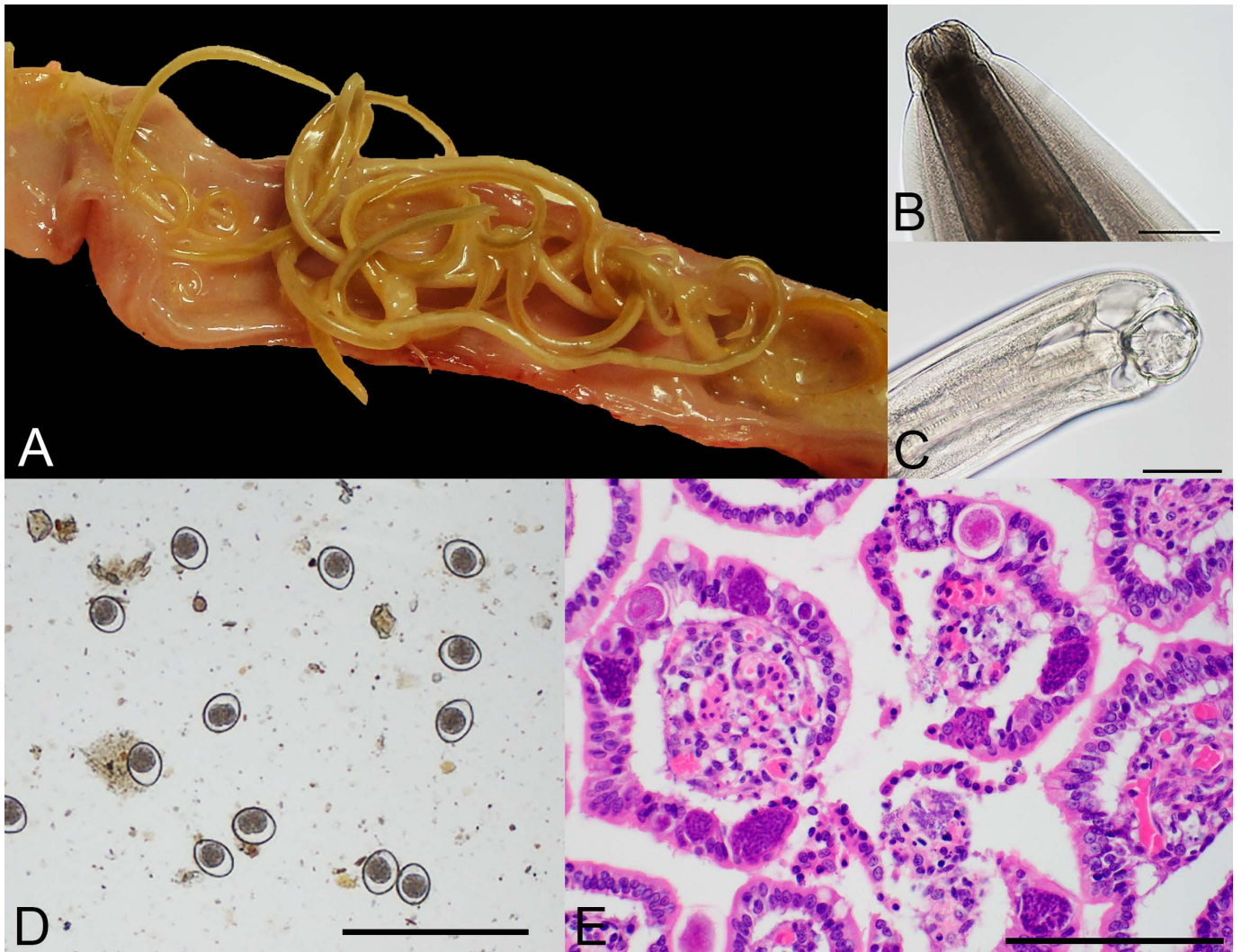




**Supplementary Figure 1.** Representative photographs of common gross lesions identified in kittens that died or were euthanized due to diarrhea. Panel A is a photograph of jejunum demonstrating the gross lesion of hyperemia. Panel B demonstrates a dilated, hyperemic jejunum and Panel C of the same section when opened demonstrates clear fluidic content and fibrin covered ulcers. Panel D demonstrates a small intestine with thickened wall. Panel E demonstrates a colon filled with melena.



**Supplementary Figure 2.** Representative photograph and photomicrographs of commonly identified parasites. Panel A demonstrates the gross appearance of ascarids (*Toxocara cati*) within the small intestine. Panel B demonstrates the anterior of the ascarid *Toxocara cati*, with its characteristic fleshy lips and cervical alae (Bar = 200  $\mu$ m). Panel C demonstrates the anterior of a hookworm (*Ancylostoma tubaeforme*) with its characteristic dorsal mouth and buccal capsule (Bar = 200  $\mu$ m). Panel D demonstrates the appearance of *Isospora felis* oocysts using fecal float centrifugation method (Bar = 200  $\mu$ m). Panel E demonstrates the appearance of unsporulated oocysts and schizonts of *Isospora sp.* within the villi of a kitten (Bar = 100  $\mu$ m).

Supplementary Table 1. Demographic data from 61 live kittens with and without clinical signs of diarrhea.

<b>Demographic Variable</b>	<b>No Diarrhea (n=33)</b>	<b>Diarrhea (n=28)</b>
<b>No. (%) kittens source 1<sup>a</sup></b>	24 (73)	23 (82)
<b>No. (%) kittens source 2<sup>b</sup></b>	9 (27)	5 (18)
<b>Age in weeks</b>	6.3 ± 1.8	7.0 ± 2.3
<b>Sex</b>	11 males 21 females 1 unknown	14 males 13 females 1 unknown
<b>Weight (range) in grams</b>	480 (280 – 909)	682 (227 – 1000)
<b>No. (%) weaned</b>	33 (100)	26 (93)
<b>Days (range) in foster care</b>	0 (1 – 30)	9 (0 – 42) ***
<b>No. (%) concurrent evidence of URD</b>	3 (9)	6 (21)
<b>No. (%) decreased appetite</b>	1 (3)	2 (7)

Data represent number (No) and percent (%) of kittens, mean ± SD for age, or median (range) for weight and days in foster care.

URD: Upper respiratory tract disease

<sup>a</sup> County Animal Center

<sup>b</sup> County SPCA

\*\*\* P < 0.001; Mann-Whitney Rank Sum test

Supplementary Table 2. Demographic data from 54 deceased kittens that were apparently healthy or had died or were euthanized due to diarrhea.

<b>Demographic Variable</b>	<b>No Diarrhea (n=20)</b>	<b>Diarrhea-associated mortality (n=34)</b>
<b>No. (%) kittens source 1<sup>a</sup></b>	20 (100)	8 (24)
<b>No. (%) kittens source 2<sup>b</sup></b>	0 (0)	26 (76)
<b>Age in weeks</b>	6.5 ± 2.5	5.6 ± 1.8
<b>Sex</b>	11 males 9 females	16 males 18 females
<b>Weight (range) in grams</b>	538 (261 – 896)	326 (163 – 598) <sup>***</sup>
<b>No. (%) weaned</b>	15 (75)	27 (79)
<b>Days (range) in foster care</b>	3.0 (0 – 11)	8.5 (0 – 37) <sup>***</sup>
<b>No. (%) concurrent evidence of URD</b>	3 (15)	7 (21)
<b>No. (%) decreased appetite</b>	0 (0)	24 (71) <sup>***</sup>
<b>Time (range) in hours from death to necropsy</b>	3.0 (1 – 24)	6.75 (1 – 24) <sup>**</sup>

Data represent number (No.) and percent (%) of kittens, mean ± SD, or median (range).

URD: Upper respiratory tract disease

<sup>a</sup> County Animal Center

<sup>b</sup> County SPCA

<sup>\*\*</sup>P<0.01, <sup>\*\*\*</sup>P<0.001; Mann-Whitney Rank Sum test

<sup>\*\*\*</sup>P<0.001 X<sup>2</sup> (appetite)

Supplementary Table 3. Gross and light microscopic lesion scores obtained from apparently healthy kittens and kittens that were euthanized or died due to severe diarrhea and on the basis of aEPEC culture and/or qPCR for *eae*.

Kitten categories	No diarrhea (n=20)	Diarrhea-associated mortality (n=34)	No diarrhea		Diarrhea-associated mortality		All Kittens	
			aEPEC – (n=11)	aEPEC + (n=9)	aEPEC – (n=16)	aEPEC + (n=18)	aEPEC – (n=27)	aEPEC + (n=27)
<b>Fecal score</b>	4 (2 – 6)	7 (2 – 7)**	5 (3.5 – 6)	4 (2 – 6)	7 (4 – 7)	7 (2 – 7)	6 (3.5 – 7)	6 (2 – 7)
<b>Gross lesions (No.)</b>	7 (35)	32 (94)***	3 (27)	4 (44)	16 (100)	16 (89)	19 (70)	20 (74)
Stomach	1 (5)	6 (1)	1 (9)	0 (0)	3 (19)	3 (17)	4 (15)	3 (11)
Small intestine	7 (35)	25 (74)*	3 (27)	4 (44)	12 (75)	13 (72)	15 (56)	17 (63)
Colon	0 (0)	9 (26)*	0 (0)	0 (0)	4 (25)	5 (28)	4 (15)	5 (19)
Perineum	0 (0)	11 (32)**	0 (0)	0 (0)	7 (44)	4 (22)	8 (30)	5 (19)
Other	0 (0)	2 (6)	0 (0)	0 (0)	1 (6)	1 (6)	1 (4)	1 (4)
<b>Microscopic lesion score</b>								
<b>Stomach</b>	0 (0 – 2)	0 (0 – 2)	0 (0 – 2)	1 (0 – 2)	0 (0 – 1)	0 (0 – 2)	0 (0 – 2)	0 (0 – 2)
<b>Small intestine</b>	3 (0 – 7)	4 (0 – 12)**	1 (0 – 5)	4 (0 – 7)	3 (0 – 11)	6 (0 – 12)**	3 (0 – 11)	5 (0 – 12)***
Villus atrophy/stunting	0 (0 – 2)	1 (0 – 3)	0 (0 – 1)	1 (0 – 2)	0 (0 – 3)	1.5 (0 – 3)	0 (0 – 3)	1 (0 – 3)
Epithelial injury	0 (0 – 2)	0 (0 – 3)	0 (0 – 2)	0.5 (0 – 2)	0 (0 – 2)	1 (0 – 3)*	0 (0 – 2)	1 (0 – 3)*
Crypt dilation/ distortion	0 (0 – 1)	1 (0 – 3)***	0 (NR)	0 (0 – 1)	0 (0 – 3)	1 (0 – 3)	0 (0 – 3)	0 (0 – 3)
IEL	0 (0 – 2)	1 (0 – 3)	0 (0 – 2)	0.5 (0 – 2)	1 (0 – 3)	1 (0 – 2)	0.5 (0 – 3)	1 (0 – 2)
Inflammatory infiltrate	1 (0 – 3)	2 (0 – 3)	1 (0 – 2)	2 (0 – 3)*	1 (0 – 3)	2 (0 – 3)*	1 (0 – 3)	2 (0 – 3)*
<b>Colon</b>	2 (0 – 6)	3 (0 – 8)*	2 (0 – 6)	2 (0 – 5)	2 (0 – 7)	4.5 (1 – 8)**	2 (0 – 7)	3 (0 – 8)*
Epithelial injury	0 (0 – 1)	0 (0 – 3)*	0 (0 – 1)	0 (NR)	0 (0 – 3)	0 (0 – 3)	0 (0 – 3)	0 (0 – 3)
Crypt dilation/distortion	0.5 (0 – 2)	1 (0 – 3)	1 (0 – 2)	0 (0 – 2)	1 (0 – 3)	1 (0 – 3)	1 (0 – 3)	1 (0 – 3)
Fibrosis/atrophy	0 (0 – 1)	0 (0 – 2)	0 (0 – 1)	0 (NR)	0 (0 – 1)	0 (0 – 2)	0 (0 – 1)	0 (0 – 2)
Inflammatory infiltrate	1 (0 – 2)	1 (0 – 3)	1 (0 – 2)	1 (0 – 2)	1 (0 – 3)	2 (0 – 3)*	1 (0 – 3)	2 (0 – 3)*
Goblet cell number	0 (0 – 2)	0 (0 – 1)	0 (0 – 1)	0 (0 – 2)	0 (0 – 1)	0 (0 – 1)	0 (0 – 1)	0 (0 – 2)
<b>Autolysis</b>	18 (90)**	15 (44)	10 (91)	8 (89)	10 (63)	5 (28)	20 (74)	13 (48)
Mild	2 (10)	7 (21)	1 (9)	1 (11)	6 (38)*	1 (6)	7 (26)	2 (7)
Moderate	5 (25)	5 (15)	3 (27)	2 (22)	3 (19)	2 (11)	6 (22)	4 (15)
Severe	11 (55)***	3 (9)	6 (55)	5 (56)	1 (6)	2 (11)	7 (26)	7 (26)

Data presented as number (No.) and percent (%) of kittens, or median (range). NR = no range. **One kitten with no diarrhea cultured aEPEC (*eae* +, *bfp* -, *stx* - *E. coli*) but *eae* was not amplified from the fecal DNA.** Because culture results are more specific for the diagnosis of EPEC, this kitten

was included in the EPEC positive group.

Proportions compared between groups by Fisher Exact test

Scores compared between groups by Mann-Whitney Rank sum test

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Supplementary Table 4. Most common gross lesions observed at autopsy in 39 kittens and corresponding light microscopic lesions observed in the same region of intestine.

Gross lesion	Histopathologic lesion	No diarrhea (n=7)	Diarrhea-associated mortality (n=32)	Total (n=39)
<b>Hyperemia (n=21)</b>		5/7 (71)	16/32 (50)	21/39 (54)
	Inflammatory infiltrate	2/5 (40)	9/16 (56)	11/21 (53)
	Expanded crypts with debris	0/5 (0)	7/16 (44)	7/21 (33)
	Villus blunting	0/5 (0)	6/16 (38)	6/21 (29)
	Congestion	0/5 (0)	6/16 (38)	6/21 (29)
	Autolysis	3/5 (60)	3/16 (19)	6/21 (29)
	No specific lesion	1/5 (20)	3/16 (19)	4/21 (19)
<b>Thickened wall (n=6)</b>		1/7 (14)	5/32 (16)	6/39 (15)
	Epithelial cell loss	0/1 (0)	3/5 (60)	3/6 (50)
	Inflammatory infiltrate	0/1 (0)	3/5 (60)	3/6 (50)
	Autolysis	1/1 (100)	1/5 (20)	2/6 (33)
	No specific lesion	0/1 (0)	1/5 (20)	1/6 (17)
<b>Dilation (n=7)</b>		0/7 (0)	7/32 (22)	7/39 (18)
	No specific lesion	0 (0)	4/7 (57)	4/7 (57)
	Epithelial cell loss	0 (0)	2/7 (29)	2/7 (29)
	Villus blunting	0 (0)	1/7 (14)	1/7 (14)
	Inflammatory infiltrate	0 (0)	1/7 (14)	1/7 (14)
	Expanded crypts with debris	0 (0)	1/7 (14)	1/7 (14)
<b>Pale/pallor (n=2)</b>		0/7 (0)	2/32 (6)	2/39 (5)
	Inflammatory infiltrate	0 (0)	1/2 (50)	1/2 (50)
	Epithelial cell loss	0 (0)	1/2 (50)	1/2 (50)
	No specific lesion	0 (0)	1/2 (50)	2/2 (100)
<b>Intussusception (n=1)</b>		0/7 (0)	1/32 (3)	1/39 (3)
	Severe coagulative necrosis of intussusceptum	0 (0)	1/1 (100)	1/1 (100)

Data represent number (No) and percent (%) of kittens. Kittens often had more than one gross and/or microscopic lesion.



Supplementary Table 5. Results of parasitological examination(s) of gastrointestinal samples obtained at the time of autopsy from 51 kittens.

Parasite identified	No. (%) of kittens							
	No diarrhea (n=20)	Diarrhea-associated mortality (n=31)	Method of parasite identification and their sensitivity					
			Gross examination	Dissection microscopy of GI content	Fecal centrifugation flotation	Fecal sedimentation	Direct microscopic examination	Histo-pathology
<b>Any parasite</b>	15/20 (75)*	13/31 (42)	5/28 (18)	26/28 (93)	26/28 (93)	21/28 (75)	5/28 (18)	7/28 (25)
<i>Toxocara cati</i>	12/20 (60)**	5/31 (16)	3/17 (18)	16/18 (89)	11/18 (61)	9/18 (50)	1/18 (6)	1/18 (6)
<i>Isospora spp.</i> #	7/20 (35)	7/31 (23)	0/14 (0)	0/14 (0)	14/14 (100)	10/14 (71)	4/14 (29)	6/14 (43)
<i>Diplydium caninum</i>	2/20 (10)	2/31 (6)	2/4 (50)	4/4 (100)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)
<i>Ancylostoma tubaeforme</i>	3/20 (15)	0/31 (0)	0/3 (0)	3/3 (100)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
<b>Other</b>	1/20 (5)	2/31 (6)	0/3 (0)	3/3 (100)	1/3 (33)	2/3 (67)	0/3 (0)	0/3 (0)
<i>Molineus barbatus</i>	1/20 (5)	1/31 (3)	0/2 (0)	2/2 (100)	1/2 (50)	2/2 (100)	0/2 (0)	0/2 (0)
<i>Aonchotheca putorii</i>	0/20 (0)	1/31 (3)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Physalopterid	0/20 (0)	1/31 (3)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
<i>Taenia taeniaformis</i>	0/20 (0)	1/31 (3)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)

Data are shown as number (%) of kittens.

\*\*P<0.01; \*P<0.05; X<sup>2</sup>

#*Isospora spp.* include: *I. rivolta* and/or *I. felis*

Supplementary Table 6. Comparison of medical records data from live and deceased kittens for factors associated with positive results of aEPEC culture and/or qPCR for *eae*.

	Live kittens		Deceased kittens		All kittens	
	aEPEC negative (n=39)	aEPEC positive (n=22)	aEPEC negative (n=27)	aEPEC positive (n=27)	aEPEC negative (n=66)	aEPEC positive (n=49)
<b>Age</b>	6.8 ± 2.1	6.3 ± 2.0	5.4 ± 1.9	6.5 ± 2.2	6.2 ± 2.1	6.4 ± 2.0
<b>Sex</b>	14 males 24 females	10 males 10 females	14 males 13 females	13 males 14 females	28 males 37 females	23 males 24 females
<b>Weight</b>	1 undocumented 529 (280 – 966)	2 undocumented 482 (227 – 1000)	1 undocumented 315 (153 – 824)	1 undocumented 347 (176 – 896)	1 undocumented 455 (153 – 966)	2 undocumented 450 (176 – 1000)
<b>Environment</b>						
Days in foster care	1 (0 – 42)	5 (0 – 28)	6 (0 – 37)	6 (0 – 21)	5 (0 – 42)	6 (0 – 28)
Weaned	36 (92)	22 (100)	20 (74)	22 (81)	56 (85)	44 (90)
Obtained from source <sup>a</sup>	30 (77)	17 (77)	17 (63)	11 (41)	47 (71)	28 (57)
Obtained from source <sup>b</sup>	9 (23)	5 (23)	10 (37)	16 (59)	19 (29)	21 (43)
Time from death to autopsy	NA	NA	4.5 (1 – 24)	4 (1.5 – 24)	NA	NA
<b>Clinical signs</b>						
Presence of diarrhea	15 (38)	13 (59)	16 (59)	18 (67)	31 (47)	31 (63)
Concurrent URD	3 (8)	2 (9)	2 (7)	3 (11)	5 (8)	5 (10)
Decreased appetite	0 (0)	3 (14)*	12 (44)	12 (44)	12 (18)	15 (31)
<b>Medications administered</b>						
Anthelmintic(s)	28 (72)	17 (77)	13 (48)	16 (59)	41 (62)	33 (67)
Anti-protozoal(s)	21 (54)	16 (73)	12 (44)	14 (52)	33 (50)	30 (61)
Antibiotic(s)	3 (8)	2 (9)	4 (15)	8 (30)	7 (11)	10 (20)
Flea/tick preventative(s)	6 (15)	5 (23)	9 (33)	10 (37)	15 (23)	15 (31)
Probiotic	10 (26)	8 (36)	4 (15)	6 (22)	14 (21)	14 (29)
<b>Other treatments</b>	3 (8)	7 (32)*	6 (22)	11 (41)	9 (14)	18 (37)**
Subcutaneous or IV fluids	3 (8)	7 (32)*	6 (22)	10 (37)	9 (14)	17 (35)*
Mirtazapine	0 (0)	0 (0)	2 (7)	7 (26)	2 (3)	7 (14)
Nutritional supplements	2 (5)	1 (5)	3 (11)	3 (11)	5 (8)	4 (8)

Data are shown as number (%) of kittens, mean ± SD, and median (range).

<sup>a</sup> County Animal Center

<sup>b</sup> County SPCA

Proportions compared between groups by  $\chi^2$  or Fisher exact test (when values < 5)

\*P < 0.05, \*\*P < 0.01

Supplementary Table 7. Detailed list of medications and treatments administered to kittens in the study.

Medication administered	Live kittens		Deceased kittens	
	No Diarrhea (n=33)	Diarrhea (n=28)	No Diarrhea (n=20)	Diarrhea-associated mortality (n=34)
<b>Anthelmintic(s)</b>	17 (52)	27 (96)	2 (10)	27 (79)
Pyrantel	10 (30)	23 (82)	2 (10)	11 (32)
Fenbendazole	6 (18)	2 (7)	0 (0)	21 (62)
Selamectin	6 (18)	5 (18)	1 (5)	9 (26)
<b>Anti-protozoal(s)</b>	11 (33)	23 (82)	1 (5)	25 (74)
Ponazuril	11 (33)	23 (82)	1 (5)	25 (74)
Sulfadimethoxine	0 (0)	1 (4)	0 (0)	1 (3)
<b>Antibiotic(s)</b>	1 (3)	4 (14)	0 (0)	14 (41)
Metronidazole	0 (0)	2 (7)	0 (0)	9 (26)
Other	1 (3)	2 (7)	0 (0)	5 (15)
<b>Flea/tick preventative</b>	6 (18)	5 (18)	1 (5)	18 (53)
Selamectin	6 (18)	5 (18)	1 (5)	9 (26)
Fipronil	0 (0)	0 (0)	0 (0)	8 (24)
Nitenpyram	0 (0)	0 (0)	0 (0)	3 (9)
<b>Probiotic<sup>a</sup></b>	2 (6)	16 (57)	0 (0)	10 (29)
<b>Other</b>	0 (0)	10 (36)	0 (0)	17 (50)
Subcutaneous or IV fluids	0 (0)	10 (36)	0 (0)	16 (47)
Vitamin supplements <sup>b-e</sup>	0 (0)	3 (11)	0 (0)	3 (9)
Mirtazapine	0 (0)	0 (0)	0 (0)	9 (26)
Chlorpromazine	0 (0)	0 (0)	0 (0)	5 (15)
Famotidine	0 (0)	0 (0)	0 (0)	4 (12)
Nutritional supplements <sup>f,g</sup>	0 (0)	0 (0)	0 (0)	4 (12)

Data shown as number and (%) of kittens

<sup>a</sup> Fortiflora, Nestlé Purina, Vevey, Switzerland

<sup>b</sup> Hi-Vite, Vetoquinol USA Inc, Fort Worth, TX 76137

<sup>c</sup> FeloVite, Vetoquinol USA Inc, Fort Worth, TX 76137

<sup>d</sup> Lixotinic, Zoetis, Parsippany, New Jersey 07054

<sup>e</sup> B-complex

<sup>f</sup> Nutri Cal, Tomlyn/Vetoquinol USA Inc, Fort Worth, TX 76137

<sup>g</sup> Glucose