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CONSENSUS STATEMENT

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ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats

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Abstract

Immune-mediated hemolytic anemia (IMHA) is an important cause of morbidity and mortality in dogs. IMHA also occurs in cats, although less commonly. IMHA is considered secondary when it can be attributed to an underlying disease, and as primary (idiopathic) if no cause is found. Eliminating diseases that cause IMHA may attenuate or stop immune-mediated erythrocyte destruction, and adverse consequences of long-term immunosuppressive treatment can be avoided. Infections, cancer, drugs, vaccines, and inflammatory processes may be underlying causes of IMHA. Evidence for these comorbidities has not been systematically evaluated, rendering evidence-based decisions difficult. We identified and extracted data from studies published in the veterinary literature and developed a novel tool for evaluation of evidence quality, using it to assess study design, diagnostic criteria for

Abbreviations: AIHA, autoimmune hemolytic anemia; C, confidence of comorbidity diagnosis score; CI, confidence interval; D, study design score; DAT, direct antiglobulin test; FeLV, feline leukemia virus; FIP, feline infectious peritonitis; FIV, feline immunodeficiency virus; I, confidence of IMHA diagnosis score; IME, integrated metric of evidence; IMHA, immune-mediated hemolytic anemia; L, likelihood of a causal link between comorbidity and IMHA score; N, number of patients with a given comorbidity; PCR, polymerase chain reaction; Q, study quality score; SAT, saline agglutination test; VCCIS, Veterinary and Comparative Clinical Immunology Society.

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IMHA, comorbidities, and causality. Succinct evidence summary statements were written, along with screening recommendations. Statements were refined by conducting 3 iterations of Delphi review with panel and task force members. Commentary was solicited from several professional bodies to maximize clinical applicability before the recommendations were submitted. The resulting document is intended to provide clinical guidelines for diagnosis of, and underlying disease screening for, IMHA in dogs and cats. These should be implemented with consideration of animal, owner, and geographical factors.

KEYWORDS
comorbidity, Delphi survey, direct antiglobulin test, erythrocyte, evidence, flow cytometry, hemolysis, iceberg model, spherocyte, veterinary and comparative clinical immunology society

1 | INTRODUCTION

Immune-mediated hemolytic anemia (IMHA) in dogs and cats is associated with high morbidity and mortality.[1–5] Pathogenic autoantibodies target erythrocyte membrane epitopes,[6,7] providing a mechanism for fraction crystallizable receptor-mediated extravascular hemolysis mediated by macrophages.[8] Complement can interact with antibodies bound to erythrocytes, facilitating extravascular hemolysis or causing intravascular hemolysis by formation of the membrane attack complex. An expeditious diagnosis that distinguishes IMHA from other causes of anemia is critical to the rapid institution of appropriate treatment. Various criteria for the diagnosis of IMHA have been described in the literature based on the documentation of immune-mediated erythrolysis or proxy markers for this phenomenon,[9–12] but little consensus exists on which criteria are required for definitive diagnosis. Furthermore, the differentiation of spontaneous IMHA from disease associated with putative trigger factors is an important first step in the diagnostic evaluation, because removal of trigger factors whenever possible is a crucial component of treatment. However, no guidelines exist for the diagnostic assessment of trigger factors, and formal assessment of the evidence for their implication in IMHA is lacking. An evidence summary would allow clinicians to better gauge the likelihood of a given comorbidity being implicated in the pathogenesis of IMHA, and would help to guide on which diagnostic tests should be performed in individual patients.

The objective of this Consensus Statement is therefore to present guidelines on both the fundamental diagnosis of IMHA and tests to screen for putative trigger factors, based on evidence, inferences from parallel data in human medicine, and expert opinion. Work contributing to this Consensus Statement was completed by members of the Consensus Panel and additional members of the relevant *Veterinary and Comparative Clinical Immunology Society* (VCCIS) task forces established in 2015.

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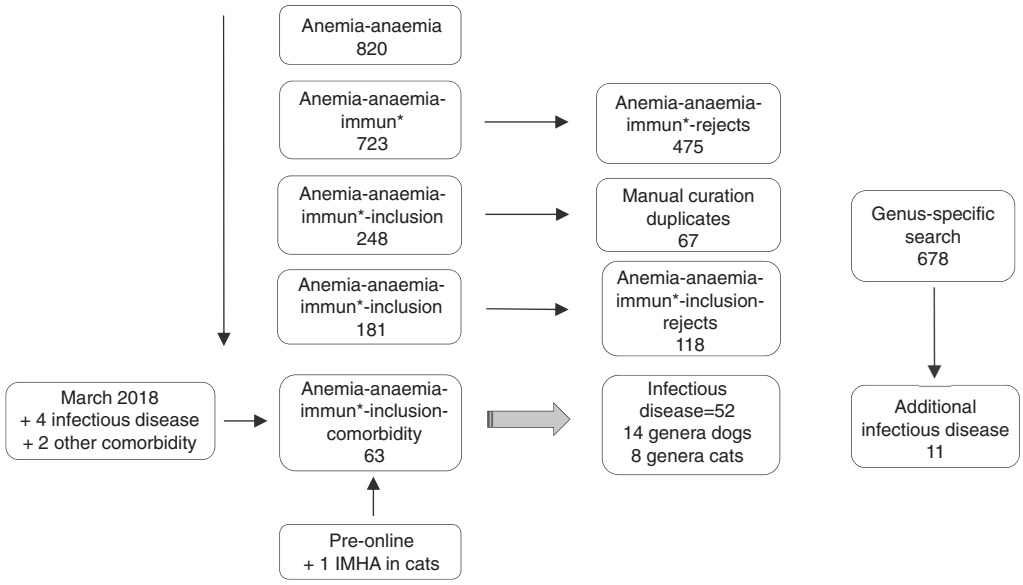

FIGURE 1 Curation of records. Papers captured by a search algorithm for anemia that met inclusion criteria (n = 248) were manually curated to remove duplicates (n = 67), after which remaining papers were screened to assess whether they mentioned comorbidities, yielding 63 papers; an additional 6 were added in March 2018. An independent search for infectious agents yielded an additional 11 papers of relevance. One additional paper was identified by examining reference lists of the captured papers. IMHA, immune-mediated hemolytic anemia

2 | MATERIALS AND METHODS

2.1 | Literature review

We searched 2 databases (Medline and Web of Science) for relevant references in April 2016 and March 2018. Standard Boolean search terms allowing lemmatization were adopted. References captured by the algorithm {(anemia OR anaemia) AND (dog OR cat) AND (immun*)}, hereafter denoted by A1, were imported into reference management software (Mendeley, Elsevier, New York; EndNote X8, Clarivate Analytics, Philadelphia), before manual screening on the basis of inclusion criteria outlined in Supporting Information S1. The reference lists of papers also were examined to capture references not cited on Medline or Web of Science. Pathogen-specific searches were conducted to capture additional references (Supporting Information S1).

2.2 | Curation of records

A total of 723 papers were captured by the search algorithm A1. Abstracts of all papers were reviewed by OAG, LK, UJ, ALM, SB, BG, RG, and JS, leading to the rejection of 475 papers because they failed to meet inclusion criteria. A further 67 duplicate papers were excluded, yielding 181 unique papers. Of these, an additional 118 papers were excluded because they did not include information on patients with potential trigger factors. Of the remaining 63 papers, 52 contained information of relevance to infectious disease, including 14 genera of microbes infecting dogs and 8 genera of microbes infecting cats. A pathogen-specific search on the basis of these genera yielded 11 additional papers meeting inclusion criteria. A search performed in March 2018, using both A1 and the pathogen-specific algorithms, yielded another 6 papers. An important paper published before the advent of online archiving was added to the list. Data therefore were extracted from 81 papers (Figure 1).

2.3 | Quality assessment

We designed a novel quality assessment and data extraction tool, which included domains to capture information on study design (D) and quality (Q), confidence of comorbidity diagnosis (C), likelihood of a causal link between comorbidity and IMHA (L), confidence of IMHA diagnosis (I), and the number of patients with a given comorbidity (N). For the purposes of this study, the term "comorbidity" included exposure to drugs, toxins, and vaccines. Additional domains captured detailed information on each of the comorbidities, including statistical inferences when available. Comorbidities were summarized in 5 broad categories: infectious disease, cancer, inflammatory disease, drugs and toxins, and vaccines. Panel members and non-panel VCCIS task force members were assigned to random pairings for the purpose of data extraction and quality assessment, dividing the total number of papers equally among all pairs. Concordance among the pairs was sought if individual members disagreed on specific observations, and all observations relating to quality assessment were confirmed by LK and OAG.

For each comorbidity identified in a paper, an integrated metric of evidence (IME) was computed as the sum of the normalized scores, weighted according to our assessment of relative importance to evidence rating, so long as only that comorbidity was present in individual patients, hence IME = $2D + Q + C + 2L + I + N$. If >1 comorbidity was present in individual patients, including those infected with >1 agent, an IME value was not calculated. Reference to the patients nevertheless was made in the narrative if they yielded insight. Score *D* was assessed after positing the question: Does the study ask whether a comorbidity induces (or is associated with) IMHA as part of

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its hypothesis or specific aims, or is the question that a comorbidity induces (or is associated with) IMHA answered by study design? If the answer was yes, a *D* score was assigned; if the answer was no, the study was designated "Descriptive Association Only" for that comorbidity and assigned an arbitrary *D* score of 1 (the lowest possible) out of a maximum of 7. A *Q* score was not computed for comorbidities assigned *Descriptive Association Only*, because general study quality in those cases was irrelevant to the question of the causal relationship between comorbidity and IMHA; *Q* scores in those cases were therefore 0. The maximum normalized score for each criterion was 1, yielding a maximum IME value of 8 and a minimum of <1. An IME value of 0 was applied when a study presented evidence against a comorbidity being associated with IMHA. For each comorbidity within a paper, the most conservative score for each of the IME criteria was applied for the relevant cohort of patients, to avoid exaggeration of evidence.

Threshold IME values were computed to allow comorbidities to be designated as negligible, low, intermediate, or high evidence for a causal relationship with IMHA. The threshold between negligible and low evidence was taken to be a hypothetical *Descriptive Association Only* study, with intermediate *C*, *L*, and *I* scores, and 1 positive case (IME = 2.95). The threshold between low and intermediate evidence was taken to be a hypothetical cross-sectional study, with a *Q* score of 28, intermediate *C*, *L*, and *I* scores, and 2-5 positive cases (IME = 4.37). Finally, the threshold between intermediate and high evidence was taken to be a hypothetical prospective cohort/case-control study, with a *Q* score of 28, high *C* score, intermediate *L* score, high *I* score (mechanistically based), and 2-5 positive cases (IME = 5.78).

The quality assessment and data extraction tool is shown in Supporting Information S2; Supporting Information S3 shows the derivation of each of the scores *D*, *Q*, *C*, *L*, *I*, and *N*.

2.4 | Delphi process

Each panel member drafted recommendations in assigned areas. The draft recommendations then were subjected to 3 rounds of Delphi review, during which each panel member was able to give written feedback on every recommendation using an online questionnaire (Survey Monkey, San Mateo, California). At the end of each round, suggestions were incorporated into the working template by 1 facilitator, and only recommendations for which consensus had not yet been reached were included in the subsequent Delphi cycle. After 3 rounds of review, unanimous consensus had been reached on the majority of recommendations. Where differences of opinion remained, these are indicated in the text of the Consensus Statement.

2.5 | Production of the consensus statement

OAG, LK, UJ, AMM, and SB were assigned ≥1 sections of the working document for further editing and incorporation of comments generated during oral presentation. These sections then were edited by the co-Chairs, before submission to ACVIM for review by all members. The draft Consensus Statement also was submitted to the European College of Veterinary Internal Medicine, American College of Veterinary Emergency and Critical Care, European College of Veterinary Emergency and Critical Care, the American College of Veterinary Pathology, and the European College of Veterinary Clinical Pathology for solicitation of comments from members. Feedback from these specialist colleges was used by the panel members to produce the final Consensus Statement.

3 | DIAGNOSIS OF IMMUNE-MEDIATED HEMOLYTIC ANEMIA

No agreed diagnostic gold (criterion) standard exists for IMHA in veterinary patients, similar to the situation in humans. The British Society for Hematology's guidelines for diagnosis of autoimmune hemolytic anemia (AIHA) recommend the direct antiglobulin test (DAT) in patients with hemolysis, but also emphasize that the test is neither specific nor 100% sensitive for AIHA.[13] The diagnostic tests described below therefore should be interpreted in combination with the results of other diagnostic tests and response to immunosuppression. The lack of a gold (criterion) standard also presents challenges when interpreting the literature. For example, although sensitivities and specificities are discussed here (either as reported directly in the referenced studies, or calculated by the panel using data within the reference), the criteria used to define cases as positive or negative for IMHA vary. Variability also exists in how tests are performed and validated, and therefore performance in 1 study may not generalize to others.

Cognizant of these caveats, 7 of 8 panel members considered the algorithm in Figure 2 appropriate for diagnosis of primary or secondary IMHA, complemented by the following explanatory notes. (One panel member considered that markers of immune-mediated disease should be assessed with caution in the absence of hemolysis.) This discussion is restricted to immune-mediated destruction of circulating erythrocytes (ie, precursor-targeted immune-mediated anemia or pure red cell aplasia are not discussed). Readers requiring additional information on immune-mediated differentiation failure or destruction at the level of the bone marrow are referred elsewhere.[14–17]

3.1 | Anemia

A spun PCV is suggested because calculated hematocrit may be unreliable when agglutination is present.[18–20] However, studies in humans have reported increased plasma trapping by abnormal erythrocytes (eg, spherocytes), which could introduce inaccuracy into the spun PCV.[21] To the best of our knowledge, the effect of agglutination or spherocytosis on spun PCV has not been investigated in veterinary species. Lack of regeneration (as assessed by reticulocyte count) does not eliminate IMHA because approximately 30% of dogs have nonregenerative anemia at presentation.[5,22]

3.2 | Signs of immune-mediated destruction

3.2.1 | Prominent spherocytosis

Spherocytes should be used as a diagnostic criterion only in dogs because feline erythrocytes do not consistently display central pallor. Interpreting

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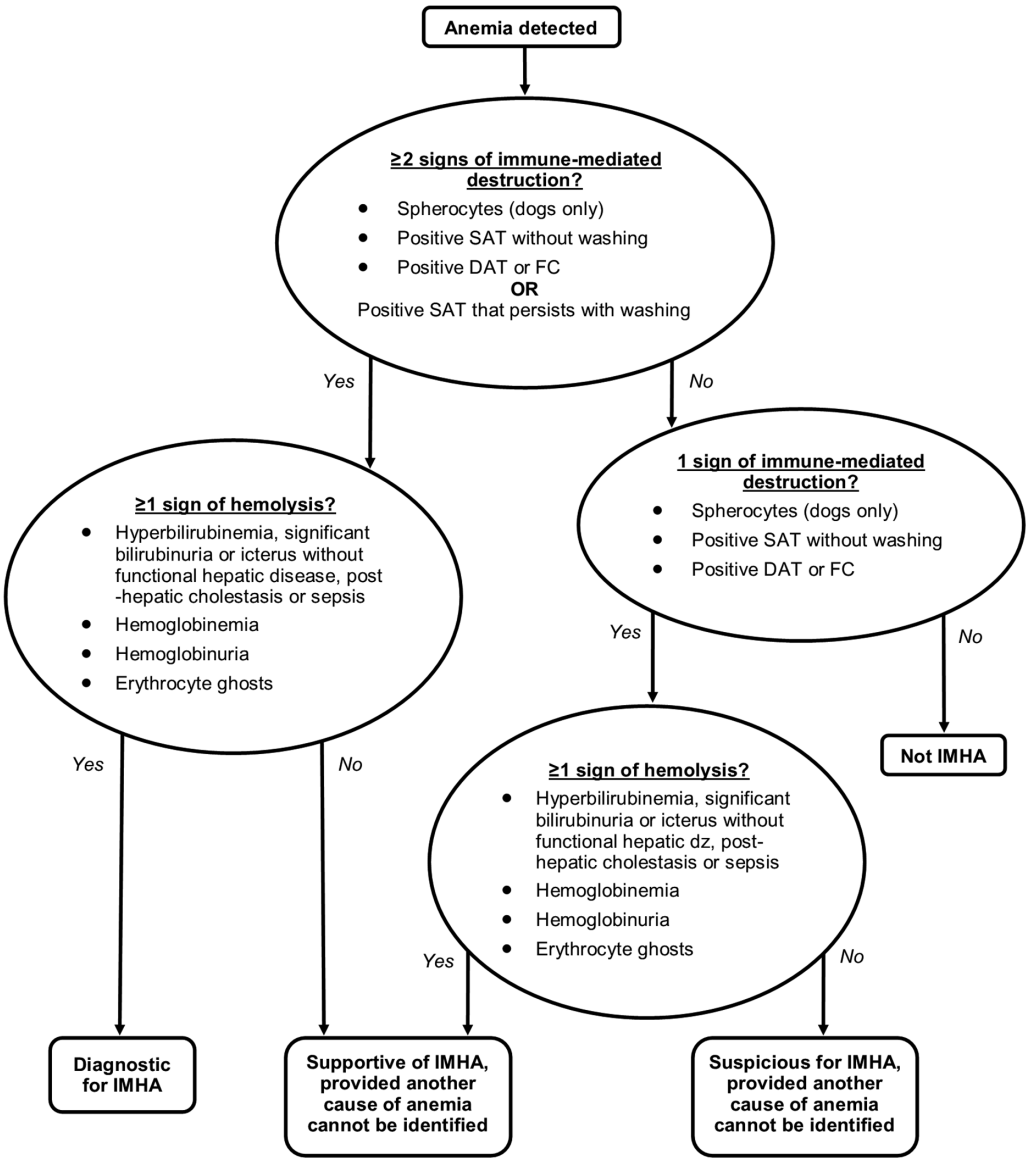

FIGURE 2 Diagnostic algorithm for immune-mediated hemolytic anemia (IMHA). Having identified anemia in a patient, biomarkers of immune-mediated destruction should next be assessed, including the saline agglutination test (SAT), direct antiglobulin test (DAT), and/or flow cytometry (FC); at least 2 should be present, or a positive SAT that persists with washing, to make a firm diagnosis of IMHA. Signs of hemolysis should then be assessed, at least 1 of which should be present for a firm diagnosis. Variations on this theme would yield a supportive or suspicious diagnosis, provided another cause of anemia is not identified. Additional abbreviations: ≥, at least; dz, disease

spherocytes after blood transfusion should be done cautiously, because stored blood products may contain high proportions of spherocytes[23] and spherocytes have been documented in human patients with hemolytic transfusion reactions.[24,25] Spherocytosis should be assessed in the monolayer of a well-made blood smear, because spherocyte-like artifacts arise toward the feathered edge and in thick areas.[26] In anemic animals, spherocytes should be confirmed in the deeper monolayer to avoid artifacts in thin areas. Spherocytosis also can induce increased osmotic fragility,[27,28] but because osmotic fragility testing is influenced by other factors (eg, hyperlipidemia,[27] erythrocyte age[29]), the panel does not advocate its routine use in the diagnosis of IMHA.

Reported causes of non-immune-mediated spherocytes, or morphologically similar pyknocytes, should be eliminated, including oxidative damage (eg, zinc[30,31] and acetaminophen[32]), envenomation,[33–37] hypersplenism (eg, hepatosplenic lymphoma),[38] pyruvate kinase deficiency,[39] disorders associated with erythrocyte fragmentation (eg, endocarditis,[40] microangiopathic hemolytic disorders including hemangiosarcoma,[41] or hemolytic uremic syndrome),[42] and dyserythropoiesis.[43] Hereditary spectrin deficiency also potentially may give rise to spherocytes if smears are made from blood stored >24 hours.[44] The percentage of spherocytes on blood smears from human patients with mutations causing hereditary spherocytosis is variable, but can be high.[45] A literature search for canine hereditary spherocytosis did not identify any cases with marked spherocytosis.

In a single study, ≥5 spherocytes/×100 oil immersion field yielded 63% sensitivity (95% confidence interval [CI], 39%-84%) (when 95% CIs were not provided by the authors, the online MedCalc Diagnostic

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test evaluation calculator [https://www.medcalc.org/calc/diagnostic_test.php] was used to calculate them) and 95% specificity (95% CI, 76%-100%) for IMHA in dogs,[27] compared with 74% sensitivity (95% CI, 49%-91%) and 81% specificity (95% CI, 59%-95%)[c] for ≥3/×100 oil immersion field.[27] A threshold of ≥5 spherocytes/×100 oil immersion field therefore could be considered supportive of a diagnosis of IMHA, but 3-4 spherocytes/×100 oil immersion field also may be consistent with IMHA provided no other cause of spherocytosis is identified. These thresholds are similar to the criteria for 1+ spherocytosis in a proposed semiquantitative grading system.[46] Where spherocyte numbers are low (versus their typical abundance in extravascular IMHA), variability among fields could be an issue: calculating mean count over several fields (eg, 10) could help establish the true extent of spherocytosis. For enrollment of cases in IMHA research, only high-quality blood smears should be used, and given the pitfalls of spherocyte recognition, examination of the smears by a board-certified clinical pathologist is advantageous.

3.2.2 | Positive saline agglutination test

Although evaluation of dried blood smears or hematology instrument scatter plots[20] can suggest agglutination, the panel does not consider these techniques adequate to confirm agglutination based on the possibility of overlapping rouleaux on blood smears and the potential for other causes of macrocytes on scattergram evaluation.[47] Saline agglutination testing performed by mixing 4 drops of saline with 1 drop of blood has a reported specificity of 100% (95% CI, 95%-100%)[c] for IMHA in dogs.[27] Mixing blood and saline 1:1 yielded a specificity of 95% (95% CI, 88%-99%)[c] based on 85 dogs without IMHA, or 85% (95% CI, 65%-96%)[c] when only anemic dogs were considered.[11] Agglutination that persists after mixing 1 drop of blood with 4 drops of saline therefore is considered adequate evidence for agglutination in most cases.[27] Considerably higher dilution ratios can aid the microscopic identification of agglutination. To decrease false positives, confirming that agglutination persists after washing erythrocytes 3 times in a 1:4 ratio with saline[11] is recommended for animals with equivocal results (eg, rare small erythrocyte clumps in an otherwise negative test), markedly increased total protein (eg, leishmaniasis, multiple myeloma, and feline infectious peritonitis [FIP]) or fibrinogen concentrations,[48] or strong rouleaux formation on blood smear examination. Based on reports of agglutination of washed erythrocytes from normal dogs at 4°C, we suggest that the saline solution should be between room temperature and 37°C.[49]

3.2.3 | Demonstration of anti-erythrocyte antibodies

Five panel members preferred the direct Coombs' test (DAT) and 3 considered flow cytometry and DAT to be equally useful. Supporting Information S4 provides recommendations for performance of these tests. If the DAT cannot be performed because agglutination persists after washing, the combination of anemia, hemolysis, and persistent agglutination is sufficient for diagnosis of IMHA. Immunochromatography offers an alternative to conventional DAT or flow cytometry, but confirming negative results by conventional DAT may be advisable because of frequent weak positive test strips in DAT-positive dogs.[11]

For DAT, sensitivity ranged from 61 to 82% for dogs[27,50] and 82% for cats[51] for studies reported between 2006 and 2016 that did not rely on DAT alone for the diagnosis of IMHA and reported sensitivity or sufficient information for its calculation. Specificity for DAT was 94%-100% for dogs[11,27,50,52] and 95%-100% for cats[9,51,53] for studies published between 2006 and 2016 that reported specificity or sufficient information for its calculation. Although small experimental studies have reported sensitivities of up to 100% for flow cytometry,[54–57] sensitivity was 67% (95% CI, 53%-79%)[c] in a larger study reporting results of routine clinical testing.[58] For studies including clinically ill negative controls, specificity for flow cytometry was 87.5% (95% CI, 47%-100%)[c54] to 92% (95% CI, 88%-95%)[c.56]

Reports of sample handling effects on flow cytometry are lacking. Storage of samples at 4°C for up to 7 days before DAT testing is acceptable unless the laboratory advises otherwise.[11] Current data, although limited, suggest that although immunosuppression does not immediately result in a negative DAT,[11,59] interindividual variability exists in the time required to become negative DAT after initiation of treatment.[11,60] For flow cytometry, anecdotal reports suggest that immunosuppression decreases the percentage of antibody-positive erythrocytes.[56] Therefore, where possible, we recommend collection of samples for DAT or flow cytometry before initiation of treatment. Large-scale studies of the effect of prior blood transfusion on DAT or flow cytometry are lacking. Based on reports of DAT-negative results for 21 dogs posttransfusion,[11] prior blood transfusion is not an absolute contraindication for testing. However, a positive DAT has been reported in a dog without signs of IMHA but with a history of multiple transfusions.[61] Furthermore, delayed serological or hemolytic transfusion reactions with positive DAT are reported in humans.[62–64] Therefore, where possible, we recommend collection of samples for DAT before blood transfusion.

A suggested advantage of flow cytometry compared with DAT is the generation of a more quantitative result, potentially allowing monitoring of therapeutic success. Statistical associations are reported between laboratory or clinical features and the percentage of antibody-positive erythrocytes.[56,58] However, the clinical value of the percentage of positive erythrocytes has not been evaluated rigorously.[56]

3.3 | Evidence of hemolysis

3.3.1 | Spherocytosis

In dogs, spherocytes (assessed as described) can provide evidence of hemolysis, consistent with evidence of this phenomenon in human erythrocytes.[65,66] The increased rigidity of spherocytes results in entrapment within the spleen and subsequent extravascular hemolysis.[28,67,68]

3.3.2 | Hyperbilirubinemia

In the absence of decreased functional hepatic mass, obstructive cholestasis, or sepsis, hyperbilirubinemia may represent evidence of hemolysis. At least 1 of the following is considered sufficient evidence for hyperbilirubinemia: icterus, total serum or plasma bilirubin concentration above

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reference interval, bilirubinuria in cats, or ≥2+ bilirubin on a urine reagent strip in dogs. Bilirubin reported for hemolyzed samples should be interpreted in combination with information regarding the likely impact of hemolysis on the assay.[69]

3.3.3 | Hemoglobinemia/Hemoglobinuria

Hemoglobinemia can be detected by visual examination of plasma or measurement of cell-free hemoglobin. When using instrument-based indicators of hemolysis, limitations of the individual method should be considered. For example, spectrophotometric hemolytic indices rely on manufacturer-specific methods and algorithms that are not directly comparable among different instruments.[70] Similarly, discrepancy between mean cell hemoglobin concentration and cellular hemoglobin concentration provided by ADVIA hematology instruments may reflect hemolysis or other sample characteristics, such as lipemia.[71]

Hemoglobinemia should only be interpreted as evidence of hemolysis after eliminating artifactual hemolysis. Common causes of in vitro hemolysis include, but are not limited to, traumatic venipuncture,[72–75] freezing, storage, and (based on studies in humans) sampling via an IV catheter[72,76,77] or post-collection injection of samples into vacutainers.[78] The likelihood of in vitro hemolysis is increased if factors that increase erythrocyte fragility are present (eg, lipemia).[79] Provided causes of myoglobinuria are absent, hemoglobinuria is considered present if urine is red and discoloration is not cleared by centrifugation, or if a positive heme reaction on urine dipstick is present in the absence of intact erythrocytes on microscopic sediment examination. Assessment of hemoglobinuria should be performed using a fresh urine sample, and anecdotally the likelihood of erythrocyte lysis in urine is increased in alkaline or poorly concentrated or hyposthenuric urine samples.

3.3.4 | Erythrocyte ghosts

Ghost cells provide evidence of intravascular hemolysis if seen on a smear made immediately after blood collection.[3,80]

4 | PUTATIVE TRIGGER FACTORS

4.1 | Infectious disease

Recent evidence suggests that any infection can trigger immune dysregulation, loss of immune tolerance, and development of immune-mediated disease in an individual patient with a genetic, epigenetic, or susceptible microenvironmental milieu at the time of infection.[81–86] However, certain organisms may cause specific immune-mediated diseases. Examples in people include *Mycoplasma pneumoniae* infection causing IMHA and *Helicobacter pylori* causing immune-mediated thrombocytopenia.[87,88] Other mechanisms such as circulating immune complex deposition and activation of immune cells through fraction crystallizable receptor engagement or delivery of immunoglobulin-bound nucleic acid to Toll-like receptors also occur during some infections.[89] Damage to target cells is another mechanism that may make a pathogen particularly likely to induce autoimmunity by increased exposure of self-epitopes that normally are sequestered or inefficiently presented to immune cells.[81,90] This proposed mechanism for the development of IMHA[91] contributes to the accelerated clearance of erythrocytes during *Plasmodium* infection in people and mice.[92] It also may occur during *Babesia gibsoni* infection in dogs and *Mycoplasma haemofelis* infection in cats.[93–97] Antibody-mediated removal is part of normal erythrocyte senescence.[98] Organisms thus may cause IMHA by amplifying normal antibody-mediated removal of aged or damaged erythrocytes.

4.1.1 | Consensus Summary Statement

Organisms identified in this review with a high and an intermediate level of evidence as a cause of IMHA are likely to induce disease by a mechanism that can trigger immune-mediated erythrocyte destruction in many patients. Further study is required to determine the role of other infections in IMHA. We emphasize that, for most studies, a low level of evidence represents a lack of studies designed to answer whether an infection is associated with IMHA, rather than studies that specifically demonstrated a lack of evidence. In addition, environmental, genetic, and epigenetic factors play a role in whether immune-mediated disease occurs in an individual patient. Therefore, clinicians should consider the possibility that any identified recent or recurrent infection may contribute to the development of IMHA. Furthermore, eliminating the possibility of infection is prudent before immunosuppressive treatment.

4.1.2 | Summary of evidence

Overall, 66 manuscripts were reviewed.[3,9,10,12,27,51,57,94–97,99–153] The IME values were calculated for 27 infectious agents or types of infection (Figures 3–5), but could not be calculated for many infectious agents either because of the way data were summarized (eg, the number of individual patients with IMHA and an infection could not be discerned) or because an individual patient with IMHA had >1 comorbidity (Supporting Information S2). In addition, most investigators did not specifically ask whether infection causes IMHA. Consensus Summary Statements are presented here for all genera of organisms for which at least 1 study had an intermediate or higher level of evidence that infection induced IMHA. All additional organisms are discussed in Supporting Information S5.

4.2 | Infections in dogs

4.2.1 | Piroplasms

Seventeen studies documented 103 cases of IMHA in *Babesia*-infected dogs.[57,93–96,99,102–104,111,120,122,128,134,141,147,151] The IME values for *Babesia* species as a whole ranged from 0.00 to 6.99, with a median of 4.55. Fifty-three percent (10/19) of the IME values demonstrated an intermediate or high level of evidence that *Babesia* causes IMHA. For

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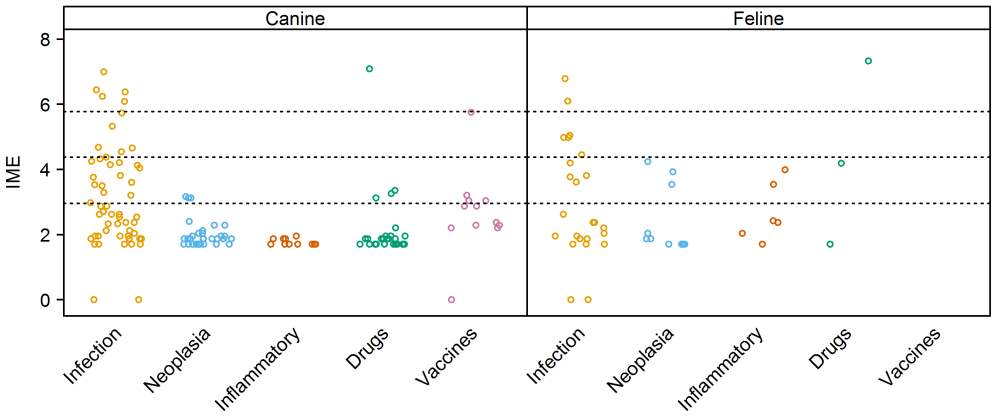

FIGURE 3 Integrated metric of evidence (IME) summary for all comorbidity categories. Horizontal dotted lines indicate the threshold IME values between negligible and low (2.95), low and intermediate (4.37), and intermediate and high (5.78) levels of evidence. Most IME values fell within the negligible and low zones of evidence, while a small number were 0, indicating evidence against that comorbidity inducing immune-mediated hemolytic anemia (IMHA). No studies of relevance to vaccination as a potential trigger for IMHA in cats could be found

3 additional studies, the number of dogs with *Babesia* and IMHA could not be determined.[105,125,136]

There is a high level of evidence that immune-mediated destruction of erythrocytes contributes to anemia in dogs infected with *B. gibsoni*. Immune-mediated hemolytic anemia was documented in 69 dogs in 9 studies,[93–96,99,102,103,111,151] with an additional study (in which the number of infected dogs with IMHA could not be determined) providing useful mechanistic insight.[125] The median IME value was 5.32, ranging from 2.54 to 6.99. For this *Babesia* species, 88% (8/9) of the studies showed intermediate (4) or high (4) IME values. Four were studies of dogs experimentally infected with *B. gibsoni*, yielding a median IME value of 6.41 and range of 6.08-6.99.[94–96,99] Natural infection with *B. gibsoni* occurs most commonly in fighting breeds.[102,125] However, mixed breed dogs used in experimental studies also develop IMHA, suggesting that the immune-mediated pathogenesis is largely driven by the parasite.[99]

Whether other species of *Babesia* cause IMHA in dogs remains unclear. One study documented IMHA in 2 chronically infected splenectomized mixed breed dogs experimentally infected with what was thought to be *B. gibsoni*, but later characterized as *Babesia conradae*,[147] yielding an IME value of 6.25. Five studies documented 13 cases of IMHA in dogs infected with *Babesia canis*, with a median IME value of 3.20 and range of 0-4.32.[57,104,111,128,134] *Babesia vogeli* was documented in 2 studies of 5 dogs with IMHA, with IME values of 5.73 and 4.14. Five cases of IMHA were documented in a study of *Babesia rossi*-infected dogs, although the authors presumed a *Babesia* species based on cytological examination of blood smears and geographic locale.[120] The IME value was 2.56. In an additional study, the

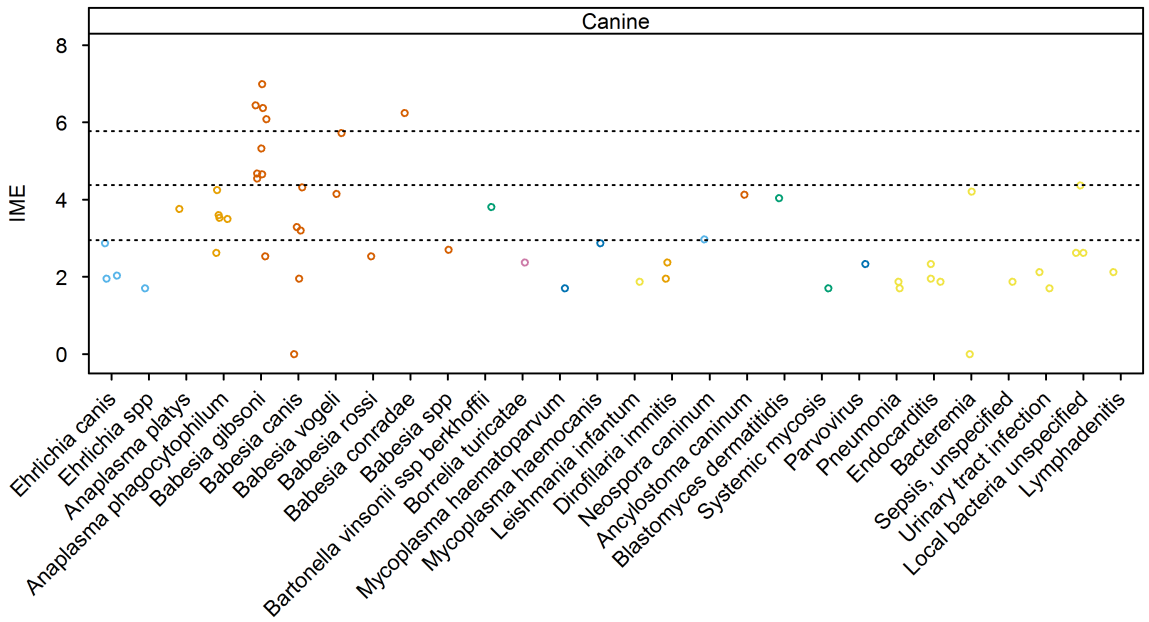

FIGURE 4 Integrated metric of evidence (IME) values for infectious agents and diseases in dogs. Horizontal dotted lines indicate the threshold IME values between negligible and low (2.95), low and intermediate (4.37), and intermediate and high (5.78) levels of evidence. spp, species (plural term)

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Babesia species was not specified, but again was likely to be *B. rossi*.[122] Nine dogs with IMHA were documented in that study, with an IME value of 2.70. Thus, the evidence for large *Babesia* species causing IMHA is lower than that for *B. gibsoni*, attributed in part to the fact that most studies were not designed to determine if an association between IMHA and infection existed. Nevertheless, differences also may exist in pathogenicity among *Babesia* species that influence the risk of IMHA. For example, 1 study found that the majority of anemic *B. vogeli*-infected dogs had IgM and IgG bound to erythrocytes, but these antibodies were not detected in dogs infected with *B. canis*.[57] In this study, eccentrocytosis, suggesting oxidative damage, was more common in *B. canis*-infected dogs. The IME value for *B. canis* in this study was 0, whereas it was 5.73 for *B. vogeli*.[57]

The mechanism of immune-mediated erythrocyte destruction during *B. gibsoni* infection has been explored. Because *Babesia* species infect erythrocytes, antibodies appropriately targeting the organisms could result in "immune-mediated" erythrocyte destruction without targeting self-antigen. However, antibodies produced during infection also appear to target erythrocyte membranes. Oxidative injury may play a role in anti-erythrocyte antibody formation.[96] Activated macrophages cause oxidative damage to uninfected as well as infected erythrocytes during *B. gibsoni* infection, a factor that may contribute to the severity of IMHA in some dogs.[96] In addition to oxidative damage, sialic acid residue removal is required to expose epitopes that are targeted by antibody.[94] Interestingly, anti-erythrocyte antibodies that developed in dogs experimentally infected with *B. gibsoni* did not attach to undamaged red blood cells in dogs that had recovered from clinical infection.[93] Furthermore, in vitro studies have shown that *Babesia*-induced antibody reactivity against erythrocytes is higher for aged and oxidized than for fresh erythrocytes.[95] Taken together, these data suggest that ongoing damage to the red cell membrane and increased exposure of epitopes that are usually "hidden" facilitates immune-mediated erythrocyte destruction. Once infection is controlled, the drive for immune-mediated destruction stops.

Like *Babesia* species, *Rangelia* and *Theileria* species are protozoan parasites that infect erythrocytes in dogs. A study of dogs experimentally infected with *Rangelia vitelli* demonstrated that a regenerative anemia suspicious for IMHA developed in infected dogs.[149] Treatment of infection resolved the anemia without immunosuppression. A retrospective case series of dogs naturally infected with *Theileria* spp. developed IMHA.[137] Dogs were treated with combined immunosuppression and imidocarb dipropionate. The authors reported resolution of hematological abnormalities during an unspecified study period. The IME values could not be calculated, because the total number of dogs with IMHA could not be discerned.

Consensus Summary Statement

The evidence that piroplasms, and in particular *B. gibsoni*, cause IMHA is intermediate to high. For *B. gibsoni*, evidence suggests that during infection, antibodies target host erythrocyte antigens exposed as a consequence of transient oxidative damage or sialic acid residue removal. Further study is needed to determine if differences in pathogenicity among species, host factors, or both mediate risk of development of IMHA in infected dogs. What is known about the mechanism of erythrocyte destruction suggests that immunosuppression should not be necessary to resolve immune-mediated erythrocyte destruction in most cases.

4.2.2 | *Anaplasma* species

Nine dogs with IMHA in 5 studies were infected with, or exposed to, *Anaplasma phagocytophilum*.[101,108,126,129,150] The median IME value was 3.53, with a range of 2.62-4.25. In addition to IMHA, platelet-bound antibodies were documented in some dogs with concurrent thrombocytopenia.[101,108] Although most dogs in these reports were treated concurrently with doxycycline and immunosuppressive corticosteroid treatment, 1 dog responded to doxycycline treatment alone,[150] whereas another dog had prednisone discontinued after 2 days.[108] One retrospective case series documented 2 dogs with acute *Anaplasma platys* infections with concurrent IMHA.[100] Both dogs had spherocytosis and positive saline agglutination and Coombs' test results supporting the diagnosis of IMHA. The IME value for this study was 3.76.

Consensus Summary Statement

The evidence that *A. phagocytophilum* causes IMHA is low. However, most studies reporting *A. phagocytophilum* in dogs with IMHA were limited to case reports or retrospective studies, and were not designed to investigate a causal relationship. The presence of IMHA and other immune-mediated conditions concurrent with this infection suggests that prospective controlled studies to examine a possible causal relationship between *A. phagocytophilum* and IMHA in dogs are warranted. The evidence that *A. platys* induces IMHA in dogs is low, but data are limited to a single retrospective case series. Further prospective, controlled studies are required to document a possible causal relationship between *A. platys* and IMHA in dogs.

Evidence that other vector-borne agents (including *Dirofilaria immitis*, *Ehrlichia* spp. *Borrelia* spp., hemotropic *Mycoplasma* spp., *Bartonella* spp., and *Leishmania infantum*), non-vector-borne protozoal pathogens (including *Neospora caninum*), and other bacterial infections induce IMHA was negligible to low (Figure 4), or could not be quantified based on how results were reported (Supporting Information S5).[105,109,125,137,145] For some of these organisms, such as *Leishmania* spp., *D. immitis*, and *Bartonella* spp., Coombs' test-positive anemia is observed commonly with infection.[105,109,145] Therefore, from a clinical perspective, it is still important to eliminate infection with these agents in a dog in which IMHA is a differential diagnosis.

4.3 | Infections in cats

4.3.1 | *Babesia felis*

Immune-mediated hemolytic anemia was documented in 9 of 56 cats infected with *B. felis* in 1 study from South Africa. Six of the 9 cats were coinfected with feline leukemia virus (FeLV).[138] Treatment for

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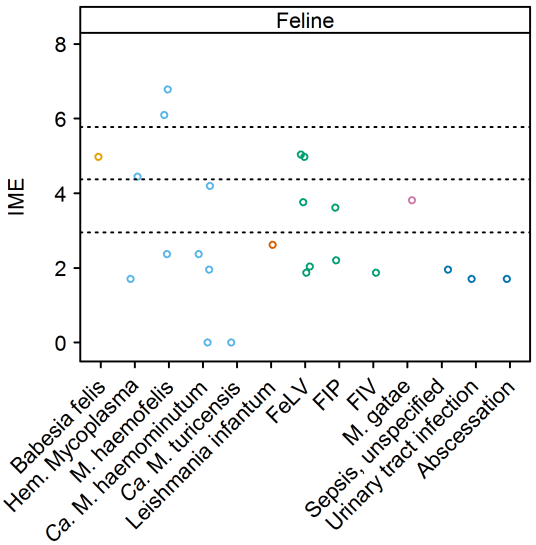

FIGURE 5 Integrated metric of evidence (IME) values for infectious agents and diseases in cats. Horizontal dotted lines indicate the threshold IME values between negligible and low (2.95), low and intermediate (4.37), and intermediate and high (5.78) levels of evidence. *Ca.*, *Candidatus*; FeLV, feline leukemia virus; FIP, feline infectious peritonitis; FIV, feline immunodeficiency virus; Hem., hemotropic; M., *Mycoplasma*

B. felis without immunosuppression resolved IMHA, yielding an IME value of 4.98.

Consensus Summary Statement

Although studies are limited, an intermediate level of evidence was found that *B. felis* causes IMHA in cats, and that treatment resolves IMHA without immunosuppression.

4.3.2 | Hemotropic *Mycoplasma* species

Seven studies documenting IMHA in 21 cats infected with hemotropic *Mycoplasma* spp. yielded IME values.[3,51,97,110,130,140,153] The median IME value was 2.37, but values differed widely among hemotropic *Mycoplasma* species, ranging from 0 to 6.78. Overall, a high level of evidence exists for *M. haemofelis* inducing IMHA in cats. Immune-mediated hemolytic anemia was documented in 15 cats in 3 studies for this species.[3,97,140] The median IME value was 6.10, with a range of 2.37-6.78. Two of the 3 studies provided high evidence[97,140]; the other study was not designed to answer whether infection causes IMHA.[3] In a study of cats experimentally infected with *M. haemofelis*, severe macrocytic Coombs' test-positive anemia and persistent auto-agglutination of erythrocytes developed. In contrast, these findings did not occur when cats were infected with the less pathogenic *Candidatus* (*Ca.*) Mycoplasma haemominutum and *Ca.* Mycoplasma turicensis species.[140] The target of anti-erythrocyte antibody that develops during *M. haemofelis* infection in cats was investigated in 1 study.[97] Serum from cats infected with *M. haemofelis* agglutinated infected and neuraminidase-treated erythrocytes but not normal erythrocytes, suggesting that, as in babesiosis, damage to erythrocytes and unmasking of antigens contribute to the pathogenesis of IMHA.[97] *Ca.* M. haemominutum infection was documented in 3 cats with IMHA over 4 studies (1 study showing no association with IMHA),[3,51,130,140] yielding a median IME value of 2.16 and range of 0-4.2.

Consensus Summary Statement

A high level of evidence was found that *M. haemofelis* causes IMHA in cats. Negligible to low level of evidence was found that the less pathogenic species *Ca. M. haemominutum* causes IMHA, and no evidence was found that *Ca. M. turicensis* induces IMHA. Whether coinfection and host immune status play roles in development of IMHA in cats infected with different hemotropic *Mycoplasma* species requires further study.

4.3.3 | Viral infections

Feline leukemia virus

Seven studies meeting inclusion criteria were identified.[3,9,110,119,138,153,154] However, other comorbidities such as erythroleukemia, myeloproliferative disease, chronic interstitial nephritis, glomerulonephritis and splenic amyloidosis, and drug administration were documented in some infected cats, precluding IME calculation.[110,138,153,154] The median IME value for the others was 3.77, with a range of 1.87-5.04.[3,9,110,119,153,154]

Consensus Summary Statement

Collectively, the evidence that FeLV infection induces IMHA is low. The observation that some FeLV-positive cats also have Coombs' test-positive anemia should prompt further investigation into whether immune-mediated erythrocyte destruction can contribute to anemia in FeLV-positive cats.

Summaries of the evidence for other infections that have been documented in cats with IMHA, including FIP, feline immunodeficiency virus (FIV), *L. infantum*, *Mycoplasma gatae*, *M. haemofelis*, soft tissue infection, and urinary tract infection are provided in Supporting Information S6.

5 | CANCER

5.1 | Cancer in dogs

Immune-mediated hemolytic anemia is a recognized paraneoplastic syndrome in people.[155–158] Chronic lymphocytic leukemia is a well-established cause of IMHA in people.[155,156] Other neoplasms have been associated with IMHA in humans, but the causal mechanisms remain elusive.[157,158] The IME values could be calculated for 13 studies (Figure 6). The global median IME value was 1.87, with a range from 1.70 to 3.12, thus representing levels of evidence that were negligible (29 IME values; 70 patients) or low (3 IME values; 3 patients). The generally low level of evidence reflects the fact that the majority of the published studies did not specifically ask whether cancer is

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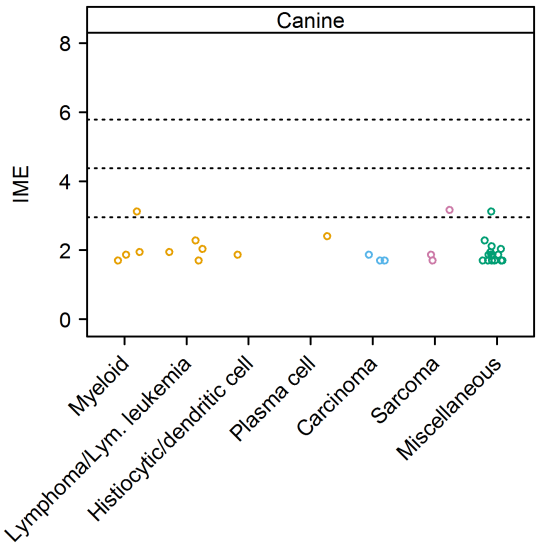

FIGURE 6 Integrated metric of evidence (IME) values for cancer types in dogs. Horizontal dotted lines indicate the threshold IME values between negligible and low (2.95), low and intermediate (4.37), and intermediate and high (5.78) levels of evidence. Miscellaneous cancer types included pheochromocytoma, unspecified abdominal, adrenal, bladder, cardiac, mediastinal, and splenic masses, other hematopoietic tumors, and unspecified neoplasia. Lym., lymphoid; Myeloid, myeloid leukemia or myeloproliferative disease

associated with canine IMHA, or if they did, were associated with low Q scores.

Two individual studies yielded low level evidence. The first described a mast cell tumor in a dog with IMHA, with an IME value of 3.12, and a pheochromocytoma in a dog with concurrent IMHA and immune-mediated thrombocytopenia, with an IME value of 3.12.[116] The second, a case report of an undifferentiated sarcoma in a Flat-Coated Retriever with IMHA, postulated that the sarcoma was a trigger for the IMHA, with an IME value of 3.17.[159] *Mycoplasma haemocanis* was identified in a splenectomized dog with cytological and clinical characteristics of acute lymphocytic leukemia.[139] Although the development of IMHA in this dog was attributed to the hemotropic *Mycoplasma* infection, involvement of the leukemia could not be excluded. A canine IMHA patient with a duodenal leiomyosarcoma had an IME value of 1.70.[10] Three further patients with hemangiosarcoma and IMHA were described, for which the IME value was 1.87.[2] A number of other papers yielded a negligible level of evidence for neoplasia as a cause of IMHA. These documented the presence of carcinomas,[2,10,12] malignant histiocytosis, and other hematopoietic tumors,[134] myeloid neoplasia,[2,10,132,134,160] multiple myeloma,[161] sarcomas,[2,10] and miscellaneous undefined tumors[2,10,134,142,143,162,163] in dogs with IMHA.

Consensus Summary Statement

Evidence of a causal link between cancer and IMHA in dogs currently is lacking in the veterinary literature, largely reflecting the fact that the majority of the published studies did not specifically ask whether cancer was associated with IMHA in dogs. Further studies are needed to determine if such an association exists. Although no evidence for a causal link exists, cancer cannot be eliminated as a potential trigger for this disease.

5.2 | Cancer in cats

Five studies reported 21 cats with neoplasia and IMHA (Figure 7). These studies provide negligible evidence for a causal link between neoplasia and IMHA, yielding a median IME value of 1.87 and a range of 1.7-4.4. No study specifically addressed this hypothesis. In a single retrospective study of 107 cats with IMHA, concurrent neoplasia was present in 16 (15%) cats.[3]

5.2.1 | Hematopoietic and lymphoid neoplasia

Eight cats for which an IME value could be calculated for lymphoma and IMHA were identified.[3,9,164] Two of the 3 studies reporting these cases did not demonstrate a causal association between IMHA and lymphoma,[3,9] and 1 study was considered to partially report or suggest causality.[164] The latter reports 2 sibling specific pathogen-free experimental cats. For both cats, lymphoma/lymphocytic leukemia was diagnosed on histological review after necropsy. However, interpretation of the histology in both cats was equivocal. The histological pattern was described as multicentric T-lymphoblastic infiltration with associated B-lymphocyte proliferation, which the authors concluded was most likely a lymphoproliferative disorder, but they did not eliminate an aberrant immune response. For 1 cat, the diagnosis of

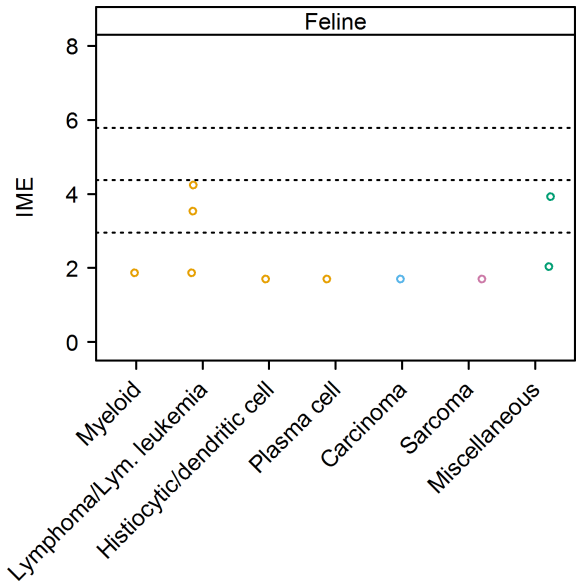

FIGURE 7 Integrated metric of evidence (IME) values for cancer types in cats. Horizontal dotted lines indicate the threshold IME values between negligible and low (2.95), low and intermediate (4.37), and intermediate and high (5.78) levels of evidence. Miscellaneous cancer types included gastrointestinal and uncharacterized neoplasia. Lym., lymphoid; Myeloid, myeloid leukemia or myeloproliferative disease

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lymphoma/lymphocytic leukemia was made within 3 weeks of the onset of IMHA, and no other potential trigger for secondary IMHA was described. For the second cat, 2 episodes of IMHA were described, 1 potentially associated with an experimental herpes virus infection and the other potentially associated with experimental FeLV infection. For the other 2 studies, neither the method of diagnosis nor the subtype of lymphoma was specified. The evidence for a causal association between IMHA and lymphoma is low, with a median IME value of 3.54 and a range of 1.87-4.24.

A single cat with multiple myeloma and IMHA was identified.[3] This study did not show a causal association between neoplasia and IMHA, and the method of diagnosis of neoplasia was unclear. The evidence for an association between IMHA and multiple myeloma was negligible, with an IME value of 1.70.

Three cats with erythroleukemia[3,153] and 3 cats with non-specified myeloproliferative disease[110] and IMHA were identified. No study demonstrated a causal association between IMHA and neoplasia. The report of 2 of the cats with erythroleukemia suggests that diagnosis was based on bone marrow cytological or histological review, or both.[3] The method of diagnosis of neoplasia was not described for the third cat with erythroleukemia.[153] For the cats with non-specified myeloproliferative disease, the diagnosis was based on bone marrow examination, but details are limited.[110] All cats with non-specified myeloproliferative disease were FeLV positive.[110] The evidence for a causal association between erythroleukemia and IMHA is negligible, with an IME value for the 1 study in which it could be assigned of 1.87.[3] Other studies of erythroleukemia or unspecified myeloproliferarative disease did not yield IME values because of the presence of comorbidities.

A single cat with histiocytic sarcoma and IMHA was identified.[3] This study did not show a causal association between IMHA and neoplasia, and the method of diagnosis of neoplasia was not described, yielding an IME value of 1.70.

5.2.2 | Solid tumors

A single case of pancreatic carcinoma[110] and a single case of anaplastic sarcoma[3] with giant cells in cats with IMHA were identified. Neither study showed a causal association between IMHA and neoplasia. The evidence for a causal association between IMHA and carcinoma, and sarcoma, was negligible, with an IME value of 1.70 in each case.

5.2.3 | Miscellaneous and minimally described neoplasia

One cat with IMHA and uncharacterized gastrointestinal neoplasia[51] and 6 cats with IMHA and uncharacterized masses[3] were identified. A causal association between IMHA and these lesions was not identified. The method of diagnosis for the presumed neoplastic lesions was not described. The evidence for a causal association between IMHA and uncharacterized gastrointestinal neoplasia is low, with an IME value of 3.92, and negligible for uncharacterized masses, with an IME value of 2.04.

Consensus Summary Statement

Currently, no strong evidence exists for a causal link between cancer and IMHA in cats; further studies are needed to determine if such an association exists. Nevertheless, retrospective evidence suggests a relatively high prevalence of concurrent cancer in cats with IMHA.

6 | INFLAMMATORY DISEASE

6.1 | Pancreatitis in dogs and cats

Inflammation that often occurs with IMHA could indirectly lead to pancreatitis by activation of neutrophils or formation of thromboemboli. Subsequent oxidative damage, ischemic events, or both then may directly damage the pancreas. Alternatively, inflammation associated with pancreatitis could lead to IMHA by indirectly inducing autoantibodies to form against erythrocytes. Autoantibodies that bind to epitopes on both exocrine pancreatic epithelium and erythrocytes also may be generated.[165] To date, none of these hypotheses has been confirmed in veterinary species. Observation of concurrent pancreatitis and IMHA has been reported in an 8-year-old female Cocker Spaniel[166] and in some retrospective studies of dogs with IMHA (Figure 8). Studies that evaluated groups of dogs with IMHA indicate that the prevalence of concurrent pancreatitis is low, between 1% (1/93 IMHA dogs[142]) and 5% (1/19 dogs[10]). Both of these studies yielded an IME value for pancreatitis of 1.70.[10,142] The Cocker Spaniel in the case report had both IMHA and pancreatitis.[166] However, cholestasis and renal failure also were present. Although cholestasis and renal failure can be complications of pancreatitis, primary organ disease could not be eliminated, precluding calculation of an IME value.

A recent study of 11 cats with IMHA showed that 3 of these cats (3/11; 27.3%) had pancreatitis,[165] yielding an IME value of 3.99. This study was designed to answer the question of whether IMHA is associated with pancreatitis.[165] Another large study of cats with IMHA indicated that 6/107 (5.6%) had concurrent cholangitis, pancreatitis, or both.[3] An IME value could not be calculated because information regarding the number of cats with pancreatitis alone was not specified.

Consensus Summary Statement

The evidence for pancreatitis causing IMHA is negligible in dogs and negligible to low in cats. Additional studies would be required to establish a causal relationship.

6.2 | Necrosis in dogs

One study attributed secondary IMHA to concurrent liver necrosis[12] and another to concurrent necrotizing inflammation of the tail,[2] both in single patients; both studies yielded an IME value of 1.70. Further evaluation of these patients was not pursued.

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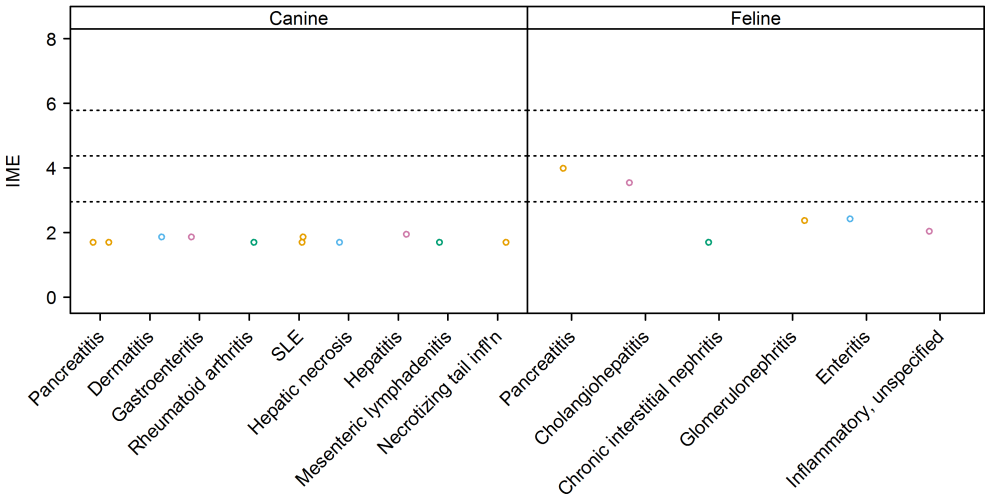

FIGURE 8 Integrated metric of evidence (IME) values for inflammatory diseases. Horizontal dotted lines indicate the threshold IME values between negligible and low (2.95), low and intermediate (4.37), and intermediate and high (5.78) levels of evidence. infl'n, inflammation; SLE, systemic lupus erythematosus

Consensus Summary Statement

The evidence for necrosis as a cause of IMHA is negligible in dogs and is not reported in cats.

6.3 | Other sources of inflammation in dogs and cats

Reports of other inflammatory processes were identified in several reports. Two studies reported 4 dogs with IMHA that were diagnosed with systemic lupus erythematosus.[2,167] Other reported inflammatory diseases in dogs with IMHA included 3 dogs with gastroenteritis, 2 dogs with dermatitis,[2] and 1 dog each with hepatitis,[143] rheumatoid arthritis,[10] and mesenteric lymphadenitis.[2] Negligible evidence was found for these inflammatory conditions inducing IMHA, with a median IME value of 1.70 and a range of 1.70-1.95. Seven of 107 (6.5%) cats with IMHA had clinical evidence of inflammation or infection that was not further classified, yielding an IME value of 2.04.[2]

Consensus Summary Statement

Anecdotal reports suggest that generalized inflammatory processes induce IMHA in dogs and cats, but direct evidence is lacking. Well-designed studies to determine whether non-infectious inflammatory processes cause IMHA are warranted.

7 | DRUGS AND TOXINS

7.1 | Dogs

Seventeen studies described dogs with IMHA that had been exposed to drugs or toxins,[1,10,12,27,34,80,116,127,135,142,143,160,168–172] but only 11 reported cases with sufficient primary data for the calculation of an IME value.[10,12,27,34,80,142,143,168–170,172] The majority of cases (35/36) were dogs exposed to antimicrobial drugs.[10,80,142,143,168,170] For these cases, IME values ranged from 1.70 to 7.09, with a median of 1.87 (Figure 9). The highest level of evidence, with an IME value of 7.09, came from 1 unblinded, randomized, prospective clinical trial in which 6 of 14 dogs given escalating doses of cefazedone acquired anti-erythrocyte antibodies.[168] The remaining reported cases were associated with low or negligible evidence to support other drugs or toxins as a cause for IMHA in dogs (Figure 9).

7.2 | Cats

Two papers describe the development of IMHA after administration of propylthiouracil to cats, with respective IME values of 7.33 and 4.19. In the first study of 105 cats, 7 cats with hyperthyroidism treated with propylthiouracil developed immune-mediated drug reactions.[173] This finding was followed by a prospective, un-blinded, non-randomized trial in which 17 healthy cats were given the same drug, causing 9 to develop Coombs' test-positive anemia.[174] One additional case report describes warfarin exposure in a cat with IMHA, with an IME value of 1.70.[110]

Consensus Summary Statement

The prevalence of drug-induced IMHA in dogs and cats is either rare or underreported. However, a lack of evidence does not preclude the possibility of a drug or toxin triggering IMHA.

8 | VACCINES

8.1 | Dogs

The most effective vaccines elicit robust immune responses only against the pathogen of interest. However, vaccines also may elicit unfavorable immune responses resulting from mechanisms such as molecular mimicry, bystander cell activation, or downregulation of self-tolerance, which contribute to autoimmunity.[175,176] For dogs, 32 papers mentioned that vaccines could be a trigger for IMHA, of which only 12 papers describe 79 clinical cases with documented temporal associations of ≤30 days between vaccine administration and IMHA.[5,22,80,107,116,143,170,177–181] The types of vaccines given to each

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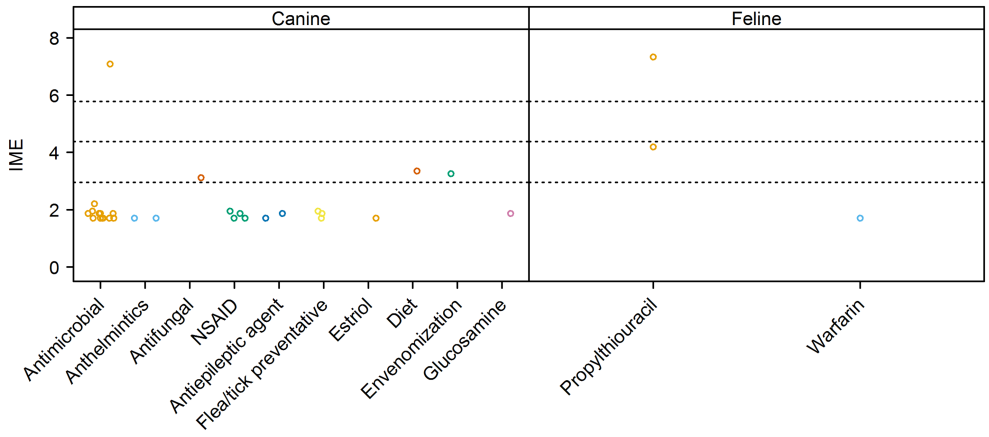

FIGURE 9 Integrated metric of evidence (IME) values for drugs. Horizontal dotted lines indicate the threshold IME values between negligible and low (2.95), low and intermediate (4.37), and intermediate and high (5.78) levels of evidence. The single antimicrobial drug yielding high-level evidence in dogs was cefazedone. NSAID, non-steroidal anti-inflammatory drug

patient were not consistently recorded. Seven papers provide negligible primary case data linking vaccination and IMHA.[22,80,116,143,170,180,181] Three papers provide evidence for a link between vaccination and IMHA in the low range, with IME values between 2.95 and 4.37.[5,177,178] No publications that provide high levels of evidence to support an association between vaccination and IMHA were found. Two studies reported intent to evaluate an association between vaccines and IMHA. In 1 retrospective study, associated with an IME value of 5.76, a difference was found in the frequency of IMHA cases diagnosed within the first month after vaccination and those diagnosed at subsequent months, whereas a similar temporal distribution was not identified in a control group.[179] A subsequent study had a similar Q score, but showed no difference in the number of cases with recent vaccination history between the IMHA and control groups, and therefore was awarded an IME value of 0.[107]

Other papers we reviewed excluded patients with a recent vaccination history, with the intent of describing only dogs with idiopathic IMHA.[182–185] In addition, some papers did not include the vaccination status of dogs with IMHA.[56,101,161,186,187] Other data we excluded from analysis included studies with an uncertain diagnosis of IMHA,[188,189] case studies in which alternative causes of IMHA were possible,[171,190] and cases from studies in which the timing of vaccination was not specified.[16] Only 1 study prospectively investigated a link between vaccination and autoimmune disease in 5 dogs, demonstrating the presence of autoantibodies after vaccine administration. However, these dogs only were followed for 21 days after vaccination and did not meet the criteria for diagnosis of IMHA.[191] With only 2 papers in the veterinary literature aiming to evaluate a link between vaccination and IMHA, and each of these respectively supporting[179] or refuting[107] an association, the question of whether vaccines trigger IMHA in dogs remains unanswered. Similarly, insufficient evidence is available to determine whether vaccination triggers autoimmune disease in people.[192] No reports of an association between vaccine administration and IMHA in cats were found.

Consensus Summary Statement

Considering the wide practice of vaccination and lack of conclusive evidence of an association with IMHA, current vaccination strategies generally are safe. Patients should be individually assessed for their own risks and benefits before vaccination. Further studies are needed to determine if and when vaccine-associated IMHA occurs in dogs and cats, and to develop better methods for the diagnosis of vaccine-associated disease.

9 | GLOBAL SCREENING RECOMMENDATIONS

9.1 | Optimal minimum database (dogs and cats)

9.1.1 | Consensus Summary Statement

A thorough history documenting vaccination, travel, exposure to fleas and ticks, flea and tick prevention, and heartworm testing and prevention is recommended. A thorough physical examination including retinal examination should be performed. Laboratory screening should include a CBC, blood film examination by a board-certified clinical pathologist (or equivalently trained hematologist), serum biochemical profile, and routine urinalysis. Urine culture and fecal flotation with centrifugation also should be considered. Abdominal radiographs are important to eliminate hemolysis caused by zinc toxicity. Imaging and other diagnostic tests to screen for cancer remain a reasonable component of a diagnostic evaluation for IMHA in dogs and cats, performed at the discretion of the attending clinician on the basis of the likelihood of cancer in the individual patient. Routine testing for pancreatitis in dogs and cats with IMHA is not recommended, unless clinical presentation suggests that it is a credible differential diagnosis.

9.1.2 | Rationale

History will help assess the likely risk of certain infections. A thorough physical examination and diagnostic imaging will help identify any potential nidus of infection or the presence of neoplastic lesions. Patterns of abnormalities identified on the CBC, serum biochemistry, and urinalysis can increase the index of suspicion for specific infectious agents that may be associated with IMHA.[193–202] This minimum database also can identify additional pathological processes (eg, proteinuria) that may require specific treatment. Although insensitive, blood

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smear examination can be useful in identifying the presence of vector-borne disease agents. Although the evidence associated with urinary tract infection as a cause of IMHA is negligible, identification and treatment of infection before immunosuppression is prudent. We refer the reader to the ACVIM consensus statement on the treatment of IMHA (in press) for further recommendations and additional discussion on the specific circumstance of treating subclinical bacteriuria in an immunosuppressed patient. The evidence that gastrointestinal parasites cause IMHA in dogs is low, but rapid resolution of IMHA with treatment and minimal immunosuppression has been described (Supporting Information S5).[121] Imaging will help identify neoplasia or a nidus of infection.

9.2 | Testing for infectious agents in dogs

9.2.1 | Consensus Summary Statement

Dogs with IMHA should be screened for infection with *Babesia* spp. using combined testing with serology and polymerase chain reaction (PCR). Repeat testing by means of PCR should be performed in all dogs originally testing negative but with a high risk of infection based on breed or exposure risk. The sensitivity of PCR and serological testing may vary depending on the laboratory and test design. Infection with other piroplasms, including *Rangelia* and *Theileria* species, should be eliminated in endemic areas. Because *D. immitis* infection is associated with anemia and positive Coombs' test results, all dogs should be screened for *D. immitis* in endemic areas or when travel to such areas has occurred. Further study to determine how and if other vector-borne disease agents cause IMHA is required before definitive screening recommendations can be made for additional organisms. However, screening for additional vector-borne pathogens, in particular *Anaplasma* spp., *Bartonella* spp., *Ehrlichia* spp., and, in endemic areas, *Leishmania* spp., should be strongly considered. Potential foci of other infections identified during initial screening should be further investigated at the discretion of the attending clinician.

9.2.2 | Rationale

The evidence that *Babesia* spp. induce IMHA is intermediate to high. Infection with *B. gibsoni* should be ruled out. Transmission of *B. gibsoni* in fighting breeds is through bite wounds and vertical transmission.[102,203] However, tick transmission by *Haemaphysalis* spp. and possibly *Rhipicephalus sanguineus* can occur, and experimental infection of mixed breed dogs results in IMHA.[99,204–208] Therefore, screening for *B. gibsoni* in all breeds with IMHA is prudent. *B. vogeli* should be ruled out in dogs with a history of exposure to *R. sanguineus*. Retired racing Greyhound dogs are at increased risk of infection because of the common occurrence of *R. sanguineus* infestations in racing kennels.[102,193,209] Testing for *B. canis* and *B. rossi* by means of serology and PCR should be performed in endemic areas. Dogs living in California and Coyote hunting dogs specifically should be screened for *B. conradae* by means of PCR (no serological test is available).[210,211] Although evidence of causation is lacking, Coombs' test-positive anemia is commonly documented in dogs with heartworm disease, bartonellosis, and leishmaniosis (Supporting Information S5).[105,109,145] General principles for optimal use of serology and PCR in diagnosing vector-borne disease are summarized in Supporting Information S7. Generally, combining PCR with serological testing enhances sensitivity.[212–214] Repeat testing, including repeating PCR on the same or additional samples, and pairwise serological testing to demonstrate a 4-fold change between acute and convalescent titers, also are necessary to document infection in many cases.[212–217]

9.3 | Testing for infectious agents in cats

9.3.1 | Consensus Summary Statement

Polymerase chain reaction testing for *B. felis* should be performed in cats from endemic areas and in those with suggestive clinical signs. Serological testing was not available at the time of writing, but combined testing would be optimal based on studies of *Babesia* species infecting dogs. Polymerase chain reaction testing for *M. haemofelis* should be performed in all cats with IMHA. Further studies are needed to determine whether infection with other hemotropic *Mycoplasma* species is associated with IMHA in immunosuppressed or coinfected cats. Testing for all 3 species is preferred when possible. All sick cats should be tested for FeLV and FIV infection, according to American Association of Feline Practitioners retrovirus management guidelines (https://www.catvets.com/guidelines/practice-guidelines/retrovirus-management-guidelines), screening all cats with IMHA for FeLV using antigen ELISA. Proviral FeLV DNA quantitative PCR testing may be helpful as a confirmatory test. Routine testing for feline coronavirus and non-hemotropic *Mycoplasma spp.* in cats with IMHA is not recommended, but appropriate diagnostic tests should be considered in cats with compatible clinical signs.

9.3.2 | Rationale

Identification of *Babesia* spp. by light microscopy of blood smears is considered insensitive for screening in cats. Polymerase chain reaction to identify parasitic DNA or RNA is recommended.[218] A high level of evidence was found that *M. haemofelis* causes IMHA in cats. Coinfection and host immune status may play a role in the development of IMHA in cats infected with the less pathogenic hemotropic *Mycoplasma spp.* In addition, coinfection with multiple hemotropic *Mycoplasma* species is common.[51,144] Therefore, infection with a less pathogenic species may signal that repeat testing for *M. haemofelis* is warranted. Non-hemotropic *Mycoplasma* infection only has been described in 1 cat with IMHA.[148] However, *M. pneumoniae* causes cold agglutinin hemolytic anemia in people, and infection with *Mycoplasma cynos* was associated with development of cold agglutinins in a dog.[135] Therefore, it should be considered as a possible trigger in cats with IMHA and other findings compatible with infection. The evidence for FeLV in association with IMHA in cats is negligible to intermediate. Polymerase chain reaction testing for proviral DNA could be considered as part of infectious disease screening. The overall evidence that FIP induces IMHA is negligible. However, given

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the immune mechanisms underlying effusive FIP, testing for FIP in cats with compatible clinical and laboratory findings is judicious.

9.4 | Drug and vaccine administration in dogs and cats

9.4.1 | Consensus Summary Statement

There is insufficient evidence to recommend withholding necessary medications for dogs and cats with IMHA. However, all medications, particularly those previously implicated in immune-mediated diseases, should be used with caution in patients with IMHA. Every patient should ideally have a complete history recorded, which includes all vaccines and drugs administered, the doses, dates, frequency, duration, and route of their administration, and information about the products being used such as manufacturer, indications, specific lot, and any adverse events. Exposure to toxins should also be documented in any dog or cat with IMHA.

9.4.2 | Rationale

Evidence for cefazedone in dogs[168] and propylthiouracil in cats[173,174] suggests that >1 class of drugs may be associated with IMHA in small animals. For most commonly prescribed medications, the evidence is negligible. Specific documentation of vaccine histories and long-term prospective studies may help determine whether vaccines can trigger IMHA. To date, approximately 8% of dogs with a diagnosis of IMHA and vaccination histories had been vaccinated within 30 days of IMHA diagnosis. However, studies comparing this prevalence to adequate controls are limited and inconclusive. Animals with IMHA are at risk for recurrence of anemia, making careful decisions about the risks and benefits of revaccinating important in every case. Animals receiving immunosuppressive treatment are less likely to mount protective immunity after routine vaccination.

10 | ICEBERG MODEL AND PROPOSED NEW NOMENCLATURE

Based on the data analyzed here, we propose a unified model for the pathogenesis of IMHA and a new system of nomenclature, in which the disease is categorized as "non-associative" and "associative" rather than "primary" and "secondary," respectively (Figure 10A,B). This clarification is needed because the word "primary" implies that all triggers have been definitively ruled out, whereas "secondary" implies causation. We propose that the term "associative" be used when a comorbidity is identified. In some cases, the comorbidity might have caused the IMHA (secondary IMHA), whereas in others it might be coincidental (primary IMHA). "Non-associative" IMHA cases are those in which comorbidities are not identified in the diagnostic evaluation, and include primary ("idiopathic") and cryptogenic cases. The latter implies that an underlying cause was not identified, perhaps because the underlying pathomechanisms are not currently understood, or the comorbidity could not be detected using available testing.

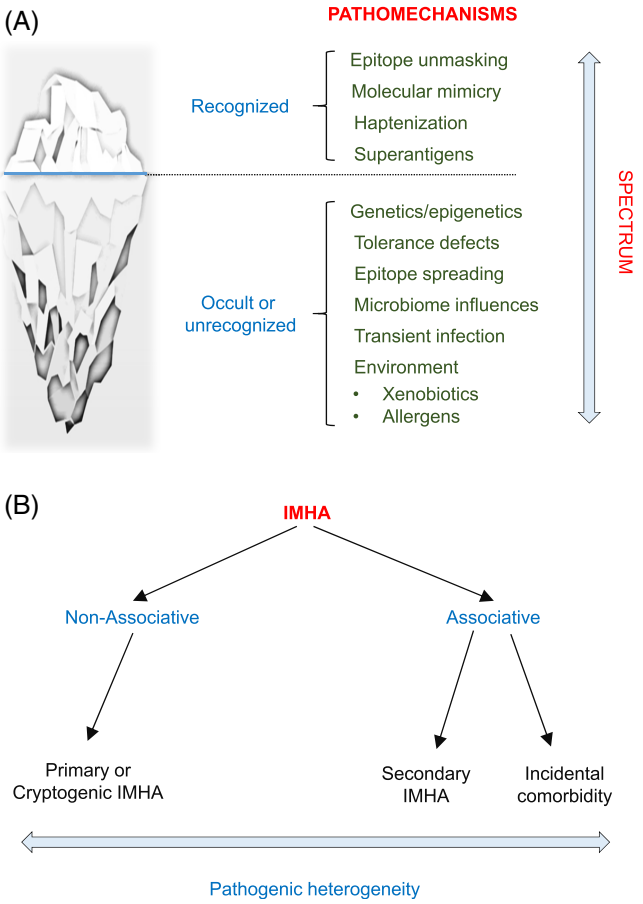

FIGURE 10 Iceberg model and proposed new nomenclature for immune-mediated hemolytic anemia (IMHA). A, The iceberg model posits that pathomechanisms underlying IMHA fall on a spectrum, both recognized (above the water level) and currently unrecognized or occult (concealed), the latter postulated to be the majority. Hypothetical occult pathomechanisms are listed. B, We propose a new nomenclature for IMHA to better reflect the heterogeneity in pathomechanisms underlying IMHA

11 | FUTURE RESEARCH DIRECTIONS

When the VCCIS task forces were formed, we began by identifying a focused question that represented an important problem in veterinary immunology, namely "What is the evidence that infection, neoplasia, drugs, vaccines, and other comorbidities cause IMHA in dogs and cats?" Our original intent was to perform a systematic review of the literature to answer this question. However, it quickly became apparent that very few studies in the veterinary literature were designed to determine if a given comorbidity causes IMHA, hence an expanded approach was used to evaluate the evidence presented in our review. There is a critical need for well-designed, prospective, case-controlled clinical studies that directly ask the question of whether infections, neoplasia, drugs, and vaccines cause IMHA. Some comorbidities are likely to cause IMHA in a large number of affected patients, such as an infection that expresses an epitope mimicking an erythrocyte antigen widely expressed in a population, or an organism that causes

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transient expression of normally hidden epitopes. Others might induce IMHA only in patients with epigenetic and genetic predisposition, or a given inflammatory context. Studies that investigate how individual comorbidities trigger IMHA, and the role of genetics and epigenetics, will help identify what diseases to screen for in all patients, and what diseases to screen for in selected patients that may be at increased risk of developing IMHA from any trigger. Mechanistic studies also will determine which comorbidities, when treated, will lead to resolution of IMHA without the need for immunosuppression. Stringent criteria for the diagnosis of IMHA and definitive diagnosis of a comorbidity must be integrated into study design in order to make meaningful observations.

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CONFLICT OF INTEREST DECLARATION

Jonathan Fogle has been paid by Merial for speaking engagements and continuing education. Linda Kidd has been a paid speaker for IDEXX and Zoetis and has occasionally consulted for IDEXX, Zoetis and Merck. All other authors had no conflicts of interest to declare.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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