

Epidemiology of Necrotizing Meningoencephalitis in Pug Dogs

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Background: Although the histopathologic features of necrotizing meningoencephalitis (NME) have been described previously, little information is available concerning the signalment, geographic distribution, seasonal onset, treatment, and survival of affected dogs.

Animals: Sixty Pugs with NME and 14 contemporaneous control Pugs with other intracranial diseases (non-NME group).

Methods: Pugs that were euthanized or died because of intracranial disease were prospectively obtained. All dogs had necropsy, histopathology, and testing for various infectious diseases and were subsequently divided into NME and non-NME groups. Signalment, geographic distribution, seasonal onset, treatment, and survival were compared between groups.

Results: In Pugs with NME, median age at onset of clinical signs was 18 months (range, 4–113 months). A greater proportion of female dogs were present in the NME group (40/60) compared with the control group (6/14). Pugs with NME had a significantly lower mean weight (7.81 kg) than control Pugs (9.79 kg) ($P = .012$). Mean survival in Pugs with NME was 93 days (range, 1–680 days), with dogs receiving any form of treatment living significantly longer than those that were not treated ($P = .003$). Anticonvulsive drugs were the only treatment significantly associated with longer survival ($P = .003$).

Conclusions and Clinical Importance: NME appears to be a common cause of intracranial signs in Pugs, based on the high proportion of NME dogs reported in this population. Pugs with NME are most commonly young adult female dogs. Although further investigation is needed to determine the optimal treatment of NME, anticonvulsive drugs appear to beneficially affect duration of survival.

Key words: Central nervous system; Encephalitis; Inflammation.

Necrotizing meningoencephalitis (NME) is a nonsuppurative inflammatory central nervous system (CNS) disease that is common in Pugs,^{1–7} with variants recognized in other breeds such as the Maltese,⁸ Chihuahua,⁹ Yorkshire Terrier,^{10–12} Pekingese,¹³ and French Bulldog.¹⁴ In Pugs, NME lesions are most common in the cerebral cortex and subcortical white matter.^{1,3} The hallmark of the disease is extensive necrosis, which varies in severity from microscopic neuronal necrosis and gliosis in the early stages of lesion evolution to gross cavitation of parenchyma in more chronic cases.^{1,3} Affected dogs have variable degrees of mononuclear inflammatory cell infiltration of the meninges and cerebral cortex.^{1,3}

The etiopathogenesis of NME in Pugs is poorly understood. Although some investigators believe that NME lesions resemble those associated with forms of viral encephalitis in other species,¹ viral involvement has yet to be demonstrated. A study of paraffin-embedded brain tissue using polymerase chain reaction (PCR) from 12 dogs with necrotizing encephalitis failed to yield DNA from herpes-, adeno-, or parvovirus.¹⁵ Several investigators have speculated that NME is either a primary

autoimmune disease or secondary autoimmune event that occurs in response to antigen exposure.^{2,15–17} Autoantibodies directed against astrocytes and glial fibrillary acid protein (GFAP) have been identified in the cerebrospinal fluid (CSF) and serum of affected dogs, which may support these hypotheses.^{17–20} Mx proteins, which are interferon-induced GTPases associated with viral and inflammatory disease, also have been demonstrated in brain tissue from Pugs with NME.²¹ Recently, a multifactorial etiopathogenesis has been advocated, with environmental influences and genetic background both hypothesized to contribute to the development of NME.^{2,15,16}

Limited data are available concerning the age, sex, and geographic background of Pugs with NME. To the authors' knowledge, information concerning treatment and survival largely has been based on small case series or clinical anecdotes. The goals of this report, therefore, were to characterize 60 Pugs with necropsy-confirmed NME to obtain data on signalment, geographic distribution of cases, seasonal onset, treatment, and survival.

Materials and Methods

Data Collection and Inclusion Criteria

Study dogs were obtained between August 2002 and February 2008 with cooperation from the Pug Dog Club of America, interested breeders, and individual Pug owners. Information concerning the study was dispersed by 1 investigator (K.G.) through electronic media and presentations at national and regional canine shows. Although Pug owners were permitted to contact the investigator, only veterinarians were allowed to determine whether a Pug met inclusion criteria. The study protocols were reviewed and approved by the animal care and use committee at Texas A&M University.

Pugs exhibiting intracranial neurologic signs that were euthanized or died because of intracranial disease were enrolled in the study by referring veterinarians contacting 1 investigator (K.G.). Enrollment was encouraged regardless of clinical diagnosis because

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the investigators wished to obtain a contemporaneous control population and avoid exclusion of cases because of antemortem misclassification. All animals were required to have necropsy and histopathology of the CNS performed to be included in the study population. Identifying information, signalment, weight, date of onset of seizures, geographic location, CSF analysis, treatment, vaccination history, and survival were obtained from participating veterinarians and entered prospectively into a dedicated database. Data were coded as present, not performed, or unavailable.

Total white blood cell (WBC) (count/ μ L), total red blood cell (RBC) (count/ μ L), and total protein (mg/dL) were noted for each dog with CSF data. Pleocytosis was defined as a cell count of >5 cells/ μ L and was classified as lymphocytic ($\geq 70\%$ of cell population), lymphocyte predominant ($\geq 50\%$ but $<70\%$ of population), large mononuclear ($\geq 70\%$ of cell population), large mononuclear predominant ($\geq 50\%$ but $<70\%$ of population), neutrophilic ($\geq 70\%$ of cell population), neutrophil predominant ($\geq 50\%$ but $<70\%$), or mixed (no cell type $\geq 50\%$ of population).²² RBC counts >0 cells/ μ L were considered increased. Treatments were categorized as antimicrobials, glucocorticoids, other immunosuppressive agents (eg, cytosine arabinoside, cyclosporine, lomustine), and anticonvulsive drugs (ACDs). For each treatment type, the specific agent or agents used were recorded. Based on available information, standard treatment for NME was considered a combination of ACDs, glucocorticoids, and other immunosuppressive agents.^{2,16} Survival was defined as the time in days between the onset of seizures and euthanasia or death because of intracranial disease. Seizure activity was selected to define disease onset in this population because it is easily recognized by laypersons and common in intracranial disorders, especially NME. In cases where seizures were not reported by clients, the 1st date a dog was examined because of intracranial neurologic signs was defined as disease onset.

Disease Confirmation

Pugs that were euthanized or died because of intracranial disease were shipped either frozen or chilled to Texas A&M University, Department of Pathobiology, for necropsy. Samples were processed routinely for histopathology. Dogs were divided into NME and non-NME groups based on the results of gross findings and histopathology. Criteria for the diagnosis of NME were based on previous reports^{1,3,4} and included gross discoloration of the cerebral cortex or subcortical white matter with loss of gray-white distinction, predominant burden in the prosencephalon, microscopic evidence of necrosis with mononuclear inflammatory cell infiltration, and absence of a demonstrable infectious etiology. Infectious disease screening was performed on brain tissue using PCR for *Ehrlichia* spp., *Borrelia* spp., *Bartonella* spp., *Toxoplasma gondii*, *Neospora caninum*, and viral etiologies (herpesvirus, adenovirus, and parvovirus). The regions of the brain (prosencephalon, cerebellum, and brainstem) with NME lesions were noted.

Dogs in the non-NME group were subclassified based on the identified etiology. Granulomatous meningoencephalomyelitis (GME) was defined by white matter predominant, nonmalacic, angiocentric lesions consisting of whorls of lymphocytes, plasma cells, large mononuclear cells, or occasional scattered neutrophils.^{3,23} Dogs diagnosed with GME had no infectious organisms isolated. Non-NME dogs with encephalitis that did not fit a particular accepted pathologic pattern and had no identifiable infectious etiology were grouped as meningoencephalitis of unknown etiology (MUE) as described previously.^{16,24–26}

Post hoc Population of Pugs with Intracranial Disease at Texas A&M University

Pugs admitted to Texas A&M University between 1992 and 2008 with intracranial localizing signs were identified to obtain prelimi-

nary information on the frequency of various diagnoses. Pugs were divided based on whether necropsy or clinical data (eg, advanced imaging of the brain, CSF analysis, infectious disease titers) were used to determine diagnosis.

Statistical Analysis

Data were summarized for cases of NME and non-NME controls by descriptive statistics. Mann-Whitney *U*-tests were used to compare medians of quantitative data, and χ^2 (or Fisher exact) tests were used to compare categorical variables between cases and non-NME controls. Within NME cases, the effect of variables on the duration of survival was assessed by the Cox proportional hazards analysis. Investigated factors included age and weight at onset of clinical signs, sex, month of onset of clinical signs, geographic location of primary residence, seasonal information, vaccination history, CSF characteristics, and administered treatments. Month of onset was categorized as winter (January-March), spring (April-June), summer (July-September), or fall (October-December). Client zip code information was used to determine the average temperature during the month of diagnosis.^a Primary location was first categorized into 7 regions based on states within the northeastern, mid-Atlantic, southeastern, midwestern, southwestern, mountain plains, and western regions. Location was subsequently dichotomized as east or west of the Mississippi River and originating from a location north of 40° latitude. Classification of client residence as urban, suburban, and rural was performed based on the reported client zip code.^b Vaccine administration at any time before onset of clinical signs was recorded as a dichotomous variable (yes/no) for each of the following: rabies, distemper combination products, leptospirosis, bordetella, Lyme disease, giardia, coronavirus, and parvovirus. Additionally, the time (in days) between most recent vaccination and onset of clinical signs was determined. Bivariable Cox proportion hazards analysis was used to evaluate each variable individually with survival time, and $P \leq .2$ was considered significant for these screening models. Vaccination history was simply evaluated as a dichotomous variable in all Cox models. Multivariable modeling of survival time included those variables that were found to be statistically significant based on the bivariable analysis as a starting point for model building. Within NME cases, survival time was compared between treatment modalities by Mann-Whitney *U*-tests. One statistical package was used for calculating mid-*P*-adjusted exact binomial confidence intervals for proportions, and χ^2 (Fisher exact) tests^c and other analyses were performed using commercially available software.^d Results were considered significant at $P \leq .05$.

Results

There were 60 Pugs with NME and 14 Pugs without NME that met inclusion criteria. All Pugs with NME had seizure activity as did 13/14 control Pugs. All dogs with NME had lesions in the prosencephalon, with 24/60 and 23/60 also having identifiable involvement of the cerebellum and brainstem, respectively. Necropsy diagnoses in the non-NME group included MUE ($n = 5$), no observed lesions (4), CNS lymphoma (2), metastatic adenocarcinoma (1), GME (1), and hemorrhagic infarction (1). The median age of NME dogs was 18 months (range, 4–113). Pugs with NME were younger than control Pugs based on mean age, but this difference was not significant (Table 1; $P = .051$). The mean body weight of Pugs with NME was significantly lower than control Pugs (9.8 kg; SD ± 2.1 ; $P = .012$). There were 10 intact female, 30 spayed female, 17 male castrated, and 3 male intact dogs with NME. Although the proportion of

Table 1. Descriptive statistics and comparison of quantitative variables between 60 Pugs diagnosed with necrotizing meningoencephalitis (NME) and 14 controls without NME from 2002 to 2008.

Variable	NME Cases		Non-NME Controls		P Value ^a
	Mean (SD)	Minimum, Median, Maximum (n)	Mean (SD)	Minimum, Median, Maximum (n)	
Age at onset (months)	27.5 (22.2)	4, 18, 113 (n=54)	52.0 (34.9)	5, 63, 100 (n=11)	.051
Weight at onset (kg)	7.81 (1.97)	3.3, 7.73, 12.3 (n=49)	9.79 (2.12)	7.4, 9.55, 14.3 (n=9)	.012
Survival after onset (days)	93.1 (138)	1, 35, 680 (n=55)	112 (173)	0, 18.5, 456 (n=10)	.548
Mean temperature month of diagnosis (°C)	13.2 (10.2)	-8.3, 15.3, 28.9 (n=46)	12.9 (9.6)	-3.3, 16.7, 23.9 (n=7)	.990
Time from most recent vaccination (days)	287 (323)	1, 161, 1,310 (n=37)	442 (545)	9, 226, 1,460 (n=8)	.550

^aBased on Mann-Whitney *U*-tests.

females in the NME group (0.67) was higher than in the control group (0.43), this difference was not significant (Table 2; $P = .10$). However, the number of female dogs with NME was significantly greater than would be expected if the true percentage was 50% in the pet population ($P = .01$).

The geographic and seasonal distribution of NME dogs was investigated. Most dogs with NME originated from the western ($n = 17$), southeastern (11), and mid-western (11) regions; dogs with NME also resided in the mountain plains (7), mid-Atlantic (4), northeastern (4), and southwestern (4) regions. The proportion of dogs in the NME group that resided in the eastern United States (0.50) was not statistically different from the proportion of dogs in the non-NME group that resided in the eastern United States (0.46). The proportion of dogs in the NME group that resided in locations north of 40° latitude (0.45) was not statistically different from the proportion of dogs in the non-NME group (0.23). The smallest proportion of dogs with NME had onset of clinical signs in the fall (October-December: 0.18), but the onset of clinical signs was not statistically different among various seasons. Seasonal onset of clinical signs was not statistically different between the non-NME and NME groups. The mean temperature at the time of disease onset was not different between the NME and non-NME groups.

Although most dogs with NME were from urban areas (72%), the proportion of NME Pugs residing in urban areas was not significantly different from that of control Pugs.

CSF analysis was performed on 14 dogs with NME. The mean CSF WBC count was 120 cells/ μ L (SD \pm 160; range, 0–540), with 12/14 dogs having CSF pleocytosis. The mean CSF RBC count was 548 cells/ μ L (SD \pm 1,558; range, 0–5,670), with 10/13 dogs having RBC counts above the reference range. The mean CSF protein concentration was 88.4 mg/dL (SD \pm 55.1; range, 25–203), with 11/14 dogs having a concentration above the reference range. The cytologic appearance of the CSF was described as lymphocytic pleocytosis ($n = 8$), mixed cell pleocytosis (2), normal (2), large mononuclear-predominant pleocytosis (1), and large mononuclear pleocytosis (1).

Vaccination history was available for 70% (42/60) of NME cases and 71% (10/14) non-NME controls. Eighty-five percent (35/41) of NME cases had a history of rabies vaccination compared with 90% (9/10) of control dogs. The percentage of NME cases vaccinated for distemper combination, leptospirosis, bordetella, Lyme disease, giardia, coronavirus, and parvovirus were 90% (37/41), 10% (4/41), 56% (23/41), 17% (7/41), 5% (2/42), 41% (17/41), and 17% (7/41), respectively. The percentage

Table 2. Descriptive statistics and comparison of categorical variables among 60 Pugs diagnosed with necrotizing meningoencephalitis (NME) and 14 controls without NME from 2002 to 2008.

Variable	NME Cases		Non-NME Controls		P Value ^a
	Proportion (n)	95% CI	Proportion (n)	95% CI	
Proportion female	0.67 (n=60)	0.541, 0.777	0.43 (n=14)	0.196, 0.689	.098
Proportion intact	0.22 (n=60)	0.126, 0.334	0.07 (n=14)	0.004, 0.305	.282
Proportion January – March	0.27 (n=55)	0.168, 0.401	0.20 (n=10)	0.035, 0.520	1.000
Proportion April – June	0.27 (n=55)	0.168, 0.401	0.40 (n=10)	0.142, 0.709	.461
Proportion July – September	0.27 (n=55)	0.168, 0.401	0.20 (n=10)	0.035, 0.520	1.000
Proportion October – December	0.18 (n=55)	0.096, 0.300	0.20 (n=10)	0.035, 0.520	1.000
Proportion from urban location	0.72 (n=50)	0.584, 0.831	0.60 (n=10)	0.291, 0.858	.468
Proportion from areas above 40° latitude	0.45 (n=60)	0.328, 0.577	0.23 (n=13)	0.062, 0.509	.145
Proportion from eastern US	0.50 (n=48)	0.373, 0.627	0.46 (n=13)	0.213, 0.726	.802
Proportion vaccinated < 1 month previously	0.11 (n=38)	0.034, 0.257	0.25 (n=8)	0.044, 0.612	.277

CI, confidence interval. Eastern United States includes all states east of the Mississippi River.

^aBased on χ^2 or Fisher exact tests.

Table 3. Descriptive statistics and comparison of survival time by treatment for 60 Pugs diagnosed with necrotizing meningoencephalitis (NME) from 2002 to 2008.

Treatment	Received Treatment		Did Not Receive Treatment		P Value ^a
	Mean (SD)	Minimum, Median, Maximum (n)	Mean (SD)	Minimum, Median, Maximum (n)	
Any treatment	100 (144)	1, 40.5, 680 (n=46)	7.4 (6.4)	3, 4, 18 (n=46)	.020
Antibiotics	95 (113)	1, 44, 384 (n=22)	91 (164)	1, 20, 680 (n=27)	.310
Corticosteroid therapy	97 (123)	1, 49, 497 (n=28)	88 (162)	1, 14, 680 (n=22)	.171
Other immunosuppressive therapy	177 (176)	7, 124, 497 (n=6)	82 (133)	1, 21.5, 680 (n=44)	.061
Anticonvulsive therapy	113 (154)	1, 44, 680 (n=38)	19 (24)	3, 6, 80 (n=11)	.016
Standard therapy	186 (195)	7, 121, 497 (n=5)	83 (132)	1, 23, 680 (n=45)	.123

^aBased on Mann-Whitney *U*-tests. Standard therapy, treatment with corticosteroids, other immunosuppressives, and anticonvulsives.

of non-NME controls vaccinated for distemper combination, leptospirosis, bordetella, Lyme disease, giardia, coronavirus, and parvovirus were 80% (8/10), 20% (2/10), 40% (4/10), 10% (1/10), 0% (0/10), 40% (4/10), and 30% (3/10), respectively.

The mean survival of dogs in the NME group was 93 days (SD \pm 138; median, 35; range, 1–680 days), whereas dogs in the non-NME group had a mean survival of 112 days (SD \pm 173; median, 18.5; range, 0–456 days). Survival time between groups was not statistically different ($P = .548$). Treatment for NME was attempted in 46/60 dogs and not performed in 6/60 dogs; treatment data were not available for 8/60 Pugs with NME. Data related to the effect on treatment on survival are presented in Table 3. The mean survival time in dogs with NME receiving treatment was 100.5 days (SD \pm 144; median, 40.5; range, 1–680 days), whereas the mean survival in dogs not receiving treatment was 7.4 days (SD \pm 6.4; median, 4.0; range, 3–18 days). ACDs were the most common treatment, and 38/60 Pugs with NME received these medications (n = 34 phenobarbital, 19 diazepam, 2 potassium bromide, 1 levetiracetam). The mean survival in Pugs with NME receiving any ACD was 113 days (SD \pm 154; median, 44; range, 1–680 days). Corticosteroids were administered to 28/60 dogs with NME, and the mean survival time in this population was 97 days (SD \pm 123; median, 49; range, 1–497 days). Eighteen dogs with NME received prednisone alone, 5 dexamethasone alone, and 5 a combination of prednisone and dexamethasone. Antibiotics were administered to 22/60 dogs with NME, and the mean survival time in this group was 95 days (SD \pm 113; median, 44; range, 1–384 days). Other immunosuppressive drugs were given to 6/60 dogs (n = 3 cytosine arabinoside, 1 lomustine, 1 cyclosporine, 1 cytosine arabinoside and cyclosporine), with a mean survival time of 177 days (SD \pm 176; median, 124; range, 7–497 days).

Bivariable Cox proportional hazards regression was performed to examine factors associated with survival in Pugs with NME. Dogs with disease onset in September-December ($P = .048$) had significantly higher rates of death compared with those in other seasonal categories (Table 4). Treatment ($P = .003$), and the administration of ACDs ($P = .003$) resulted in significantly longer survival than no treatment and no ACD administration, respectively. A multivariable model could not be fitted to these data because of small sample size and colinearity between predictor variables.

Analysis of the post hoc population from Texas A&M University yielded 6 necropsy-confirmed cases of intracranial disease (4 NME, 1 histoplasmosis, 1 normal necropsy) and 10 presumptive cases of intracranial disease (7 NME, 2 hydrocephalus, 1 unknown). Only 1 dog from the necropsy-confirmed group was included in the prospective study population. The proportion of Pugs from Texas A&M University with intracranial disease diagnosed with NME was 11/16.

Discussion

Since the 1980s, NME has become an increasingly well-recognized cause of encephalopathy in Pugs. Although the histopathologic features of this disease have been characterized, critical epidemiologic information remains unreported.^{1,2,16} The aim of this prospective case study was to search for commonalities in signalment, geographic distribution, seasonal onset of clinical

Table 4. Bivariable Cox proportional hazards regression for the prediction of survival time in 60 Pugs diagnosed with necrotizing meningoencephalitis from 2002 to 2008.

Variable	Parameter Estimate (β)	P-Value (Wald)	Hazard Ratio (95% CI)
Female	0.397	0.180	1.49 (0.83, 2.66)
Onset during September - December	0.744	0.048	2.11 (1.01, 4.41)
MRI performed	-0.563	0.101	0.57 (0.29, 1.12)
Received any treatment	-1.532	0.003	0.22 (0.08, 0.60)
Received anticonvulsive treatment	-1.113	0.003	0.33 (0.16, 0.69)
Received other immunosuppressives	-0.602	0.173	0.55 (0.23, 1.30)
Received standard therapy	-0.597	0.212	0.55 (0.22, 1.41)
History of leptospirosis vaccination	-0.707	0.193	0.49 (0.17, 1.43)
History of borreliosis vaccination	-0.731	0.108	0.48 (0.20, 1.18)
History of giardia vaccination	-1.868	0.069	0.15 (0.02, 1.15)
History of parvovirus vaccination	0.855	0.053	2.35 (0.99, 5.59)

signs, treatments, and outcomes in a population of 60 Pugs with histopathologically confirmed NME.

Based on data from this report, NME appears to be a very common cause of intracranial neurologic signs in Pugs. Pugs with NME represented 60 of 74 (81%) dogs in our population, which was constructed using inclusion criteria that encouraged the submission of Pugs that were euthanized or died because of intracranial disease, regardless of clinical diagnosis. A post hoc population of Pugs obtained from Texas A&M University admissions with confirmed or clinically diagnosed intracranial disease indicated NME was present in 69% (11/16) of identified dogs. Although the authors believe these data suggest a very high proportion of Pugs that are euthanized or die because of intracranial disease have NME, incidence may be different in Pugs that survive despite neurologic impairment.

The mean age at onset of Pugs with NME described here was 27.5 months (range, 4–113). This finding is consistent with Cordy and Holliday's original report of 17 Pugs with NME that had a mean age of onset of 29 months.¹ Pugs with NME had a lower mean age than control Pugs without NME (mean, 52 months \pm 34.9), although the difference was not significant ($P = .051$). This observation may suggest that age does not discriminate NME from other intracranial diseases in Pugs. However, the non-NME control Pugs may not be representative of Pugs with all intracranial disorders because of the method in which cases were collected or because the sample size may have been too small to identify a significant difference between groups. The age of Pugs with NME, for instance, appears to be younger than that which typically is reported for granulomatous meningoencephalitis (GME), another common noninfectious inflammatory disease of the CNS. In a study of 42 cases of histopathologically confirmed GME, the mean age of affected dogs was 55 months (range, 6–144 months).²⁷ Age ranges from 3 smaller studies on GME were <1 year–13 years,²⁸ 4–10 years,²⁹ and 3–8 years.³⁰

Pugs with NME more frequently were female (40/60) than were controls (6/14), but this difference was not significant ($P = .10$). The proportion of females in the NME group, however, was significantly higher than a null assumption of a 50:50 male to female population ($P = .01$). The high proportion of females in the NME group is interesting, because autoimmune encephalopathies seem to be more common in females across several species. Several studies have shown that females are affected more commonly with GME than are males, with 1 large report indicating that 68% of cases were intact or spayed female dogs.^{23,27} Females also are predisposed to developing multiple sclerosis (MS) and experimental allergic encephalitis (EAE), which are autoimmune demyelinating diseases of humans and rodents, respectively.^{31–33} The mechanisms for female overrepresentation in EAE and MS are unclear, but may relate to sex steroid-associated alterations in T-helper 1 cytokines, suppression of T-regulatory cells, X-chromosome susceptibility alleles, or other factors.^{31–33} Interestingly, local dysregulation of T-cell behavior also is believed to be important in the development of GME.³⁴ Although the lesion distribution

and histopathology of NME are distinct from GME, MS, and EAE, the similar sex bias associated with all 4 diseases may indicate a degree of overlap in etiopathogenesis. The high proportion of female Pugs in the NME group lends further support to the hypothesis that immune system dysfunction plays a role in lesion development.

Compared with control Pugs, those with NME had a significantly lower body weight. Low body weight in Pugs with NME, like the high proportion of females in this group, may support underlying immune dysregulation because cytokines associated with various autoimmune diseases can result in loss of lean muscle mass and cachexia.^{35,36} Alternatively, intracranial lesion burden may differ between Pugs with NME versus controls, leading to a direct effect on dietary intake. Injuries to the diencephalon, for example, have been shown to impact appetite.³⁷

The seasonal onset and geographic distribution of NME have not been investigated previously. Although both seasonal and geographic information is available for some forms of infectious encephalitis in dogs, such as rabies, to the authors' knowledge, these data have not been obtained for other idiopathic inflammatory CNS diseases such as steroid-responsive meningitis (SRMA), GME, and various tremor syndromes.³⁸ Although there was no significant difference in the proportion of dogs with onset of NME during each season or the average outdoor temperature at onset between NME dogs and controls, the population investigated in this study was relatively small. Immune-mediated encephalitides in other species may have a seasonal bias in onset. In MS, for example, exacerbations are common during certain seasons because of weather-associated alterations in immunocompetence or monthly variations in exposure to viruses that may trigger immune-mediated inflammation.^{39,40}

Geographic location also was evaluated for its potential to contribute to the development of NME. Pugs with NME most commonly were from the western ($n = 17$), southeastern (11), and midwestern (11) regions, with the fewest dogs originating from the mid-Atlantic (4), northeastern (4), and southwestern (4) regions. When geographic case distribution was dichotomized into the eastern and western United States and above and below 40° latitude, no significant differences between groups were identified. Although the lack of geographic influence in case distribution may be genuine, the small number of NME cases in this report and the means of case recruitment necessitate cautious interpretation of results. The incidence of immune-mediated encephalopathies in other species, including MS, may vary based on the geographic latitudes where patients live.⁴¹ Although a high proportion of Pugs with NME resided in urbanized areas (72%), this was not significantly different than the proportion of control dogs from urbanized zones (60%). The high proportion of both cases and controls that resided in urbanized areas may simply be reflective of the number of Pugs in these regions, differences in client values between rural and urbanized areas, or a difference in environmental factors that influence the development of intracranial disease in general.

Vaccination status was examined in NME and non-NME groups, because antigen exposure has been speculated to play a role in the development of various encephalitides, including NME.^{1,2,16} The proportion of dogs vaccinated within 1 month of disease onset and the duration between vaccination and onset were not significantly different between groups. Furthermore, only a small proportion of NME dogs (0.11) were vaccinated within 1 month of onset. Pugs with NME and a history of parvovirus vaccination had a higher death rate (hazard ratio = 2.35; Table 4), but this result was not significant ($P = .053$). If parvovirus vaccination is involved in the pathogenesis of NME, mechanisms are most likely immune mediated because dogs in this report had no identifiable parvovirus in brain tissue as assessed by PCR. In humans, vaccination has been associated with various immune-mediated encephalopathies.⁴²⁻⁴⁴ Measles virus vaccine, for example, has been speculated to predispose individuals to MS, whereas mumps vaccine has been associated with a form of postvaccinal aseptic meningitis.^{42,43} Despite these associations, the risk of autoimmune encephalitis after vaccination is believed to be exceedingly low in humans, in some cases on the order of 1 case per 1,000,000 vaccinations.⁴² The lack of clear association between vaccination and NME in this report is therefore not unexpected, especially considering the small sample size.

Data from CSF analysis were available from 14/60 Pugs with NME. The mean CSF WBC cell count was 120 cells/ μL (range, 0–540), with 12/14 dogs having CSF pleocytosis. Most dogs (8/14) had lymphocytic pleocytosis, with the remainder having mixed cell or large mononuclear pleocytosis. These data are similar to what has been described in 12 Pugs with NME, where pleocytosis was detected in all dogs (mean, 374 cells/ μL ; range, 71–630) and inflammation was predominantly lymphocytic.¹ The CSF results in this report also resembled what has been reported in Yorkshire Terriers and Maltese with NME. In 5 Yorkshire Terriers with NME, pleocytosis was detected in 4 dogs (range, 12–76 cells/ μL) and characterized as lymphocytic or large mononuclear.¹¹ In a population of 3 Maltese dogs with NME, CSF WBC counts ranged from 50 to 247 cells/ μL . Results of CSF analysis from other causes of noninfectious meningoencephalomyelitis in dogs, such as GME and SRMA, may be somewhat different than NME in Pugs. In 1 report, mean cisternal CSF WBC count from 17 dogs with GME was 800 cells/ μL (range, 9–5,400); most dogs had lymphocytic or large mononuclear pleocytosis.³⁰ In another study, 16/16 dogs with GME had pleocytosis, with 8 having WBC counts ranging from 15 to 80 cells/ μL , 5 having WBC counts ranging from 81 to 1,000 cells/ μL , and 3 having WBC counts >1,000 cells/ μL .⁴⁵ Dogs with SRMA typically have neutrophilic pleocytosis, with many animals having >1,000 cells/ μL .⁴⁵ Although CSF results cannot definitively distinguish NME from other disorders, marked pleocytosis and a high proportion of neutrophils are not characteristic. The lack of marked CSF pleocytosis in some Pugs with NME and absence of CSF pleocytosis in 2/14 Pugs with NME are interesting, especially considering

data that suggest relatively consistent involvement of the leptomeninges.^{1,3}

The mean survival for Pugs with NME was 93 days (range, 1–680 days), with dogs that received any form of treatment having significantly longer mean survival times (101 days) than those that received no treatment (7.4 days). Because only Pugs with necropsy-confirmed NME were included in our analysis, survival data may be biased toward Pugs with more severe disease and therefore falsely shortened. When treatments were divided into antimicrobials, ACDs, corticosteroids, and immunosuppressive drugs, only the administration of an ACD was significantly associated with prolonged survival. The administration of ACDs may be directly neuroprotective and enhance owner perceptions of quality of life. Prolonged seizure activity induces neuronal apoptosis via the activation of both extrinsic (eg, cell-surface receptors) and intrinsic (eg, organelle) triggers.⁴⁶ Seizures also may damage neurons through glutamate-mediated excitotoxicity and the induction of proinflammatory cytokines.^{47,48} Although providing ACDs may help prevent development of these lesions, decreasing seizure frequency also will improve perceived quality of life and hence survival. In a recent report on idiopathic epilepsy, inadequate seizure control was recognized as a reason for decreased quality of life in 5/12 dogs.⁴⁹ The inclusion of dogs that did not receive treatments in comparison groups may have skewed results toward certain treatments having a favorable effect on survival. If dogs were not treated because euthanasia was deemed most appropriate, then short survival in this group may have confounded the comparison because untreated dogs had a worse prognosis (ie, confounding by indication to treat).

Given the relatively small number of dogs receiving treatments ($n = 46$) and the method of data acquisition, the lack of effect of certain interventions must be interpreted cautiously. Corticosteroids and other immunosuppressive drugs seem to exert a beneficial effect on survival in dogs with other forms of encephalitis that may have an immune-mediated basis, such as GME and SRMA.^{23,24,26,28,50,51} Although the mean survival in 6 Pugs with NME in this report receiving other immunosuppressive drugs was 177 days (range, 7–497 days), this survival time was not significantly different from the population of Pugs that did not receive immunosuppressive therapy ($P = .061$).

Based on our data, NME appears to be a common cause of intracranial signs in Pugs. Pugs with NME seem to be predominantly young adult, female dogs. Although NME lesions were most common in the prosencephalon (60/60 dogs) as has been previously reported,¹ a high proportion of Pugs also had involvement of the brainstem (24/59) and cerebellum (23/58). There was no obvious seasonal or geographic distribution, although most cases identified were from the western, midwestern, and southeastern regions of the United States. There was no apparent relationship between vaccination and NME, although data concerning hazard ratio and parvovirus vaccination suggest the need for additional investigations. CSF analysis typically identifies a lymphocytic pleocytosis (mean WBC count 120 cells/ μL ; range,

0–540). Mean survival time in this population of necropsy-confirmed cases was 93 days.

Footnotes

^a <http://www.weatherreports.com>

^b <http://depts.washington.edu/uwruca/>

^c Epi Info, version 6.04, CDC, Atlanta, GA

^d SPSS version 15.0, SPSS Inc, Chicago, IL

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