Supporting Information 5: Overview of the evidence evaluation process for comorbidities
The goal of the comorbidity evidence evaluation is to:

1. Summarize the evidence that a given comorbidity causes ITP
2. Make screening recommendations based on that evidence.

You will be assigned approximately 30 papers from which to extract data for the evidence evaluation process. The process will be nearly identical to the methodology we used for the ACVIM Consensus Statement for the Diagnosis of IMHA which can be found here https://onlinelibrary.wiley.com/doi/pdfdirect/10.1111/jvim.15441. The evidence evaluation process will consist of the following steps:

1. **Extract data** from your assigned manuscripts into the Data Extraction Spreadsheet. Please see the excel spreadsheet attachment and instructions below. Each manuscript will be assigned to two evidence evaluators. The manuscripts will be made available to you by Erin Eldermire, using a manuscript sharing app called "Box".
2. The spreadsheet filled out by each evaluator will be compiled by Domain chairs into one spreadsheet. Any discrepancies in assignment of study design will be resolved by your Domain Chairs (Oliver Garden or Linda Kidd).
3. The completed spreadsheet will be turned over to the statistician, Dr. Ruby Chang. She will calculate and summarize the integrated metric of evidence (IME) values (see attachment and the ACVIM Consensus Statement for the Diagnosis of IMHA above for how IME is calculated) for each comorbidity.
4. Evaluators will be assigned to **write an evidence summary** based on the IME value and other information extracted in the spreadsheet (see example from the IMHA consensus below).
5. The evidence summary statements will be edited by Domain Chairs and sent out for Delphi review for consensus among evaluators.
6. Screening recommendations will be developed based on the evidence summaries and finalized by Delphi review.

During the process, from the articles you review, **please select one key reference ≥ 5 years old (a paper that you feel would almost invariably be cited by subsequent papers on the subject) and send that title to Andrew Mackin (Mackin@cvm.msstate.edu)** who will perform a quick search for more recent literature that has cited this manuscript. This is just a fail-safe mechanism to make sure we are not missing any important papers that we did not identify during our initial screening process.

As you evaluate your articles, **please peruse their references lists and if you find a useful reference that has been missed, please alert your Domain Chairs!** (This has occurred during some trial runs).

Additional information on filling out the Data Extraction Spreadsheet:
Assigning study design and extracting data on study quality:

Part of the IME value is the study design quality score "Q", and metrics associated with it (Columns entitled "General Quality Assessments". Descriptions of study designs can be found in the "study design guidelines" tab in the spreadsheet.

Important note: Assigning the study design can be tricky sometimes. The question as to whether a comorbidity causes ITP is most directly asked and answered by studies designed in the form of a PECO question

- P= population of dogs or cats
- E= exposure to associative disease, i.e. comorbidity
- C= comparison to a lack of exposure to comorbidity
- O= outcome, i.e. the development of ITP

Studies structured in this way are very straightforward in terms of the data extraction and evidence evaluation process. However, **many studies do not directly ask and answer this question as a part of their hypothesis, objectives, or the study design**. The first part of the evidence evaluation process in the spreadsheet is to assign study design. There are dropdown menus in the first columns of the spreadsheet that you can choose from. **If a study does not hypothesize that a comorbidity causes ITP or directly ask or answer whether a comorbidity causes ITP, but it does describe dogs or cats with a comorbidity and ITP somewhere in the results, then the study design is assigned "Descriptive Association"** category. Because the study design does not apply to our question in that instance, you can leave all of the General Quality Assessment columns (G-Q) blank. For example, see Shropshire et al "Validation of a clinically applicable flow cytometric assay for the detection of immunoglobulin associated platelets in dogs *Vet Immunol and Immunopath* 2018."This study's objective was to develop a flow cytometric assay for the detection of platelet-associated immunoglobulin. They hypothesized that the direct assay would be superior to the indirect assay. The study design has no relevance to our question. Yet, in the study the positive samples were prospectively enrolled client owned animals with ITP. One dog in that group had presumptive secondary ITP following a recent vaccination that resolved without long-term immunosuppressive therapy. Because the study design did not have to do with our question, the choice for study design would be "Descriptive association" and you can skip filling out the General Quality Assessment cells but extract the rest of the data. Note that a case report is a **case report** and not a descriptive association study if the authors *hypothesized* that a comorbidity caused ITP. We will review this more in the orientation meeting.

Extracting data for other scores and other information:
In addition to the Q (Quality) and Study Design score for each comorbidity, there are scores for the robustness of the diagnosis of the comorbidity, a causality score, a diagnosis of ITP score and a number of cases with comorbidity score that go into the IME calculation. For example, for infectious disease diagnosis, culture or PCR evidence (direct detection) receives a higher score than serologic testing, while "all other references to infection" receives the lowest score. Please note that the ITP diagnosis score will be derived from the ITP diagnostic algorithms the consensus panel developed (attached).

You can find the scale on how these are scored under the "minimum work-ups" tab the "Causality" score tab and the "ITP diagnosis" tab on the spreadsheet. There are also hyperlinks to these guidelines embedded within the column title cells for your reference.

- The data is extracted by category of comorbidity as you move horizontally across the spreadsheet. When you come across a paper with more than one co-morbidity (like two different infectious disease agents or some with neoplasia and others with infection) **please duplicate the row** in the spreadsheet and then extract the data so that they are separated by comorbidity.

- Please also note that for the inflammatory comorbidities we do not have a robustness of diagnosis scale developed for conditions other than sepsis. Please just enter the method of diagnosis free form and we will develop a way to score them post hoc.

- Columns BE-BI question the number of dogs with an infectious comorbidity and ITP. The most important column to fill out is BE, the number of patients with the comorbidity and ITP, which is used in the IME calculation. The rest may not be reported in the study and that is fine. If they are, we can use them to calculate relative risk and it can inform the overall evidence evaluation. There are similar cells for each comorbidity and the most important cell to fill out is highlighted in orange.

- Note that we have an additional non-PICO question that data will be extracted for and that is: What is the role of immunosuppression/is it necessary or harmful in the face of a reversible comorbidity? Does it vary with the infecting agent/comorbidity or individual patient? (Post hoc note to readers: Results of the non-PICO question are presented in the companion manuscript "ACVIM Consensus on Treatment of Immune Thrombocytopenia".)

- Finally there is the last column, which asks you to summarize your impression of the important take away points for the study.

Evidence Summary Instructions (after data extraction finalized and IME scores calculated)
For your assigned comorbidities please write a brief evidence summary narrative and a more concise summary statement for your comorbidity using the format detailed below. Then, please write a very brief (one to two sentence) statement regarding screening recommendations for patients with ITP for the comorbidity following the format described below. The concise summary statements and the compiled screening recommendations will be edited by the domain chairs and sent out for Delphi review to all evidence evaluators and the consensus panel. We hope to get at least one round of Delphi prior to the Forum (those go quickly), therefore we ask return these by 9 am pacific Tuesday June 14th if at all possible. If you cannot complete yours before then please let us know and prioritize those you feel are most important/have the most evidence for causing ITP or perhaps those that had an unexpectedly low level of evidence first, i.e. the ones that are most interesting to you and clinically relevant.

As a reminder, our PECO question for comorbidities is:

- P= population of dogs or cats
- E= exposure to associative disease, i.e. comorbidity
- C= comparison to a lack of exposure to comorbidity
- O= outcome, i.e. the development of ITP

e.g. In dogs, what is the effect of infection with *E. canis* compared to a lack infection with *E. canis*, on the development of ITP?

We also have extracted data for the non-pico question of "What is the role of immunosuppression/is it necessary or harmful in the face of a reversible comorbidity? Does it vary with the infecting agent/comorbidity or individual patient?." This question will be addressed after the forum we are not addressing it now.

Attached to this email please find:

1. The compiled, consolidated data extraction spreadsheet. The nonhighlighted entries are those for which IME values could be calculated. The IME scores and overall value are included in columns GL-GS. The pink entries do not have IME scores but may have valuable information for your narratives.
2. Descriptive statistics sheet. This contains the IME values showing the median, range and the number of studies documenting negligible (T0), low(T1), intermediate (T2) and high (T3) levels of evidence as a cause for ITP. Note the IME "N" here is referring to the number of studies receiving and IME value for that comorbidity.
3. A description of how IME scores and the overall IME value is calculated.
4. Graphs depicting the IME values for each comorbidity group and comorbidity.
5. The IMHA consensus on the diagnosis of IMHA for reference

The process:

1. Write the evidence narrative.

To write the evidence narratives examine/search/sort the data extraction spreadsheet for the organism/comorbidity.
Manuscripts for which an IME value could be calculated have been consolidated into one set of scores and any differences between reviewers were reconciled by a domain chair. Comments regarding the reconciliation in column GK. For the non-IME papers, each evidence evaluator's entry has been left separate for your information.

There will be manuscripts where not all of the components of the IME value could be extracted. For example, if the number of dogs (n) meeting our criteria (algorithm) for ITP could not be discerned it did not receive an IME value. Some of these manuscripts had a nonquantifiable but high level of evidence however, and are worthy of highlighting in the evidence summaries. For example, see Trepanier 2003 where an ITP score could not be calculated based on the way the data was presented but it is still a very informative manuscript.

The general flow of the evidence summary paragraph should begin with the number of studies documenting the comorbidity in the data extraction spreadsheet (those that met the studies inclusion criteria). NOTE there are a few theses and review articles in the spreadsheet, these should NOT be included in the overall "n" as they do not meet our inclusion criteria, but they may be informative to you for background information.
Then state the range and median of IME values for the comorbidity as a whole. Percent (n/total number of studies) IME values falling into negligible, low, intermediate and high levels of evidence based on IME values and quartiles listed in the descriptive statistics spreadsheet follow. Next, the number of studies where an IME could not be calculated is mentioned. Then a narrative summarizing your impression of the overall evidence based on both the IME values and the studies where IME value could not be calculated. The Evaluator Conclusions column GJ will be very helpful here. If applicable, summarizing detail of studies that documented immunologic confirmation or studies exploring mechanisms follow the IME value summary. For infectious diseases, summarizing IME values and evidence by species of organism would be included here.

2. Write the consensus summary statement to be used in the Delphi.

For the Consensus summary statement create a very brief summary statement encompassing the body of evidence you reviewed for the comorbidity.

Overall the evidence that [Comorbidity X] causes ITP in [population studied] is [negligible/low/intermediate/high] and if appropriate, a brief summary of additional details on species or mechanism or other important conclusions. One for dogs and one for cats please.

3. Write draft screening recommendations for the comorbidity.

For screening recommendations please use the following format:

- IN [RELEVANT POPULATION] SCREENING FOR [COMORBIDITY} IS [RECOMMENDED / SHOULD BE CONSIDERED / CAN BE CONSIDERED / IS NOT RECOMMENDED]

Example Evidence Summary from ACVIM Consensus on the Diagnosis of IMHA

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 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 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.