCL), dogs typically presented with signs of lameness, difficulty in moving, and/or joint pain. The diagnosis was confirmed by orthopedic examination, radiographic evidence, and/or surgery. In Dachshunds and Corgis, where intervertebral disc disorders (IDD) is a concern, the diagnosis included herniation, rupture, extrusion, protrusion, fracture, compression, stenosis, or spinal cord injury. For cancers (LSA, HSA, MCT, OSA, MC), the diagnosis was based on the presence of a tissue mass, lumps on the skin or enlarged lymph nodes, and confirmed by chemical panels, appropriate blood cell analyses, imaging, histopathology, and/or cytology. PYO was confirmed by ultrasonic evidence and/or post-surgically after removal of the uterus. UI was confirmed by clinical signs of abnormally frequent urination, urinalyses and exclusion of urinary tract infection and/or other disease. If a diagnosis was listed in the record as "suspected" based on some clinical signs but not confirmed, the case was excluded from the analysis for that specific disease, but the dog was included in other disease analyses.

Although body condition scores have been reported to be a factor in the occurrence of joint disorders (3, 15), our previous studies on the retrievers and German Shepherd Dog found no significant relationship when body condition scores were compared between dogs with and without a joint disorder. Therefore, in the current paper the body condition score is not reported for each breed.

Statistical Analyses

Survival analysis was used to test for differences with respect to the hazard of a disease in the neutered and intact groups, while adjusting for the differences in time at risk for a disease. The groups were initially compared using a Kaplan Meier life table analysis. Post-hoc comparisons among the subgroups were based on least squares means of the hazard within each subgroup. For comparisons where the Kaplan Meier test showed significance at the p <0.05

level, both the log-rank and Wilcoxon tests were used for further analyses. Because joint disorders are expected to be seen at a similar risk throughout a dog's lifespan, regardless of age, the log-rank test was used initially for the joint disorders. If the log-rank test did not show significance but the Wilcoxon test did for joint disorders, the Wilcoxon test result was reported with significance level and an asterisk. The reverse rule of thumb was used with cancers where the first test examined was the Wilcoxon test, since the risk of cancer is expected to be higher in older dogs. If the Wilcoxon test did not show significance but the log-rank test did for cancers, the log-rank test result was reported with significance level and an asterisk. For all statistical tests, the two-tailed statistical level of significance was set at $p < 0.05$ and reported as either $p < .05$ or $p < 0.01$. Each breed was analyzed separately, and there were no statistical comparisons between breeds. However, the overall findings with each breed allow for some general comparisons.

Data Presentation

For each breed represented on a separate page in Appendix 1, the numbers of intact and neutered males and females are given. In the tables, the percentage of dogs with each of the diseases and the percentage having at least one of the joint disorders and at least one of the cancers (except MC) was calculated for intact males and intact females as well as those neutered at various age ranges. Statistical analyses compared the occurrences of joint disorders and cancers between each neuter period and intact dogs. If the comparison was significant at either the $p < 0.05$ or $p < 0.01$ level, the data were bolded and the p-value was given. The detailed datasets are available online (Figshare, doi: 10.6084/m9.figshare.7231010). Three breeds for which findings have been previously published (Golden Retriever, Labrador Retriever, German Shepherd Dog) are included to present an overall picture in the same Appendix 1. The data for these three breeds were expanded through 11 years of age, to provide continuity among breeds and diseases.

For each breed, a short paragraph summarizes the main findings on joint disorders (HD, CCL, ED), cancers (LSA, HSA, MCT, OSA) for both males and females, and MC, PYO and UI for females. For Dachshunds and Corgis, the occurrence of IDD is listed for both sexes. Survival analyses were not done on IDD occurrence because the condition represented so many different disease diagnoses. Also included in the breed summary information is a suggested guideline for neutering age for males and females to avoid increasing the risks of a disease under consideration. When there was no noticeable occurrence of an increase in joint disorders or cancers with neutering, the guideline statement was made that those wishing to neuter should decide on the appropriate age (or briefly stated as choice in **Table 1**). When neutering at <6 months was associated with an increased disease risk but no increased risk was evident with neutering beyond 6 months, the default recommended guideline was neutering beyond, 6 months.

Results

The breed-by-breed findings are presented in four different formats. One format, seen in this section below, is a short paragraph for each breed. The occurrence of the joint disorders and the cancers followed is reported for the intact and neutered dogs, and the increase in the two disease types over that of the intact dogs, if significant, is reported. Other findings are also mentioned if appropriate, such as IDD occurrence in Dachshunds and Corgis. A second format, represented in **Table 1**, is a very brief summary of spaying and neutering guidelines based on findings regarding joint disorders and cancers for each breed, allowing the reader to quickly scroll through the various breeds. In the third format, the data-based findings, with statistical

Suggested guidelines for age of neutering: 35 breeds

	Males					Females				
	Leave intact	Choice	Beyond 6 months	Beyond 11 months	Beyond 23 months	Leave intact	Choice	Beyond 6 months	Beyond 11 months	Beyond 23 months
Australian Cattle Dog		✓						✓		
Australian Shepherd		✓					✓			
Beagle				✓			✓			
Bernese Mt. Dog					✓		✓			
Border Collie				✓					✓	
Boston Terrier				✓			✓			
Boxer					✓					✓
Bulldog		✓					✓			
Cavalier King Charles Spaniel		✓					✓			
Chihuahua		✓					✓			
Cocker Spaniel			✓							✓
Collie		✓							✓	
Corgi			✓				✓			
Dachshund		✓					✓			
Doberman Pinscher	✓									✓
English Springer Spaniel		✓							✓	
German Shepherd					✓					✓
Golden Retriever				✓		✓				
Great Dane		✓					✓			
Irish Wolfhound					✓		✓			
Jack Russell Terrier		✓					✓			
Labrador Retriever			✓						✓	
Maltese		✓					✓			
Miniature Schnauzer		✓					✓			
Pomeranian		✓					✓			
Poodle (Toy)		✓					✓			
Poodle (Miniature)				✓			✓			
Poodle (Standard)					✓		✓			
Pug		✓					✓			
Rottweiler				✓				✓		
Saint Bernard		✓						✓		
Shetland Sheepdog		✓								✓
Shih Tzu		✓								✓
West Highland White Terrier		✓					✓			
Yorkshire Terrier		✓					✓			

Summary of spaying and neutering guidelines based on findings regarding increased risk of joint disorders and cancers. The term "choice" means there was no increased risk for any age.

Table 1. *Suggested Guidelines by Breed for Age of Neutering.*

notations for each breed, are reported in Appendix 1. In the fourth format, the raw data allowing the reader to perform their own calculations, if desirable, is available in Figshare.

The mean age of last entry was calculated for intact and neutered males and females for each breed and presented in Appendix 2. Across all breeds the mean age of last entry in the record for neutered males was 5.5 years (range 3.71–6.54), for neutered females 5.7 years (range 4.21–6.97), for intact males 4.9 (range 4.15–7.11), and intact females 4.7 (range 3.41–6.32). Upon perusal of the data, it is evident that the mean age of data entry for intact dogs was younger than that of neutered dogs, especially for females, where there is disparity of almost 1 year. To address the issue of whether the lower age of last entry for intact dogs could have resulted in a lower rate of disease occurrence in intact dogs in either joint disorders or cancers, we examined data of dogs where the last entry was at 8 years or beyond. We looked at three breeds with the largest databases (Golden Retrievers, Labrador Retrievers, and German Shepherd Dogs) and where there were significant differences in disease diagnoses between early neutered and intact dogs. Using these parameters, the occurrences of joint disorders in Golden Retrievers for those neutered at ≤ 6mo. vs. intact, in males,

there was a 6-fold difference (18% vs. 3%) and in females 3-fold (25 vs. 8%). For male Labrador Retrievers, the figures were 22 vs. 8% and in females 33 vs. 10%. For male German Shepherd Dogs, the figures were 33 vs. 2% and for females, 29 vs. 9%. For cancers in female Goldens, the figures were 26 vs. 14%. The incidence figures, although not sufficient for meaningful statistical analyses, are consistent with the larger database where all ages are included. Thus, while the age of the last visit is a limitation for analyses on late-occurring cancers and joint disorders, the examples chosen for dogs seen at the age of 8 years or beyond are consistent with the overall results presented here; these results appear to represent what would be seen in the general situation.

General Findings

Looking at the occurrences of these joint disorders and cancers, it is clear that most breeds are unaffected for these diseases by age of neutering. Vulnerability to joint disorders associated with neutering is generally related to body size. Small-dog breeds – Boston Terrier, Cavalier King Charles Spaniel, Chihuahua, Corgi, Dachshund, Maltese, Pomeranian, Poodle-Toy, Pug, Shih Tzu, Yorkshire Terrier – do not appear to have an increased risk in joint disorders with neutering compared to the breeds of larger size. However, in the breeds of larger body size there were differences among the breeds with the two giant breeds – Great Danes and Irish Wolfhounds – showing no indication of increase in one or more joint disorders with neutering at any age.

Although the occurrence of MC was tracked, the female mean age at the last hospital visit for all breeds ended short of the reported, late-onset mean age of MC occurrence in intact female dogs. Thus, the low occurrence of MC in intact females (typically under 6 percent) cannot be expected to represent the actual incidence over a female's lifetime. When the percentage of MC was calculated for only those dogs seen through 8 years of age or older (including cases diagnosed past the 12th birthday), the results did not appear appreciably different than the percentages seen using the study age range. However, the number of dogs seen through age 8 or beyond was fairly small, so the analysis results might change with an increased sample size of these older dogs.

The following are brief summaries for each of the breeds along with suggested guidelines for age of neutering. See Appendix 1 for the complete data set, including statistical analyses for each breed.

Australian Cattle Dog

The study population was 61 intact males, 58 neutered males, 48 intact females, and 70 spayed females for a total of 237 cases. In this sample, 5 percent of intact males and 2 percent of intact females were diagnosed with one or more joint disorders. Neutering males was not associated with any increased risk in joint disorders, but there was an association with spaying females at <6 mo. where the risk of a joint disorder increased to 15 percent ($p < 0.05$). The occurrence of cancers was low for males and females left intact (0 and 3 percent, respectively). There were no evident occurrences of the cancers in dogs neutered at various ages. The occurrence of MC in intact females was 6 percent and in those spayed at 2–8 years, 6 percent. For females left intact, 4 percent were reported with PYO. UI was not reported in any of the spayed or intact females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males, those wishing to neuter should decide on the appropriate age. In females, the increased risk of a joint disorder with spaying occurred only at the <6 mo. range, so the suggested guideline is spaying at, or beyond, 6 months.

Australian Shepherd

The study population was 93 intact males, 135 neutered males, 76 intact females, and 136 spayed females for a total of 440 cases. In this sample, 3 percent of intact males and 4 percent of intact females were diagnosed with one or more joint disorders. Neutering males and females was not associated with any evident increased risk in joint disorders. The occurrence of cancers was 9 percent for intact males and, in contrast, only about 1 percent for intact females. Neutering males did not appear to be associated with an overall increased risk of cancers above the rather high level of intact males. However, spaying females at 6–11 mo. and at 2–8 years was associated with a 7–8 percent risk in cancers which may have reached significance with a larger sample size. The occurrence of MC in intact females was zero, but was 8 percent in females spayed at 2–8 years. For females left intact, 5 percent were reported with PYO. UI was reported in just 1 percent of early-spayed females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males, those wishing to neuter should decide on the appropriate age. The guideline for females is the same while also maintaining vigilance for the cancers which may be associated with spaying beyond 6 months, or else leaving the female intact and being vigilant for MC.

Beagle

The study population was 42 intact males, 82 neutered males, 45 intact females and 87 spayed females for a total of 256 cases. Just 2 percent of intact males were diagnosed with one or more joint disorders, but with neutering at 6–11 mo. joint disorders increased 7-fold to 15 percent, which may have reached significance with a larger sample size. None of the females left intact or spayed had a joint disorder. None of the intact males or females was diagnosed with any of the cancers followed. There was no evident increased occurrence of cancers in neutered males and females. There was no occurrence of MC in intact or late-spayed females. There was 1 case of PYO in intact females (2 percent). UI was reported in only 2 percent of early-spayed females.

For males, in light of a possible increase in joint disorders for those neutered at 6–11 mo., the suggested guideline is to delay neutering males until beyond a year of age. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered females, those wishing to neuter should decide on the appropriate age.

Bernese Mountain Dog

The study population was 59 intact males, 74 neutered males, 37 intact females, and 65 spayed females for a total of 235 cases. The percentage of intact males with at least one joint disorder was 4 percent and for intact females, 11 percent. Neutering males any time prior to 2 years of age was associated with a significant increase in at least one joint disorder to 23–24%, about a 6-fold increase over intact males ($p < 0.01$). Spaying females before 6 mo. increased the likelihood of a joint disorder to over 3-fold that of intact females, but this did not reach significance. The occurrence of one or more of the cancers followed was 9 percent for both intact males and intact females. There was no evident increase in cancer risk in males related to neutering, but with females, spaying at <6 mo. was associated with a 2-fold increase above that of intact females. There was no occurrence of MC in females, whether left intact or neutered at any age, and a 5 percent occurrence of PYO in intact females. There was no occurrence of UI in intact or spayed females. Reflecting the increased risk of joint disorders for males, the suggested guideline for neutering males is delaying neutering until well-beyond 2 years. Lacking a significant occurrence of increased joint disorders or cancers in neutered females, those wishing to neuter should decide on the appropriate age.

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Border Collie

The study population was 105 intact males, 85 neutered males, 88 intact females, and 121 spayed females for a total of 399 cases. In this sample 2–3% of intact males and females were diagnosed with one or more joint disorders, and neutering males and females was not associated with any evident increased risk in joint disorders. The occurrence of one or more of the cancers followed in intact males was 2 percent and none for females left intact. For males, there was a significant increased risk in one or more of the cancers to 13 percent with neutering at 6–11 mo. ($p < 0.05$), and for females there was a significant increase in the cancers to 11 percent with spaying at 6–11 mo. ($p < 0.01$). The occurrence of MC in intact females was just 1 percent, and for PYO, 4 percent. UI was reported in just one spayed female. The suggested guideline for neutering, given the significant risk of cancers, is holding off neutering of both sexes until beyond a year of age.

Boston Terrier

The study population was 75 intact males, 67 neutered males, 54 intact females, and 96 spayed females for a total of 291 cases. None of the intact or neutered males or females was diagnosed with one or more joint disorders. For cancers, the story is a bit different in that 5 percent of intact males were diagnosed with one or more cancers and 10 percent of males neutered at <6 mo., and 12 percent of males neutered at 6–11mo. had cancers ($p < 0.01$, the two neuter periods combined). For females, 2 percent of intact females had one or more of the cancers and with spaying, there was no evident increase of cancers. The occurrence of MC in intact females was 2 percent and for PYO, 7 percent. UI was 2 percent in early-spayed females. In light of the significant increase in cancers in males with neutering through 11 months of age, the suggested guideline for males is delaying neutering to beyond a year of age. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered females, those wishing to neuter should decide on the appropriate age.

Boxer

The study population was 220 intact males, 203 neutered males, 128 intact females, and 210 spayed females, for a sample size of 761 cases. Males and females left intact had just a 2 percent occurrence of joint disorders, with neutered males and females showing no apparent increase in this measure. The occurrence of one or more of the cancers followed in intact males was 17 percent, and for intact females, 11 percent. Neutering males before 2 years significantly raised the risk of a cancer over that of intact males to 32 percent ($p < 0.01$). The same pattern of increase in cancers was seen in spaying females with up to 20 percent of females having one or more of the cancers with spaying done before 2 years, an increase that was not significant, but with an expanded database may have been. There was no occurrence of MC in intact females. PYO was diagnosed in 2 percent of intact females. Just 1 percent of spayed females were diagnosed with UI. Given the risk of increased cancers, the suggested guideline for both sexes is to delay neutering until beyond 2 years of age.

Bulldog

The study population was 198 intact males, 156 neutered males, 90 intact females, and 114 spayed females for a sample of 558 cases. The occurrence of joint disorders in intact males was 7 percent and 5 percent in intact females. Neutering at <6 mo. raised the incidence to 15 percent for males and to 18 percent for females, which did not reach significance for either. The cancers followed occurred at the 6 to 7 percent level in intact males and females. There were no significant increases above this with neutering males or females. The occurrence of MC in females left intact was 1 percent and 2 percent

with spaying at 2–8 years. There was a 2 percent occurrence of PYO in intact females and no UI in early spayed females. Lacking a significant occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age, but some people may wish to be cautious in view of the possible apparent risk in joint disorders.

Cavalier King Charles Spaniel

The study population was 51 intact males, 72 neutered males, 87 intact females, and 76 spayed females, for a sample size of 286 cases. For males and females left intact, the occurrences of one or more joint disorders were just 4 and 1 percent, respectively, and for both sexes neutering was not associated with any increase in this measure. The occurrences of cancers in intact males were 2 percent and zero for intact females. For both sexes neutering was not associated with any increase in this measure. The occurrence of MC in females left intact was zero. The occurrence of PYO was 2 percent in intact females. There was no occurrence of UI in spayed females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Chihuahua

The study population was 261 intact males, 189 neutered males, 298 intact females, and 289 spayed females for a total sample of 1,037 cases. For both males and females, neither those left intact, nor those neutered at any age had a noteworthy occurrence of a joint disorder. The cancers followed in both intact and neutered males and females were <5 percent with no evident increase with neutering at any age. The occurrence of MC in females left intact was 1 percent, and in females neutered at 2–8 mo., 4 percent. In intact females, PYO was diagnosed in 2 percent. There was no UI diagnosed in any of the spayed females. Lacking a noticeable occurrence of increased joint disorders or cancers with neutering in either sex, those wishing to neuter should decide on the appropriate age.

Cocker Spaniel

The study population was 71 intact males, 112 neutered males, 61 intact females, and 127 spayed females, for a sample size of 369 cases. The occurrence of at least one joint disorder was seen in 1 to 3 percent of the intact males and females. Neutering males at <6 mo. was associated with a significant increase of this measure to 11 percent ($p < 0.01$). Spaying females was not associated with an increase in joint disorders. The occurrence of one or more of the cancers followed was 6 percent in intact males with no increase with neutering. Although there was no occurrence of cancers in intact females, this measure rose significantly to 17 percent in females spayed between 1 and 2 years of age ($p < 0.01$), entirely due to MCT. For females left intact, 11 percent were diagnosed with MC and 5 percent with PYO. None of the spayed females developed UI. The suggested guideline for males is neutering beyond 6 months of age. Given the increased cancer risk for females spayed at a year of age, the suggested guideline is delaying spaying until beyond 2 years of age.

Collie

The study population was 29 intact males, 26 neutered males, 24 intact females, and 37 spayed females, for a sample size of 116 cases. The occurrence of at least one joint disorder was seen in 7 percent of the intact males and in none of the intact females. None of the neutered males or females had a noteworthy occurrence of a joint disorder. The occurrence of one or more of the cancers followed was 11 percent for intact males and none for the intact females. There was no evident increase of cancers in males with neutering, and in females, there was an increase of cancer to 40 percent in those spayed at

<6 mo., which may have reached significance with a larger sample size. For females left intact, 4 percent were diagnosed with MC, and 16 percent were diagnosed with PYO. Of females spayed at 6–11 mo., 13 percent had UI. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males, those wishing to neuter a male should decide on the appropriate age. For females, given the apparent risks of cancers with spaying at <6 mo. and UI with spaying at 6–11 mo., the guideline is to delay spaying until the female is a year old.

Corgi (Welsh), Pembroke and Cardigan

The study population was 42 intact males, 78 neutered males, 50 intact females, and 70 spayed females, for a total sample size of 240 cases. Although these are two breeds, they vary only a little in size, so these two breeds are combined for statistical analyses and display of data. The occurrence of at least one joint disorder in intact males was 5 percent and for intact females 6 percent. There was no significant increase in this measure in males or females with neutering. This is one of the breeds where intervertebral disc disorders are a concern, and in 3 percent of intact males and 8 percent of intact females, IDD was reported. In males neutered before 6 months, the occurrence of IDD reached 18 percent, and in females there was no increase with neutering. The occurrence of one or more of the cancers followed was 5 percent in intact males and 6 percent in intact females. In neutered males and females, there was no evident increase in cancers. For females left intact, the occurrence of MC was 8 percent, and there was zero occurrence of PYO. There was no diagnosis of UI in spayed females. The suggested guideline for age of neutering for males, given the increase in IDD with neutering at <6 mo., is beyond 6 months. Lacking a noticeable occurrence of increased joint disorders, IDD, or cancers with neutering females, those wishing to neuter a female should decide on the appropriate age.

Dachshund

The study population was 177 intact males, 170 neutered males, 99 intact females, and 212 spayed females, for a total sample size of 658 cases. Joint disorders were basically absent in males and females, left intact or neutered. This is a breed plagued by intervertebral disc disorders, and in this sample 53 percent of intact males and 38 percent of intact females were diagnosed with a form of IDD. There was no evident increase in this measure with neutering of males or females. The occurrence of the cancers followed was <1% in both intact males and females, with no indication of an increased risk with neutering. For females left intact, the occurrence of MC was 1 percent and for PYO, 4 percent. None of the spayed females developed UI. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Doberman Pinscher

The study population was 109 intact males, 91 neutered males, 53 intact females, and 108 spayed females, for a sample size of 358 cases. The percentage of intact males with at least one joint disorder was 2 percent and 0 percent for intact females. There was no evident increase in this measure with neutering males. For females, spaying within 11 months resulted in an increase in joint disorders of 11 percent, which did not reach significance. The occurrence of one or more of the cancers followed for both intact males and intact females was 2 percent. In neutered males at the 1 year and 2–8 year periods, there was a non-significant increase in occurrence of cancers to 6 percent and 13 percent, respectively. For females, there was no noteworthy increase in cancers with spaying at any time. The occurrence of MC in females left intact was 2 percent and 4 percent for those spayed at 2–8 years. There

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was a 7 percent occurrence of PYO in intact females. UI was a significant risk in females spayed at any age up to 2 years, ranging from 25 percent in the females spayed at <6 mo. (p <0.01) to 19 percent for those spayed between 1 and 2 years (p <0.05). The suggested guideline, based on fragmentary results, for males is to leave the male intact or neuter before 1 year of age to avoid the possible increased risk of cancers seen in those neutered beyond a year of age. For females, the suggested guideline, also based on limited data, given the risk of UI in early spayed females, and the possible increased risk of a joint disorder, is to consider delaying spaying until beyond 2 years of age.

English Springer Spaniel

The study population was 52 intact males, 57 neutered males, 37 intact females, and 66 spayed females for a total sample of 212 cases. In males and females left intact, the occurrence of one or more joint disorders was 5 and 8 percent, respectively. Among males and females neutered at various ages, there were no noteworthy increases in joint disorders. The cancers followed occurred in the intact males and females at a 6 percent level, and neutering at any age was not associated with any evident increase in this measure in either sex. In intact females, MC was diagnosed in 6 percent, and for those spayed at 2–8 years, 15 percent. PYO was not reported in any of the intact females. Spaying females at 6–11 mo. was associated with a 13 percent occurrence of UI, which may have reached significance with a larger sample size. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males, those wishing to neuter should decide on the appropriate age. For females, given the increased risk of UI in those spayed before 1 year, the suggested guideline is to delay spaying until a year of age.

German Shepherd Dog

The study population was 514 intact males, 272 neutered males, 173 intact females, and 298 spayed females for a total of 1,257 cases. In males and females left intact, the occurrence of one or more joint disorders was 6 and 5 percent, respectively. Neutering males at <6 mo., 6–11 mo. and 1–2 years was associated with increased risks of this measure to 19, 18 and 9 percent, respectively (p <0.01). Spaying females at <6 mo. and 6–11 mo. was associated with a 20 and 15 percent level of increased risk (p <0.01), and spaying at 1–2 years with a 5 percent risk level (p <0.05). The occurrence of one or more of the cancers followed for intact males and females was 3 percent and 2 percent, respectively. Neutering at the various ages was not associated with any appreciable increased risk in cancers followed. The occurrence of MC in intact females was 5 percent and for those spayed at 2–8 years, 6 percent. Of intact females, 3 percent were reported with PYO. UI ranged up to 9 percent for females spayed from <6 mo. through 1 year of age (p <0.05–0.01). The suggested guideline for males, given the risks of joint disorders, is delaying neutering until over 2 years of age. For females, with the same joint issues as males plus the risks of UI, the suggested guideline is delaying spaying until over 2 years of age.

Golden Retriever

The study population was 318 intact males, 365 neutered males, 190 intact females, and 374 spayed females for a total of 1,247 cases. In intact males and females, the level of occurrence of one or more joint disorders was 5 percent and 4 percent, respectively. Neutering males at <6 mo. and at 6–11 mo. was associated with risks of 25 percent and 11 percent, respectively (p <0.01). In females, spaying at <6 mo. and at 6–11 mo. was associated with risks of 18 percent and 11 percent (p <0.01, when combined). The occurrence of one or more of the cancers followed in intact males was a high 15 percent and for intact females 5 percent. Neutering males at <6 mo. and at 6–11 mo. was associated with increased risks

of cancers to 19 and 16 percent, respectively (p <0.01). Spaying females at <6 mo. and at 6–11 mo., was associated with increases in cancers to 11 and 17 percent, respectively (p <0.05, when combined) and spaying at 1 year and at 2–8 years was associated with increased risks of 14 percent (p <0.01, when combined). The occurrence of MC in intact females was 1 percent and for those spayed at 2–8 years, 4 percent. For females left intact, 4 percent were reported with PYO. No cases of UI were reported in females spayed at any age. The suggested guideline for males, based on the increased risks of joint disorders and cancers, is delaying neutering until beyond a year of age. The suggested guideline for females, based on the increased occurrence of cancers at all spaying ages, is leaving the female intact or spaying at one year and remaining vigilant for the cancers.

Great Dane

The study population was 90 intact males, 103 neutered males, 69 intact females, and 91 spayed females for a total sample of 353 cases. This is a giant breed where one might expect a high risk of joint disorders. However, both intact males and females have low levels of joint disorders, just 1 and 2 percent, respectively. For both males and females, there was no evident increase in this measure with neutering. The occurrence of one or more of the cancers followed in intact males was 6 percent and for intact females, 3 percent. There was no evident increase in this measure of cancers with neutering in either sex. In intact females, MC was diagnosed in just 2 percent and PYO in 6 percent. In early-spayed females, no UI was reported. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age. However, given the large body size, and physiology of late musculoskeletal development, neutering well-beyond year 1 should be considered.

Irish Wolfhound

The study population was 30 intact males, 19 neutered males, 21 intact females, and 16 spayed females for a total of 86 cases. Even with the small number of cases, this breed was chosen for analyses because of the large body size: challenging the Great Dane for height, and where one might expect an increased risk of joint disorders. In this sample, 7 percent of intact males and none of the intact females had a joint disorder. No joint disorders were seen in neutered males or females. With the intact males and females, the incidences of one or more cancers were 8 percent and 21 percent, respectively. With neutering males at 1 year, there was an increase in cancer occurrence to 25 percent (p <0.05). There was no evident increase in cancers in neutered females above the relatively high level in intact females. There was no occurrence of MC in intact females or those spayed late. For females left intact, 5 percent were reported with PYO. UI was not reported in any of the spayed or intact females. The suggested guidelines for males given the increased occurrence of cancers around at ages 1–2 years, is neutering beyond 2 years. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered females, those wishing to neuter should decide on the appropriate age. However, given the large body size, and physiology of late musculoskeletal development, some may want to consider neutering females well-beyond year 1.

Jack Russell Terrier

The study population was 92 intact males, 87 neutered males, 84 intact females, and 113 spayed females for a total sample of 376 cases. As in other small dogs, joint disorders were rare; none of the intact males, and just 2 percent of intact females had one or more joint disorders. Neutering was not associated with any increase in this measure in either sex. In intact males, 3 percent, and in intact females none, had one or more of the cancers followed. There

was no evident increase in cancer occurrence in either sex with neutering at any age. In females left intact, MC was seen in 1 percent, as was PYO. In those spayed at 2–8 years, MC was diagnosed in 3 percent. UI was not diagnosed in any females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Labrador Retriever

The study population was 714 intact males, 381 neutered males, 400 intact females, and 438 spayed females for a total of 1,933 cases. One or more joint disorders were reported in 6 percent of both intact males and intact females. This measure was significantly increased to 13 percent for males neutered before 6 mo. ($p < 0.01$). In females spayed at <6 mo. and 6–11 mo., the risk of a joint disorder was 11–12 percent for each period ($p < 0.01$, spay periods combined). The occurrence of cancers followed was 8 percent and 6 percent, respectively, for intact males and females. Neutering at the various ages was not associated with any evident increased risk in the cancers. The occurrence of MC in intact females was 1 percent and for those spayed at 2–8 years, 2 percent. For females left intact, 2 percent were reported with PYO. UI was reported at a low rate (2–3%) in females spayed at various ages though 1 year. Given the significant occurrence of joint disorders in males neutered at <6 mo., the suggested guideline for males is neutering beyond 6 months. For females, given the increased risks of joint disorders with spaying through 11 months of age, the suggested guideline is delaying spaying until beyond a year of age.

Maltese

The study population was 49 intact males, 72 neutered males, 65 intact females, and 86 spayed females for a total sample of 272 cases. As mentioned in Appendix 1, the Maltese and Chihuahua vie for the smallest breeds and the Great Dane and Irish Wolfhound for the largest, but all four breeds share a low predisposition to joint disorders. For the Maltese in both sexes, there was no occurrence of joint disorders in either those left intact or neutered. Virtually the same picture emerges with cancers, with only one of 64 intact females being diagnosed with a cancer. There was no occurrence of MC in the intact females and only one case among the 19 females spayed at 2–8 years. PYO was seen in none of the intact females. UI did not occur in any of the females.

Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Miniature Schnauzer

The study population for this small-dog breed was 47 intact males, 63 neutered males, 25 intact females and 96 spayed females for a total sample of 231 cases. There was virtually no occurrence of any joint disorders in males or females either left intact or neutered. The incidence of cancers in intact males was 4 percent and in females, zero percent. There was no indication of cancer increase related to neutering in either sex. There was no occurrence of MC in any of the females left intact or spayed, and a 4 percent occurrence of PYO in intact females. None of the females was diagnosed with UI. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Pomeranian

The study population was 84 intact males, 69 neutered males, 65 intact females, and 104 spayed females for a total sample of 322 cases. As with other dogs of small body size, both males and females had no occurrences of joint disorders in either those left intact or neutered. With regard to cancers, for both males and females left intact, the occurrence of cancers was zero, and there was no indication of increased cancer risk related to neutering in ei-

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ther sex. There was just one case of MC in females left intact, and 7 percent with PYO. None of the females was diagnosed with UI. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Poodle, Toy

The study population was 49 intact males, 53 neutered males, 58 intact females, and 78 spayed females for a total sample of 238 cases. While the AKC registers all the Poodle varieties as the same breed, the three main varieties are dealt with separately here because of differences in size. In intact males, 4 percent had one or more joint disorders and in intact females there was no occurrence of a joint disorder. In neutered males and females, there was no evident increased risk of a joint disorder. There was a 2 percent occurrence of cancers in intact males and none in intact females. In neutered males and females, there was no noteworthy occurrence of cancers. In intact females, there was only a single case of MC and no case of PYO in intact females and no occurrence of UI in spayed females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Poodle, Miniature

The study population was 41 intact males, 60 neutered males, 30 intact females, and 69 spayed females for a total sample of 199 cases. The AKC registers the Toy, Miniature, and Standard Poodle varieties, all as the same breed. However, because of differences in size, the varieties of Poodles are dealt with separately here. There was no occurrence of a joint disorder in intact males or females. However, in males neutered at 6-11 mo., there was a significant 9 percent occurrence of joint disorders ($p < 0.01$), reflecting CCL. In spayed females, there was no occurrence of a joint disorder. In intact males and females, there was a 5 and zero percent occurrence of cancers, respectively. There was no indication of increased cancer occurrence related to neutering in either sex. The only occurrence of MC in females was one female that had been spayed at 2–8 years. Of intact females, 6 percent developed PYO. Just one female spayed at <6 mo. developed UI. The suggested guideline for males, based on the significant occurrence of a joint disorder with neutering at 6-11 mo., is delaying neutering until a year of age. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered females, those wishing to neuter should decide on the appropriate age.

Poodle, Standard

The study population was 47 intact males, 88 neutered males, 53 intact females, and 87 spayed females for a total sample of 275 cases. The AKC registers the Toy and Miniature, along with the Standard Poodle, as all being Poodles. However, because of differences in size, the varieties of Poodles are dealt with separately here. There was a 2 percent occurrence of joint disorders in both intact males and females. In males neutered at <6 mo., there was a non-significant increase to 8 percent, and in spayed females, there was no occurrence of joint disorders. The occurrences of cancers in intact males and females were 4 and 2 percent, respectively. In males neutered at 1 year of age, the occurrence of one or more cancers rose to a significant 27 percent ($p < 0.01$), all due to the increased risk of LSA. In females, there was no significant increase in cancers with spaying. There was a 4 percent occurrence of MC, and a 2 percent occurrence of PYO in the females left intact. Just one female spayed beyond 2 years later developed UI. The suggested guideline for males, based on the occurrence of one or more cancers with neutering at 1 year, is to delay neutering until 2 years of age. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered females, those wishing to neuter should decide on the appropriate age.

Pug

The study population was 96 intact males, 106 neutered males, 63 intact females, and 118 spayed females for a total sample of 383 cases. In intact males and females, the occurrences of joint disorders were zero and 2 percent, respectively. In neutered males and females, there was no evident increased occurrence of joint disorders. The level of occurrence of one or more cancers in intact males was 6 percent and in intact females, 8 percent. Neutering males and females did not lead to any evident increase in risk of a cancer. There were no cases of MC in females left intact or spayed at any time, and there was a 5 percent occurrence of PYO in the intact females. None of the females was diagnosed with UI. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

Rottweiler

The study population was 315 intact males, 152 neutered males, 143 intact females, and 239 spayed females for a total sample of 854 cases. Joint disorders are a major concern in this breed with 8 percent of intact males and 16 percent of intact females having one or more joint disorders. In males, neutering at <6 mo. and at 6-11 mo. resulted in 10 percent and 22 percent occurrences (combined $p < 0.05$). In females, spaying at <6 mo. resulted in a significant 43 percent occurrence ($p < 0.05$), the main joint disorder being CCL. The cancers followed occurred in the intact males and females at 16 and 11 percent, respectively. These relatively high occurrences of cancers in intact males and females were not increased by neutering at any age. Of females left intact or spayed at 2–8 years, 8 and 5 percent were diagnosed with MC, respectively. In intact females, 12 percent were diagnosed with PYO. With regard to UI, 1 percent of intact females had UI, and in females spayed at <6 mo. and 6-11 mo., 4 and 6 percent, respectively had UI. The suggested guideline for males, given the risk of joint disorders for those neutered at 6-11 mo. or earlier, is neutering beyond a year of age. For females, given the increased risk of joint disorders with neutering at <6 mo., the suggested guideline is spaying beyond 6 months.

Saint Bernard

The study population was 26 intact males, 27 neutered males, 18 intact females, and 23 spayed females for a total sample of 94 cases. This breed was chosen because of the large size. In intact males and females, the occurrences of one or more joint disorders were 8 percent and 6 percent, respectively. While there was no evident increase in joint disorders with neutering males, in females spayed at <6 mo., joint disorders increased to a significant 100 percent ($p < 0.01$). The cancers followed occurred in intact males and females at 4 and 11 percent, respectively. With neutering males and females, there were no noteworthy increases in cancers. There was no occurrence of MC in either the intact or spayed females. In intact females, PYO was diagnosed in 15 percent There was no occurrence of UI in spayed females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males those wishing to neuter should decide on the appropriate age. The suggested guideline for females given in the increased risk of joint disorders with neutering at <6 mo., is neutering beyond 6 months. However, given the large body size, some may wish to consider neutering well-beyond 1 year of age.

Shetland Sheepdog

The study population was 31 intact males, 30 neutered males, 20 intact females, and 52 spayed females for a total sample of 133 cases. There were no joint disorders in intact males and just one in the intact females. In neutered males, the only joint disorder was in one of the males neutered at <6 mo. and in females there was no joint disorder associated with spaying. The occurrence

of cancers in intact males was 6 percent and in intact females, zero. There were no evident increases in cancers in neutered males or females. There was no occurrence of MC in intact or spayed females and a 14 percent occurrence of PYO in intact females. Spaying at 6-11 mo. resulted in a 6 percent occurrence of UI, but at 1 year a 33 percent occurrence. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males, those wishing to neuter should decide on the appropriate age. However, to avoid the high level of UI occurrence in females, one could consider spaying females at, or beyond, 2 years.

Shih Tzu

The study population was 104 intact males, 112 neutered males, 77 intact females, and 139 spayed females for a total sample of 432 cases. In this small-dog breed there were no occurrences of joint disorders in either intact or neutered males and females, revealing virtually no vulnerability in this regard. There was no occurrence of the cancers followed in intact males and females. In neutered males there was no occurrence of cancers. However, in females, the occurrence of cancers for those spayed at 6-11 mo. was 7 percent and at 1 year this measure reached a significant 18 percent ($p < 0.01$). MC occurred in 3 percent of intact females. PYO occurred in 5 percent of intact females. UI was not reported in any females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males, those wishing to neuter should decide on the appropriate age. The picture is very different for spaying females where the increased risk of cancers started with spaying at 6-11 mo., reaching 18 percent with spaying at year 1. The suggested guideline for females is to delay spaying until the female is 2 years of age. Another possibility is to spay a female a month or two before 6 months to avoid the increased risk of cancers.

West Highland White Terrier

The study population was 35 intact males, 33 neutered males, 28 intact females, and 46 spayed females for a total sample of 142 cases. Just one intact male had a joint disorder, and other than this, no joint disorders were reported in intact females or in neutered males or females. None of the intact males or females had any of the cancers followed. There were no noteworthy occurrences of the cancers in neutered males or females. There were no occurrences of MC in either intact or neutered females, and a 7 percent occurrence of PYO in intact females. The occurrence of UI was 14 percent for females spayed at <6 mo. and 6 percent at 6-11 mo. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age. However, for females, one could consider delaying spaying until a year of age to avoid the risk of UI.

Yorkshire Terrier

The study population was 134 intact males, 178 neutered males, 144 intact females, and 229 spayed females for a total sample of 685 cases. There were no joint disorders reported in intact males, and in intact females, just 1 percent. In neutered males and females there were no noteworthy occurrences of joint disorders. In intact males and intact females, just 1 percent were reported with at least one of the cancers followed. In both neutered males and females, none of the cancer occurrences was noteworthy. In intact females, the occurrence of MC was 1 percent as was the occurrence with spaying at 2–8 years. PYO was reported in 7 percent of intact females. No UI was reported in any of the intact or spayed females. Lacking a noticeable occurrence of increased joint disorders or cancers in neutered males or females, those wishing to neuter should decide on the appropriate age.

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Discussion

Since the reporting from this center of increased risks of joint disorders and some cancers in Golden Retrievers, Labrador Retrievers, and German Shepherd Dogs (11–13), the appropriate age of neutering has become a common point of discussion (16–18). With the evidence-based information on the risks, if any, of joint disorders, cancers, PYO and UI associated with neutering at different ages for males and females of various as dog breeds, dog owners, and their veterinarians, can use this information to select an age for neutering for the long-term health of their companion dogs on a case-by-case basis.

The overall major finding from the present study is that there are breed differences – and sometimes sex differences – with regard to the increased risks of joint disorders and cancers associated with neutering at various ages. For example, with the Boston Terrier, neutering females at the standard 6 month age did not increase the risks of joint disorders or cancers over that of dogs left intact, but with males, neutering before a year of age was associated with a significant increase in cancers. The opposite effect with genders was seen in the Cocker Spaniel where neutering at 6 months was not associated with an increase in joint disorders or cancers in males, but in females there was a significant increase in risk of cancers to 17 percent with neutering before 2 years.

Another important finding that holds across several breeds is that with the small-dog breeds – Cavalier King Charles Spaniel, Chihuahua, Corgi, Dachshund, Maltese, Pomeranian, Poodle-Toy, Pug, Shih Tzu, Yorkshire Terrier –the occurrences of joint disorders were close to zero in both the intact and neutered males and females. In these small-dog breeds, the occurrence of cancers was low in both those kept intact and neutered. Two exceptions were the Boston Terrier and Shih Tzu where there was there a significant increase in cancers with neutering.

As noted in the results section, the mean date of last entry per patient in the hospital record ranged from about 4.5 to 5.5 years, which means the data especially represent rather early-occurring joint disorders and cancers. The perspective taken here is that it is the early occurring joint disorders and cancers that are the most impactful on the human caregivers, both emotionally and financially, as well as their dogs. To just delay neutering by a year or so to lower the risk of a joint disorder or cancer in those breeds where the issue is relevant, is a noteworthy goal, making it worthwhile to discuss appropriate ages to neuter with caregivers who have a new puppy.

A suggested guideline for the use of the data presented here for those who may wish to focus on a breed or two, is to first scroll through **Table 1** to peruse the breeds for a brief look at the neutering guidelines for the breeds of interest. The next step could be to refer to summary paragraphs in the Results section, which present the major findings with a suggested guideline for neutering age. Then for a third step, one could turn to Appendix 1 for detailed joint disorder and cancer tabular data as well as data on MC, PYO, and UI. Our intention is to offer readers data-based information to make case-by-case decisions about age of neutering. As is clearly evident in the breed-specific data presented, one cannot make a generalization for all dogs about age of neutering guidelines.

As mentioned, this study involved 35 breeds, counting the three varieties of Poodles as three breeds. Thus, most breeds registered by AKC or other comparable agencies were not covered. The breeds chosen were the most popular, and with the largest dataset in our records, or were included to sample the largest range of breed sizes as was feasible. Hence, some of the largest breeds (e.g., Great Dane, Irish Wolfhound) and smallest breeds (Miniature Schnauzer, West Highland White Terrier) were included despite lower numbers of patient records. While with some of the most popular breeds there

were over 1,000 cases in the database, most breeds ended up with 200 to 500 cases which was sufficient for statistical analyses where the impact of neutering was substantial.

A suggestion for those interested in a breed not covered in this study is to find a breed or two closest genetically to the breed of their interest in order to get an estimate of the various disease risks, if any, associated with neutering. However, one needs to bear in mind that even genetically related breeds may vary a great deal. An example is seen when comparing Golden and Labrador Retrievers, using the data from this study, where in the Labrador, there was no increase in cancer risk above that of intact dogs with neutering, but in the female Golden, the risk of a cancer with neutering increased to 2–4 times that of the 5 percent level of intact females. The popular Poodle breed provides another example, where there are three major varieties in size, the Standard, Miniature, and Toy. In the Standard, neutering males at 1 year was associated with a highly significant increase in the risk of a cancer (mainly LSA) to over six times that of intact males, whereas in the Miniature, there was no increase in cancers with neutering but a significant increase in joint disorders (mainly CCL) with neutering at 6-11 mo.

A likely mechanism by which early neutering may lead to a joint disorder is related to disturbance of the closure of the long-bone growth plates by gonadal hormone secretion as the animal approaches maturity (19, 20). We have proposed that neutering much before the closure of growth plates allows the long bones to grow a little longer than normal, and may sufficiently disturb joint alignments in some neutered dogs to lead to a clinically-apparent joint disorder.

Given the frequency with which early neutering is performed in dogs, it seems surprising that osteoporosis has not been examined given that in humans, chronic loss of gonadal hormones is associated with osteoporosis (21). It could be that the wolf ancestor of the dog had one breeding season and that the bone structure of mature dogs was not as affected by seasonal fluctuations of gonadal hormones as with a permanent gonadal hormonal loss in humans.

One of the frequently mentioned advantages of early neutering of female dogs is protection against MC (22). There may be important genetic, breed-line differences in the occurrence of MC that are not portrayed in our database. However, relevant to the discussion of MC is the recent meta-analysis of published studies on neutering females and MC, finding that the evidence linking neutering to a reduced risk of MC is weak (23). In the data gathered in this study, through 11 years of age, the occurrence of MC in females left intact was rarely above 6 percent and frequently 2 percent or less. For those neutered at <6 months, there was, as expected, no occurrence of MC. Obviously with most cases of intact females not followed through 11 years, and with the 12-year cut-off for those that were followed, many occurrences of MC were missed. However, it seems reasonable, that if MC was a common occurrence in intact females that this disease would have been more frequent in the intact females followed. Further, a very late onset of MC would seem less disturbing to pet owners than the much earlier onsets of joint diseases and other cancers.

For males, there is some concern that neutering beyond puberty will increase the likelihood of a problem behavior such as aggression. However, studies show that while neutering males can reduce aggression to people or other dogs in about 25 percent of males, neutering prior to puberty is no more effective in preventing this problem than is neutering in adulthood in resolving the problem (24, 25).

This paper deals primarily with the risks of diseases that are seen within a given breed and sex. Comparisons between breeds are difficult to interpret, in part because of differences in developmental and physiological factors be-

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tween breeds including those between smaller and larger breeds. In the text we have reported the occurrences of various diseases in percentages but in statistical analyses the actual data are used. When disease incidence is particularly low in one or more neutering subgroups, the ability to detect significant differences will be low, but there still could be differences which may or may not have been evident in the statistical analyses.

There are at least two major limitations to this study. First, relatively few breeds are covered compared to those included in the various breed registries of kennel clubs and canine organizations. This limitation was necessary so as to apply the same diagnostic criteria for diseases covered across all breeds, using the same database, and the necessity of having sufficient cases for analyses. Second, no information is available as to the reasons the owners or others chose to neuter, or not to neuter their dogs. In California, the vast majority of dogs are neutered, and since 2005 it is legally required for dogs to be neutered prior to adoption from an animal shelter or humane society (26); many breeders impose the same requirement.

In conclusion, the data presented should provide to veterinarians and interested puppy caregivers data-based information for the best age for neutering to avoid increasing the risk of joint disorders and some cancers beyond that of leaving the dog intact. Readers can note that an elevated risk for a joint disorder or cancer occurs in relatively few of these breeds. In other words, with most breeds or sexes, neutering can apparently be done without referral to a particular age, at least with regard to the joint disorders or cancers covered in this study. Of course, individual factors must be taken into account. For puppies of mixed breed, another paper that is currently in press provides data-based information dealing with age of neutering and the risk of one or more joint disorders as a function of the dog adult weight category (27). This information can also help inform decisions on age of recommended neuter in purebred dogs where the breed is not covered in our data.

Data Availability Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: (Figshare, doi: 10.6084/m9.figshare.7231010).

Author Contributions

BH, LH, and AT: conceived and designed study, collected and complied, and analyzed data. NW: statistical analyses. BH, LH, AT, and NW: drafted and edited manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.00388/full#supplementary-material

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Tres suplementos alimentarios que ayudan a mantener la función cardíaca

La adición de determinados suplementos aminoacídicos y componentes derivados de aminoácidos a la dieta de las mascotas puede ayudar al tratamiento de determinadas patologías. Además, la deficiencia de algunos de ellos puede ser la causa directa de enfermedades del corazón.

(R) evolution

PEQUEÑOS ANIMALES

Taurina

La taurina es uno de los aminoácidos libres más abundantes. Se encuentra en elevadas concentraciones en los tejidos del músculo cardiaco, músculo esquelético, sistema nervioso central y plaquetas. Actúa en numerosos procesos metabólicos, ejerciendo diversas funciones:

- Antioxidación
- Actividad en las células fotorreceptoras de la retina
- Estabilización de las membranas neuronales
- Desarrollo del sistema nervioso
- Reducción de la agregación plaquetaria
- Reproducción
- Actividad miocárdica[1]:
  - Modulación de las concentraciones de calcio en los tejidos y su disponibilidad.
  - Inactivación de los radicales libres y cambio de la osmolaridad celular.
  - Efectos en la osmorregulación del miocardio.
  - Otros mecanismos específicamente relacionados con la función miocárdica incluyen la N-metilación de los fosfolípidos de la membrana celular, efectos directos en las proteínas contráctiles e interacciones con el sistema renina-angiotensina-aldosterona.

¿Qué ocurre en casos de deficiencia de taurina?

En **gatos**, la taurina es un aminoácido esencial y su deficiencia puede causar miocardiopatía dilatada (MCD), degeneración de la retina y anomalías reproductivas. Existen evidencias de que la MCD causada por su deficiencia puede ser reversible con la suplementación de este aminoácido[2,3].

En **perros**, hasta hace unos años, la taurina no se consideraba un aminoácido esencial ni se conocía su papel en el desarrollo de la MCD[4]. Sin

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embargo, diversos estudios han demostrado que sí lo es en perros alimentados con dietas restrictivas en proteína y que, al igual que los gatos, pueden desarrollar MCD secundaria a la deficiencia de taurina[5].

L-carnitina

La L-carnitina es un derivado aminoacídico que se obtiene de la proteína de la dieta o por síntesis endógena en el hígado, siendo la lisina y la metionina los aminoácidos precursores. La síntesis requiere hierro, vitamina C y vitamina B6 como cofactores. El músculo esquelético y el cardiaco son los lugares donde se almacena hasta el 95-98% de la carnitina del cuerpo.

Entre las funciones de la carnitina, la más importante es la de cofactor de algunas enzimas necesarias para el transporte de ácidos grasos de cadena larga al interior de las mitocondrias, donde se oxidan para la generación de energía para el corazón, que obtiene de esta manera aproximadamente el 60% de su producción de energía total.

¿Qué es la miocardiopatía dilatada (MCD)?

Se trata de una enfermedad del corazón muy habitual, progresiva y, en gran medida, irreversible, que puede conducir a fallo cardiaco congestivo o muerte súbita. Es la segunda enfermedad cardiaca más habitual en perros, con una prevalencia superior al 50% en algunas razas10. La nutrición está actualmente aceptada como un importante adyuvante a la terapia médica en perros y gatos con MCD.

¿Qué ocurre en casos de deficiencia de L-carnitina?

La deficiencia de L-carnitina puede ser un trastorno primario o secundario.

- Las deficiencias primarias pueden aparecer por defectos genéticos en la síntesis, transporte, absorción o degradación. En personas se han asociado con cardiomiopatías.
- Las deficiencias secundarias son más comunes en pacientes que siguen dietas restrictivas

Se ha demostrado en perros que la deficiencia de L-carnitina puede favorecer el desarrollo de MCD en perros. Además, varios estudios[6,7,8,9,10] han puesto de manifiesto que suplementar con carnitina mejora el tiempo de supervivencia de perros con MCD.

Hidrolizado de levadura de cerveza

La levadura de cerveza es un subproducto de la industria cervecera que puede ser un ingrediente beneficioso en la alimentación de las mascotas, ya que aporta el contenido nutricional que necesitan los perros y gatos[11]. Esterilizada y sin poder leudante, es una levadura inactiva compuesta por el organismo unicelular Saccharomyces cerevisiae.

Su administración tiene efectos beneficiosos sobre la salud intestinal y la función inmune de los perros, estimulando las respuestas Th1 y, en consecuencia, la inflamación. Además, mejoran la palatabilidad de las dietas[12]. Esto resulta especialmente útil para los perros con poco apetito a consecuencia de una enfermedad crónica.

Es una fuente proteínica rica en aminoácidos esenciales y vitaminas del grupo B:

- Los aminoácidos ayudan a la mascota a construir y mantener sus músculos, huesos, sangre, órganos, sistema inmunitario y pelaje y uñas. En particular, la arginina es un aminoácido esencial que reacciona con el oxígeno para producir óxido nítrico. El óxido nítrico relaja los músculos lisos de los vasos sanguíneos y reduce la presión arterial[13]. La hipertensión puede contribuir a las cardiopatías y a la insuficiencia cardíaca crónica, por lo que es conveniente controlar la tensión arterial de cualquier perro sospechoso de padecer una cardiopatía.
- Las vitaminas del grupo B contribuyen a la función cerebral, la fuerza muscular, la producción de glóbulos rojos y la digestión de los animales.

Por otro lado, se ha demostrado que estimula la producción de determinados marcadores de defensa antioxidantes, lo que ayuda a mejorar la salud cardiovascular de los animales[14].

- A medida que progresa la insuficiencia cardiaca congestiva, aumenta el daño a las células cardiacas por la formación de radicales libres. Los estudios realizados en perros con insuficiencia cardíaca congestiva han demostrado que estos pacientes presentan un aumento de oxidantes reactivos y una disminución de antioxidantes a medida que progresa la enfermedad[15].
- En perros con fallo cardiaco, la oxigenación y el metabolismo celular no funcionan de forma apropiada, lo que conlleva la producción de elevadas cantidades de radicales libres. Los radicales libres son responsables de los principales daños celulares, lo que se denomina estrés oxidativo[16].

Adicionalmente, la levadura de cerveza contiene sodio, calcio, magnesio y potasio. Muchos de los medicamentos utilizados para tratar las cardiopatías disminuyen los niveles sanguíneos de potasio y magnesio.

o Unos niveles inadecuados de potasio y magnesio pueden favorecer las arritmias cardiacas y debilitar las contracciones del músculo cardiaco[17].

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Dogs as a Natural Animal Model of Epilepsy

Los perros como modelo animal natural de la epilepsia

Palabras clave: convulsiones, medicamentos anticonvulsivos, farmacocinética, EEG intracraneal, neuroestimulación receptiva, estado epileptico, epilepsia canina

Keywords: *seizures, antiseizure medications, pharmacokinetics, intracranial EEG, responsive neurostimulation, status epilepticus, canine epilepsy*

https://www.frontiersin.org/articles/10.3389/fvets.2022.928009/full

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La epilepsia es una enfermedad neurológica común tanto en humanos como en perros domésticos, lo que convierte a los perros en un modelo de traducción ideal de la epilepsia. En ambas especies, la epilepsia es una enfermedad cerebral compleja que se caracteriza por una predisposición duradera a generar convulsiones epilépticas espontáneas recurrentes. Además, al igual que en los seres humanos, el estado epiléptico es una de las emergencias neurológicas más comunes en perros con epilepsia. En ambas especies, la epilepsia no es una sola enfermedad, sino un grupo de trastornos que se caracterizan por una amplia gama de signos clínicos, edad de inicio y causas subyacentes.

Epilepsy is a common neurological disease in both humans and domestic dogs, making dogs an ideal translational model of epilepsy. In both species, epilepsy is a complex brain disease characterized by an enduring predisposition to generate spontaneous recurrent epileptic seizures. Furthermore, as in humans, status epilepticus is one of the more common neurological emergencies in dogs with epilepsy. In both species, epilepsy is not a single disease but a group of disorders characterized by a broad array of clinical signs, age of onset, and underlying causes. Brain imaging suggests that the limbic system, including the hippocampus and cingulate gyrus, is often affected in canine epilepsy, which could explain the high incidence of comorbid behavioral problems such as anxiety and cognitive alterations. Resistance to antiseizure medications is a significant problem in both canine and human epilepsy, so dogs can be used to study mechanisms of drug resistance and develop novel therapeutic strategies to benefit both species. Importantly, dogs are large enough to accommodate intracranial EEG and responsive neurostimulation devices designed for humans. Studies in epileptic dogs with such devices have reported ictal and interictal events that are remarkably similar to those occurring in human epilepsy. Continuous (24/7) EEG recordings in a select group of epileptic dogs for >1 year have provided a rich dataset of unprecedented length for studying seizure periodicities and developing new methods for seizure forecasting. The data presented in this review substantiate that canine epilepsy is an excellent translational model for several facets of epilepsy research. Furthermore, several techniques of inducing seizures in laboratory dogs are discussed as related to therapeutic advances. Importantly, the development of vagus nerve stimulation as a novel therapy for drug-resistant epilepsy in people was based on a series of studies in dogs with induced seizures. Dogs with naturally occurring or induced seizures provide excellent large-animal models to bridge the translational gap between rodents and humans in the development of novel therapies. Furthermore, because the dog is not only a preclinical species for human medicine but also a potential patient and pet, research on this species serves both veterinary and human medicine.

Introduction

Domestic dogs (*Canis lupus familiaris*) provide an ideal model for translational medicine as they have the most phenotypic diversity and known naturally occurring diseases of all land mammals

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other than humans (1). Dogs share an evolutionary history and high amount of ancestral genetic sequence with humans, as well as the characteristics of our environment (2). The level of sophistication of the healthcare system for dogs in Europe and the United States is second only to that of humans. Thus, data related to dog health presents many opportunities to discover insights into health and disease outcomes in both dog and human populations. In fact, naturally occurring diseases in companion animals are often similar—and sometimes identical—to human diseases concerning the disease etiology, progression, and how that disease responds to medical intervention or treatment (1, 3, 4). In addition, dogs are the main non-rodent species in preclinical drug development, particularly in the evaluation of pharmaceutical safety, pharmacokinetics, and efficacy (5–7). Concerning translational neuroscience, it is important to note that unlike the lissencephalic brains of mice and rats, the brains of both dogs and humans are gyrencephalic (2).

Epilepsy is the most common medical neurologic disease of dogs (8). While reference to using dogs with naturally occurring epilepsy as a potential comparative model of the underlying basis and therapy of epilepsy was made in the 1970's (9, 10), we were the first to perform comparative pharmacokinetic studies on anti-seizure medications (ASMs; previously termed antiepileptic drugs) in dogs (11–24). We proposed epileptic dogs as a natural model of human epilepsy in research and drug development some 40 years ago (24–26) followed by numerous studies in this species, including the first controlled clinical drug trial in epileptic dogs (27). This review will highlight the usefulness of dogs with naturally occurring or induced seizures as a large animal model of epilepsy with a focus on pharmacology and drug development. In this respect, it is important to note that research on this species serves both veterinary and human medicine, as the epileptic dog is not only a preclinical species for advancing knowledge and treatment for humans, but also a potential patient as a pet. To emphasize the biomedical and societal importance of this aspect, we will use the development of the ASM imepitoin for canine epilepsy as an example.

Epilepsy in Dogs

Epidemiology of Epilepsy in Dogs

In both dogs and humans, epilepsy is a complex brain disease characterized by an enduring predisposition to generate recurrent epileptic seizures. Epilepsy is not a single disease but a group of disorders characterized by a broad array of clinical signs, age of onset, and underlying causes (28). The true prevalence of epilepsy in dogs is unknown and has been estimated to be 0.6–0.75% in the general dog population (29, 30), which is similar to the prevalence of epilepsy in humans (28). In certain dog breeds predisposed to idiopathic epilepsy, considerable higher prevalence rates are reported than those estimated for the general dog population, which is one of the reasons a genetic component is suspected in certain canine breeds (31).

The International Veterinary Epilepsy Task Force (IVETF) divides epilepsy into the categories of structural epilepsy (due to acquired or inherited structural brain alterations) and idiopathic epilepsy (32). Idiopathic epilepsy is defined as a disease in its own right where no structural cerebral pathology is suspected (or seen) and in many cases, a genetic component may be involved (32). In this respect, the terminology of the IVETF differs from the terminology of the International League Against Epilepsy (ILAE) for human epilepsy, in which "idiopathic" has been replaced by "genetic" and "unknown etiology" (33). Based on seizure types, epilepsies are classified into focal, generalized, generalized and focal, and unknown (33). At the next level, the ILAE differentiates numerous epilepsy syndromes by a distinctive clinical pattern and electroencephalographic (EEG) features (34), which is not possible yet in

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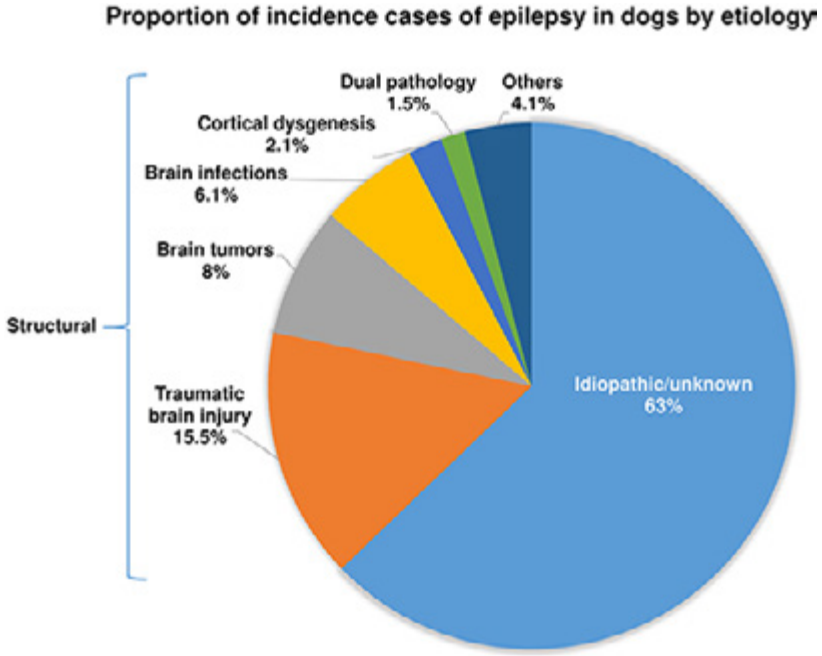
canine epilepsy because of the limitations of EEG analyses in dogs (see below). Well-recognized examples of epilepsy syndromes in humans include childhood absence epilepsy, juvenile myoclonic epilepsy (JME), and benign epilepsy with centrotemporal spikes (33). A final level of diagnosis of the ILAE classification scheme establishes that the primary etiology and epilepsy diagnosis have been determined (33). This level of diagnosis opens the gateway to a precision-medicine approach that reflects current scientific efforts (35). In medicine, the ability to make an etiological diagnosis is rapidly increasing with the revolution in genetics and other fields such as neuroimaging. Numerous new etiological diagnoses are emerging, particularly pediatric epileptic encephalopathies (36, 37). One of the best-known examples is the Dravet syndrome, which is caused by a known mutation of the sodium channel gene SCN1A (35). Because of the limited availability of EEG-video, genetic, and brain imaging data, canine classification of epilepsy is mainly based on presumed etiology.

In 2013, we published the outcome of a large retrospective study in 1,000 dogs referred to the Department of Small Animal Medicine and Surgery of our University in Hannover over 11.5 years (38). As shown in Figure 1, 63% of the dogs were categorized as having idiopathic or unknown etiology, and 37% had a structural etiology. Within the group of structural or acquired epilepsy, dogs with traumatic brain injury (TBI) formed the largest subgroup. More recently, similar data were reported by Hall *et al.* (39). Based on a retrospective study on 900 dogs undergoing magnetic resonance imaging (MRI) for seizures, structural lesions were identified as a cause of seizures in 45.1% of cases, and no structural lesions were identified in 54.9% of cases. In the structural epilepsy group, TBI was less often identified as a cause of acquired epilepsy than in our study, which may be due to the differences in study design or case population (39). A similar figure of 46% of epileptic dogs having structural causes was obtained in a prospective study by Podell *et al.* (40).

Importantly, the epidemiologic data on predisposing causes of epilepsy in dogs shown in Figure 1 (38) are very similar to respective studies in humans with epilepsy (41–43). Based on the relative proportion of etiologies identified in a large population-based study out of Rochester, Minnesota, U.S.A., over 50 years (44), 65% of the patients were categorized as "idiopathic/cryptogenic" and 35% as symptomatic. In the latter group, head trauma was identified as the cause of epilepsy in 6% of the population, stroke in 10%, brain tumors in 6%, infections in 3%, degenerative

Figure 1. *Presumed causes of recurrent epileptic seizures in dogs with epilepsy. See Steinmetz et al. (38) and text for further details.*



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causes in 4%, and congenital brain alterations in 8%, respectively. This remarkable similarity with the predisposing causes of epilepsy in dogs shown in **Figure 1** is a strong argument for the suitability of epileptic dogs as a translational model of epilepsy. However, it is important to note that in human medicine the percentage of "cryptogenic" epilepsies (now termed epilepsies with unknown etiologies) is progressively declining in recent years because of the frequent use of high-resolution MRI and the advent of modern technologies to identify genetic causes, such as next-generation sequencing (42, 45, 46). It is to be expected that similar advances will take place concerning epilepsy in dogs in the future. The recent MRI-based study of Hall *et al.* (39) is a good example because the percentage of dogs without obvious structural lesions was only 54.9 percent of cases, which is ~8% lower than in the 2013 study of Steinmetz *et al.* (38).

Dog breeds, which have been identified as being predisposed to idiopathic epilepsy, include the Australian Shepherd, Belgian Tervueren, Belgian Shepherd, Border Collie, Irish Wolfhound, Labrador Retriever, Petit Basset Griffon Vendeen, Finnish Spitz Dog, and Italian Spinone (31, 47). Even though pedigree analysis has strongly suggested genetic influence in these breeds, the identification of the affected genes has been quite difficult (47–49). Up to date, only a few monogenic epilepsies have been identified in dogs that parallel epilepsies in humans regarding epilepsy onset and seizure types (47). Thus, in contrast to the genetics of inherited human epilepsies, where modern techniques such as high-throughput sequencing have led to the identification of a progressively increasing high number of epilepsy syndromes, including the epileptic encephalopathies, with known genetic basis (36, 42, 45, 50–52), this area of research is in its infancy in canine epilepsy.

Seizure Types in Dogs With Epilepsy

According to the ILAE, epileptic seizures are divided into focal onset, generalized onset, and unknown onset (53). Generalized onset seizures are subdivided into motor (e.g., generalized tonic-clonic) and non-motor (e.g., absence) seizures. Focal onset seizures may secondarily generalize to generalized tonic-clonic seizures. In principal, these seizure types are also observed in epileptic dogs. In the past, generalized tonic-clonic seizures were often considered the most frequent type of seizures in canine epilepsy, but accumulating evidence suggests that focal onset seizures are the major seizure onset form in canine epilepsy (32, 54, 55). As in humans, generalized tonic-clonic seizures may have a generalized onset or arise by secondary generalization after focal onset seizures. In dogs, seizure type (e.g., focal vs. generalized) should not be used as an isolated variable to predict the presence of structural epilepsy, although focal (partial) seizures often suggest a structural etiology (40, 56). In general, the type of epilepsy and seizures is an important factor for the prognosis of therapy (56). Structural epilepsies with focal onset seizures have a poorer prognosis than idiopathic epilepsies with generalized onset seizures (57). Focal onset seizures may be very subtle and can be easily missed by the dog's owner, particularly when they occur at the night. More complex focal seizures may manifest as bizarre behavior, such as unprovoked aggression, running uncontrollably, or rhythmic barking (32). Furthermore, structural epilepsy with focal onset seizures may be associated with a pre-ictal phase, i.e., is a period of altered behavior in which the dog may hide, appear nervous, or seek out the owner. Although the literature on ictal semiology of focal seizures in dogs is limited, similarities have been found with regard to the distribution and semiology of focal seizures between dogs and humans (58). As in humans (28), focal seizures with or without secondary generalization seem to be the most frequent type of seizures in dogs with epilepsy, associated with a poor prognosis of treatment (55).

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EEG Studies in Dogs With Epilepsy

In a clinical setting, non-invasive scalp EEG recording by a standardized electrode arrangement is a key method in the evaluation of epilepsy in humans, guiding primary diagnosis, epilepsy classification, and treatment (59, 60). In contrast, the EEG has never been established as a routine laboratory test for the diagnosis of canine epilepsy, at least in part because non-invasive scalp EEG recording is compromised by artifacts due to the thick muscles on the dog's skull (61). To overcome this problem, subdermal needle scalp electrodes have been used in specific neurological referral hospitals, but this necessitates immobilization of the dog by deep sedation or anesthesia, which is likely to affect interictal and ictal EEG recordings (62). To reduce this problem, when sedation or general anesthesia was used for EEG electrode placement, ambulatory EEG recording may extend beyond recovery to a normal mentation state (63). The IVETF (32, 56) has recognized and described the importance of the EEG in epileptic dogs and noted that the development of a standardized EEG protocol is an urgent priority for veterinary neurology, not the least to promote resective epilepsy surgery in the future. Indeed, surgical removal of the epileptic focus is the only available cure for epilepsy (64), but is not yet used in dogs with drug-resistant epilepsy (DRE) because it is difficult to accurately localize the origin of seizures in the brain of this species (65, 66).

There are various reports of EEG recordings in epileptic dogs, but most likely due to the sedation or anesthesia used, the detection rates of EEG alterations in most reports were low (66). It is unlikely that EEG recordings in epileptic dogs can be used to characterize seizures unless novel implantable EEG devices and continuous EEG monitoring become available. The group of Brian Litt at the University of Pennsylvania and collaborators have developed a novel implanted device to wirelessly record and analyze continuous intracranial canine EEG (67). When using this device for continuous intracranial EEG (iEEG) monitoring in six conscious (non-anesthetized) dogs with naturally occurring epilepsy over 5 months, Davis *et al.* (67) demonstrated previously uncharacterized intracranial seizure onset patterns in these animals that are strikingly similar in appearance to human focal onset epilepsy. In a subsequent yearlong study with this device in four epileptic dogs, Ung *et al.* (68) found significant temporal variability in seizures and interictal bursts after electrode implantation that required several weeks to reach a steady-state. These findings, comparable to those reported in humans implanted with the NeuroPace Responsive Neurostimulator System (RNS) device (see below), suggest that transient network changes following electrode implantation may need to be taken into account when interpreting or analyzing iEEG during evaluation for epilepsy surgery. Once a steady-state was reached, multiple seizure types were observed in each dog, with significant temporal variation between types (68). Seizures typically occurred in clusters, and isolated seizures were rare (see below for a more detailed discussion of this iEEG device).

Morita *et al.* (69) used continuous EEG recording with subcutaneous electrodes every 1–3 months under sedation with xylazine in epileptic Shetland Sheedogs and found that an epileptic focus was initially detected in the frontal lobe, particularly the internal area, and that paroxysmal foci developed diffusely in other lobes of affected dogs with recurrent convulsions. These examples illustrate the usefulness of continuous EEG recordings in canine epilepsy in localizing the onset of seizures and characterizing their evolution.

Status Epilepticus in Dogs With Epilepsy

Status epilepticus (SE), the condition of ongoing seizures or repetitive seizure activity without recovery of consciousness between seizures, is one of the

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more common neurological emergencies with a risk of high mortality or morbidity in people (70). Most frequently, SE is characterized by generalized convulsive tonic-clonic seizures, whereas non-convulsive SE is less frequent. SE may occur in patients with previous epilepsy or acute disorders of the CNS (71). Common causes of SE in human patients with epilepsy include low ASM levels or abrupt termination of treatment with ASMs. SE requires immediate i.v. treatment with an ASM to reduce mortality (72). However, not all patients will respond to initial treatment. The two most important variables that influence the drug response of SE are the underlying etiology and the duration of SE (73). Concerning SE duration, the longer the SE persists (typically ~0.5–1 h), the more likely is the SE to be unresponsive to drug therapy, the higher the mortality, and the worse the long-term consequences are in survivors. Based on treatment response, SE is divided into four stages: early, established, refractory, and super-refractory (74). Initial i.v. treatment with benzodiazepines (BDZs) has become the standard of care for early SE. When treatment fails ("established SE"), a second-line ASM is injected. If this treatment fails, too, SE is defined as refractory, potentially necessitating anesthetic agents to terminate SE (75). Refractory SE occurs in 23–43% of patients with SE and is associated with short-term fatality rates between 16 and 39% (75). Super-refractory SE is defined as seizure activity >24 h despite treatment with anesthetic agents. This includes cases in which seizures recur with an attempted withdrawal of the anesthetics (76). Effective treatment of SE is critical as morbidity and mortality increase dramatically the longer convulsive SE persists.

It has been estimated that nearly 60% of epileptic dogs may—at some point in their lifetime—experience one or more SE events (77). SE may be the first manifestation of a seizure disorder in dogs (78). It results from the failure of endogenous termination of an isolated seizure. The prognosis for dogs with SE is quite poor—up to 25% of affected dogs will not survive hospital discharge (78, 79). SE can lead to permanent brain damage (e.g., neuronal cell necrosis, network reorganization, gliosis) and severe systemic complications (e.g., cardiorespiratory collapse, shock, acidosis, and electrolyte imbalances) (80). Cluster seizures may be a precursor of SE and are defined as two or more seizures within 24 h. However, they differ from SE because, during cluster seizures, patients regain consciousness, or return to baseline CNS function, between seizures (81). As in humans, the main goals of treatment of SE or cluster seizures in dogs are to halt seizure activity, prevent further seizures, identify the cause of the seizures, and manage any complications (79). Effective ASMs in canine SE are the same as are used in humans with SE, making canine SE a translational platform for human therapeutic trials (77).

SE is typically treated by i.v. administration of ASMs in a hospital setting. As in humans, i.v. BDZs are the first-line treatment of SE in dogs. However, when i.v. access is not available for emergency treatment, intramuscular, rectal, intranasal, buccal or sublingual administration may be useful. Charalambous *et al.* (82) performed a randomized parallel-group clinical trial on intranasal midazolam vs. rectal diazepam for the management of canine SE and found that intranasal midazolam is a quick, safe, and effective first-line medication for controlling SE in dogs and appears superior to rectal diazepam. However, in 30% of the dogs, intranasal midazolam did not terminate the SE. In this respect, it is important to note that the subtypes of SE [early (BDZ-responsive) SE, established SE, refractory SE, super-refractory SE] described above for people have been applied to dogs (80). Furthermore, as in humans, the longer the SE persists before the onset of treatment, the higher the likelihood of drug resistance (83). Combinatorial therapies may be more effective to interrupt SE than single drug treatment (84).

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Sudden Unexpected Death in Epilepsy (SUDEP) in Dogs With Epilepsy

Sudden unexpected death in epilepsy (SUDEP) has been defined in persons with epilepsy as "the sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death of a patient with epilepsy with or without evidence of a seizure, excluding documented status epilepticus, and in which postmortem examination does not reveal a structural or toxicological cause of death" (85). SUDEP typically occurs in patients with poorly controlled epilepsy. Although SUDEP is relatively rare, it contributes to the reduced life expectancy of patients with drug-resistant epilepsies (86, 87). Each year, roughly 1 in every 1,000 adults and 1 in 4,500 children with epilepsy will die from SUDEP. The underlying cause of SUDEP is unknown. The condition may be due to an abnormality of breathing, cardiovascular dysfunction, arousal deficits, or a combination of these (87). In dogs, SUDEP is thought to be uncommon but may be underrecognized (78). Probable SUDEP has been documented in a large cohort of dogs with idiopathic epilepsy (88).

Comorbidities in Dogs With Epilepsy

Comorbidities such as mood and psychiatric disorders or deficits in learning and memory may be present before the onset of epilepsy, may constitute an aspect of the epilepsy syndrome, or occur as a consequence of epilepsy in people (89). Indeed, some common mechanisms, such as structural and functional alterations in the limbic system, might underlie both epilepsy and comorbidities (89). In humans, psychiatric disorders, such as anxiety, depression, psychosis, attention-deficit/hyperactivity disorder (ADHD), and cognitive decline are common comorbidities of epilepsy (28, 90). The prevalence of psychiatric disorders in people with epilepsy is higher than in either the general population or patients with other chronic medical diseases (91).

In epileptic dogs, a variety of comorbid behavioral changes have been reported, including anxiety and defensive aggression, psychosis-like symptoms (e.g., barking without apparent cause, chasing shadows or light spots, aimless pacing and staring into space), ADHD-like symptoms, and cognitive alterations (92, 93). However, abnormal behaviors such as anxiety, restlessness, irritation, and attention-seeking may also constitute prodromal signs that precede the onset of a seizure or post-ictal signs, indicating an involvement of the limbic system. Furthermore, focal seizures with a sensory or psychic component often manifest as behavioral changes, including anxious behaviors, restlessness, pacing, and seeking out their owner (58).

Treatment of Epilepsy in Dogs

ASMs, previously referred to as anticonvulsant or antiepileptic drugs, are the mainstay of symptomatic epilepsy treatment in humans and dogs (72, 94). The goal of epilepsy therapy is the complete elimination of seizures, which, however, is not always achievable, with a secondary goal to reduce the severity and frequency of seizure events (see below). Currently, about 30 ASMs are available for epilepsy therapy in humans; however, not all are suitable for therapy in dogs. The main reason for this is pharmacokinetic species differences. As shown in Table 1, most ASMs are much more rapidly eliminated in dogs than in humans, making maintenance of therapeutic drug levels in dogs difficult if not impossible. Only three ASMs, phenobarbital, imepitoin, and potassium bromide, have been approved for epilepsy therapy in dogs in Europe, and only one (primidone) in the U.S. Potassium bromide is only approved in Europe as add-on therapy in

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dogs in which treatment with phenobarbital or imepitoin failed. As in humans, epileptic dogs have to be treated daily and lifelong with an ASM, because the treatment only symptomatically suppresses the seizures. Treatment with too low doses or abrupt termination of treatment may lead to life-threatening SE (see above).

In dogs that are resistant to the approved drugs, ASMs that are approved for the treatment of human epilepsy can be tried as add-on medication, provided the half-life is long enough to allow maintenance of effective drug levels (cf., Table 1). A variety of such ASMs has been tried as add-on therapy (or monotherapy) in epileptic dogs, mostly with limited success (24, 57, 94). However, levetiracetam has been successfully used for "pulse" treatment for cluster seizures and shortly before generalized convulsive seizures that are predicted by behavioral alterations (93, 99).

Phenobarbital, primidone, and BDZs (e.g., clobazam, clonazepam, and diazepam) lead to tolerance (loss of efficacy) and physical dependency upon chronic treatment of dogs; so the drug dose has to be increased during the 1st weeks of treatment (100). This tolerance is mainly due to the adaptation of the $GABA_A$ receptor to the continuous presence of these drugs (functional tolerance); in the case of phenobarbital and primidone, metabolic tolerance

ASM	Half-life (h)		Perceived mechanism of action
	Human	Dog	
Acetazolamide	10–15	?	Carbonic anhydrase inhibitor
Brivaracetam	7–8	?	SV2A modulator
Cannabidiol (CBD)	24–48	11–19	Unknown (CBD does not act on CB1 or CB2 receptors)
Carbamazepine	25–50[a,b]	1–2[a,b]	Modulator of voltage-gated sodium channels
Cenobamate	50–60	?	Modulator of voltage-gated sodium channels plus PAM at $GABA_A$ receptors
Clobazam	16–50	~1.5	Agonist at the BZD-BS of the $GABA_A$ receptor
Clonazepam	18–50	1–3	Agonist at the BZD-BS of the $GABA_A$ receptor
Diazepam	24–72[a] (DMD = 40–130)	1–5[a] (DMD = 4)	Agonist at the BZD-BS of the $GABA_A$ receptor
Eslicarbazepine acetate	10–20	?	Modulator of voltage-gated sodium channels
Ethosuximide	40–60	11–25	Modulator of voltage-gated calcium channels
Felbamate	14–22	4–8	Mixed
Fenfluramine	13–30	2–4	Increase of serotonin release
Fosphenytoin (a prodrug of phenytoin)	~7–15 min[a] (phenytoin 15–20[b,c])	~3 min[a] (phenytoin 2–6[b,c])	Modulator of voltage-gated sodium channels
Gabapentin	5–7	3–4	Modulator of α2δ subunit of calcium channels
Imepitoin*	~8	2–6	Partial agonist at the BZD-BS of the $GABA_A$ receptor
Lacosamide	13	2–2.5	Modulator of voltage-gated sodium channels
Lamotrigine	21–50	2–5	Modulator of voltage-gated sodium channels
Levetiracetam	6–11	4–5	SV2A modulator
Oxcarbazepine	1–2.5[a] (MHD = 8–14)	~4[a] (MHD = 3–4)	Modulator of voltage-gated sodium channels
Perampanel	70	5	Inhibitor of glutamate receptors of the AMPA subtype
Phenobarbital*	70–100[b]	25–90[b]	Partial agonist at the barbiturate-BS of the $GABA_A$ receptor
Phenytoin	15–20[b,c]	2–6[b,c]	Modulator of voltage-gated sodium channels
Potassium bromide*	~300	~600	Potentiation of GABA
Pregabalin	6	6–7	Modulator of α2δ subunit of calcium channels
Primidone**	6–12[a] (PB = 70–100)	4–12[a,b] (PB = 25–90)	Acts *via* metabolism to PB, which is a partial agonist at the barbiturate-BS of the $GABA_A$ receptor
Retigabine (ezogabine)	6–8	3–10	Activator of voltage-gated potassium (K_v7) channels
Rufinamide	6–10	~10	Mixed
Stiripentol	5–13	?	PAM at $GABA_A$ receptors
Sulthiam	2–16	?	Carbonic anhydrase inhibitor
Tiagabin	5–8	1–2	Inhibitor of GAT1 GABA transporter
Topiramate	20–30	3–4	Mixed
Valproate	8–15[a]	1–3[a]	Mixed
Vigabatrin	5–7[d]	?[d]	Inhibitor of GABA degradation
Zonisamide	60–70	~15	Mixed

Data are from previous reviews of Löscher (95, 96) and have been revised and updated for the present review. "?" indicates that no data were found. If not noted otherwise, all half-lives are in hours. Note that dogs eliminate most ASMs much more rapidly than humans, which has to be considered when using such drugs for chronic studies in dogs. ASMs that are approved for canine epilepsy therapy in Europe are marked by an asterisk while ASMs that are approved for canine epilepsy therapy in the US are marked by two asterisks. In addition, the perceived main mechanism of action of ASMs is shown for each drug. "Mixed" indicates that the ASM acts by more than one mechanism. For details see Rogawski et al. (97) and Sills and Rogawski (98).

BS, binding site; BZD-BS, benzodiazepine binding site; DMD, desmethyldiazepam; MHD, monohydroxy derivative; PAM, positive allosteric modulator; PB, phenobarbital; SV2A, synaptic vesicle glycoprotein 2A.

[a] Active metabolites.

[b] Shortens on continuing exposure to the drug (because of enzyme induction).

[c] Non-linear kinetics (half-life increases with dose).

[d] Duration of action independent of half-life because of irreversible inhibition of GABA degradation.

Table 1. *A comparison of elimination half-lives of antiseizure medications (ASMs) in humans and dogs.*

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(by induction of liver enzymes) contributes to the overall loss of efficacy. With such drugs, abrupt termination of treatment may lead to life-threatening SE. In contrast, imepitoin, which differs from phenobarbital and BDZs, acts only as a partial low-affinity agonist at the $GABA_A$ receptor and has no tolerance or dependency liability (101). A further advantage of treatment with imepitoin is that, in contrast to phenobarbital and potassium bromide, no therapeutic drug monitoring (by determination of drug plasma levels) is needed during therapy (101). In this respect, it is interesting to note that therapeutic plasma levels of phenobarbital in epileptic dogs are in the same range (10–40 µg/ml) as those in persons with epilepsy (102).

If ASMs fail to suppress or, at least, ameliorate seizures, there are several additional options to treat the DRE, including the ketogenic diet and vagus nerve stimulation (VNS) (65). However, to my knowledge, only limited proof of evidence is available for such treatments in dogs (see below). In human medicine, precision medicine is being developed for specific types of genetic epilepsies with known etiology (35), but this type of therapy is not available for dogs, yet.

In medicine, many seizure-free patients consider withdrawal of ASMs, both when seizure control is achieved by medication alone, or once they became seizure-free following epilepsy surgery. However, about 30–50% of seizure-free patients who are withdrawn from ASMs will experience seizure recurrence (103). To our knowledge, we were the first to examine how often reinstitution of therapy in people will promptly control epilepsy as before (104). Although seizure control was regained within ~1 year in half of the cases, it took some patients as many as 5–12 years. In addition, in 19% resuming medication did not control epilepsy as before, and chronic DRE with many seizures was seen in up to 23% of patients with a recurrence (104). After our initial report, similar figures have been reported in numerous clinical studies (103). More recently, we examined the same issue in canine epilepsy (105). Following ASM withdrawal, 36% of the epileptic dogs remained seizure-free, but 64% suffered from seizure recurrence, of which only 43% could regain seizure freedom after resuming ASM therapy. Thus, this dog study reflected similar findings in human patients and questioned whether the risk of seizure recurrence is worth the benefit of stopping treatment.

Randomized Controlled Trials in Epileptic Dogs

Approval of novel ASMs for epilepsy in humans depends on several randomized controlled trials (RCTs), typically performed as add-on therapy in patients with focal epilepsy that is refractory to standard treatments (106). In contrast, in dogs RCTs are also possible in animals with newly diagnosed epilepsy. Furthermore, an added advantage of RCTs in dogs is that US Food and Drug Administration (FDA) agreement is not needed for canine studies unless the drug is being developed for approval in dogs (8). Proof of efficacy by appropriately designed RCTs is available for phenobarbital, potassium bromide, and imepitoin (57). For the latter drug, several RCTs have been performed both in dogs with newly diagnosed epilepsy and in ASM-resistant dogs (57, 101). To our knowledge, we were the first to compare primidone and phenobarbital in a controlled trial in epileptic dogs, showing that phenobarbital is superior to primidone (27), which led to abandoning primidone as a drug of first choice in canine epilepsy. Furthermore, we demonstrated that major ASMs used in humans such as carbamazepine, phenytoin, and valproate are not effective in epileptic dogs because their short half-lives in this species (**Table 1**) do not allow to maintain effective plasma concentrations during chronic treatment (24).

In contrast, only a few RCTs have been performed in dogs for ASMs that are only approved for human patients (57). As an example, Munana *et al.*

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(107) conducted a randomized, placebo-controlled, blinded crossover trial on levetiracetam in dogs resistant to phenobarbital and potassium bromide. Levetiracetam was repeatedly reported to be effective in small non-controlled trials, but in the RCT levetiracetam was not more effective than placebo (107). Nevertheless, as described above, levetiracetam is used as a pulse treatment for seizure prevention in epileptic dogs that are resistant to chronic ASM treatment. For this indication, levetiracetam has the advantage that it is much less sedative than BDZs that are otherwise used for such short and transient pulse treatment. Furthermore, intermittent or pulse treatment with levetiracetam avoids the development of tolerance (loss of efficacy) that has been observed during chronic treatment with this drug in dogs (108) and, initially, in kindled rats (109).

Munana *et al.* (110) performed similar small RCTs in drug-resistant epileptic dogs with dietary modification and surgical implants, again without any significant difference from placebo. Interestingly, as in humans, a positive response to placebo administration, manifesting as a decrease in seizure frequency, was observed in epileptic dogs (107, 110). This needs to be considered when evaluating open-label studies in dogs that aim to assess the efficacy of ASMs, as the reported results might be overstated (110). There are several explanations for placebo effects on seizure frequency in humans or dogs with epilepsy, including "regression to the mean," anticipation, classical conditioning, and the natural history of the disease (110, 111). Regression to the mean is a statistical term used to describe the natural fluctuations of seizures that occur over time in a drug trial that typically has a duration of a few months. Epilepsy is a waxing and waning disorder, and fluctuations in seizure frequency are common throughout the disease (112). Dog owners are most likely to seek a change in therapy for their pet (or inclusion of the dog in a drug trial) when seizures are under poor control. Over the short term, improvement in the seizure frequency is probable, regardless of the treatment administered. Thus, drug trials without placebo control may erroneously attribute an improvement in seizure frequency to the drug treatment, whereas in fact, it is because of the effect of time. An alternative to a placebo group is the use of a pseudo-placebo group that is treated with an ASM at a low subtherapeutic dose (113). Furthermore, the superiority of a drug can be demonstrated using a comparative design against a standard ASM.

The latter design was used in a more recent RCT that compared the effectiveness of monotherapy with levetiracetam vs. phenobarbital in dogs with newly diagnosed epilepsy; phenobarbital was effective but levetiracetam was not, even when administered three times daily to take account of the short half-life of this drug in dogs (114). In an RCT to assess the effect of oral cannabidiol administration in addition to conventional ASMs treatment on seizure frequency in dogs with intractable epilepsy, the proportion of responders was similar between the cannabidiol and placebo groups (115). In contrast, a multicenter RCT on a ketogenic medium-chain triglyceride (MCT) enriched diet administered as an add-on dietary supplement had a positive effect on seizure control and behavior in dogs with ASM-resistant epilepsy (116). Furthermore, an RCT on repetitive transcranial magnetic stimulation (rTMS) yielded positive effects on seizure frequency in dogs with DRE (117). Similarly, an RCT on the efficacy of phenobarbital or potassium bromide as add-on ASMs for controlling dogs refractory to a maximum dose of imepitoin resulted in an improvement in seizure management in the majority of the dogs (118).

Drug Resistance in Epileptic Dogs

DRE occurs when a person has failed to become (and stay) seizure-free with adequate trials of two ASMs (119). Numerous studies suggest that epilep-

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sy fails to be controlled with ASMs in about one-third of adults and ~20–25% of children (28). This condition is also referred to as intractable, medically refractory, or pharmacoresistant epilepsy. Patients with such DRE have increased risks of premature death, injuries, psychosocial dysfunction, and reduced quality of life, so the development of more effective therapies is an urgent clinical need (120). In epileptic dogs, the percentage of drug resistance may even be higher (≥50%) than in humans (121). This may be because the drugs [phenobarbital, primidone (*via* its major active metabolite phenobarbital), imepitoin, potassium bromide] that are approved for the treatment of canine epilepsy all act as positive allosteric modulators (PAMs) at the same target (the $GABA_A$ receptor), whereas the many more ASMs approved for humans act by diverse mechanisms (**Table 1**) (98). Thus, a patient resistant to one mechanistic category of ASMs (e.g., $GABA_A$ receptor PAMs) can be switched to another mechanistic category (e.g., ion channel modulators), thereby enhancing the therapeutic armamentarium, whereas this is not possible in epileptic dogs. As in humans, drug resistance continues to be a major clinical problem in the therapeutic management of canine epilepsies with substantial implications for quality of life and survival times (121).

The mechanisms underlying drug resistance in canine epilepsy are only poorly understood. Seizure density and the occurrence of cluster seizures have been linked with a poor response to ASMs (121). Moreover, evidence exists that the genetic background and alterations in epigenetic mechanisms might influence the efficacy of ASMs in dogs with epilepsy (121, 122). Only insufficient data are available in epileptic dogs to support prominent hypotheses of drug resistance in human epilepsy, e.g., the transporter, target, and network hypotheses (120), which will be discussed in more detail below.

Importantly, before defining an epilepsy as drug resistant, pseudo-resistance should be excluded. The main reason for pseudo-resistance in epileptic dogs is poor owner compliance in medical treatment of their pets (123, 124). Another reason may be that the dog is not epileptic but rather has a paroxysmal dyskinesia disorder (125), which, without EEG and knowledge about clinical differences, can be falsely diagnosed as epilepsy (126).

Pathogenesis of Epilepsy in Dogs

As shown in Figure 1, a variety of brain insults can induce epileptogenesis, i.e., the process underlying the development of epilepsy (Figure 2). In addition, gene mutations underlying inherited epilepsies induce this process. Mainly based on data from rodent models of epilepsy, epileptogenesis is characterized by a variety of structural, molecular, and functional changes in the brain, including inflammatory processes, blood-brain barrier (BBB) disruption, neurodegeneration, synaptic sprouting, plastic changes in ion channels and receptors, and the resultant development of neuronal hyperexcitability in affected brain regions (Figure 2). However, not all patients with the brain insults shown in Figures 1, 2 will develop epilepsy; so biomarkers to predict epilepsy in patients at risk are urgently needed (127). Furthermore, currently, no therapies are available that halt or modify these processes to prevent epilepsy in patients at risk (128). If such therapies would become available, they could also be used to prevent secondary epileptogenesis, i.e., the process leading from newly diagnosed epilepsy to chronic epilepsy, which is often refractory to ASMs (**Figure 2**).

Neuroimaging Studies

Except for a few genetic epilepsies, the causes of canine epilepsy are poorly understood. Although the introduction of the MRI as a diagnostic tool of epileptic dogs has disclosed a variety of structural and functional brain abnormalities in such animals (38, 39, 132–

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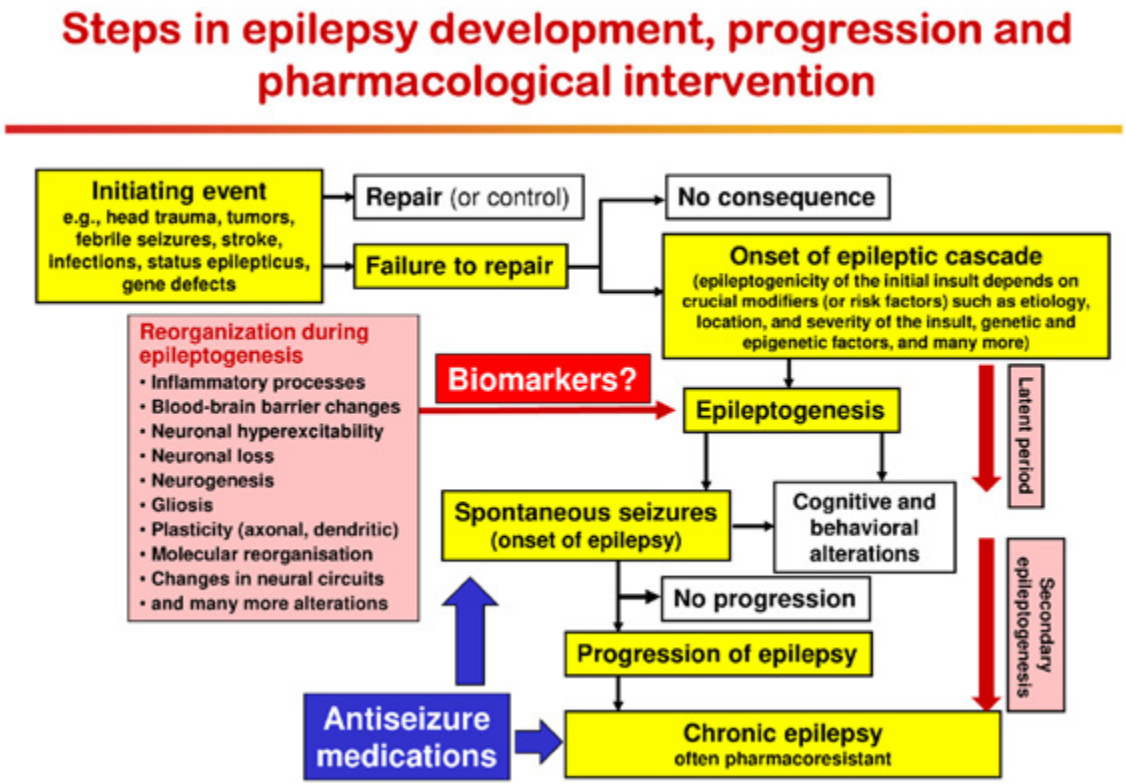

Figure 2. *Epileptogenic processes and risk factors involved in the development of epilepsy after acute brain insults. Possibly depending on crucial modifiers or risk factors, the same brain injury can be epileptogenic or not. Immediately after brain injury, early (or provoked) seizures may occur; these acute symptomatic seizures are not indicating epilepsy but may increase the risk of developing epilepsy. In the majority of patients, brain insults do not cause epilepsy. The term epileptogenesis includes processes that render the brain susceptible to spontaneous recurrent seizures and processes that intensify seizures and make them more refractory to therapy (progression or "secondary epileptogenesis"). During epileptogenesis, multiple brain alterations occur, including altered excitability of neurons and/or neuronal circuits, activation of microglia, astrocyte dysfunction, alterations in expression and function of receptors and ion channels (in part recapitulating ontogenesis), loss of neurons, neurogenesis, axonal and dendritic sprouting, gliosis, inflammatory processes, and more. It is important to note that some of these alterations may be related to post-injury repair or recovery and not suited as targets to halt the epileptogenic process. The "latent period" is the time from the initiating epileptogenic brain injury to the first onset of spontaneous clinically obvious seizures. This latent period, during which the epileptogenic processes take place, may last days to months to years. The figure has been modified from previous versions (129–131).*

136), this by itself does not explain the exact molecular causes of spontaneous recurrent seizures as observed in epilepsy. Furthermore, epileptic dogs, including those with "idiopathic" epilepsy, may have heterogeneous underlying pathologies, including subtle structural changes that cannot be identified on conventional visual inspection of brain MRI.

In a recent peri-ictal MRI study in 81 dogs with suspected idiopathic epilepsy, the most common brain areas affected were the hippocampus (39/81), cingulate gyrus (33/81), and piriform lobes (32/81) (135). This may suggest that, similar to humans, the limbic system (or mesial temporal lobe) is particularly affected in epileptic dogs. This possibility is substantiated by several other MRI studies in large numbers of epileptic dogs (132–134, 136, 137). However, in contrast to human patients, from which epileptic tissue for electrophysiologic and molecular studies can be obtained during epilepsy surgery by resection of epileptogenic focal tissue, such resective surgery is in its infancy in veterinary medicine (66, 138–140).

The use of functional MRI and magnetic resonance spectroscopic imaging (MRSI) in dogs will be discussed in separate sections below.

More recently, positron emission tomography (PET) has been used in epileptic dogs (141, 142). Non-invasive nuclear imaging by PET and single-photon emission computed tomography (SPECT) has significantly contributed to epileptic focus localization in human neurology for several decades (143). Because molecular radiotracer imaging by PET or SPECT offers functional insight into brain alterations, such techniques have the potential for a better understanding of the pathophysiology of epilepsy. Neuro-nuclear imaging in dogs may also serve to identify an epileptic focus in MRI-negative epilepsy. Joint efforts in Finland have led to two recent publications supporting that F-18-fluoro-deoxy-glucose (F-18-FDG) PET for identification of the epileptic focus region as widely used in presurgical evaluation in human patients is translatable to veterinary patients. In juvenile Lagotto Romagnolo dogs with focal-onset epilepsy, Jokinen *et al.* (141) identified regions with reduced glucose metabolism in the cerebral cortex associated with EEG abnormalities. A second study performed by the same group prospectively evaluated adult Finnish Spitz dogs with focal idiopathic epilepsy by EEG and F-18-FDG and found abnormalities by visual analysis in 9/11 dogs with occipital cortex findings most consistent with the epileptic status (142).

Brain Tissue Studies

In a postmortem study in an epileptic Shetland Sheedogs, neuronal loss and gliosis were found in the limbic system, including the cingulate gyrus, amygdaloid nucleus, dorsal and ventral parts of the hippocampus, and dorsomedial nucleus of the thalamus (144), which is in line with postmortem findings in human patients with epilepsies originating in the limbic system (145). In a subsequent study in a larger group of epileptic Shetland Sheedogs that died in SE, neurodegeneration and astrocytosis were found predominantly in the cingulate cortex and internal area of the frontal cortex (69). In addition to neurodegeneration, neurogenesis has been reported in the dentate gyrus of an epileptic dog (146), resembling the aberrant neurogenesis in this region reported in humans with temporal lobe epilepsy (TLE) (147, 148). However, in a group of six epileptic dogs of different breeds, which were euthanized because of frequent and severe drug-resistant seizures, no loss of neurons in the dentate hilus and no axonal sprouting were determined, indicating the absence of TLE pathology (149). This is not surprising because only one of the six dogs exhibited focal seizures. Neuron loss in the hippocampus of dogs with epilepsy has been described previously in case reports (144, 150, 151) and a colony of research Beagles (152). Potschka *et al.* (153) described obvious pathomorphological alterations in canine hippocampal tissue from dogs with both idiopathic as well as symptomatic epilepsy, which would be consistent with data from MRI analyses described above. However, whether TLE exists in dogs remains a matter of debate. Suspected hippocampal sclerosis from MRI scans and volumetry (see above) requires to be substantiated by tissue studies (154).

Does Mesial Temporal Lobe Epilepsy Exist in Dogs?

In humans, the most common type of epilepsy in adults is mesial TLE (mTLE), an epilepsy syndrome that is characterized by focal (complex partial) seizures originating from the mesial temporal lobe and pathologic lesions, such as hippocampal sclerosis and neurodegeneration in other regions of the temporal lobes (155). For many decades, the limbic system in the temporal lobes, including the hippocampal formation and parahippocampal areas such as the piriform, perirhinal, and entorhinal cortices, have been known to play a crucial role in the development of seizures and epilepsy (156–163). The hippocampus is considered by many to be the generator of mTLE. mTLE is typically associated with hippocampal sclerosis, a neuropathological condition with severe neuronal cell

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loss and gliosis in the hippocampus, specifically in the CA1 (Cornu Ammonis area 1) region and subiculum of the hippocampus proper and in the hilus of the dentate gyrus (164). In addition to neuron loss, aberrant sprouting of dentate granule cell mossy fibers in mesial TLE is thought to underlie the creation of aberrant circuitry that promotes the generation or spread of spontaneous seizure activity (163, 165). Surgical removal of the sclerotic hippocampus in drug-resistant patients often improves or even cures TLE (120).

The mechanisms by which hippocampal lesions and the associated neuronal network changes within and beyond the hippocampus can lead to enhanced seizure susceptibility and development of recurrent seizures have been the topic of intense research, both in rodent models of mTLE and by using resected tissue from mTLE patients (145). Indeed, in most mTLE patients the seizures originate in this region. However, a very long-standing question and a subject of ongoing debate are whether hippocampal sclerosis plays a role in the development of the epileptic focus or whether it is the consequence of repeated seizures (166, 167).

As discussed above, the relevance of temporal lobe pathology remains a matter of debate in canine epilepsy (54, 56, 153, 154). There have been several reports in the veterinary literature suggesting that mTLE also occurs in dogs. However, in the absence of convincing ictal or interictal EEG abnormalities to confirm that the seizure activity is in the temporal lobe, and with the absence of pathology similar to the human disease (hippocampal sclerosis), there is no definitive evidence that some types of canine epilepsy are actually analogous to TLE in humans. However, several of the MRI and brain tissue data described above strongly indicate an involvement of the hippocampus and other temporal lobe regions in canine epilepsy. Furthermore, many epileptic dogs have a focal seizure presentation that is very similar to that described in humans with mTLE including excessive salivation, staring off, dilated pupils, and facial twitching (32). In line with this, reflecting features of human mTLE, an association between the presence of unilateral epileptic EEG discharges and a decrease in the unilateral hippocampal volume has been described in canine epilepsy (133).

Brain Microdialysis Studies on GABA and Glutamate

Epilepsy is broadly characterized by aberrant neuronal excitability. Glutamate is the predominant excitatory neurotransmitter in the adult mammalian brain; thus, much of past epilepsy research has attempted to understand the role of glutamate in seizures and epilepsy (168). Glutamate has been implicated in both the initiation and propagation of seizures as well as brain damage that can occur following prolonged or repeated seizures. Gamma-aminobutyric acid (GABA), the most common inhibitory neurotransmitter in the brain, usually suppresses seizure activity. It has long been thought that epilepsy and its increased propensity for recurrent spontaneous seizures are due to an imbalance between glutamatergic excitation and GABAergic inhibition in the brain (169, 170). However, this outdated idea ignores the complexity of the GABAergic and glutamatergic systems in the brain (171). Indeed, experience with GABA indicates that certain neurotransmitters may have either anticonvulsant or proconvulsant effects depending on the neuronal networks, the age, and the pathology involved (172–174).

Despite this complexity of brain neurotransmitter functioning, numerous studies using intracerebral microdialysis of extracellular amino acids in the epileptic focus of human patients undergoing epilepsy surgery have shown marked increases in glutamate release interictally and, more markedly, during seizures (175–180). Extracellular GABA levels were either unchanged or increased during seizures. However, when the release of GABA in the human hippocampus was stimulated by glutamate, it was markedly decreased in

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epileptogenic hippocampi, in contrast with contralateral, non-epileptogenic hippocampi (181). Intracerebral microdialysis has also been used in epileptic dogs (129). In epileptic Shetland Sheedogs, high values for extracellular glutamate levels were detected in the frontal and parietal lobes in association with an increased number of spikes and sharp waves during hyperventilation. In the cerebrum of Shetland Sheedogs that died of SE, immunohistochemistry using antibodies against glutamate and glutamate transporters (GLT-1 and GLAST) disclosed a decrease of GLT-1 in the cerebral cortex and lateral nucleus of the thalamus (129). These data indicate that the astrocytic uptake of glutamate by GLT-1 is altered in these epileptic dogs, which would explain the increase in extracellular glutamate levels. The GLT-1 findings are of interest because this astrocytic glutamate transporter regulates extracellular glutamate homeostasis in the brain and GLT-1 dysregulation is thought to contribute to the development of epilepsy (182).

Magnetic Resonance Spectroscopic Imaging Studies on Brain GABA Levels

One inherent problem in measuring extracellular amino acids during epilepsy surgery is the lack of adequate non-epileptic controls. Magnetic resonance spectroscopic imaging (MRSI) can be used to determine GABA in the brain of epilepsy patients vs. controls (183). Indeed, by using MRSI, Petroff *et al.* (184) reported that persons with mTLE had lower occipital lobe GABA levels than did subjects without epilepsy. MRSI has also been used to study the effect of ASMs that act by potentiating GABAergic transmission on GABA levels, showing that the GABA aminotransferase (GABA-T) inhibitor vigabatrin increases GABA levels in patients with epilepsy (185). In apparent contrast, valproate did not increase significantly GABA concentrations in the occipital lobe of adult patients with complex focal seizures (186). Comparable MRSI studies on brain GABA levels in epileptic dogs are not available but the technique has been evaluated in non-epileptic dogs to measure postictal perturbations of cerebral metabolism following induction of seizures by pentylenetetrazole (PTZ) (187). One disadvantage of measuring brain levels of GABA or glutamate by MRSI is the low spatial resolution of the technique and the fact that only regions such as the occipital lobe can be assessed.

Studies on CSF GABA and Glutamate Levels

Another technique to assess extracellular brain levels of amino acids is to determine them in the cerebrospinal fluid (CSF). Close dose-dependent correlations between ventricular or cisternal CSF GABA levels and brain GABA concentrations have been reported following the administration of drugs that elevate brain GABA content (188–190), indicating that CSF GABA levels may reflect brain GABA metabolism and GABA release into the extracellular space. An example in dogs is shown in Figure 3A, in which we compared GABA levels in the brain cortex, CSF, and plasma of an anesthetized dog following administration of valproate, demonstrating impressive parallelism of the GABA alterations. Furthermore, using a seizure threshold model in untreated dogs, we found a highly significant positive correlation between CSF GABA and seizure threshold (Figure 3B), indicating that the concentration of GABA in CSF is related to GABAergic activity in brain compartments involved in the regulation of seizure excitability (192). Moreover, when we kindled dogs by repeated administration of the $GABA_A$ receptor antagonist PTZ, the progressive increase in seizure severity was associated with a decrease in CSF GABA levels, which was prevented by the ASM phenobarbital (195). These data thus

suggested the usefulness of CSF GABA measurements in clinical investigations of brain GABAergic function. In line with this suggestion, Wood *et al.* (193) reported that the mean lumbar CSF GABA concentration among 21 medicated epilepsy human patients with intractable seizures was significantly lower than that of 20 unmedicated normal volunteers (**Figure 3C**). This prompted us to perform a similar study on epileptic dogs (194). As shown in Figure 3D, epileptic dogs exhibited a similar decrease in CSF GABA than previously observed in humans with epilepsy. We also determined CSF GABA in unmedicated epileptic dogs and found no difference to medicated dogs with epilepsy (Figure 3D). Treatment consisted of either primidone or phenobarbital, which are not known to affect brain GABA levels. The similar outcome of CSF GABA studies in epileptic dogs and humans was about the first direct evidence that epileptic dogs may serve as a translational model for the human disease.

In another study in cooperation with pediatric neurologists, we determined CSF GABA levels in children with epilepsy (196–198). Untreated children had significantly lower CSF GABA levels than controls [120 (range 91–159) pmol/ml vs. 174 (range 95–316) pmoles/ml; $P < 0.02$]. The same was true for ASM-treated children with epilepsy when the ASM valproate, which has been reported to increase GABA metabolism (191), was excluded (198). Indeed, valproate was found to increase CSF GABA levels in children with epilepsy by about 100% (197), which is similar to the CSF (and brain) GABA increase with valproate observed in dogs (**Figure 3A**). A significant decrease in CSF GABA was also found in children with febrile seizures (199).

However, some studies did not report significant decreases in CSF GABA in persons with epilepsy (200–204). At least in part, this could be due to the methods used to determine the low CSF GABA levels, which are known to be sensitive to artifactual increases during sampling, storing, and thawing of CSF samples and GABA analysis (205). More recent studies with modern analytical methods such as electrospray tandem mass spectrometry (ESI-MS/MS) confirmed the initial CSF GABA findings in children and adult persons with epilepsy (206, 207). Similarly, our findings on low CSF GABA in epileptic dogs were confirmed by subsequent studies (208, 209). Interestingly, Podell and Hadjiconstantinou (210) reported that low concentrations of CSF GABA correlate to a reduced response to phenobarbital therapy in epileptic dogs, indicating that low initial CSF GABA is a biomarker of subsequent response to treatment. In humans with epilepsy, the GABA-T inhibitor vigabatrin was found to increase CSF GABA levels (211–214), which was predicted by our studies in dogs (189, 190). Vigabatrin nonresponders had a less marked CSF GABA increase than responders (215). Similarly, treatment of seizures by a ketogenic diet was found to increase CSF GABA in epileptic human patients, with higher GABA levels in responders than non-responders during the diet (216).

In addition to GABA, glutamate levels were measured in the CSF of both dogs and humans with epilepsy. In both species, increases in CSF glutamate concentrations were reported (69, 204, 208, 217–220), although some studies did not confirm these findings (201–203). Such inter-study differences in the outcome of CSF amino acid levels in epilepsy may be due to varying experimental design, patient populations, and ASMs, or the lack of adequate controls.

Plasma GABA as a Biomarker of Drug Effects

Lumbar puncture for CSF sampling is an invasive method with ethical constraints. Thus, we examined whether drug-induced alterations in the brain and CSF GABA levels are reflected in the plasma. As shown in **Figure 3A**, surprisingly, the increase in cortical and CSF plasma levels upon treatment of dogs with GABA elevating drugs such as valproate was reflected

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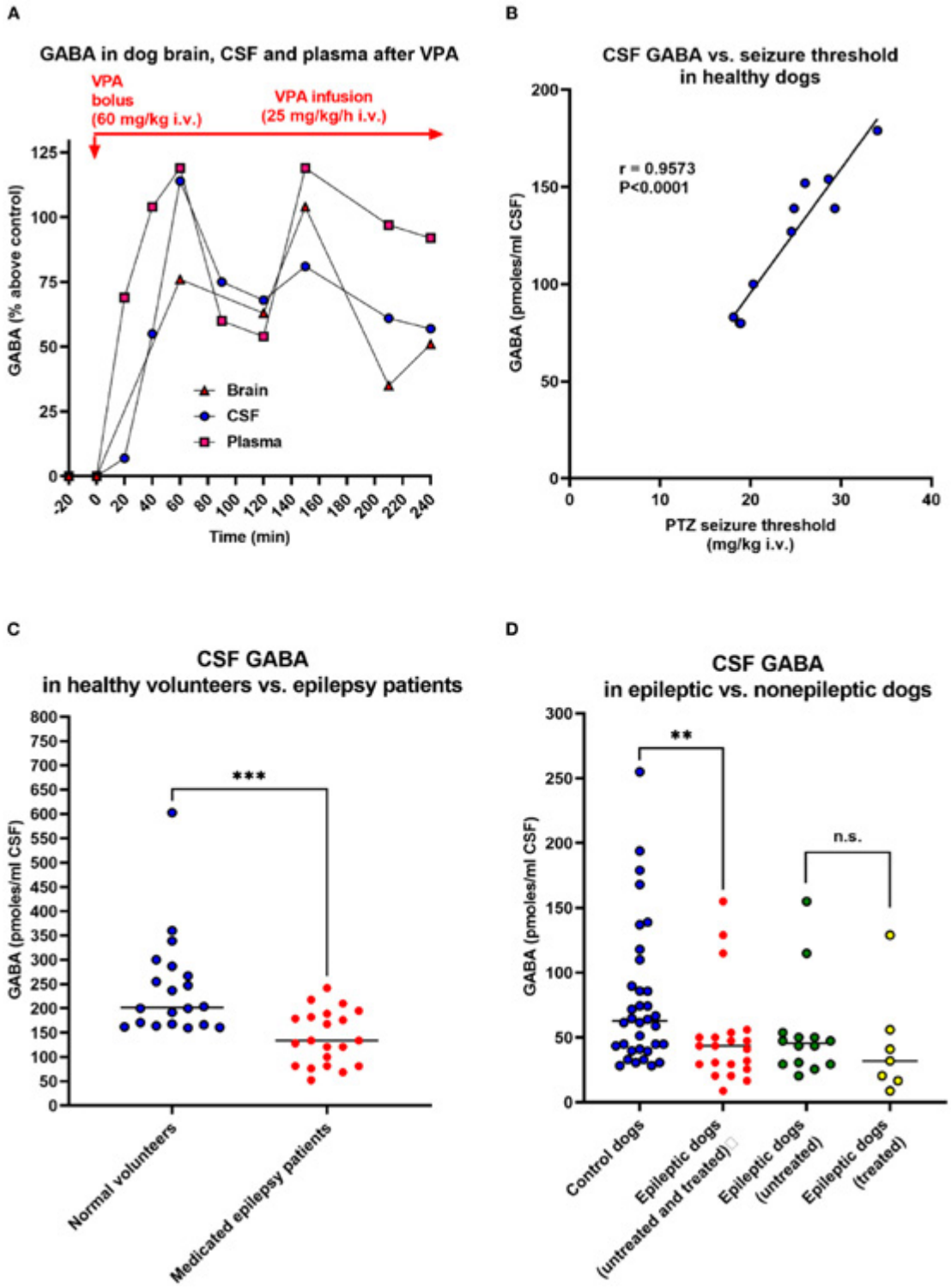

Figure 3. *Relationship between CSF GABA concentrations and neuronal excitability. In the dog studies shown in (**A,B,D**), CSF was withdrawn from the subarachnoidal space by a suboccipital puncture during anesthesia. (A) GABA levels in the cerebral cortex, CSF, and plasma during the administration of the antiseizure drug valproate (VPA) in an anesthetized dog. Similar experiments were performed with vigabatrin and other GABA-T inhibitors to investigate the relationship between GABA levels in the brain parenchyma and those in CSF and plasma. Unexpectedly, these experiments showed that plasma GABA alterations reflect respective alterations in the brain and CSF. Also, note the correlation between brain and CSF GABA alterations. VPA is thought to increase GABA synthesis (191), which explains the GABA increases in dogs and other species, including humans (see text). Data are from Löscher (190). (**B**) Correlation between CSF GABA levels and pentylenetetrazole seizure threshold in 10 healthy dogs. Data are from Löscher (192). (C) CSF GABA levels in 20 adult unmedicated healthy volunteers and 21 adult epilepsy patients. All patients had more than three seizures a day despite chronic treatment with ASMs (phenytoin, phenobarbital, or primidone). Data are shown as individual lumbar CSF GABA levels and median; the significant inter-group difference is indicated by asterisks (P = 0.0003). Data are from Wood et al. (193). (**D**) CSF GABA levels in 34 adult healthy control dogs and 21 adult epileptic dogs. The CSF GABA levels in the epileptic dogs are also shown separately for untreated (n = 14) and treated (n = 7) dogs, respectively. Data are shown as individual CSF GABA levels and median; the significant inter-group difference is indicated by asterisks (P = 0.0075). CSF GABA levels in treated (phenobarbital or primidone) and untreated dogs did not differ significantly. Only one of the seven treated dogs was seizure-free at the time of CSF sampling. Data are from Löscher and Schwartz-Porsche (194).*

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by plasma GABA levels. Similar findings were reported by us for vigabatrin (189, 190). This prompted us to evaluate plasma GABA as a diagnostic tool for the treatment of human epilepsy patients with valproate and vigabatrin. In both healthy volunteers and epilepsy patients, subchronic treatment with valproate dose-dependently increased plasma GABA levels (221, 222).

Interestingly, a cross-sectional study of epilepsy patients with vigabatrin add-on treatment showed that vigabatrin responders had a significantly higher plasma GABA level than non-responders and controls (223, 224). The possibility of using the plasma GABA increase caused by vigabatrin as a biomarker for the antiseizure response to this drug in patients with drug-resistant focal epilepsy prompted us to perform a prospective clinical study to evaluate changes in plasma GABA concentration in relation to clinical response during vigabatrin treatment of epilepsy (225). Vigabatrin responders had a significant increase in mean plasma GABA both after short-term and long-term treatment, whilst non-responders had no significant changes in GABA levels.

However, while plasma GABA levels parallel drug-induced increases in the brain and CSF GABA, they do not reflect disease-associated alterations in GABA concentrations in the brain (226), although recent studies reported an association between plasma GABA levels and posttraumatic stress disorder symptoms (227, 228). Furthermore, Saleem *et al.* (229) reported that the plasma levels of GABA and glutamate were significantly higher in patients with DRE compared to healthy controls, but, at least in part, this could be a consequence of the treatment of epilepsy patients with ASMs such as valproate.

To our knowledge, plasma GABA has not yet been evaluated as a potential biomarker in dogs with epilepsy. In our dog experiments with PTZ, plasma GABA levels did not reflect the alterations in CSF GABA in the absence of treatment with ASMs (190, 195). However, as shown in **Figure 3A**, drug-induced increases in CSF GABA levels of dogs by ASMs such as valproate or vigabatrin were reflected by plasma GABA levels (189, 190, 195). This can be explained by the fact that the GABA degrading enzyme GABA-T is also present in peripheral tissues and blood platelets (230). Furthermore, the GABA synthesizing enzyme glutamate decarboxylase (GAD) is present in some peripheral tissues (230). Thus, drugs such as valproate and vigabatrin that affect GABA-T and/or GAD will increase GABA both in the periphery and the CNS.

Platelet GABA-T as a Biomarker

Similar to our studies in dogs and humans, experimental studies in rodents have shown that the increase in the brain or CSF GABA concentration induced by vigabatrin or other GABA-T inhibitors, is paralleled by an increase in plasma GABA concentration (188, 231, 232). Interestingly, in children with untreated epilepsy, the activity of GABA-T in platelets was reported to be significantly lower than in healthy controls (233). Surprisingly, patients receiving valproate in monotherapy had a significantly higher GABA-T activity than both the control group and the untreated children with epilepsy (233). As expected, treatment with vigabatrin reduced GABA-T activity in platelets (234). In rats, it was shown that platelet GABA-T reflected the inhibition of GABA-T and increase in GABA levels in the brain after treatment with vigabatrin (235).

Another study in adult patients with focal epilepsy reported that the activity of GABA-T in platelets was increased, but all patients received ASMs such as valproate (236). In apparent contrast to the increased platelet GABA-T, this enzyme was not increased in hippocampal tissue resected during epilepsy surgery. In a study on medicated patients with JME and refractory focal epilepsy, the mean activity of platelet GABA-T in JME patients was significantly higher than in control subjects, whereas focal epilepsy patients did not significantly differ from controls (237). In the latter study, also the GABA uptake into plate-

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lets was measured, showing a significant decrease in GABA uptake in both groups of epilepsy patients. Based on the outcome of a study that evaluated GABA and its metabolism and function in platelets as compared to neurons, Kaneez and Saeed (238) proposed that platelets could be further developed to be used as a peripheral model to study neuronal GABAergic function and its abnormality in diseases such as epilepsy.

The Role of Neuroinflammation

An emerging field in epilepsy research is the assessment of neuroinflammation as a critical process during epileptogenesis as well as in chronic epilepsy (143). PET radioligands of the mitochondrial transmembrane protein TSPO (also known as peripheral BDZ receptor) can be utilized to visualize activated brain resident microglia and brain invading macrophages (239). In addition to patients, TSPO imaging is widely used in animal models of brain diseases (240) but, to our knowledge, not yet in dogs with epilepsy. However, neuroinflammation is routinely being investigated in canine epilepsy by other diagnostic methods (241). Indeed, inflammatory diseases of the CNS are important causes of seizures in dogs (242) and, as in humans, neuroinflammation may be involved in epileptogenesis and ictogenesis, i.e., the processes leading to epilepsy and seizures, respectively. For instance, high-mobility group box 1 (HMGB1), a key mediator of neuroinflammation with increased levels in patients with epilepsy, is significantly increased in the blood serum of epileptic dogs (243). Similarly, dogs with epilepsy had increased levels of interleukin (IL)-1β in serum regardless of the underlying cause of the disease (244). In the CSF of epileptic dogs, significantly higher tumor necrosis factor (TNF)-α and IL-6 concentrations were found (245).

The Role of the Blood-Brain Barrier

Non-invasive brain imaging methods are also useful to detect alterations in the blood-brain barrier (BBB), which are a hallmark of epilepsy (246). Increased permeability of the BBB leading to extravasation of blood compounds like albumin and subsequent albumin-induced alterations in the brain parenchyma is considered to be a crucial factor for the development of epilepsy (246, 247). *In vivo* imaging approaches to visualize a leaky BBB are based on the detection of contrast agents or radiotracers which do not cross the intact BBB (143). Contrast-enhanced MRI is an established technique to diagnose BBB leakage after epileptogenic insults (248). The latter technique was recently used in 46 epileptic dogs and 6 healthy controls (249). BBB dysfunction (BBBD) was found in 37% of epileptic dogs. The mean BBBD severity score of the piriform lobe in epilepsy dogs was significantly higher compared to control. Furthermore, a significantly higher CSF to serum albumin ratio was found in dogs with BBBD relative to dogs with intact BBB. Brain immunohistochemistry in dogs that were euthanized at the owner's request due to uncontrolled seizures suggested active transforming growth factor (TGF)-β signaling and neuroinflammation in the piriform cortex, showing increased levels of serum albumin colocalized with glial acidic fibrillary protein (GFAP) and phosphorylated Smad 2 (pSMAD2; a downstream signal of activated TGF-β signaling) in an area where BBBD had been detected by MRI. The authors of this landmark study concluded that the involvement of the piriform lobe seen by their MRI protocol emphasizes the possibility of using dogs as a translational model for the human disease (249). One limitation in using imaging methods such as MRSI, PET, or SPECT in dogs is that anesthesia is necessary to achieve immobility of the subject for neuroimaging, which can considerably influence the results of functional brain imaging results (143).

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Genetic Causes of Epilepsies

Compared to structural, biochemical, and immunological alterations in dogs with epilepsy, much less is known about the genetic causes of epilepsy in dogs. In humans, innovations centered around novel technologies, analytics, and collaboration have led to remarkable progress in gene discovery (45, 250), which has increased our understanding of the causes of epilepsy (**Figure 4A**). A key difference between the paradigm in the 1990's and today's understanding of epilepsy is that we now have the confidence to leave the term "idiopathic" behind for human epilepsy (42, 250). More than 80 genes are considered as epilepsy genes, i.e., genes that cause epilepsies or syndromes with epilepsy as the core symptom (36). Additional some 800 genes are epilepsy-related or putatively associated with epilepsy. The functions of epilepsy genes are shown in **Figure 4B**. Most epilepsy genes lead to functional changes in ion channels and cause epileptic channelopathies (37). However, as shown in **Figure 4B**, various other functional changes, including alterations in receptors for GABA and glutamate, may be caused by epilepsy genes (36). This has resulted in a variety of epilepsy syndromes for which the genetic basis is known (50). The new genomic era now directly affects clinical care toward precision medicine (250). However, monogenetic epilepsies are rare; for most patients, epilepsy is regarded as a complex disorder associated with multiple genes and external environmental factors.

In dogs, many studies of breeds with "idiopathic epilepsy" have failed to identify genes or loci of interest (47, 49, 251). Gene discovery in dogs with progressive myoclonic epilepsies (PMEs) has been more successful, with eight known genes; six of these are orthologous to corresponding human disorders (48, 49). In 2016, Hayward *et al.* (252) undertook the largest canine genome-wide association study (GWAS) to date, with a panel of over 4,200 dogs genotyped at 180,000 markers, to accelerate mapping efforts. Among these results, the authors found additional candidate genes related to epilepsy in Irish Wolfhounds using 34 cases and 168 controls. In a more recent study that explored the pathogenesis of canine epilepsy using a systems genetics method with Hayward's *et al.* data (252), combining both GWASs and gene interactions, Cui *et al.* (253) reported 26 significant subnetworks correlated with canine epilepsy. Combined with gene ontology (GO) enrichment analysis, Cui *et al.* (253) identified three additional genes that were omitted by the GWAS analysis. Thus, as in medicine, advances in genetic sequencing technologies and bioinformatics are likely to increase the identification of genes and genetic disorders that are associated with epilepsy in dogs. A recent example in this regard is a study on severe early-onset epilepsy in several litters of Parson Russel terriers (254). Combined homozygosity mapping and genome sequencing revealed an in-frame 6-bp deletion in the nuclear-encoded pitrilysin metallopeptidase 1 (*PITRM1*) encoding for a mitochondrial protease involved in mitochondrial targeting sequence processing and degradation. The functional consequences of the mutation were modeled in yeast and showed impaired growth in permissive conditions and an impaired respiration capacity. Postmortem examination revealed an acute diffuse forebrain-predominant necrotizing polioencephalopathy affecting mainly the pyramidal cell layers of the hippocampus proper and entorhinal cortex (254), suggesting an involvement of the limbic system as suggested previously for other types of epilepsy in dogs (see above).

Another interesting recently discovered genetic epilepsy in dogs was described by Wielaender *et al.* (255, 256). They discovered a novel genetic myoclonic epilepsy in juvenile Rhodesian Ridgeback dogs, characterized by vigorous myoclonic seizures that occur mainly during relaxation periods. More than one-third of affected dogs develop generalized tonic-clonic seizures in the course of the disease and 35% are reported to be photosensitive.

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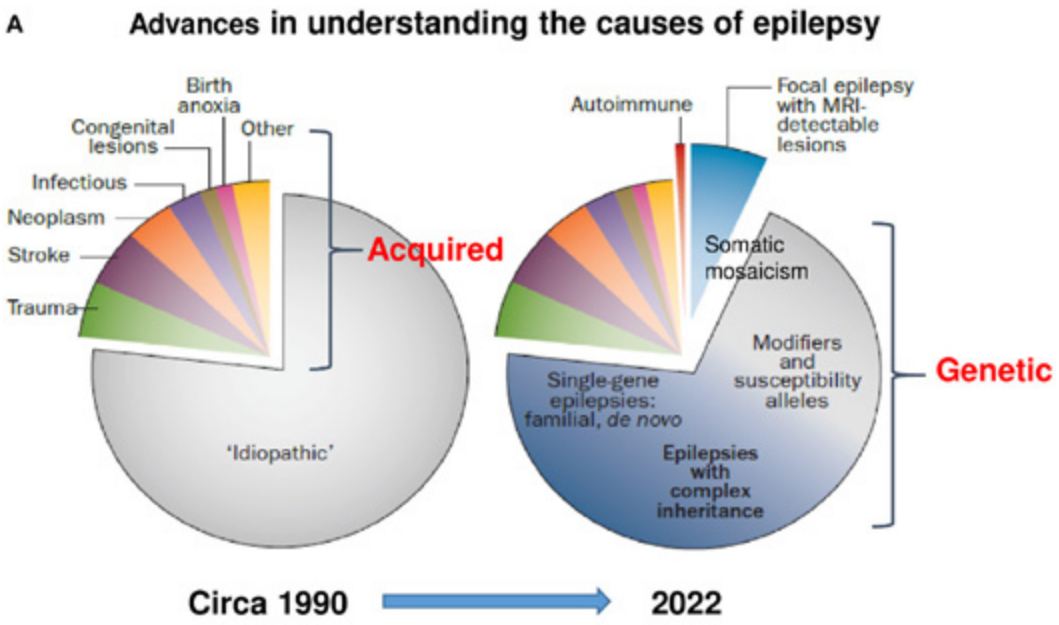

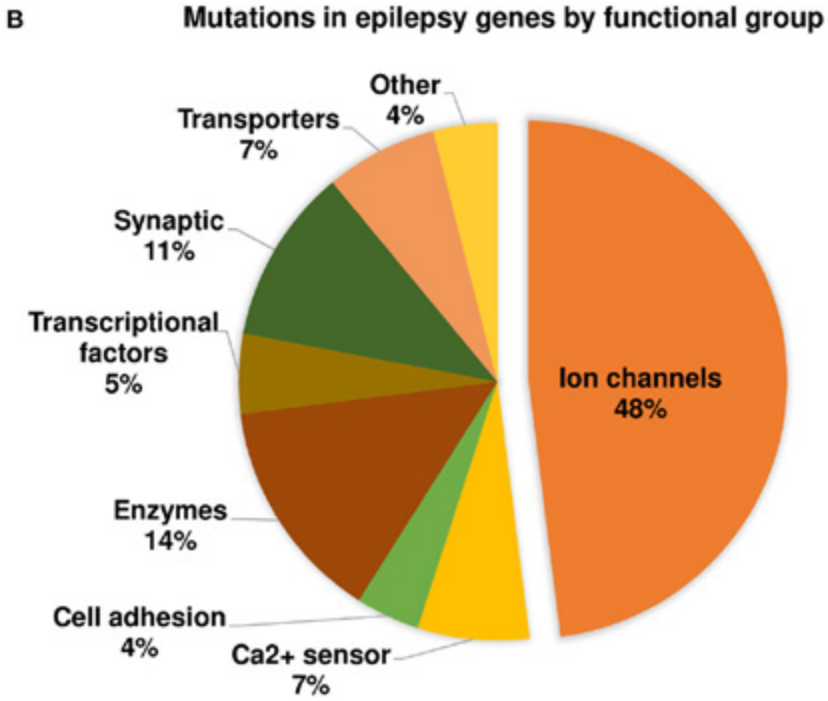

Figure 4. *Advances in understanding the causes of human epilepsy. (**A**) Left graph: Till the ~1990's, the majority of epilepsies were characterized as "idiopathic." Right graph: Today, epilepsy of unknown cause comprises a much smaller proportion, owing to the discovery of autoimmune epilepsies, epilepsies with lesions that are only detectable by MRI, and, most importantly, the reclassification of many epilepsies previously considered idiopathic as having a genetic cause. The exact proportions of monogenic and complex or polygenic epilepsies remain uncertain. Based on Thomas and Berkovic (42) and modified recently by Jeff Noebels. (**B**) Mutations identified in human epilepsy genes by gene function. Modified from Simkin and Kiskinis (37).*

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By combining a GWAS and next-generation sequencing analyses using whole-exome and whole-genome resequencing, Wielaender *et al.* (255) identified a fully penetrant recessive 4-bp deletion in the DIRAS family GTPase 1 (DIRAS1) gene with an altered expression pattern of DIRAS1 protein in the affected brain, including cholinergic forebrain nuclei. However, as in humans, monogenetic epilepsies in dogs are rare, and most idiopathic epilepsies in dogs are either complex disorders associated with multiple genes or are due to as yet overlooked structural alterations as suggested by the data of numerous MRI studies discussed above.

Spontaneous Recurrent Seizures in Large Inbred Beagle Colonies

Koestner and Rehfeld (257) reported the occurrence of spontaneous generalized convulsions in a large inbred Beagle colony. Redman and Weir (258) found that the incidence of epileptic dogs in a similar colony was 6%. Through selective breeding, this incidence could be increased to 66% (259). A detailed EEG analysis of 10 epileptic Beagles by Wiederholt (260) disclosed limbic hyperactivity in the hippocampus and amygdala, which was associated with psychomotor-like episodes of excessive lipping, smacking, chewing, and drooling, indicating focal-onset seizures. Brain and CSF levels of glutamate did not differ between epileptic Beagles and their non-epileptic siblings (261). In postmortem analyses, neurodegeneration was determined particularly in the hippocampus and cingulate cortex (262).

In addition to idiopathic epilepsy in Beagle dogs, Hegreberg and Padgett (263) described a form of familial epilepsy and its relationship to a similar condition in man, i.e., PME (or Lafora disease), a rare late-onset neurological storage disease characterized by deposits of polyglucosans (Lafora bodies) in the brain and caused by an autosomal recessive genetic defect resulting in myoclonus as well as focal and generalized seizures (264). In addition to Beagles, PME has been described in several other breeds including the Basset hound, Chihuahua, French Bulldog, Pointer, Miniature Poodle, Miniature Dachshund, and Welsh Corgi (49, 265). More recently, PME in Beagle dogs has been characterized in more detail (266).

In a large series of analyses in 68 epileptic Beagle dogs in an epilepsy-prone colony, the most common areas of neuronal damage were the hippocampus, amygdala, piriform cortex, cerebral cortex, basal nuclei, claustrum, septal nuclei, and dorsal thalamic nuclei (152). In addition, intraneuronal inclusions identical to Lafora's bodies were detected in thalamic nuclei of only six dogs (152). Using MRI with voxel-based morphometry, we compared local differences in gray matter volume between 5 healthy Beagles and 10 Beagles with either idiopathic or structural epilepsy (267). Epileptic Beagles displayed statistically significant reduced gray matter volume in the olfactory bulb, cingulate gyrus, hippocampus, and cortex, especially in temporal and occipital lobes.

Epileptic Beagle dogs have only rarely been used for drug testing because the spontaneous recurrent seizures necessitate continuous (24/7) video-EEG monitoring. Instead, as described below, seizures have been induced experimentally in epileptic and non-epileptic Beagle dogs and other dog breeds.

Experimentally Induced Seizures in Non-Epileptic Dogs

Apart from epilepsy eventually occurring in large colonies of experimentally used Beagle dogs (see above), dogs with epilepsy are typically privately owned pets, which restricts their use as a translational model, because the owners are often not willing to allow invasive experiments or to give away their animals for research purposes (95). In theory, this problem could be resolved by using epileptic dogs from large in-

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bred Beagle colonies (see above); however, the high prime and maintenance costs of dogs in the numbers necessary for selection and breeding of epileptic sublines limit the usefulness of this species for experimental studies (95). Furthermore, the naturally occurring seizures in dogs cannot be elicited at will by an investigator, which makes any scientific studies time-consuming, especially when the seizure frequency is low. However, seizures or SE can be experimentally induced in non-epileptic dogs with many similarities to spontaneous seizures in epileptic dogs. In this regard, we have used PTZ-induced seizures in dogs as a model for drug testing, but seizures can be induced at will by many other convulsive agents in dogs.

As illustrated by the development of imepitoin and also VNS, dogs in which seizures are induced chemically or electrically are a valuable tool both for developing new treatments for canine epilepsy and humans with epilepsy. For canine epilepsy, drug testing in non-epileptic dogs both serves to demonstrate the anti-seizure effect of a new therapy and for dose-finding for first clinical trials in epileptic dogs, which is nicely demonstrated by imepitoin (268). As described in more detail below, following the demonstration of the anti-seizure effect of imepitoin in rodent seizure models, we demonstrated its anti-seizure efficacy in dogs using the PTZ seizure threshold, followed by first clinical trials in dogs with epilepsy (268). The effect on PTZ seizures in non-epileptic dogs correctly predicted its anti-seizure efficacy in epileptic dogs and simplified dose finding in the first clinical trials. This is a huge advantage compared to dose finding when trying to translate preclinical data to first clinical trials in people.

Seizures Induced by Pentylenetetrazole in Dogs

PTZ, also known as pentetrazol and metrazol, is a CNS stimulant that is widely used experimentally to study seizure phenomena and to identify pharmaceuticals that may alter seizure susceptibility (269). PTZ acts predominantly by antagonizing GABAergic inhibition *via* an effect at the picrotoxin binding site of the chloride ionophore of the $GABA_A$ receptor (270). Because of its stimulatory effects on the brain stem, PTZ has clinically been used as a circulatory and respiratory stimulant and, before the invention of electroconvulsive therapy, for convulsive therapy in persons with major depression (269).

The timed i.v. PTZ infusion seizure threshold test in conscious dogs illustrated in **Figure 5** has been developed to test the loss of efficacy (tolerance) developing during prolonged treatment of BDZs such as diazepam (271). In this test, PTZ is infused at a rate of 10 mg/kg per min in 3 ml/min by an infusion pump. The convulsive threshold is defined as the amount of PTZ (in mg/kg body weight) inducing the first generalized myoclonic twitch, at which the infusion is stopped to avoid the development of more severe seizures. In Beagle dogs, the PTZ threshold is typically ~15 mg/kg i.v. At this dose, PTZ induces characteristic paroxysmal discharges in the EEG of dogs (275, 276). An anti-seizure effect is indicated if the PTZ seizure threshold is increased after pretreatment with an ASM or experimental drug, whereas a proconvulsant effect is indicated by a decrease in the PTZ seizure threshold compared to the control threshold. We have used this test in different dog breeds, including Beagles, extensively for determining the development of tolerance to BZDs and related drugs and to testing novel antiseizure compounds (see below). Furthermore, a slow infusion of PTZ is used as a proconvulsant reference compound when employing the dog EEG in safety pharmacology to evaluate proconvulsant risk of test compounds (276, 277). The advantage of using PTZ for induction of seizures is its rapid onset of action, thus allowing to determine seizure threshold during timed i.v. infusion, which is not possible with several other convulsant agents studied in this respect (269, 275).

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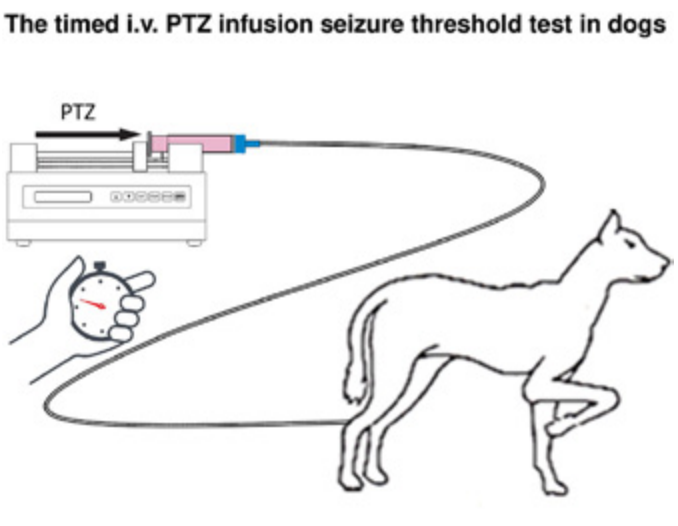

Figure 5. *Schematic illustration of the timed i.v. pentylenetetrazol (PTZ) seizure threshold test in an unrestrained dog. Before beginning the experiments, it is important to habituate the dogs to persons involved in the experiments as well as to the rooms and to handling. For seizure threshold determination, a 3% solution of PTZ (in 0.9% NaCl) is continuously infused at a rate of 3 ml/min by an infusion pump via a thin, flexible plastic catheter of about 1 m length, connected by a sharp cut-off end of an injection needle to the cephalic vein at a hind leg. The infusion is terminated immediately after the occurrence of the first generalized twitch (initial myoclonus), which usually takes on average 120 s after the onset of PTZ infusion. Before the myoclonic twitch, dogs typically exhibit tremors as a sign of increasing neuronal excitability. During PTZ infusion, the animal is only slightly restricted (or, in trained dogs, not restricted at all). The threshold dose of PTZ (in mg/kg body weight) is calculated from the infusion rate, the bodyweight of the animal, and the time necessary to produce the first myoclonic twitch (which occurs together with the first paroxysmal EEG activity). Typical PTZ seizure thresholds are in the range of ~15 mg/kg PTZ but may vary with the breed, sex, and age of the dogs. The potency of drugs to increase seizure threshold can be determined (and compared) by calculating the doses required to increase the threshold by 20% (TID20) or 50% (TID50), testing a range of doses in groups of dogs (see **Figure 7**). The same dogs can be repeatedly used at intervals of at least 1 week to avoid kindling (see text). Dogs rapidly adapt to the method and do not show any signs of discomfort or anxiety before or after the threshold determination. Before any drug experiments, the PTZ seizure threshold is determined once per week until reproducible and stable thresholds are obtained in all dogs. The figure was modified from Löscher (269). For details see Löscher et al. (274).*

When the timed i.v. PTZ infusion seizure threshold test is repeatedly used at weekly intervals in the same dogs, no kindling-like increase in seizure response is seen. However, more frequent use leads to a kindling-like effect in that the dogs respond with increasingly more severe seizures to the same dose of PTZ (195).

In addition to PTZ, other $GABA_A$ receptor antagonists such as picrotoxin, bicuculline, and penicillin have been used to induce seizures in dogs (275, 278, 279). During i.v. infusion of PTZ or bicuculline in dogs, the most frequent site of the first observed ictal EEG changes was the lateral geniculate body, whereas the neocortex and hippocampus were involved later (279). Furthermore, the glycine receptor antagonist, strychnine, has been used for the induction of seizures in dogs, for instance during the development of VNS (280).

Electrically Induced Seizures in Dogs

Over several decades, electrical induction of seizures in healthy dogs has been used for different purposes, including neuropathological studies (281), studies on electroconvulsive therapy and depression (282), cardiovascular and neurochemical responses (283), and pharmacological evaluation of experimental drugs (259, 284). For the latter purpose, the maximal electroshock seizure (MES) test, which was developed for ASM screening in mice and rats, was adapted to the dog and pharmacologically characterized by phenobarbital (285). Furthermore, the threshold version of the MES test has been used in dogs and compared with the PTZ seizure threshold (259). However, compared to the timed i.v. PTZ infusion seizure threshold test, the MES test is much less frequently used as a seizure test in dogs, at least in part because the tonic seizures induced by high-current electrical stimulation are much more severe and potentially lethal compared to the short and transient myoclonic seizures induced by PTZ. Interestingly, both the median con-

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vulsive current (determined by electric induction) and the PTZ seizure threshold were considerably lower in epileptic vs. non-epileptic Beagles (259). A decreased seizure threshold is thought to be involved in the mechanisms leading to spontaneous recurrent seizures in epilepsy (130).

Focal Seizures Induced by Topical Penicillin Applied to the Cerebral Cortex in Dogs

In 1945 Walker *et al.* (286) demonstrated that when penicillin was brought into direct contact with the brain, it produced electroencephalographic and clinical epileptiform manifestations, both in animals and man. The convulsive action of penicillin has since been confirmed in numerous experimental and clinical studies (278, 287–289). Topical penicillin applied to the cerebral cortex produces an epileptic focus in several species, including dogs. The type of focal seizures depends on the exact location of the focus. The convulsive and epileptogenic effects of penicillin result from the blockade of $GABA_A$ receptors (289). The penicillin model has been one of the most important models for answering questions about the neuronal basis of epilepsy, including the discovery of the paroxysmal depolarization shift and the analysis of the spread of seizure activity from an epileptogenic focus (288).

In dogs, the penicillin model was used to study the antiseizure effects of magnesium; the effects observed in dogs were then translated to non-human primates (290). Furthermore, the dog penicillin model has been used to study the effects of electrical stimulation of the ninth cranial nerve (the glossopharyngeal nerve) for seizure control in comparison to VNS (291), which will be discussed in more detail below.

Focal Seizures Induced by Kainate in Dogs

Hasegawa *et al.* (292) injected the glutamate receptor antagonist, kainate, into the amygdala of Beagle dogs (292). Intra-amygdala or intrahippocampal injection of kainate is a widely used model of TLE in rodents that is characterized by a limbic SE, followed, after a latent period, by frequent spontaneous recurrent seizures that are either focal or generalized convulsive (293). Kainate-treated dogs showed limbic seizures that started from the ipsilateral amygdala and developed into complex focal SE, which lasted for 1–3 days (292). However, in contrast to rodents, the dogs showed no spontaneous seizures during the 2-month observation period. Upon necropsy, severe neuronal loss was observed in the amygdala and hippocampus.

In a subsequent study on this dog model, diffusion-weighted MRI was used to characterize the temporal development of the lesions (294). Furthermore, glutamate and GABA were repeatedly determined in the CSF (295). During the acute phase (3, 6, 12, and 48 h after the onset of SE), CSF-glutamate was significantly increased, while CSF-GABA was decreased, although not significantly. In the chronic phase, both CSF-glutamate and CSF-GABA were significantly lower than normal at 72 h after the onset of SE, and their levels returned to normal at 2 months (295).

The Kindling Model of Temporal Lobe Epilepsy in Dogs

Kindling is an animal model of TLE produced by focal electrical stimulation of limbic brain areas such as the amygdala, hippocampus, or piriform cortex (296). Furthermore, as discussed for PTZ above, kindling can be induced chemically. The term "kindling" refers to the phenomenon whereby repeated electrical stimulation of a limbic brain region *via* an implanted depth electrode initially only induces focal paroxysmal EEG activity (so-called "afterdischarges") without overt clinical seizure activity (296). Subsequent stimulations induce the progressive development of focal and later secondary generalized convulsive seizures until the animal is "fully kindled" and responds with the

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same maximal seizure severity and duration upon stimulation (297). Once developed, the enhanced sensitivity to the initial subconvulsive electrical stimulus is permanent, which is the consequence of enduring molecular and functional brain alterations (296, 298). Since its introduction in 1969 by Goddard *et al.* (299), kindling has become one of the most widely used animal models of epilepsy, particularly because the mechanisms involved in kindling are thought to be relevant for epileptogenesis (300). Furthermore, the amygdala kindling model of TLE in rats has been instrumental in the preclinical development of various ASMs (301, 302). Electrical kindling has been demonstrated in numerous species, including non-human primates and humans, but is usually being performed in rats (296).

In 1979, Wauquier *et al.* (303) adapted the amygdala kindling model to Beagle dogs. Kindling in dogs occurred rapidly and did not show the five Racine stages seen in rats. Following once daily stimulation with currents of 50–700 µA, mastication was evoked in the first sessions, but generalized tonic-clonic seizures developed rapidly in all dogs with an intermediate stage of facial clonus, head nodding, and profuse salivation. Fully kindled seizures began with facial clonus, head nodding, and salivation, and were followed by opisthotonos, lifting of the contralateral forepaw, falling over backward, clonicity of the hind legs, tonic extension of the forelegs and hindlegs, quiescence, myoclonic jerking or running seizures; and terminated with wet dog shaking. Following kindling, spontaneous seizures were seen occasionally in all animals, which is an important difference from rodents, which develop spontaneous seizures only after several 100 amygdala stimulations (304). Four of eight kindled dogs died after developing SE. The kindled seizures were completely suppressed by phenobarbital and diazepam, while clonazepam was only partially effective, and phenytoin was ineffective (303).

EEG alterations during amygdala kindling in dogs have been described by Thompson and Galosy (305). Furthermore, kindling has been induced in dogs by repeated electrical stimulation of the olfactory cortex or the anterior piriform cortex (306, 307), which is particularly sensitive to electrical kindling (161, 308).

The Dog as a Model of Birth Asphyxia and Neonatal Seizures

Birth asphyxia, or impaired gas exchange during the perinatal period, which leads to progressive hypoxia, hypercarbia, and acidosis, is a significant global health problem, responsible for >1 million neonatal deaths each year worldwide (309, 310). Those who survive often suffer from a range of health issues including neonatal seizures and hypoxic-ischemic encephalopathy (HIE). HIE following birth asphyxia is the most common cause of acquired perinatal brain injury and may lead to neurologic sequelae such as epilepsy later in life (311). Neonatal seizures, which typically occur during the first 48 h after birth, are thought to contribute to mortality and morbidity following birth asphyxia (312). Currently, therapeutic hypothermia is the only standard treatment for infants with moderate to severe HIE and it has been shown to reduce both mortality and morbidity (313). However, therapeutic hypothermia has several limitations, so novel therapies for HIE are urgently needed (311). Similarly, currently used therapies (e.g., phenobarbital) for neonatal seizures have limited efficacy (312). Animal models of birth asphyxia, HIE, and neonatal seizures are important to explore cellular and molecular mechanisms, assess the potential of novel therapeutic strategies, and characterize the functional and behavioral correlates of injury (314, 315).

The dog has been used as a "large animal" model for birth asphyxia and neonatal seizures (316–318). For instance, in a series of landmark studies, Duffy *et*

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al. have used newborn dogs for studying the effects of asphyxia on cerebral blood flow and metabolism (319–321). Similarly, brain metabolism after induced seizures was investigated in neonatal dogs (316, 322). The "Beagle puppy model of perinatal asphyxia" has been used to study new therapeutic approaches, including calcium channel blockers, glutamate receptor antagonists, non-steroidal anti-inflammatory drugs, and thromboxane synthesis inhibitors (317, 323–327). More recently, neonatal encephalopathy with seizures was described as an autosomal recessive disease of Standard Poodle puppies (328). However, this condition was not associated with birth asphyxia.

Use of Dogs as a Translational Model in Epilepsy Research and Antiseizure Drug Development

As shown by the review on canine epilepsy above, epilepsy in dogs is similar to human epilepsy in its epidemiology and spontaneity, and its response and resistance to therapy. The similarities make the canine model a promising animal model for testing new therapies, including neurodevices. The advantages of the naturally occurring canine model include that (1) disease surveillance in dogs is second only to that of people; (2) inbreeding in purebred dog breeds makes the genetics easier to determine; (3) drug studies can be done at a lower cost than in people without the need for regulatory approval; (4) full-sized epilepsy monitoring device prototypes can be used in dogs; and (5) dog owners may be willing to try higher risk or unproven therapies if they are on the edge of euthanizing the dog because of poor seizure control (8). The results of canine comparative epilepsy studies are not only of potential translational benefit for people but often also directly help improve the outcome and quality of life for pet's afflicted with epilepsy. The ASM imepitoin, which was initially developed for human epilepsy but—based on dog studies—was approved for the treatment of canine epilepsy, is a good example in this respect (see below).

However, as yet, canine epilepsy is an underutilized model, which has several reasons, including the lack of public awareness about this model in research and therapy development, the marked dog-to-human differences in drug elimination, the less thorough classification of canine epilepsy and epileptic seizures, the lack of routine video-EEG recording, and insufficient knowledge about the etiology of canine epilepsy (8, 61, 153, 329). Nevertheless, as discussed above, there is a relative surge of proof-of-concept studies and RCTs of therapies in naturally occurring epilepsy in the dog in the past ~15 years. These canine studies in the areas of genetics, drug therapy, dietary therapy, implantable EEG devices, and therapeutic devices show proof of concept that canine epilepsy can be a very good model for comparative research for many, but not all, facets of epilepsy, which will be discussed below.

In addition to dogs with naturally occurring epileptic seizures, seizures may be induced by chemical or electrical stimulation in healthy dogs, which increases the applicability of the dog model for the evaluation of new treatments. An important example in this regard is the development of VNS, which was based on a series of studies in dogs with induced seizures (280), which will be described in more detail below.

In the following, I will highlight some of the areas in which the use of dogs, either with naturally occurring or induced seizures, has proved useful in translational research.

Epileptic Dogs as a Model for Evaluation of Novel Anti-seizure Drugs

Patterson (8) suggested that the canine translational model of epilepsy may well prove to be an excellent intermediate step for confirming preclin-

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ical data in rodent models just before initiating human studies. The probably best example in this regard is imepitoin, the first partial "BDZ receptor" agonist developed for the treatment of epilepsy (101). As illustrated in **Figure 6**, the $GABA_A$ receptor exhibits several binding sites, including the GABA recognition site and different recognition sites for BDZs and barbiturates (330). *Via* these sites, BDZs and phenobarbital potentiate the inhibitory action of GABA, thus acting as PAMs. Imepitoin, which is not a BDZ, also acts as a PAM *via* the BDZ site (previously termed "BDZ receptor"), but with much lower affinity and intrinsic efficacy than BDZs (101). Because the anticonvulsant effect of drugs that act *via* the BDZ site occurs at low receptor occupancy, imepitoin exerts potent antiseizure effects in various animal models, but, in contrast to BDZs, does not induce sedation, ataxia, or hypnosis at high doses and lacks tolerance and dependence liability.

We showed in the timed i.v. PTZ infusion seizure threshold test in dogs that imepitoin dose-dependently increases the PTZ seizure threshold in about the same dose range as phenobarbital (**Figures 7A,B**), whereas abecarnil, which acts as a high-affinity subtype-selective agonist/partial agonist at the BDZ recognition site, was more potent but not more effective in this model (**Figure 7C**) (268, 273). Based on the unique pharmacological profile of imepitoin, we also performed chronic studies in dogs and repeatedly determined the anti-seizure effect during treatment (268). Prolonged oral administration with twice-daily dosing of imepitoin with either 5 or 40 mg/kg over 5 weeks was not associated with loss of antiseizure efficacy in the PTZ dog model (**Figure 7D**). In contrast, as shown in **Figure 7D**, both diazepam and clonazepam lost efficacy during prolonged treatment in dogs.

The unique properties of imepitoin in dogs prompted us to perform a prospective open-label trial in dogs with newly diagnosed epilepsy (268). The data from the acute and chronic experiments with imepitoin in the PTZ model (**Figure 7**) were used for choosing doses of imepitoin for the treatment of epileptic dogs. Imepitoin markedly reduced seizure frequency and severity without a significant difference from standard treatments (phenobarbital or primidone) but was much better tolerated than the standard drugs. In dogs with chronic DRE, most dogs exhibited a reduction in seizure frequency and severity during add-on treatment with imepitoin (268). The anti-seizure efficacy and favorable tolerability of imepitoin in epileptic dogs were subsequently confirmed in several RCTs (57, 101) and the drug was approved in Europe for the treatment of canine epilepsy in 2013.

Our dog studies on imepitoin were initially thought to complement the preclinical data before initiating clinical trials in humans. As predicted by the dog experiments, imepitoin proved to be highly tolerable in phase 1 pharmacokinetic and tolerability studies in human volunteers. However, further development for humans was terminated because of pharmacokinetic differences between smokers and non-smokers (101). Instead, it was decided to develop this compound as an ASM for dogs with epilepsy, because two of the scientists (Chris Rundfeldt and Wolfgang Löscher) involved in the development of imepitoin are veterinarians and successfully argued that there is an urgent need for novel ASMs in veterinary medicine.

The β-carboline abecarnil, which as imepitoin did not lose efficacy during prolonged treatment in dogs (**Figure 7D**), was initially developed as a non-sedative ("anxioselective") anxiolytic drug for humans (331, 332). However, abecarnil also exerts broad antiseizure efficacies in a wide variety of seizure models, including PTZ seizures in dogs and photically induced seizures in epileptic baboons (273, 274, 333). Abecarnil appears to be a full agonist at α1 and α3 subunit-containing $GABA_A$ receptors but is a partial agonist at other receptor isoforms. Abecarnil was not observed to cause significant alterations in motor activity, with the

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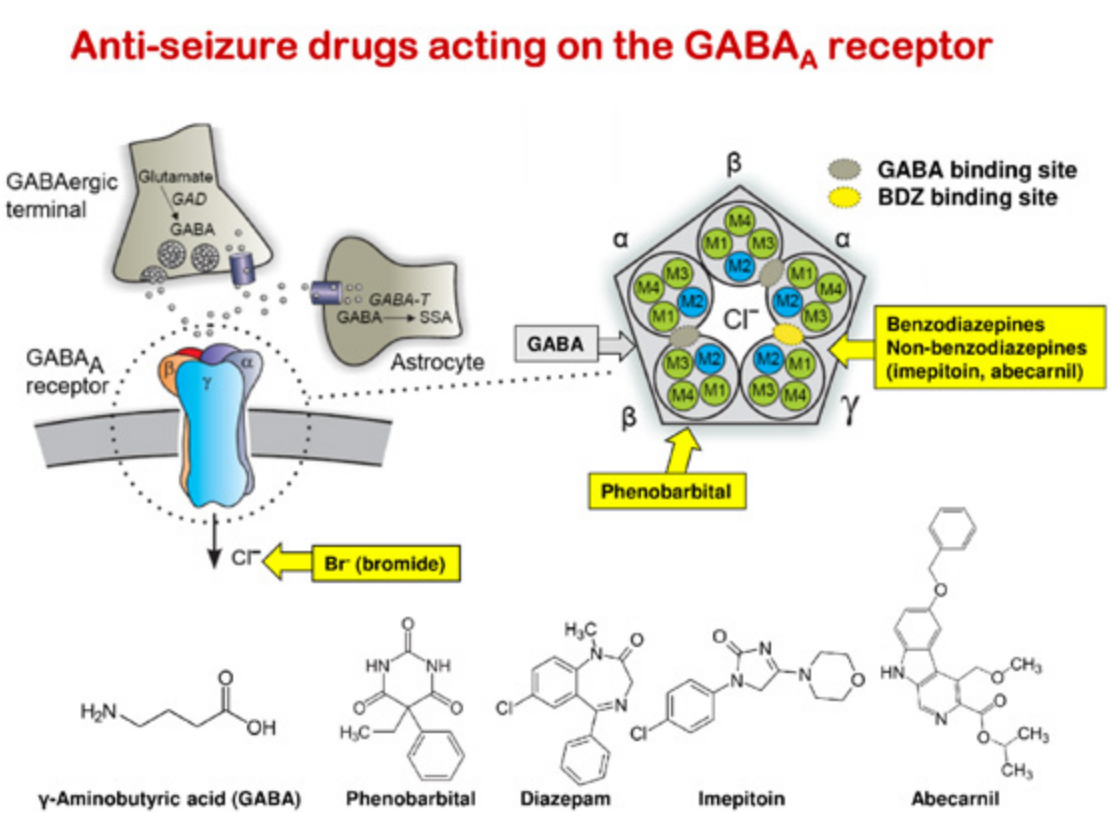

Figure 6. *Simplified schemes of the inhibitory GABAergic synapse and the structure of the GABAA receptor illustrating the site of action of benzodiazepines (BZDs) and other drugs acting via this site. The left part of the figure illustrates a GABAergic synapse showing synthesis, vesicular packaging, release, uptake, and degradation of GABA in GABAergic nerve terminals; uptake into astrocytes; and the pentameric subunit structure of a typical GABAA receptor complex in the postsynaptic membrane, consisting of α-, β, and γ-subunits. Components of the GABAergic synapse shown include glutamic acid decarboxylase (GAD), the enzyme that catalyzes the decarboxylation of glutamate to GABA; GABA-containing synaptic vesicles (circles containing GABA molecules); the GAT-1 GABA transporter (cylinders); and conversion of GABA to succinic semialdehyde (SSA) by GABA transaminase (GABA-T). The right part of the figure illustrates schematically the pentameric structure of the GABAA receptor within the plane of the neuronal membrane showing the relative positions of the transmembrane domains. Subunit interfaces are formed by M3 and M1. The interfacial locations of the two GABA and one BZD recognitions sites are shown. By binding to the BZD site, BZDs (e.g., diazepam, midazolam, and others), β-carbolines (e.g., abecarnil), and imidazolone derivatives (e.g., imepitoin) act as positive allosteric modulators of GABA leading to increased chloride channel opening frequency, increased chloride influx, and, consequently, to increased hyperpolarization of the membrane and thus inhibition of the postsynaptic neuron. Barbiturates such as phenobarbital also bind to the GABAA receptor to potentiate GABA, but the exact binding site is less well-established. Bromide ions (as produced by administration of potassium bromide) enhance GABAergic inhibition but the mechanism is distinct from that of BDZs and barbiturates in that Br- ions compete with Cl– ions for GABA-gated Cl– channels and, at high concentrations, enter the neuron through these channels, thereby inducing a lasting hyperpolarization of the neuronal membrane. Once Br– ions have entered a neuron, they can only very slowly be eliminated from the neuron, explaining the poor therapeutic ratio and risk of intoxication with potassium bromide. The figure was modified from Rundfeldt and Löscher (101). For comparison, also the chemical structures of GABA, phenobarbital, a BDZ (diazepam), imepitoin, and abecarnil are shown.*

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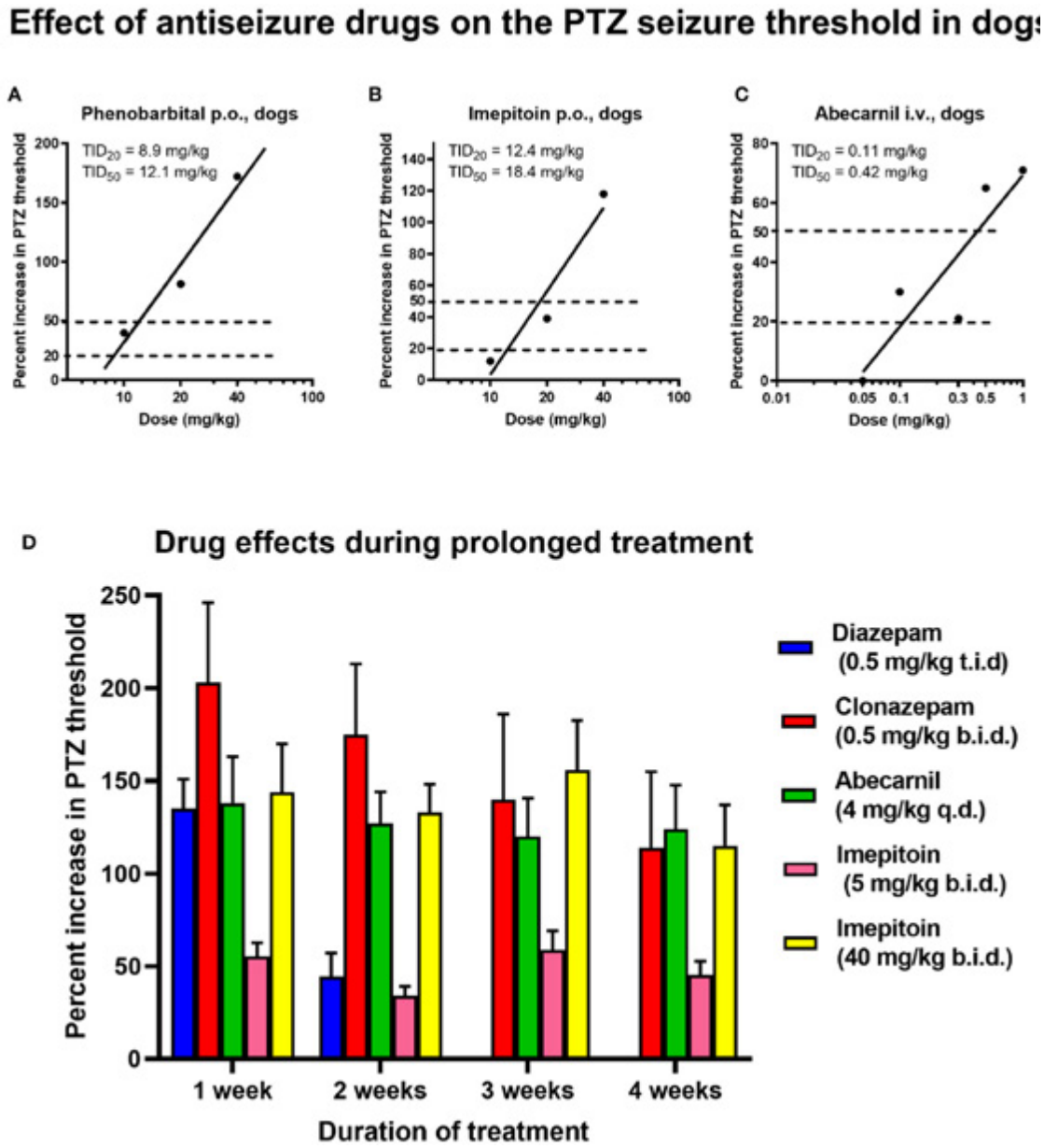

Figure 7. *Effect of antiseizure drugs on the timed i.v. pentylenetetrazol (PTZ) seizure threshold test in dogs. (**A**) Effect of different doses of phenobarbital on the PTZ seizure threshold, shown as percent increase above control threshold. Each symbol presents the percent increase in seizure threshold in a group of 6–7 dogs. By nonlinear regression analysis, the doses increasing the seizure threshold by 20% (TID20) and 50% (TID50) were calculated. (**B**) Effect of different doses of imepitoin on the PTZ seizure threshold. Other details as in (A). (C) Effect of different doses of abecarnil on the PTZ seizure threshold. Other details as in (A). (D) Alterations in antiseizure efficacy during prolonged treatment in dogs. Four drugs were compared in groups of 4–7 dogs: diazepam, clonazepam, abecarnil, and imepitoin. These drugs were administered daily over 4 weeks. The first PTZ seizure threshold was determined after 1 week of treatment. Control thresholds were repeatedly determined in each dog before the onset of treatment. Data are shown as the drug-induced mean percent increase (± SEM) above control thresholds. Note the rapid decline of antiseizure efficacy of diazepam, indicating the development of tolerance. Tolerance, although less marked, was also observed with clonazepam, but not with imepitoin or abecarnil. Data are from Frey et al. (271), Scherkl et al. (272), Löscher et al. (273), and Löscher et al. (171, 274).*

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anxiolytic and antiseizure activities of abecarnil typically manifested at doses 3–1,000 times less than those inducing sedation/ataxia and myorelaxation (332). More recently, we evaluated its antiseizure potential in a placebo-controlled pilot study in persons with epilepsy and found marked efficacy (334), which had been correctly predicted by the dog PTZ model (**Figures 7C,D**).

Epileptic Dogs as a Model for the Dietary Management of Seizures

Ketogenic diets, which are high in fat and low in carbohydrates, have been used to treat DRE since the 1920's (65, 335). The exact antiseizure mechanism of this diet is not clear, but the ketogenic diet leads to increases in circulating ketones, which may contribute to the efficacy in treating pharmacoresistant seizures (97). Despite a positive effect on seizure frequency when used as an adjunct treatment, most patients discontinue the diet because of its unpalatable and restrictive features. In the last 20 years, new variants of the classical ketogenic diet have emerged (335). Furthermore, the microbiota-gut-brain axis has evolved as a potential target for the ketogenic diet (336). Epileptic dogs have been used both for evaluating novel variants of the ketogenic diet and for studying the potential role of gut microbiota (116, 337–340).

Epileptic Dogs as a Model for Novel Therapeutic Devices

Concerning devices, the advantage of dogs vs. rodent models of epilepsy is that full-sized epilepsy device prototypes can be evaluated in dogs. As described above, the experimental basis for VNS therapy was a series of studies in the 1980's on the effects of cervical vagal stimulation on seizures induced in dogs (280). In these experiments in mixed breed dogs, seizures or tremors, respectively, were induced by i.v. injection of boluses of strychnine or PTZ at 1- to 4-min intervals until sustained muscle activity was observed electromyographically. Vagal stimulation terminated seizures in 0.5–5 s. Zabara (280) suggested that these results may form the basis of a new therapeutic approach to epilepsy. In line with this suggestion, the results of VNS in dogs were confirmed in a monkey model and shortly thereafter in the first VNS implant in an adult patient with DRE (341). After positive data from two RCTs, the VNS Therapy® System received FDA approval in 1997 for use as adjunctive therapy in reducing the frequency of focal-onset seizures which are refractory to ASMs (341). The first clinical trial using this device in dogs with DRE was published in 2002 (342), reporting up to a 50% reduction in seizure frequency in four of nine dogs. For exploring the neurochemical effects of VNS in dogs, the VNS Therapy® System was implanted in 8 Beagle dogs and levels of serotonin (5HT), norepinephrine, and dopamine were quantified in the CSF after 1 h of sham, standard, and microburst VNS (343). Rapid cycling standard and microburst VNS caused a significant increase of norepinephrine levels in the CSF, whereas no significant changes were detected in 5HT or dopamine levels. These data support previous findings indicating that VNS influences the locus coeruleus-norepinephrine system (344).

In the first long-term evaluation of VNS therapy in a dog with drug-resistant epilepsy, a 5-year-old male Shetland Sheepdog was treated with VNS for 1 year (345). During this period, stimulation parameters were repeatedly adjusted to optimize stimulation intensity while avoiding adverse effects. The frequency of generalized tonic-clonic seizures was reduced by 87% throughout the period of VNS. The owner reported that the dog regained his personality and quality of life.

More recently, Robinson *et al.* (346) examined the feasibility and efficacy of non-invasive VNS (nVNS) as an adjunct treatment for DRE in dogs. nVNS was found to be safe and easy to administer with mild adverse events. Out of 14 epileptic dogs, nine achieved a reduction in seizure frequency and four were considered responders with a 50%

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or greater reduction in seizures from baseline to the final treatment period (346).

In addition to VNS, several other types of invasive or non-invasive neurostimulation are available for adjunct therapy for persons with DRE (65). One of the most widely used techniques is RNS, which was approved by the FDA in 2013 (65). Whereas, the total volume of resection surgeries decreased in recent years, RNS implantations have increased by over 100% in persons with DRE (347). RCTs in adult DRE patients have shown that closed-loop RNS to the seizure focus *via* bilateral hippocampal electrodes reduces the frequency of disabling seizures, is well-tolerated, and is acceptably safe (348, 349). The NeuroPace RNS® System, which continuously records the iEEG, recognizes and responds to each patient's unique brain patterns, providing personalized stimulation and preventing seizures before they start. RNS or other types of deep brain stimulation are not yet routinely available for the treatment of DRE in dogs, but dogs played a decisive role in developing the iEEG devices and machine learning algorithms needed to develop closed-loop RNS for human patients. Recently, a novel implantable neural stimulating and recording device was reported to prevent SE events in a dog with severe DRE (350).

Data generated by continuous iEEG monitoring in patients demonstrated that without such monitoring many seizures are missed by the patients or their relatives and caregivers (351). This is certainly similar to seizure documentation such as seizure diaries used by owners of epileptic dogs, which may form a significant bias in trials on new therapies. Indeed, seizure counts based on seizure diaries are often inaccurate and underestimated (351, 352). Similar to the situation in human epilepsy, the unpredictability of seizures plays a major part in the management of canine epilepsy, and dog owners have a strong desire to know when a seizure occurs (353). Automated seizure detection by reliable seizure detection devices would be important to guide treatment decisions or monitor outcomes in clinical trials in both human and canine patients.

Epileptic Dogs as a Model for Novel Implantable EEG Devices

In 2011, Davis *et al.* (67) described a novel implanted device to wirelessly record and analyze continuous (24/7) iEEG and tested this device in six unsedated epileptic dogs over 5 months (see above). For iEEG monitoring, two electrode arrays with 16 intracranial sensors were placed parallel to the dura in the subdural space to record the iEEG from both hemispheres (**Figure 8**). The intracranial sensors were coupled to an implanted, rechargeable, subclavicular acquisition and transmission unit, which continuously telemeters iEEG data to an external processing unit for real-time data storage, analysis, and communicating analysis results to caregivers. A seizure detection algorithm, trained on human iEEG data, was deployed on real-time canine iEEG (67).

This device was then used in subsequent studies for mining continuous iEEG in focal canine epilepsy (68, 355). One goal of these studies was to investigate interictal bursts and their electrographic relationship to seizures. Another goal was to focus on the challenges presented by new devices that continuously monitor and process human EEG data over long periods. This work has evolved and improved steadily over recent years, embodied in devices to detect, predict, and respond to seizures in several new implantable devices (348, 349). The large archive of continuous data analyzed for this project (up to 14 months of continuous EEG recording in epileptic dogs) required rigorous, automated methods, including machine learning, for detecting and processing EEG activity. The data obtained further validated canine epilepsy as a promising model of human epilepsy and generated a set of continuous iEEG data of unprecedented length for analysis (68, 355).

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Apart from iEEG recording, interesting novel mobile (ambulatory) devices are being developed for continuous scalp or subscalp EEG monitoring in human patients with epilepsy (356, 357). One of these devices uses a subcutaneous (a.k.a. subscalp or subdermal) needle electrode (the UNEEG SubQ™; UNEEG Medical A/S, Lynge, Denmark), which is placed through a minimally invasive surgical procedure under the scalp and contains an inductive coil for transfer of power and data (358). In connection with an external recorder (the 24/7 EEG™ SubQ) combined with a complete data infrastructure and analytics software, the implanted electrode provides continuous (24/7) and automated electrographic seizure detection for up to 15 months (358–360). Several similar subscalp systems have been developed and tested in humans, including the use of such systems for seizure forecasting (359, 361). The size of the subcutaneous needle electrode or subscalp device of the UNEEG system would be ideally suited for continuous EEG recordings in epileptic dogs, which we plan to explore soon. Short-term wireless video-EEG recordings from unsedated epileptic dogs *via* subdermal wire electrodes substantiated the feasibility of such an approach (362).

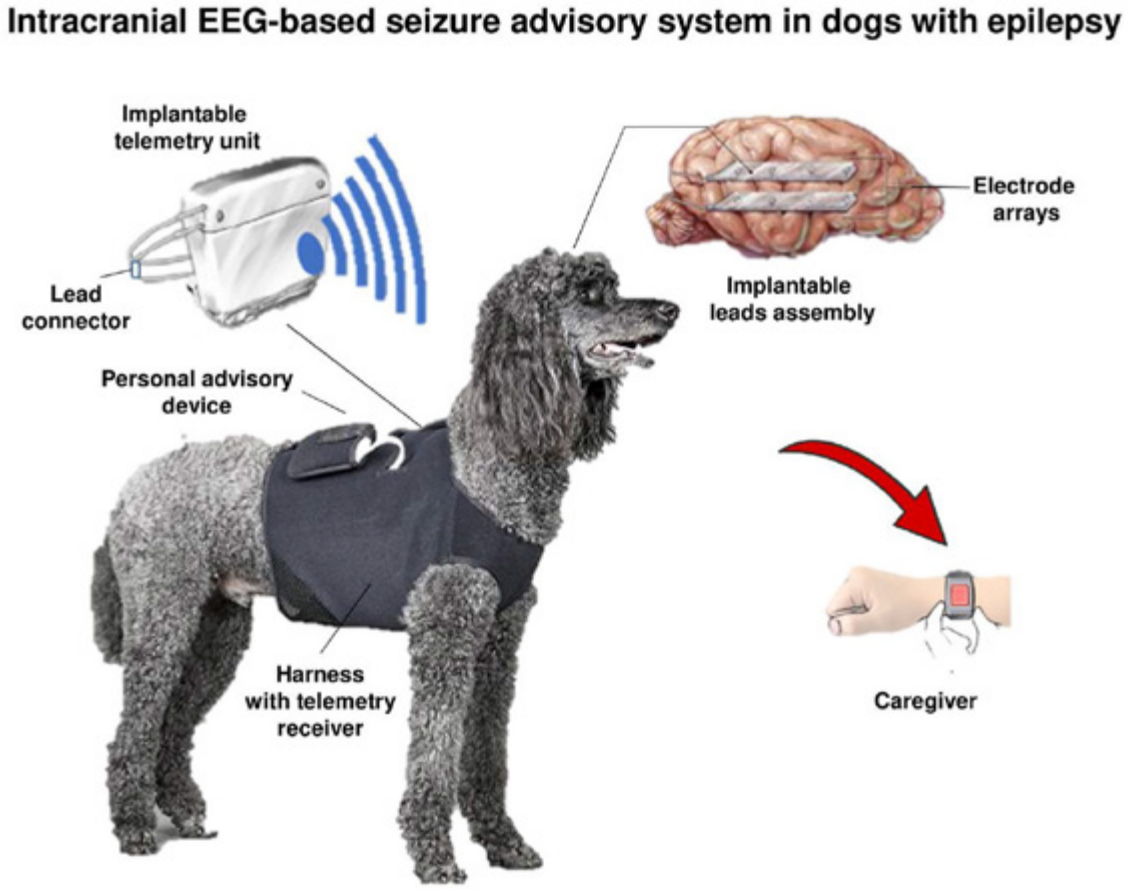

Figure 8. *Schematic of a dog with an implanted ambulatory NeuroVista Seizure Advisory System (SAS). The implantable device for recording and storing continuous iEEG includes: An Implantable Lead Assembly (ILA) placed in the subdural space, an Implantable Telemetry Unit (ITU), and a Personal Advisory Device (PAD). The ILA, which acquires 16 channels of iEEG, detects and relays electrical activity in the brain to the ITU. The ITU receives data from the implantable leads, predicts seizure activity using an algorithm, and sends a wireless alert to the PAD. The PAD sends a wireless alert to the caregiver, which may lead to accelerated intervention and administration of seizure-stopping medication (see text). All iEEG data are stored on a flash drive and uploaded weekly via the internet to a central data storage site. Modified from Coles et al. (354).*

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Epileptic Dogs as a Model for Seizure Forecasting

Predicting the occurrence of epileptic seizures using machine learning algorithms operating on iEEG or scalp EEG data has the potential to improve the lives of patients living with seizures (363, 364). However, progress toward reliable seizure forecasting has been hampered by a lack of open access to long-duration EEG recordings with an adequate number of seizures for investigators to rigorously compare algorithms and results. In 2014, a large-scale international seizure prediction competition was run on a standard data science contest portal, involving a combination of short-term human iEEG data (with 942 seizures recorded over >500 days) and long-term iEEG data in dogs (348 seizures recorded over 1,500 days) (365, 366). Data from these studies demonstrated the feasibility of seizure forecasting in canine and human epilepsy. Since then, long-duration iEEG recordings from epileptic dogs have been used to further improve the deep-learning algorithms developed for seizure forecasting (367–370). Importantly, the strongest evidence that seizure prediction is possible comes from long-term recordings in epileptic dogs, further substantiating canine epilepsy as a translational model. These studies have shown that seizure prediction performs better than chance in all dogs studied.

Epileptic Dogs as a Model of Drug-Resistant Seizures

As described above, as in humans, drug resistance is the major clinical problem in the therapeutic management of canine epilepsies. Several RCTs with ASMs in dogs with newly diagnosed epilepsy have shown that at least 50% of the animals do not achieve seizure control (121). Thus, epileptic dogs are an ideal model to study mechanisms of epilepsy (95, 121, 153, 371). To our knowledge, epileptic dogs are the only non-rodent model of epilepsy that allows the selection of animals that respond and do not respond to drug treatment and thus would seem to represent an ultimate tool to study why and how epilepsy becomes intractable. Dogs with ASM-resistant seizures are often euthanized, making postmortem brain studies possible. In this regard, a biobank for canine brain tissue would be useful because this can provide neurologists with a new insight into epileptic brain morphology and identify underlying causes such as cortical dysplasia, which might be overlooked in veterinary medicine and be vital factors for the development of drug-resistance (47).

A detailed examination of dogs with intractable generalized tonic-clonic seizures indicated several differences between resistant and non-resistant animals (95, 121, 372). Dogs with intractable epilepsy have a higher seizure frequency and more severe seizures (including SE and seizure clusters) than epileptic dogs that respond to ASMs, which is consistent with data from prognostic evaluation in humans (121) and in line with the intrinsic severity hypothesis of Rogawski and Johnson (373).

Furthermore, differences in breed distribution were seen in ASM responders and non-responders, indicating that genetics are involved in the medical intractability of canine epilepsy (372). The duration of time for which epilepsy had been present before treatment seemed not to be of major importance for the efficacy of ASM therapy in epileptic dogs.

More recently, the role of the "transporter hypothesis" of DRE has been addressed in epileptic dogs (121). For several major ASMs, drug absorption, distribution, and elimination are affected by drug efflux transporters such as P-glycoprotein (Pgp), which are regulated by promiscuous drug-sensing nuclear receptors and may be overexpressed in patients with epilepsy (120). Efflux pumps such as Pgp are also expressed at the BBB, thus critically reducing functionally relevant drug levels at target sites in the epileptogenic tissue, which may contribute to ASM resistance. Both preclinical and clinical studies have found increased expres-

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sion of Pgp and other efflux transporters in the area of the epileptic focus of ASM resistant individuals (120, 374). Such increased transporter expression may be a result of frequent seizures or genetic factors or both. For instance, SE in dogs has been reported to increase Pgp in the canine brain (375) and polymorphisms in the Pgp-encoding *ABCB1* gene have been associated with seizure outcomes in Collies with epilepsy (122, 376). Increased BBB expression of Pgp may thus be involved in ASM resistance in dogs, although several other mechanisms certainly contribute to this phenomenon (120). Add-on treatment with the Pgp inhibitor verapamil in dogs with phenobarbital-resistant epilepsy failed to improve seizure control (377). However, verapamil is not a selective Pgp blocker but mainly acts as a blocker of voltage-gated calcium channels, which complicates the interpretation of studies with this drug. Thus, clinical studies with more selective Pgp inhibitors are needed (121). Overexpression of Pgp in the brain and its inhibition can be visualized by PET imaging (378).

Another popular hypothesis of ASM resistance, the "target hypothesis," suggests that the ASM targets in the brain are altered in DRE (120). Although attractive, the clinical evidence for this mechanism is very limited. Furthermore, the fact that most ASM-resistant patients are resistant to several ASMs acting on different therapeutic targets undermines the general utility of the target hypothesis (374). Recently, it has been suggested that epigenetic mechanisms and protein-protein interactions may result in alterations of diverse drug targets in epileptogenic brain regions, thus explaining why most ASM-resistant patients are resistant to several ASMs acting on different therapeutic targets (121, 379). Seizures and epilepsy induce epigenetic changes in the transcriptome of proteins (including drug targets) by DNA methylation, which may underlie the resistance of several ASMs. However, this interesting hypothesis needs further exploration in both animal models and human and canine epilepsy.

Status Epilepticus in Dogs as a Model for Novel Treatments

In 2011, Leppik *et al.* (77) suggested that naturally occurring canine SE may become a translational platform for evaluating the safety and efficacy of interesting compounds with anti-SE activity in rodent studies before their eventual use in human trials. This proposal was based on a randomized, placebo-controlled, double-masked study on i.v. levetiracetam in dogs that presented with convulsive SE to the University of Minnesota Veterinary Medical Center. Dogs that did not become seizure-free with diazepam were enrolled and treated with either levetiracetam or placebo, resulting in a 56% response rate for levetiracetam compared to 10% for placebo (77). Details of the full trial, which also included dogs with acute repetitive seizures (cluster seizures), were published in 2012 (380). Seizure etiologies identified were idiopathic epilepsy (n = 10), inflammatory CNS disease (n = 4), intracranial neoplasia (n = 2), hepatic encephalopathy (n = 1), and two dogs had no cause determined. Leppik *et al.* (77) concluded that—in contrast to rodent models of electrically or chemically induced SE—a test species having naturally occurring SE similar to that in humans and closer in terms of pharmacokinetic characteristics and body size would be very useful. A further advantage is that—in contrast to humans—placebo-controlled studies in dogs are possible because FDA-approved treatments for canine SE are not available. The promising efficacy of levetiracetam in diazepam-refractory (established) canine SE translated to efficacy in established human SE (381).

In 2015, a multi-center proof-of-principle RCT on established canine SE treated with i.v. fosphenytoin, a prodrug of phenytoin, was published (382). Fosphenytoin was significantly more effective than placebo at phenytoin plasma levels within the therapeutic concentration range for people. In a subsequent study, the same group evaluated

the pharmacokinetics of i.v. topiramate in dogs with naturally occurring epilepsy (383). Topiramate is not yet approved for i.v. administration, but—based on data from nasogastric tube administration—may be an interesting option for treatment of established SE (384). In this regard, it is interesting to note that we evaluated the safety and pharmacokinetics of a new cyclodextrin-based i.v. formulation of carbamazepine in dogs (385) and demonstrated its efficacy in a mouse model of convulsive SE (386). In 2016, the FDA approved a cyclodextrin-based i.v. formulation of carbamazepine for use in humans.

Recently, Vuu *et al.* (387) reported the pharmacokinetics, pharmacodynamics, and safety of a new cyclodextrin-based i.v. formulation of allopregnanolone in healthy and epileptic dogs to develop this formulation for early treatment of SE. Allopregnanolone, a neurosteroid that modulates synaptic and extrasynaptic $GABA_A$ receptors, was shown to be effective in a mouse model of established SE (388). In the dog study, the rapid onset of effect of allopregnanolone after i.v. infusion suggested that this drug may be useful in the early treatment of SE (387). Overall, these various studies establish the dog as a viable clinical model of human SE.

Induced Seizures in Dogs as a Model for Novel Treatments

As described above, induced seizures in healthy dogs have been widely used to evaluate new ASMs and therapeutic devices. The most frequently used model has been the timed i.v. PTZ infusion seizure threshold test, which was instrumental in the preclinical development of novel ASMs such as imepitoin. However, the PTZ model is not susceptible to all antiseizure therapies as illustrated by the lack of VNS to increase the PTZ seizure threshold in dogs (343). In this respect, it is important to note that PTZ acts as a $GABA_A$ receptor antagonist and, as such, is particularly sensitive to ASMs (such as BDZs, imepitoin, and phenobarbital) that act as PAMs at this receptor. Thus, PTZ cannot replace seizure tests, such as the MES model, which are sensitive to ASMs acting by various other mechanisms. Similar to the MES test in rodents (389), Territo *et al.* (285) have shown that clonic-tonic seizures in the MES test in dogs can be dose-dependently suppressed by phenobarbital. The authors suggested that MES is a useful model for evaluating generalized convulsions in canines and may provide a tool for a dose selection of novel pharmaceutical compounds before first clinical trials in humans. In a subsequent study, Territo *et al.* (284) used the MES dog model to further evaluate the antiseizure efficacy of ameltolide, which acts by modulating voltage-dependent sodium channels of presynaptic neurons.

In addition to using the timed i.v. PTZ infusion seizure threshold test for determining ASM efficacies in dogs, this test can be used to determine whether loss of efficacy (tolerance) develops upon chronic administration. Examples are illustrated in Figure 7D. Furthermore, this test can be used to determine whether withdrawal hyperexcitability occurs after rapid termination of treatment. Withdrawal symptoms, including a decreased seizure threshold, spontaneous seizures, or, eventually, SE, are typical for drugs (such as BDZs, barbiturates, and opioids) that induce physical dependence during chronic treatment. For such drugs, withdrawal symptoms do, of course, also occur when no seizures are induced during prolonged treatment, but induction of seizures allows determining tolerance and dependence in the same experiment (100). For drugs that act *via* the BDZ binding site of the $GABA_A$ receptor, as an alternative to abrupt termination of treatment, injection of drugs (such as flumazenil) that act as antagonists at this site has been used to precipitate withdrawal symptoms (390). The dog model has been useful to demonstrate that tolerance and dependence can be abolished by developing compounds, such as imepitoin or abecarnil, that act only as partial or subtype-selective agonists at the $GABA_A$ receptor (101).

The Dog as a Model for Pharmacokinetic Studies

As shown in Table 1, most ASMS are eliminated more rapidly by dogs than by humans, which restricts the use of ASMs in canine epilepsy. Despite the dog-to-human differences in elimination kinetics of many ASMs (Table 1), both healthy and epileptic dogs are used to study certain pharmacokinetic aspects of ASMs. For instance, we have used dogs to study the kinetics of CNS entry of several ASMs (16, 18, 23). Similarly, we included dogs when evaluating species differences in the metabolism and plasma protein binding of ASMs (13–15, 391) and characterized the pharmacology of active metabolites, such as the main active metabolite of valproate, in dogs (392). Furthermore, as described above and shown in Table 1, we have determined the pharmacokinetics of various ASMs in dogs as a basis for dose and dosing interval selection for chronic treatment in epileptic dogs (11, 13–17, 19–24, 393). More recently, we studied in dogs whether switching from brand name to generic formulations of phenobarbital is associated with differences in effective drug levels (394).

Numerous other groups have used healthy or epileptic dogs for pharmacokinetic studies, including recent studies on i.v. fosphenytoin (382, 395), topiramate (383), and allopregnanolone (387). Such studies are important to further characterize species differences in drug pharmacokinetics and provide a basis for rational therapy of canine epilepsy or SE. Furthermore, data from invasive studies on drug BBB penetration in dogs can be translated to humans, because BBB characteristics are similar across mammalian species such as dogs and humans (396).

Conclusion

As shown in this review, because of impressive epidemiological, clinical, and pharmacological similarities, naturally occurring canine epilepsy is an excellent model for human epilepsy. The same is true for canine SE. Even though dogs metabolize most ASMs more rapidly than humans (Table 1), effective plasma levels during chronic epilepsy therapy are remarkably similar, for instance as shown for phenobarbital. Drug resistance is a major problem in both canine and human epilepsy. Similar comorbidities occur in both species. Furthermore, the body sizes of dogs are closer to humans than are rodent body sizes, which makes dose conversion between dogs and humans more accurate. Scaling factors for human equivalent doses are ~1.8 for dogs but ~12 and ~6 for mice and rats, respectively (397). Importantly, dogs are large enough to accommodate therapeutic or iEEG devices designed for humans. Studies in epileptic dogs with such devices have reported ictal events that showed remarkable similarity to human seizures (68, 355). Background EEG and interictal bursts of epileptiform discharges in these animals were also indistinguishable from human iEEG recordings. This work provided a rich dataset of unprecedented length for studying seizure periodicities and developing new methods for seizure forecasting (363).

However, there are also limitations of the epileptic dog as a translational model (329). Despite the efforts of the IVETF to standardize the diagnosis of epilepsy and the assessment of therapeutic responses, the classification of seizures and epilepsy in dogs is controversial. At least in part, this is because the EEG is not a standard procedure in the diagnostic management of canine patients with suspected epilepsy. Furthermore, the use of modern but expensive imaging techniques such as MRI is not a routine procedure in veterinary medicine, although their use is steadily increasing. Concerning studies on drugs or devices in epileptic dogs, such studies are very much affected by the dogs' owner motivation and reliability. Clinical trials in epileptic drugs are as elaborate and time-consuming as trials in human patients, but much less expensive and less limited than studies in

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humans. Thus, in drug or device development, epileptic dogs should only be used for translational purposes if the additional value is important for the decision of whether to initiate or continue testing in humans. Relevant examples in this regard are studies on novel treatments of SE or novel iEEG devices and seizure forecasting algorithms. Laboratory dogs, such as Beagles, with naturally occurring epilepsy, are an alternative to pets, but the cost and effort to maintain such dogs, along with ethical considerations, pose limitations to experimental epilepsy research in such animals (329).

In summary, dogs with naturally occurring or induced seizures provide excellent large-animal models to bridge the translational gap between rodents and humans in the development of novel therapies. Furthermore, because the dog is not only a preclinical species for human medicine but also a potential patient and pet, research on this species serves both veterinary and human medicine.

Dedication

This review is dedicated to my late colleagues and friends Drs. Hans-Hasso Frey and Dieter Schmidt who initiated my interest in epileptic dogs as a translational model in the 1970's and performed several studies with me in this species.

Author Contributions

WL wrote this review and agrees to be accountable for all aspects of the work.

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Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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