Supplementary Information 5: Evidence Narratives for Additional Canine Infections

Vector-borne agents

Bartonella spp.

One cross-sectional study describing the clinicopathologic abnormalities in 24 dogs seropositive to *Bartonella vinsonii ssp. berkhoffi* was identified. Three of 4 anemic dogs tested were Coombs'-positive,[1] yielding an IME value of 3.81.

Consensus Summary Statement: Based upon this single study, the overall evidence that *Bartonella spp.* induce IMHA is low. As the range of pathogenesis for this organism is still emerging, further prospective, controlled studies are needed to address this question.

Borrelia spp.

One retrospective study described 1 dog with IMHA that was seropositive for *B. burgdorferi, A. phagocytophilium,* and *R. rickettsii*.[2] Owing to the co-exposure status, evidence for *B. burgdorferi* as a cause of IMHA could not be evaluated. In a separate study, a Coombs'-positive anemia was documented in a dog with *Borrelia turicatae* spirochetemia,[3] yielding an IME value of 2.37.

Consensus Summary Statement: Based on isolated case reports, the overall evidence that *Borrelia spp*. infection induces IMHA is negligible. Considering that *Borrelia* infection in dogs is associated with other immune-mediated conditions (notably polyarthritis),[4] future prospective,

controlled studies to investigate the relationship between IMHA and *Borrelia* infection may be warranted.

Dirofilaria immitis

Three studies were identified and evaluated.[5-7] One prospective case-control study asking if *D. immitis* infection was associated with IMHA demonstrated that dogs with heartworm disease were significantly more likely than heartworm-negative dogs to be anemic and Coombs'-positive.[7] However, anemia in heartworm-positive dogs was not correlated with a positive Coombs' test result. Whether the lack of correlation was because there is more than one mechanism for anemia in heartworm-infected dogs or because binding of IgG, IgM or complement to erythrocytes did not contribute to anemia could not be determined. Owing to the way data were summarized, the specific number of dogs with IMHA could not be determined; an IME value could not be assigned. The IME values for the other studies, which each mentioned *D. immitis* infection in a dog with IMHA, were 1.95 and 2.37 respectively.[5,6]

Consensus Summary Statement: The overall evidence that *Dirofilaria immitis* infection can induce IMHA is low. Further investigation into whether *D. immitis* causes IMHA is warranted.

Ehrlichia spp.

Four studies had IME values assigned.[8-11] A total of 6 dogs with IMHA exposed to *Ehrlichia* species (5 for *E. canis* and one unspecified species) were documented. The median IME value for *E canis* was 2.04, with a range of 1.95-2.87.

Consensus Summary Statement: The overall evidence that *Ehrlichia* induces IMHA is negligible. However, a role for ehrlichiosis in canine IMHA cannot be ruled out. Further prospective, controlled studies are needed to determine if *Ehrlichia spp*. can induce IMHA in individual patients.

Hemotropic *Mycoplasma spp.*

Three studies that documented infection with hemotropic *Mycoplasma spp.* in 3 individual dogs with IMHA met inclusion criteria for this review. One study identified a dog infected with *M. haemocanis* and another identified a dog infected with *Ca.* M. haematoparvum. IME values for these two studies were 2.87 and 1.70 respectively.[12,13] Coombs'-positive anemia was described in a dog with T cell lymphoproliferative disease also infected with a novel hemotropic *Mycoplasma*.[14] Owing to the presence of an additional comorbidity, an IME value could not be assigned. Only one study designed to ask specifically whether IMHA is associated with natural hemotropic *Mycoplasma* infection in dogs was identified. In that study based in the United Kingdom, the blood of 227 anemic and non-anemic dogs, including that from 37 dogs with IMHA, was tested using PCR targeting *Ca.* M. haemotoparvum. No dog, with or without anemia or IMHA, tested positive, suggesting that this *Mycoplasma sp.* is not a common cause of infection in that region; therefore, no conclusions regarding the role of *Mycoplasma* infection as a trigger for IMHA in dogs could be made.[15]

Consensus Summary Statement: The evidence for hemotropic *Mycoplasma spp.* causing IMHA in dogs is low. Further study is required to determine whether *Mycoplasma spp.* can contribute to the pathogenesis of IMHA alone, in immune-compromised patients, or during co-

infection with other agents, and whether there are species differences in pathogenicity that impact the development of IMHA.

Leishmaniasis

Anemia and positive Coombs' test results are both common in dogs with leishmaniosis. In a study by Ciaramella *et al*, 5 of 25 dogs with leishmaniosis were Coombs'-positive.[16] Anemia was also common; however, the number of dogs with both a Coombs'-positive anemia and leishmaniasis could not be discerned from the way the data were reported, precluding the assignment of an IME value. One additional study identified IMHA in 2 dogs with leishmaniasis without other comorbidities, yielding an IME value of 1.87.[11]

Consensus Summary Statement: The overall evidence that *Leishmania spp*. induce IMHA is negligible to low. Given the inflammatory nature of this disease and the potential association with IMHA, further controlled studies are warranted.

Non-vector-borne protozoal infections

Neospora caninum

Neospora caninum infections have been detected in a dog with IMHA treated with immunosuppressive drugs for 2.5 weeks[17] and in a dog with IMHA plus immune-mediated thrombocytopenia immunosuppressed for 4 weeks.[18] Both dogs were suspected to have subclinical neosporosis, with latent parasites reactivated after immunosuppression. One dog was

concurrently diagnosed with a *Klebsiella pneumonia* urinary tract infection;[17] an IME value could not be assigned. The IME value for the other study was 2.97.[18]

Consensus Summary Statement: The evidence for *Neospora caninum* inducing IMHA is negligible. However, the role for subclinical neosporosis in canine IMHA cannot be ruled out.

Gastrointestinal parasites

Ancylostoma caninum

A retrospective case series described 3 cases of IMHA in dogs that were infected with *A. caninum*.[19] IMHA resolved in all 3 dogs when treated concurrently with fenbendazole and a 4 week tapering course of prednisone. *A. caninum* was considered a possible causative agent for IMHA in this report, based on the lack of other identified trigger factors in these dogs. Additionally, IMHA in each dog resolved after antiparasitic treatment in addition to a relatively short immunosuppressive treatment period. The IME value for this study was 4.13.

Consensus Summary Statement: The evidence for *Ancylostoma caninum* inducing IMHA is low. However, evidence is currently limited to only a single case series in which IMHA in each of 3 dogs resolved after anti-parasitic treatment in addition to a relatively short course of immunosuppressive treatment. *A. caninum* is a possible causative agent for canine IMHA, but further studies are needed to confirm the pathogenic nature of this agent or other gastrointestinal parasites in IMHA.

Viral infections

Canine parvovirus

Parvovirus B19, an erythrovirus, has been associated with IMHA in people.[20,21] One study by Jackson *et al* was evaluated.[22] This study was classified as a retrospective case series. Of 55 dogs with IMHA, 1 dog was identified as having canine parvovirus, yielding an IME value of 2.33.

Consensus Summary Statement: The link between viral infection and IMHA has not been widely investigated in dogs. Given the lack of relevant studies, the role of any virus in canine IMHA cannot be ruled out. Further studies to determine whether viral exposure in dogs induces IMHA in dogs may be warranted.

Bacterial infections

Endocarditis

Three separate retrospective studies describe 4 dogs diagnosed with endocarditis and IMHA.[15,22,23]. Treatment was not described in these reports; therefore, it is difficult to identify the causative nature of endocarditis in IMHA pathogenesis. The median IME value was 1.95, with a range of 1.87-2.33.

Localized bacterial infections

Local bacterial infections have also been reported in dogs with IMHA. Nineteen dogs with IMHA were described as having various localized bacterial infections.[8,17,23-27] The reported infections included 3 cases of pneumonia;[23,28] 1 case of bacterial otitis (*Proteus* spp, *Staphylococcus intermedius*, and beta-hemolytic *Escherichia coli*-positive culture);[29] 1 case of mixed agent otitis and skin lesions (*Malassezia* spp, *Proteus mirabilis*, *Pseudomonas aeruginosa* identified on skin scrapings and culture);[26] 3 urogenital tract infections;[23,28] and 2 cases of bacterial lymphadenitis (unknown organisms).[8] An additional dog with IMHA, eventually diagnosed with neosporosis after immunosuppression, was diagnosed with a concurrent *Klebsiella pneumoniae* urinary tract infection at the time of an IMHA relapse.[17] Eight dogs had unspecified localized infections.[24,27] For infectious agents in which IME values could be assigned, the median was 2.12, with a range of 1.70-4.37.

Consensus Summary Statement: The evidence for systemic or localized bacterial infections inducing IMHA was negligible. However, the reports of bacterial infection and concurrent IMHA have largely been limited to case reports or other studies that were not designed to determine whether bacterial infection is a cause of IMHA in dogs. Therefore, a role for bacterial infections in canine IMHA cannot be ruled out.

Mycoplasma cynos

One retrospective case report described a dog with *Mycoplasma cynos* pneumonia diagnosed with IMHA.[30] While the dog had only marginal anemia (hematocrit of 0.37 L/L) and lacked evidence of hemolysis, cold agglutinins were detected, raising suspicion for a diagnosis of

IMHA. Cold agglutinins are commonly detected in people with *Mycoplasma* pneumonia.[31,32] The cold agglutinins were no longer detected after 8 weeks of enrofloxacin treatment for *M. cynos,* suggesting a role of *M. cynos* in the pathogenesis of their development. The dog was also treated with a beta-lactam antibiotic, which cannot be ruled out as the underlying cause of the cold agglutinins; an IME value could not be assigned.

Systemic infections and sepsis

Two dogs were reportedly treated for systemic bacterial infections originating in the respiratory tract 4 weeks prior to being diagnosed with IMHA.[24] One retrospective study specifically investigated whether bacteremia was associated with IMHA in dogs.[33] In that study, 12/12 dogs diagnosed with IMHA had negative aerobic and anaerobic blood cultures. However, the study performed cultures on only a small number of dogs and in general, blood culture is considered relatively insensitive for diagnosing bacteremia.[34,35] The IME value for systemic infections inducing IMHA in these studies ranged from 0.00[33] to 4.20.[24] Sepsis with concurrent IMHA was reported in 3/93 (3.2%) dogs in one study,[10] yielding an IME value for sepsis of 1.87. Outcome information for these patients was not given, and there was no direct evidence for sepsis causing IMHA.

Consensus Summary Statement: The evidence for *Mycoplasma cynos* inducing IMHA is low. Based on a single case report of cold agglutinin-positive anemia in a dog and the observation that *Mycoplasma spp.* cause cold agglutinins in other species, further studies are needed to determine whether *Mycoplasma spp.* cause IMHA in some dogs.

Fungal infections

Two retrospective studies each documented 1 case of fungal infection in dogs with IMHA. In one report, the dog was diagnosed with *Blastomyces dermatitidis*, while in the second study the dog was reported to have systemic mycosis, but the agent was not specified.[10,24] The IME values for these reports were respectively 4.04 and 1.70. The evidence for fungal infections causing IMHA is negligible to low, although neither of these studies was designed to identify a potential causal relationship.

Consensus Summary Statement: Studies identifying fungal infection as a cause of IMHA are limited. Currently, there is a negligible to low level of evidence that fungal infection can cause IMHA.

References

1. Breitschwerdt EB, Blann KR, Stebbins ME, et al. Clinicopathological abnormalities and treatment response in 24 dogs seroreactive to Bartonella vinsonii (berkhoffii) antigens. J Am Anim Hosp Assoc 2004;40:92-101.

2. Mazepa AW, Kidd LB, Young KM, et al. Clinical presentation of 26 anaplasma phagocytophilum-seropositive dogs residing in an endemic area. J Am Anim Hosp Assoc 2010;46:405-412.

3. Whitney MS, Schwan TG, Sultemeier KB, et al. Spirochetemia caused by Borrelia turicatae infection in 3 dogs in Texas. Vet Clin Pathol 2007;36:212-216.

4. Krupka I, Straubinger RK. Lyme borreliosis in dogs and cats: background, diagnosis, treatment and prevention of infections with Borrelia burgdorferi sensu stricto. Vet Clin North Am Small Anim Pract 2010;40:1103-1119.

5. Carr AP, Panciera DL, Kidd L. Prognostic factors for mortality and thromboembolism in canine immune-mediated hemolytic anemia: a retrospective study of 72 dogs. J Vet Intern Med 2002;16:504-509.

6. Kidd L, Geddings J, Hisada Y, et al. Procoagulant microparticles in dogs with immune-mediated hemolytic anemia. J Vet Intern Med 2015;29:908-916.

7. Werner LL, Halliwell RE, Jackson RF, et al. An investigation of the role of immunologic factors in anemia associated with canine heartworm disease. Vet Immunol Immunopathol 1984;7:285-292.

8. Piek CJ, Teske E, van Leeuwen MW, et al. Good agreement of conventional and gel-based direct agglutination test in immune-mediated haemolytic anaemia. Acta Vet Scand 2012;54:10.

9. Holloway SA, Meyer DJ, Mannella C. Prednisolone and danazol for treatment of immune-mediated anemia, thrombocytopenia, and ineffective erythroid regeneration in a dog. J Am Vet Med Assoc 1990;197:1045-1048.

10. Wang A, Smith JR, Creevy KE. Treatment of canine idiopathic immune-mediated haemolytic anaemia with mycophenolate mofetil and glucocorticoids: 30 cases (2007 to 2011). J Small Anim Pract 2013;54:399-404.

11. Engelbrecht R, Kohn B, Leibold W, et al. Clinical findings, diagnostics and treatment results in primary and secondary immune-mediated hemolytic anemia in the dog. Kleintierpraxis 2002;47:265-278.

12. Bunch SE, Metcalf MR, Crane SW, et al. Idiopathic pleural effusion and pulmonary thromboembolism in a dog with autoimmune hemolytic anemia. J Am Vet Med Assoc 1989;195:1748-1753.

13. Mitchell KD, Kruth SA, Wood RD, et al. Serum acute phase protein concentrations in dogs with autoimmune hemolytic anemia. J Vet Intern Med 2009;23:585-591.

14. Sykes JE, Bailiff NL, Ball LM, et al. Identification of a novel hemotropic Mycoplasma in a splenectomized dog with hemic neoplasia. J Am Vet Med Assoc 2004;224:1946-1951, 1930-1941.

15. Warman SM, Helps CR, Barker EN, et al. Haemoplasma infection is not a common cause of canine immune-mediated haemolytic anaemia in the UK. J Small Anim Pract 2010;51:534-539.

16. Ciaramella P, Oliva G, Luna RD, et al. A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by Leishmania infantum. Vet Rec 1997;141:539-543.

17. Fry DR, McSporran KD, Ellis JT, et al. Protozoal hepatitis associated with immunosuppressive therapy in a dog. J Vet Intern Med 2009;23:366-368.

18. Magana A, Sanchez F, Villa K, et al. Systemic neosporosis in a dog treated for immune-mediated thrombocytopenia and hemolytic anemia. Vet Clin Pathol 2015;44:592-596.

19. Lobetti RG, Schoeman T. Immune-mediated haemolytic anaemia: possible association with Ancylostoma caninum infection in three dogs. J S Afr Vet Assoc 2001;72:52-54.

20. Lehmann HW, von Landenberg P, Modrow S. Parvovirus B19 infection and autoimmune disease. Autoimmun Rev 2003;2:218-223.

21. Puigvi L, Baumann T, Fernandez S, et al. Massive erythrophagocytosis by peripheral monocytes and neutrophils in parvovirus-B19 autoimmune hemolytic anemia. Ann Hematol 2017;96:881-882.

22. Jackson ML, Kruth SA. Immune-mediated hemolytic anemia and thrombocytopenia in the dog: a retrospective study of 55 cases diagnosed from 1979 through 1983 at the Western College of Veterinary Medicine. Can Vet J 1985;26:245-250.

23. Warman SM, Murray JK, Ridyard A, et al. Pattern of Coombs' test reactivity has diagnostic significance in dogs with immune-mediated haemolytic anaemia. J Small Anim Pract 2008;49:525-530.

24. Feldman B. Demographics of canine immune-mediated haemolytic anaemia in the southeastern United States. Comp Clin Path1996;6:42-45.

25. Klein MK, Dow SW, Rosychuk RA. Pulmonary thromboembolism associated with immune-mediated hemolytic anemia in dogs: ten cases (1982-1987). J Am Vet Med Assoc 1989;195:246-250.

26. Mellor PJ, Roulois AJ, Day MJ, et al. Neutrophilic dermatitis and immune-mediated haematological disorders in a dog: suspected adverse reaction to carprofen. J Small Anim Pract 2005;46:237-242.

27. Paes G, Paepe D, Meyer E, et al. The use of the rapid osmotic fragility test as an additional test to diagnose canine immune-mediated haemolytic anaemia. Acta Vet Scand 2013;55:74.

28. Piek CJ, van Spil WE, Junius G, et al. Lack of evidence of a beneficial effect of azathioprine in dogs treated with prednisolone for idiopathic immune-mediated hemolytic anemia: a retrospective cohort study. BMC Vet Res 2011;7:15.

29. Foster AP, Sturgess CP, Gould DJ, et al. Pemphigus foliaceus in association with systemic lupus erythematosus, and subsequent lymphoma in a cocker spaniel. J Small Anim Pract 2000;41:266-270.

30. Pinkos AC, Friedrichs KR, Monaghan KN, et al. Transient cold agglutinins associated with Mycoplasma cynos pneumonia in a dog. Vet Clin Pathol 2015;44:498-502.

31. Ali NJ, Sillis M, Andrews BE, et al. The clinical spectrum and diagnosis of Mycoplasma pneumoniae infection. Q J Med 1986;58:241-251.

32. Lee SC, Youn YS, Rhim JW, et al. Early serologic diagnosis of Mycoplasma pneumoniae pneumonia: an observational study on changes in titers of specific-IgM antibodies and cold agglutinins. Medicine (Baltimore) 2016;95:e3605.

33. Miller SA, Hohenhaus AE, Hale AS. Case-control study of blood type, breed, sex, and bacteremia in dogs with immune-mediated hemolytic anemia. J Am Vet Med Assoc 2004;224:232-235.

34. Heilmann RM, Xenoulis PG, Barr JW, et al. Comparison of PCR and conventional blood culture to analyze blood from dogs with suspected sepsis. Vet J 2013;198:714-716.

35. Paolucci M, Landini MP, Sambri V. Conventional and molecular techniques for the early diagnosis of bacteraemia. Int J Antimicrob Agents 2010;36 Suppl 2:S6-16.