TITLE: ASSOCIATION OF VACCINATION AND MENINGOENCEPHALITIS OF UNKNOWN ORIGIN

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Abstract

Background

Meningoencephalitis of unknown origin (MUO) is an umbrella term used to refer to different types of non-infectious meningoencephalitis. The etiology remains unknown. The clinical response to immunosuppressive therapy has supported a dysregulation of the immunologic response as one of the possible main factors inducing this disease.

Hypothesis/objectives

The aim of this study was to assess the risk of developing MUO after vaccination.

Animals

Fifty-six canine patients from Northwest Veterinary Specialists and University of Tennessee were included in this retrospective study.

Material and Methods

A retrospective study of medical records of dogs with clinical history, neurological examination, imaging and cerebrospinal fluid analysis findings compatible with MUO was performed. The association between diagnosis of MUO and vaccination was assessed via the Kolmogorov-Smirnov one sample test and the Clopper-Pearson confidence interval for proportions; when 16 months of vaccination history was available, a case-crossover analysis was performed assessing the risk of developing MUO within 60 days, as well as in the third- and fourth-months post-vaccination.

Results

Both statistical approaches failed to prove vaccination as a trigger for developing MUO.

Conclusions and clinical importance

There is no evidence of association between vaccination and MUO. These results support the findings already reported in other suspected immune-mediated diseases in dogs and humans. To the authors’ knowledge this is the first study assessing vaccination as a potential trigger for MUO in dogs.

# Introduction

Meningoencephalitis of unknown origin (MUO) is a wide term which include different types of non-infectious inflammatory CNS disease. These group include steroid responsive meningitis (SRMA), and eosinophilic meningoencephalitis (EME), granulomatous meningoencephalitis (GME) and necrotizing encephalitis (NE) that includes necrotizing leucoencephalitis (NLE) and necrotizing meningoencephalomyelitis (NME)(1) .Although all of these diseases are included under the name of menongoencephalitis of unknown origin, SRMA and EME are normally treated as separated entities as they both have distinctive clinical characteristics. The rest of the diseases require histopathology for definitive diagnosis and differentiation. (1-3)

The etiology of canine MUO remains unclear. Hypotheses include triggering of an excessive immunologic response, genetic predisposition, environmental factors and exposure to infectious agents. (4) The theory behind an excessive immunologic response has been supported by the response of non-infectious meningoencephalitides to immunosuppressive or immunomodulatory therapies. (5)

Experimental allergic encephalomyelitis (EAE) has been induced in rodents by immunization with adjuvanted myelin antigens by stimulating T cell-mediated immunity to myelin antigens. In Moon´s Study 58% of dogs developed EAE after receiving a subcutaneous injection of canine brain homogenate.(6,7) Under ideal conditions vaccination should be able to trigger both innate and adaptative immune system responses.(8)It has been hypothesized that vaccination may trigger a non-desirable immunoreactivity by diverse molecular mechanisms that could potentially lead to autoimmune activation.(9,10) The development of immune-mediated disease has been associated with a dysregulation and dysfunction of the adaptive and innate systems. The activation of the immune system outside the central nervous system, will trigger a neuroinflammatory response that can still affect the central nervous system despite the presence of the blood-brain barrier which will be disrupted secondary to the cellular damage produced. Therefore, the neuroinflammatory response will affect the cellular components that are normally protected by the blood-brain barrier. (2,11-14)

The aim of this study was to further investigate if a temporal correlation could be found between vaccination and the development of MUO.

# Material and Methods

The medical records of all dogs that were diagnosed with suspected MUO at North West Veterinary Specialists from August 2015 to December 2019 and from University of Tennessee from February 2002 to January 2009 were reviewed. Cases were selected based on inclusion criteria for the diagnosis of canine MUO suggested by Granger et al. in order to standardize the cases selected and trying to achieve a diagnosis with confidence (1).Specifically, dogs were included if they were >6 months old, had a neurological examination compatible intracranial lesions (either single, multifocal or diffuse) this lesions needed to be confirmed by magnetic resonance imaging (MRI). All the cases included had cerebrospinal fluid (CSF) analyses consistent with inflammation meaning increased protein count more than 27mg/ml for atlantooccipital sampling and more than 35mg/ml for lumbar sampling.(Reference ranges stablished by the laboratory). Increased white blood cell count: more than 6 num/µL irrespectively from sampling site. and/or pleocytosis suggestive of inflammation on cytological examination performed by a specialist in clinical pathology). All the cases included on the study should have available vaccination data up to two years prior to developing MUO

All dogs underwent MRI using a 0.4 T magnet (Hitachi APERTO Grande, Hitachi Medical Systems, Steinhausen, Switzerland), a 1T magnet (Siemens MAGNETOM Harmony, Siemens Medical Solutions, Malvern, USA) or a 1,5T magnet (Siemens MAGNETOM Symphony, Siemens Medical solutions, Malvern ,USA) and the images were reviewed by a specialist in veterinary diagnostic imaging. MR sequences varied, but always included dorsal, sagittal and transverse T2-weighted images, transverse T2-fluid attenuated inversion recovery and T1-weighted images before and after paramagnetic contrast injection of gadobutrol (Gadovist, 1.0 mmol/ml solution, Bayer AG, Germany).

Exclusion criteria were dogs showing clinical signs compatible with only optic neuritis. Dogs with clinical signs, MRI findings and CSF analysis suggestive of meningomyelitis (rather than meningoencephalitis) ,eosinophilic meningoencephalitis as these entity has been suspected to have a different physiopathology. Dogs with MRI studies that did not display characteristic lesions (T2-weighted hyperintensity with single or multifocal lesions with or without meningeal enhancement)(4) and finally all cases for which data regarding vaccination history were unavailable.

Fifty-six dogs with suspected diagnosis of MUO were originally included and the cases were distributed based on the vaccination availability in two groups to approach the hypothesis using two different statistical methods. The diseases against being immunized had not been taken in consideration. The only consideration was the date of the vaccination.

We identified all dogs that had been vaccinated within the 365 days antecedent to diagnosis. Dogs were grouped by time from vaccination to diagnosis into 2-month groups (under the hypothesis that immune triggering likely occurs mostly within the immediate 2-month window following vaccination). All 2-month windows were then compared using two approaches: First, a Kolmogorov-Smirnov one sample test which is a “goodness of fit” test for a set of events against a theoretical distribution. In this case (under the null hypothesis that vaccination is causally unrelated to the development of MUO) each 2-month window should have a similar proportion of diagnoses.Therefore, each 2-month window would account for approximately 16.7% of the cases. We used an online calculator to perform this analysis (<http://vassarstats.net/ksm.html>, accessed on July 21, 2020).

We then examined each 2-month window independently using the Clopper-Pearson exact confidence interval for a proportion. In this analysis, we examined the number of cases vaccinated in a 2-month window against the entire study population to create a proportion, and compared each one independently against a theoretical proportion of 0.167. Both 95% and 99.17% confidence intervals were evaluated, the latter to account for increased familywise error rate. As an exact method, Clopper-Pearson interval calculation is preferred to asymptotic estimation methods when using small sample sizes, and produces relatively conservative estimates. A confidence interval that does not include the proportion expected under the null hypothesis (i.e., 0.167) is analogous to a statistically significant difference from the expected proportion at the specified alpha level (0.05 for a 95% confidence interval, 0.0083 for a 99.17% confidence interval). This analysis was performed using commercially available statistical software (SAS 9.4) (15)

Next, a case-crossover analysis was performed, as previously described. (16) This type of analysis allows estimation of risk when a brief exposure causes a transient increase in the risk of a rare acute-onset outcome. The method compares the hazard of a dog having been vaccinated in a defined window of time leading up to the diagnosis of MUO to the hazard of having been vaccinated in a historical window of time when the patient did not develop disease. The comparison window was defined as one year prior to the case window (i.e., the window leading up to the diagnosis of MUO). We examined a subset of our sample population, with sufficient vaccination history data, to determine the risk of developing MUO within a window of 30, 60, 90 and 120 days after vaccination. (figure 1). The case-crossover analysis was performed as a stratified Cox proportional hazard regression with ties handled by Breslow’s method in SAS 9.4 (SAS Institute, Cary, NC). Because each dog serves as its own control, consideration of most covariates (sex, breed, etc.) is obviated.

There was no need of ethical approval as it is a study of retrospective nature.

# Results

Forty dogs met the inclusion criteria of vaccination within the year preceding the diagnosis of MUO. The population included 17 neutered males, 11 spayed females, 5 intact females and 7 intact males (40 per cent female and 60 per cent male) aged between 6 months and 13 years (median age 3.9 years).

The dog breeds included six Chihuahuas, three crossbreed dogs, two Jack Russell terriers, four French bulldogs, four pugs, four West Highland white terriers, three shih tzu, three Pomeranians, one of each of the following breeds: bichon frisé, cocker spaniel, miniature poodle, Shetland sheepdog and Staffordshire bull terrier.

In general, the diseases being immunized against included canine adenovirus type 1 and type 2, canine distemper, canine parvovirus, canine parainfluenza, *Leptospira interrogans* and *Leptospira kirschneri*, and *Bordetella bronchiseptica.* The most recent vaccination record was used, in some cases were multivalent vaccine, in others were monovalent.The disease being immunized was not taken in consideration

All but three dogs in the study showed mixed or lymphocytic pleocytosis on CSF with absence of infectious agents on cytologic CSF examination. One dog showed haemic contamination of the CSF and was receiving immunosuppressive therapy at presentation; one dog had severe blood contamination of the CSF; and one dog showed a neutrophilic pleocytosis with absence of infectious agents. These three dogs had neurological signs and imaging findings compatible with MUO, and all improved with immunosuppressive therapy. The dog showing neutrophilic pleocytosis on CSF analysis tested positive for canine distemper virus on serology but was negative by PCR on blood; he had been vaccinated 4 months prior to the diagnosis of MUO, and responded positively to corticosteroid therapy. Five months after starting corticosteroid therapy at immunosuppressive regime, this dog showed clinical improvement and a repeat MRI exam revealed improvement of the intra-axial lesions.

Only twenty-eight dogs (64%) were tested for both *Toxoplasma gondii* and *Neospora caninum* either on serology (12/40, 30%) or CSF-PCR (16/40, 40%) and all of them were negative. The remaining 12 dogs (30%) were not tested for infectious diseases but only underwent routine CSF analysis suggestive of inflammatory disease (cytology and protein count). Of these 12 dogs, 10 improved on immunosuppressive therapy and 2 were euthanized within 24-48 hours because of marked progressive neurological deterioration. This two dogs were not excluded because the history, the CSF results and the MRI findings were suggestive of MUO and in between 33-56% of the cases they deteriorate despite immunosuppressive therapy (15,16). Finally, in 7/40 dogs (17.5%) PCR for canine distemper virus was negative.

We failed to observe a difference between observed and expected proportions using the Kolmogorov-Smirnov one-sample test (p=0.215). Specifically, the most proximate 2-month window did not have a higher-than-expected proportion of cases (P0=0.125). When examining each 2-month window individually via confidence intervals, the period from 60 to 120 days prior to diagnosis had a higher proportion of cases than expected (0.35 [95% CI 0.206-0.517, 99.17% CI 0.168-0.570]; expected proportion under the null hypothesis 0.167,). The interval from 0 to 60 days did not differ from the expected proportion (0.125 [95% CI 0.042-0.268, 99.17% CI 0.027-0.320] vs 0.167). (Table 1)

Forty-four dogs with suspected MUO were included in the case-crossover analysis (vaccination history longer than 16 months from date of diagnosis of MUO). The population included 17 neutered males, 15 spayed females, 5 intact females and 7 intact males (46% female and 54% male), with a median age of 4.33 years.

The dog breeds included eight Chihuahuas, five crossbreed dogs, four Jack Russell terriers, four French bulldogs, four pugs, three West Highland white terriers, three shih tzus, two Pomeranians, two bichon frisé, two cocker spaniels, one of each of the following breeds: Border terrier, great Dane, miniature poodle, papillon, Shetland sheepdog, Border collie and dachshund. All but the same three dogs noted previously in the study showed mixed or lymphocytic pleocytosis with absence of infectious agents on cytologic CSF examination.

Only Twenty-nine dogs (66%) were tested for for *T. gondii* and *N. caninum* either on serology (10/44, 23%) or CSF-PCR (19/44, 43%)and all tested negative. Of the remaining 15 dogs (34%), 12 dogs (27%) were not tested for infectious diseases but only underwent routine CSF analysis. Of these 12 dogs, 10 dogs improved on immunosuppressive therapy and 2 were euthanized within 24-48 hours because of marked progressive neurological deterioration. The remaining 3 dogs improved on immunosuppressive therapy and 2 of these dogs tested negative for canine distemper virusand 1 tested negative for *Cryptococcus neoformans* by CSF-PCR. Finally, 6/44 dogs (14%) were tested for PCR for canine distemper virus and the PCR was negative in all of them.

The case-crossover analysis assessing the hazard of developing MUO following vaccination failed to identify a link between vaccination and the onset of MUO in all time windows evaluated. Initially only the 0-60- and 61-120-day periods were assessed, but based on the findings of the Clopper-Pearson confidence interval analysis, the 61-90- and 91-120-day windows were subsequently assessed. Hazard ratios less than 1 in this analysis were indicative of increased hazard of MUO in the post-vaccinal period; hazard ratios ranged from 0.29 to 0.50, with associated p-values ranging from 0.1182 to 0.5715 (Table 2).

# Discussion

Results of this study found little evidence to corroborate that recent vaccination was associated with a subsequent diagnosis of MUO in dogs. Multiple statistical methods failed to identify a strong association with vaccination. While the analysis based on confidence intervals for proportions suggested an increased proportion of MUO diagnoses for dogs vaccinated 60 to 120 days prior to diagnosis, the case-crossover analysis and Kolmogorov-Smirnov one sample test found no significant evidence of an association. The case-crossover analysis, due to both enhanced statistical efficiency and inherent control of most potential covariates, stands as perhaps the most robust analysis of these data and suggests that the association noted via the Clopper-Pearson method may be subject to unmeasured confounding.

Several studies have assessed vaccination as a potential trigger of immune-mediated diseases, such as immune-mediated hemolytic anemia in dogs.(16,19,23) .The latest American College of Veterinary Internal Medicine consensus statement indicates that there is insufficient evidence to conclude that vaccination is implicated in the development of immune-mediated hemolytic anemia.(18)Jeffry et al evaluated constituents of the faecal microbiota and brain disease and found that one fourth of dogs with MUO were been vaccinated within the month prior to having developed clinical signs, compared  to none in the control group.(19) Barnes et al evaluated also the vaccination as a potential trigger for MUO in 24 dogs and could not find any statical difference with the control cases (20)

Similarly, little evidence exists in humans that vaccination routinely triggers immune-mediated disorders such as Guillain-Barré Syndrome.(26,28) Several human studies concluded that there is not enough evidence correlating vaccination and the development of autoimmune disease. (25-39)

Based on the observations of Duval and Giger as well as Jeffery et al., we hypothesized that vaccination would trigger MUO within the first 60 days. No such association was identified in the current study, although this could be due to the relatively small sample size. It is notable that no dogs developed MUO within 15 days of vaccination, and only 2 developed disease within the first month. However, we found that a higher-than-expected proportion of dogs was vaccinated 60-120 days prior to the diagnosis of MUO, a finding that held true when corrected for multiple testing. As previously noted, this is likely a result of unmeasured confounding. Other possibilities include: an effect of the small sample size: as the relatively small number of cases (n=40) that developed MUO within 365 days of vaccination could result in a “false positive” observation, rather than a true association, or either the possibility that vaccination may occasionally trigger MUO between 2- and 4-months post-vaccination. While the latter is considered less likely in light of the case-crossover findings, a larger retrospective study or cohort study could provide clarity.

All dogs in this study that underwent serological testing for *T. gondii* and *N. caninum* tested negative. This is consistent with the observations of a recent study performed in the United Kingdom that found a very low prevalence of active *T. gondii* and *N. caninum* (0.25 and 2.25% respectively) and little evidence for antigenic exposure for these diseases in dogs (4 and 7% respectively)(1-3,17,40).

Because of the retrospective nature of our study, and the fact that most dogs improved with immunosuppressive therapy, we could not confirm the diagnosis of MUO histopathologically. However, most studies of MUO rely on characteristic imaging abnormalities, cerebrospinal fluid tap, signalment, and response to immunosuppressive therapy for a presumptive diagnosis, considering the major limitations to achieving a definitive diagnosis by brain biopsy. Although we consider this to be a major limitation, we are reasonably confident that the diagnoses were as accurate as those of prior studies utilizing Granger´s guidelines.

The current study had no control group (unvaccinated dogs that developed MUO), so a case-control study could not be performed. However, few client-owned dogs in the UK or USA have never received any vaccinations, so such studies might be both ethically and logistically difficult, if not impossible, to perform. The case-crossover analysis provides a practical alternative in which each dog serves as its own control, thereby minimizing confounding and bias while simultaneously increasing statistical efficiency without the need to recruit additional control subjects. This inherent control of confounding encompasses all time-invariant variables like breed and sex, precluding the need to include them in the model.

In conclusion, we found little evidence for association of vaccination immediately prior to a diagnosis of MUO in dogs.

# 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*.

# Author Contributions

*MR, PCC, and LG contributed to conception and design of the study. LG, LM, WT and LVZ contributed to collect the cases and LVZ organized the database. MR and PCC performed the statistical analysis. LVZ wrote the first draft of the manuscript. LVZ, LM, MR, PCC, and LG wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version*

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## Supplementary Figures

# Figure 1: Flow chart showing case selection

## Supplementary Tables

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Period | Observed frequency | Observed proportion | 95% CI of proportion | 99.17% CI of proportion | Expected proportion |
| <61 days | 5 | 0.125 | 0.042-0.268 | 0.027-0.320 | 0.167 |
| 61-120 days | 14 | 0.350 | **0.206-0.517** | **0.168-0.570** | 0.167 |
| 121-180 days | 9 | 0.230 | 0.108-0.385 | 0.081-0.439 | 0.167 |
| 181-240 days | 7 | 0.175 | 0.073-0.328 | 0.052-0.381 | 0.167 |
| 241-300 days | 5 | 0.125 | 0.042-0.268 | 0.027-0.320 | 0.167 |
| 301-365 days | 0 | 0.000 | **0.000-0.088** | **0.000-0.128** | 0.167 |

**Table 1:** Clopper-Pearson confidence intervals of a single proportion for each 2-month period

|  |  |  |  |
| --- | --- | --- | --- |
| Period | Hazard ratio | 95% CI | P value |
| 0-60 days | 0.50 | 0.045-5.514 | 0.5715 |
| 61-90 days | 0.50 | 0.09-2.73 | 0.4236 |
| 91-120 days | 0.40 | 0.08-2.06 | 0.2734 |
| 61-120 days | 0.29 | 0.06-1.38 | 0.1182 |

**Table 2.** Summary of the Case-crossover analysis

# Data Availability Statement

The datasets [GENERATED/ANALYZED] for this study can be provided upon request.