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ORIGINAL RESEARCH

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Prevalence of interfering antibodies in dogs and cats evaluated using a species-independent assay

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Background: Interfering antibodies in human serum and plasma are known to react with mammalian antibodies in immunoassays and cause false-positive test results. Although this phenomenon was recently shown in companion animals, knowledge regarding immunoassay interference in veterinary medicine is very limited.

Objectives: The aims of this study were to set up a species-independent immunoassay procedure to detect interference in serum samples, to screen for interference in a cross-section of canine and feline patient samples from an animal hospital, and to determine if the detected interference could be neutralized using an immunoassay based on nonmammalian reagents.

Methods: A 2-site sandwich-type interference assay was set up using commercially available mouse reagents. A total of 369 serum samples from 320 dogs and 263 samples from 218 cats were analyzed using the interference assay. Multiple samples were submitted from 36 dogs and 39 cats. Nineteen samples identified as interference-positive were analyzed in an assay using chicken antibodies.

Results: Interference was detected in samples from 28 dogs (9%) and 10 cats (5%) screened with the interference assay. Except for 1 cat, consistent results were obtained for all 75 dogs and cats that submitted more than 1 sample. The interference was eliminated when analyzed in the chicken-based assay ($P < .001$).

Conclusions: Substances with reactivity toward mouse IgG can be detected in serum samples from dog and cat patients using a 2-site interference assay. The detected substances are most likely interfering antibodies, possibly originating from immunization with other mammalian species.

KEYWORDS
ELISA, heterophilic antibodies, immunoassay, interference, rheumatoid factors

1 | INTRODUCTION

Immunology-based laboratory tests, such as endocrinology tests, are widely used in veterinary clinical practice. Laboratory tests are vulnerable to many types of interferences, and immunoassays are no exception.[1] In addition to visually detectable interference from sample hemolysis, lipemia, and bilirubinemia,[2,3] for instance, immunoassays could be affected by interfering antibodies in serum and plasma. Such antibodies commonly affect sandwich ELISAs by cross-linking the assay capture and detection antibodies, leading to increased signals. In the worst-case scenario, the outcome is the misinterpretation of test results and misdiagnosis, as has been reported in human medicine.[4,5]

A distinction is often made between interfering antibodies of iatrogenic and noniatrogenic origin. In people, iatrogenic interfering antibodies stem from in vivo diagnostic or therapeutic treatments with monoclonal mouse antibodies. Noniatrogenic interfering antibodies include heterophilic antibodies and rheumatoid factors that occasionally cross-react with assay antibodies.[6,7] Heterophilic antibodies are found in people with no history of in vivo antibody treatments, and rheumatoid factors are autoantibodies, usually of the IgM class.

Although most research on immunoassay interference involves human medicine studies, a few recent studies have shown that interfering antibodies in canine serum affected BNP (B-type natriuretic peptide), and possibly AMH (anti-Müllerian hormone) assay results.[8,9] Despite the potentially harmful impact on patient care, knowledge about antibody interference within veterinary medicine remains very limited. If the situation is comparable to that in human medicine, antibody interference might be a problem in veterinary medicine as well. Screening studies have shown that interfering antibodies can perturb the testing of several analytes,[10,11] and recent case reports of human antibody interference in multiple hormone assays have been published.[12-15] To screen a population for interfering antibodies, a method that is customized to detect antibody interference is needed. Often a 2-site immunoassay is used, with a combination of capture and detection antibodies that do not cross-link with any known substance so that only molecules with irregular cross-linking abilities (ie, interfering antibodies) can generate a signal.[7]

There are several ways of eliminating interfering antibodies in a sample. Assay manufacturers often incorporate mammalian immunoglobulin in the sample incubation buffer, attempting to neutralize anti-mammalian antibodies. Another option is to base the assay on reagents that interfering antibodies do not react with, such as chicken IgY, which shows no cross-reactivity with mammalian IgG[16] and has been shown to eliminate the effects of iatrogenic and noniatrogenic interfering antibodies in human samples.[16,17]

The aims of this study were to set up a species-independent interference assay for detecting interfering antibodies, to use it to screen a population of dogs and cats visiting an animal hospital, and to evaluate if the interference can be eliminated by using an assay based on chicken reagents.

2 | MATERIALS AND METHODS

2.1 | Animals

The serum used in this study was collected from dogs and cats admitted to the University Animal Hospital in Uppsala, Sweden. Written consent was obtained from owners of all animals, and the study was performed in accordance with national animal welfare regulations (SJVFS 2015:38). Serum was frozen at −20°C for up to 6 months until analysis. All samples were thawed at room temperature (RT) and vortex-mixed before analysis. Exclusion criteria were clearly visible signs of hemolysis, bilirubinemia, or lipemia. No inclusion criteria were applied.

2.2 | Interference assay

Flat-bottom, 96-well polystyrene microtiter plates (446612; Thermo Fisher, Hemel Hempstead, Hertfordshire, UK) were coated overnight at 4°C with 2 μg/mL purified mouse IgG (I5381; Sigma Aldrich, St. Louis, MO, USA) diluted in 0.05 M $NaHCO_3$, pH 9.6. Coated plates not used on the following day were stored bottom-up for a maximum of 1 month at −20°C until use. The plates were washed 3 times with 200 μL 0.05% Tween-20 in PBS (phosphate-buffered saline, 0.01 M phosphate buffer, 0.137 M NaCl, 0.0027 M KCl, pH 7.4). Serum samples, standards, and positive and negative controls were analyzed in duplicate and incubated for 90 minutes at RT.

After another 3 washes, the plates were incubated with a 1:10, 000 dilution of monoclonal HRP (horseradish peroxidase)-conjugated mouse anti-human carcinoembryonic antigen (CEA) IgG (MBS592181 Mybiosource, San Diego, CA, USA) for 90 minutes, washed again, and finally incubated with 3,3′,5,5′-tetramethylbenzidine (TMB) (T8665; Sigma Aldrich) for approximately 8 minutes in the dark before the reaction was stopped with 100 μL H_2SO_4 (S5814; Sigma Aldrich). The optical density (OD) was spectrophotometrically measured at 450 nm in a Multiskan EX plate reader (model number 355; Thermo Fisher). Plates were manually checked for dust, air bubbles, or other particles that can lead to light scattering prior to reading. All incubations were made with 50 μl volumes except for the TMB, of which 100 μL was added.

The samples were assayed undiluted. Chicken anti-mouse IgG[18] (Immunsystem AB, Uppsala, Sweden) was used as the standard with PBS dilutions starting at 1:1600 of the stock solution and then serially diluted 2-fold to a final number of 7 dilutions. Two wells on each plate were incubated with PBS and a detection antibody, representing the 0-concentration of the standard. Samples that had tested negative during trial runs, consisting of checkerboard titrations, and patient samples not subsequently included in the screening runs were pooled and used as negative controls. The chicken anti-mouse IgG was diluted 1:1600 in the pooled negative control sera and this solution represented the positive control. Appropriate dilutions for reagents were determined by checkerboard titration, where an OD of approximately 2.0, corresponding to the highest point of the standard curve, was desired.[19]

2.3 | Interference elimination

Nineteen interference-positive samples with sufficient volume (from 15 dogs and 3 cats, with 2 samples from 1 cat collected on different occasions) were selected. A 96-well microtiter plate was coated, 1 column at a time, with 2 μg/mL of purified mouse IgG diluted in 0.05 M $NaHCO_3$ or with 2 μg/mL nonimmunized chicken IgY (Immunsystem AB) diluted in 0.01 M PBS. Only 1 plate was used, in which column 1 was coated with mouse IgG, column 2 with chicken IgY, column 3 with mouse IgG, and so on. The samples were added row by row: 50 μL of the sample to the mouse IgG-coated well in column 1 and 50 μL to the adjacent chicken IgY-coated well in column 2, until all samples were allowed to react with the mouse IgG and chicken IgY, respectively. The rest of the protocol was identical to the one described above.

2.4 | Statistical analysis

All statistical analyses were performed using the R Software version 3.3.3 (R Core Team, Vienna, Austria).

Differences in prevalence, sex, breed, neutering status, and diagnosis between the interference-positive and interference-negative groups (for both dogs and cats) were tested using a 2-sample test for equality of proportions (2-tailed). Differences in age between positive and negative groups were tested with the Wilcoxon rank-sum test because the age data were not normally distributed.

In the interference elimination study, the mouse IgG and chicken IgY-assayed samples were compared using the Wilcoxon signed-rank test. Statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Dogs

The screening included 369 samples from 320 individual dogs. Multiple samples were assayed for 42 of the dogs; 36 of them twice, 5 of them 3 times, and 1 of them 4 times. All multiple samples were collected on different occasions. Ninety-six different breeds were represented. The median age of the dogs was 6.5 years, interquartile range (IQR) 3-9 years. There were 130 intact males, 37 castrated males, 107 intact females, and 46 spayed females. The dogs were allocated into 13 different categories based on diagnosis or reason for admittance to the hospital (Table 1).

Of the included dogs, 77 (24.1%) were treated with immunosuppressant medication such as glucocorticoids (n = 74), ciclosporin (n = 2), or mycophenolic acid (n = 1) at the time of sampling or within 1 month prior to sampling. Five of the dogs received blood transfusions at some point prior to sampling.

3.2 | Cats

We assayed 263 samples from 218 cats of 17 different breeds. Multiple samples were assayed for 39 of the cats; 33 of them twice and 6 of them 3 times. All multiple samples were collected on different occasions. The median age was 7 years, IQR 3-12 years. There were 27 intact males, 108 castrated males, 20 intact females, and 63

TABLE 1 Dogs screened for interfering antibodies by disease category

	Count % of total in category			
Disease category	Positive (n = 28)	Negative (n = 292)	Overall (n = 320)	Treated with immunosuppressants (n = 77)
Digestive disorder	7 8.1%	79 91.9%	86	21 24.4%
Bone, muscle, and joint disorder	5 11.9%	37 88.1%	42	3 7.1%
Neoplastic disease	4 12.5%	28 87.5%	32	4 12.5%
Skin/claw/eye/ear disorder	1 3.1%	31 96.9%	32	13 40.6%
Reproductive disorder	0 –	22 100%	22	3 13.6%
Renal/urinary disorder	4 19%	17 81%	21	4 19%
Neurologic disorder	1 5.6%	17 94.4%	18	1 5.6%
Respiratory disorder	2 12.5%	14 87.5%	16	4 25%
Intoxication	2 15.4%	11 84.6%	13	0 –
Endocrine disorder	1 11.1%	8 88.9%	9	7 77.7%
Systemic autoimmune disease	0 –	9 100%	9	9 100%
Cardiovascular disorder	0 –	8 100%	8	4 50%
Other disorders[a]	1 8.3%	11 91.7%	12	4 33.3%

Disease categories are sorted in descending order according to number of dogs. Immunosuppressants include glucocorticoids (n = 74), ciclosporin (n = 2), and mycophenolic acid (n = 1).

[a]Clinical signs not clearly related to a specific organ (eg, "lethargy" or "inappetence").

spayed females. The diagnostic classification rationale was the same as for dogs, but there were no cats diagnosed with cardiovascular disorders or systemic autoimmune disease, resulting in 11 final categories (Table 2). Twenty-six of the included cats (11.9%) were treated with glucocorticoids at the time of sampling or within 1 month prior to sampling.

3.3 | Interference ELISA

Using a 4-parameter logistic curve-fitting model (4PL), a standard curve was obtained (Figure 1). The cutoff point was determined by calculating the mean OD of the duplicates of the seventh point on the standard curve. It thus corresponds to a low, reproducible signal originating from interfering antibody reactivity. For each run, the cutoff point was required to be greater than the assay LoD (limit of detection), as determined by the formula LoD = 0-calibrator + 2 SD (where 25 repeat measurements of the 0-calibrator were made). The inter-assay CV for the cutoff point was 9.1%. Acceptable intra-assay CV for all controls, calibrators, and samples was set to <20%.[20]

A relative OD value for each sample was calculated by dividing the mean OD of the sample by the cutoff value. The cutoff limit for a positive result was >1 for all runs. Similarly, the positive control was required to be >1 and the negative control <1 in each run.

3.4 | Interfering antibodies in dogs and cats

In total, 9% of the dogs (28/320) and 5% of the cats (10/218) were interference-positive. There was no significant difference in the prevalence of interference between dogs and cats ($P = .06$). The relative OD for positive samples ranged between 1.005 and 2.019 for the dog cohort and between 1.049 and 2.081 for the cat cohort (Figure 2).

The median age of interference-positive dogs was 7 years, IQR 3.75-9 years. There were 11 intact males, 3 neutered males, 10 intact females, and 4 spayed females of 24 different breeds. A 5-year-old male Lagotto Romagnolo hospitalized for kidney failure that was sampled twice, 13 days apart, was positive for interference on both occasions. Seven of the interference-positive dogs (24.1%) were treated with glucocorticoids within a month prior to sampling. All 5 recipients of blood transfusions were interference-negative.

The median age of interference-positive cats was 8.5 years, IQR 6.5-10.5 years. There were 6 neutered males, 1 intact female, and 3 spayed females of 5 different breeds: Domestic Shorthair (n = 5), Domestic Longhair (n = 2), and 1 each of Burmese, Norwegian Forest cat, and Ragdoll. An 8-year-old spayed female Domestic Shorthair with acute cystitis was sampled 3 times over a span of 8 days, all samples being interference-positive. An 8-year-old spayed female Norwegian Forest cat admitted to the animal hospital for

TABLE 2 Cats screened for interfering antibodies by disease category

	Count % of total in category			
Disease category	Positive (n = 10)	Negative (n = 208)	Overall (n = 218)	Treated with immunosuppressants (n = 26)
Digestive disorder	4 6.6%	57 93.4%	61	7 11.5%
Renal/urinary disorder	1 2.4%	40 97.6%	41	0 –
Endocrine disorder	1 4.3%	22 95.7%	23	3 13%
Skin/claw/eye/ear disorder	0 –	21 100%	21	4 19%
Respiratory disorder	1 5.9%	16 94.1%	17	7 41.2%
Bone, muscle, and joint disorder	0 –	10 100%	10	0 –
Neoplastic disease	1 9.1%	10 90.9%	11	2 18.1%
Neurologic disorder	0 –	10 100%	10	2 20%
Reproductive disorder	1 25%	3 75%	4	0 –
Intoxication	0 –	3 100%	3	0 –
Other disorders[a]	1 5.9%	16 94.1%	17	1 5.9%

Disease categories are sorted in descending order according to number of cats. Immunosuppressants include glucocorticoids (n = 26).
[a]Clinical signs not clearly related to a specific organ (eg, "lethargy" or "inappetence").

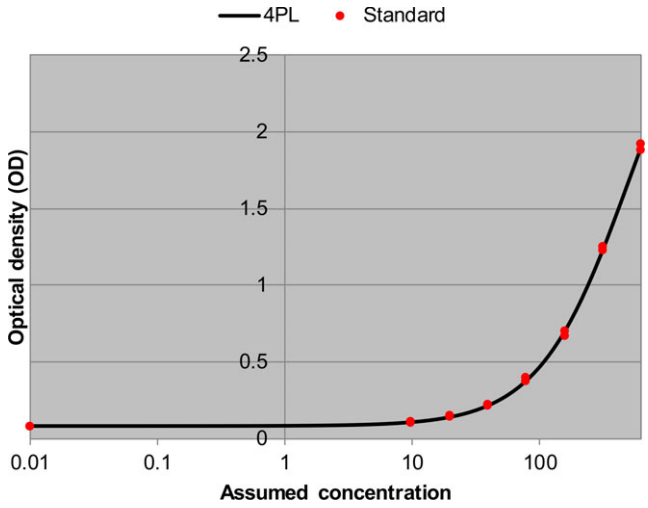

FIGURE 1 A representative standard curve from one of the ELISA runs in the screening experiment. The *x*-axis represents an assumed concentration of the standard. Point 7 corresponds to the positive cutoff level. Points 1–6 were included to ensure that the assay consistently measured interference of varying degrees. The curve assumes a 1 mg/mL concentration of anti-mouse IgG in the stock solution. The 0-concentration of the standard has been entered as 0.01 to fit on the curve. 4PL, 4-point logistic regression

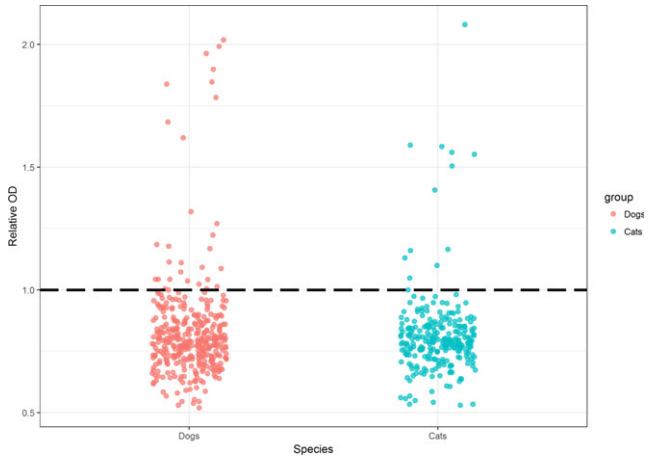

FIGURE 2 Dog (n = 369) and cat (n = 263) samples were screened with a 2-step sandwich ELISA using nonimmunized mouse IgG as the capture antibody. An HRP-conjugated mouse anti-CEA antibody was used as the detection antibody. Cutoff level is indicated by the dashed horizontal line. The results are presented on a relative scale and calculated according to the formula: (mean sample OD)/(mean cutoff OD). There was no significant difference in prevalence of interference between dogs and cats ($P = .06$)

constipation and investigation of hepatomegaly was positive for interference on the first visit, but negative on a scheduled follow-up visit 5 months later. Two of the interference-positive cats (20%) were treated with glucocorticoids within a month prior to sampling.

There was no significant difference in age, sex, breed, neutering status, or diagnostic category between interference-positive and interference-negative dogs and cats.

3.5 | Interference elimination

Wells coated with chicken IgY produced significantly less signal ($P < .001$) compared with those coated with mouse IgG. When compared to the cutoff point, all 19 assayed samples were negative with chicken IgY as the capture antibody. Five samples also tested negative when mouse IgG was used for coating, despite previously testing positive in the screening experiment (Figure 3).

4 | DISCUSSION

We present an in-house species-independent ELISA for detecting interfering antibodies based on commercially available reagents. The method used in this study is based on the principle that the anti-IgG antibody in the samples forms a bridge between the capture and detection antibodies. An anti-human CEA antibody was selected for detection because it does not match the specificity of the capture antibody and because conjugated anti-CEA antibodies are widely available and maintain their reactivity well over time, making the experiment easily repeatable.

The interference ELISA was used to screen serum samples from dogs and cats visiting a university animal hospital. The prevalence of interfering antibodies in this population was 9% for dogs and 5% for cats. This is the first published interference screening of samples from animals. There are several studies on the prevalence of interfering antibodies in people using different methods and study populations and with varying results (numbers range from <1% to 80%).[21-23] It is important to note that the presence of interfering antibodies in a sample does not automatically lead to interference in an immunoassay used for clinical diagnosis. Immunoassay interference depends on many factors, including host species of the immunoassay antibodies and species-specific reactivity of the interference-causing antibodies in the samples. The multifactorial nature of immunoassay interference is one of the reasons for comparing the OD of the samples in the present study rather than the concentration. Furthermore, because interfering antibodies are a heterogeneous mixture of different antibody classes and subclasses with varying properties,[24] there is no universal calibrator for unknown samples.

Interference in the present study was most likely caused by interfering antibodies. Samples with visible signs of hemolysis, lipemia, and bilirubinemia—all known sources of "matrix effects" in assays[2,3]—were excluded at a preanalytical stage. Freezing the samples at −20°C inactivates the complement system contained within the sample and eliminates interference by complement activation.[25] A considerable number of dogs and cats in the screening experiment (75 in total) were sampled (and assayed) on multiple occasions. With one exception, the test results were consistent. Because the samples from the only test subject with inconsistent results (a Norwegian Forest cat) were collected 5 months apart, this finding is consistent with the reported transience of heterophilic antibodies.[26] The high degree of repeatability suggests that various potential error sources

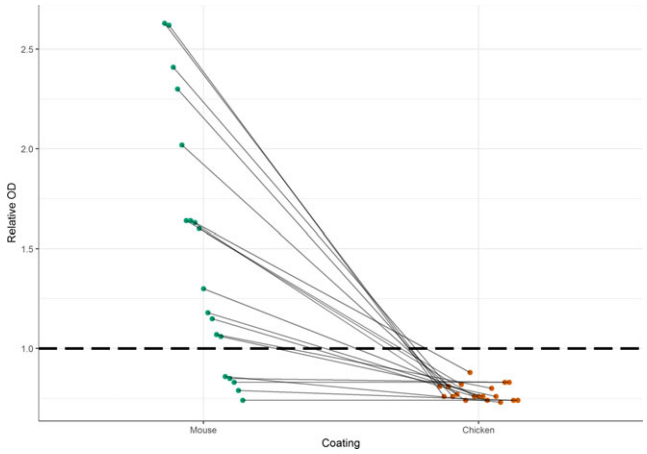

FIGURE 3 Reactivity against nonimmunized mouse IgG and chicken IgY using an HRP-conjugated mouse anti-CEA antibody for detection. Positive cutoff level is indicated by the dashed horizontal line. The results are presented as: (mean sample OD)/(mean cutoff OD)

such as environmental factors in the laboratory and operator errors did not influence the results significantly. However, there was no statistical analysis performed on this data because of lack of statistical power (only 3 positive subjects contributed multiple samples).

The strongest indication that the interference was caused by interfering antibodies comes from the results of the elimination experiment. We were able to demonstrate a significant decrease in signal and elimination of reactivity in all 19 samples studied by switching the capture antibody from mouse IgG to chicken IgY. Using chicken IgY as the capture antibody in a 2-step sandwich ELISA has previously been shown to eliminate antibody interference in human serum.[16,17] The biological explanation is that mammalian and avian species are phylogenetically different and their antibodies do not cross-react.[27] Because interference elimination using avian antibodies specifically targets interfering antibodies, the results indicate (1) the presence of anti-mammalian antibodies with reactivity toward mouse IgG in the interference-positive samples and (2) that chicken IgY can be used to eliminate interference in nonhuman specimens as well.

Five of the 19 samples previously identified as interference-positive were not positive when assayed against mouse IgG in the elimination experiment. Varying results upon repeated testing are sometimes observed in samples with heterophilic antibodies, and may even increase the suspicion of interference.[7] Heterophilic antibodies are generally of low affinity, which implies a low association rate (long time to bind). Considering this, a longer sample incubation time than 90 minutes might be needed to allow for the completion of antigen-antibody binding and the stabilization of results. However, the sample incubation time was selected to reflect the incubation times used in commercial assay protocols, which are generally 1-2 hours. Second, it has been suggested that heterophilic antibodies compensate for low affinity by binding to stacked capture antibodies.[28] The stacking creates a tight space in multiple binding sites, which enhances binding and increases avidity. If the stacking is a prerequisite for optimal heterophilic antibody binding conditions, they are likely to be prone to high CV, because the physical orientation of capture antibodies and formation of stacks can be assumed to be unique for each microtiter well. Multiple runs and a high CV re-assaying routine in the elimination experiment would probably be a good strategy for dealing with this problem but would require more sample material than available.

The interfering antibodies in the positive samples are most likely to be of noniatrogenic origin. Iatrogenic antibodies are unlikely to be present because the administration of monoclonal antibody therapy in veterinary practice is rare. However, dogs and cats share environments and lifestyles with human beings, and thus they also share routes believed to induce heterophilic antibodies. These routes include direct physical contact with other species as well as indirect contact via vaccinations and food ingestion.[29,30] The interference assay tested reactivity toward mouse IgG, but it is possible that the test-positive subjects had been immunized by other species because cross-reactivity between IgG from different mammalian species is known to happen.[31,32] We reviewed journal data of the screened dogs and cats to identify possible risk factors for interference, but we found no significant correlation with parameters such as age, sex, breed, neutering status, and diagnosis. These results are consistent with the unknown and essentially "random" etiology of heterophilic antibodies. This argument is based on the assumption that the mechanism behind heterophilic antibody formation does not differ between species.

It is also possible that rheumatoid factors account for some of the detected interference. Age, gender, and autoimmunity are suggested risk factors for rheumatoid factor interference in people[10] due to the overrepresentation of women,[33] the increasing prevalence of rheumatoid arthritis with age,[33,34] and the high prevalence of rheumatoid factors in patients with rheumatoid arthritis (estimated to be 70%). However, rheumatoid factors can also be found in approximately 5%-10% of the general human population.[35] Rheumatoid factor testing has sometimes been used for diagnosing rheumatoid arthritis in dogs, but prevalence data are uncertain because of lack of test standardization.[36] Immune-mediated joint disorders are rare in cats, but rheumatoid factor tests have mostly been positive in reports of cats with rheumatoid-like arthritis.[37,38]

The fact that we saw no association between interference and known risk factors might be due to low statistical power. Only 9 dogs (all of which tested negative for interference) were classified with autoimmune disease, and all were treated with glucocorticoids. Because glucocorticoid administration has been shown to cause overall immunoglobulin deficiency,[39,40] the treatments might also affect the risk of antibody-induced interference. However, dogs and cats treated with immunosuppressant medication were not underrepresented among the interference-positive test subjects. Unfortunately, we were not able to account for the dose and duration of treatment because this information was not available in all cases. Finally, because we do not have access to a full medical history for all dogs and cats, there might be chronic underlying diseases present

in addition to the ones registered. This raises the possibility of a potential correlation between autoimmune disease and interference going undetected.

5 | CONCLUSION

Using a species-independent 2-site interference assay, we detected interference in serum samples from 9% of dogs and 5% of cats in a cohort of patients admitted to an animal hospital. The interference was probably caused by interfering antibodies in the samples, most likely heterophilic antibodies or rheumatoid factors. Switching the capture antibody from mouse IgG to chicken IgY eliminated interference in all samples tested, indicating that the interfering antibodies may have originated from direct or indirect contact with mice or other mammalian species.

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REFERENCES

1. Kroll MH, Elin RJ. Interference with clinical laboratory analyses. *Clin Chem*. 1994;40:1996-2005.
2. Weber TH, Kapyaho KI, Tanner P. Endogenous interference in immunoassays in clinical chemistry. A review. *Scand J Clin Lab Invest Suppl*. 1990;201:77-82.
3. Findlay JWA, Smith WC, Lee JW, et al. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. *J Pharm Biomed Anal*. 2000;21:1249-1273.
4. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. *Lancet*. 2000;355:712-715.
5. Ghosh S, Howlett M, Boag D, Malik I, Collier A. Interference in free thyroxine immunoassay. *Eur J Intern Med*. 2008;19:221-222.
6. Kricka LJ. Human anti-animal antibody interferences in immunological assays. *Clin Chem*. 1999;45:942-956.
7. Bolstad N, Warren DJ, Nustad K. Heterophilic antibody interference in immunometric assays. *Best Pract Res Cl En*. 2013;27:647-661.
8. Holst BS. Diagnostic possibilities from a serum sample-Clinical value of new methods within small animal reproduction, with focus on anti-Mullerian hormone. *Reprod Domest Anim*. 2017;52:303-309.
9. Solter PF, Oyama MA, Sisson DD. Canine heterophilic antibodies as a source of false-positive B-type natriuretic peptide sandwich ELISA results. *Vet Clin Pathol*. 2008;37:86-95.
10. Bjerner J, Nustad K, Norum LF, Olsen KH, Bormer OP. Immunometric assay interference: incidence and prevention. *Clin Chem*. 2002;48:613-621.
11. Bolstad N, Warren DJ, Bjerner J, et al. Heterophilic antibody interference in commercial immunoassays; a screening study using paired native and pre-blocked sera. *Clin Chem Lab Med*. 2012;50:409-409.
12. Censi S, Cavedon E, Fernando SW. Calcitonin measurement and immunoassay interference: a case report and literature review. *Clin Chem Lab Med*. 2016;54:1861-1870.
13. Mongolu S, Armston AE, Mozley E, Nasruddin A. Heterophilic antibody interference affecting multiple hormone assays: is it due to rheumatoid factor? *Scand J Clin Lab Invest*. 2016;76:240-242.
14. García-González E, Aramendía M, Álvarez-Ballano D, Trincado P, Rello L. Serum sample containing endogenous antibodies interfering with multiple hormone immunoassays. Laboratory strategies to detect interference. *Pract Lab Med*. 2016;4:1-10.
15. Revet I, Boesten LSM, Linthorst J, et al. Misleading FT4 measurement: assay-dependent antibody interference. *Biochem Med*. 2016;26:436-443.
16. Larsson A, Mellstedt H. Chicken antibodies – a tool to avoid interference by human anti-mouse antibodies in ELISA after invivo treatment with murine monoclonal-antibodies. *Hybridoma*. 1992;11:33-39.
17. Larsson A, Karlsson-Parra A, Sjoquist J. Use of chicken antibodies in enzyme immunoassays to avoid interference by rheumatoid factors. *Clin Chem*. 1991;37:411-414.
18. Hjortberg M, Jin M, Larsson A, Nilsson BO. Immunoglobulins and monoclonal anti-blastocyst antibodies in the mouse uterine secretion during the early pre-implantation period. *J Reprod Immunol*. 1991;1991:277-287.
19. Crowther JR. *The ELISA Guidebook*, 2nd ed. New York, NY: Humana Press; 2009:79-110.
20. DeSilva B, Smith W, Weiner R, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. *Pharm Res*. 2003;20:1885-1900.
21. Lipp RW, Passath A, Leb G. The incidence of non-iatrogenic human anti-mouse antibodies and their possible clinical relevance. *Eur J Nucl Med*. 1991;18:996-997.
22. Thompson RJ, Jackson AP, Langlois N. Circulating antibodies to mouse monoclonal immunoglobulins in normal subjects–incidence, species specificity, and effects on a two-site assay for creatine kinase-MB isoenzyme. *Clin Chem*. 1986;32:476-481.
23. Ward G, McKinnon L, Badrick T, Hickman PE. Heterophilic antibodies remain a problem for the immunoassay laboratory. *Am J Clin Pathol*. 1997;108:417-421.
24. Ismail AA. Identifying and reducing potentially wrong immunoassay results even when plausible and "not-unreasonable". *Adv Clin Chem*. 2014;66:241-294.
25. Carlander D, Larsson A. Avian antibodies can eliminate interference due to complement activation in ELISA. *Ups J Med Sci*. 2001;106:189-195.
26. Kazmierczak SC, Catrou PG, Briley KP. Transient nature of interference effects from heterophile antibodies: examples of interference with cardiac marker measurements. *Clin Chem Lab Med*. 2000;38:33-39.
27. Hadge D, Ambrosius H. Evolution of low molecular weight immunoglobulins–IV. IgY-like immunoglobulins of birds, reptiles and amphibians, precursors of mammalian IgA. *Mol Immunol*. 1984;21:699-707.
28. Bjerner J, Bormer OP, Nustad K. The war on heterophilic antibody interference. *Clin Chem*. 2005;51:9-11.

29. Ismail AAA, Walker PL, Cawood ML, Barth JH. Interference in immunoassay is an underestimated problem. *Ann Clin Biochem*. 2002;39:366-373.
30. Selby C. Interference in immunoassay. *Ann Clin Biochem*. 1999;36:04-721.
31. Esteves MB, Binaghi RA. Antigenic similarities among mammalian immunoglobulins. *Immunology*. 1972;23:137-145.
32. Mehta PD, Reichlin M, Tomasi TB Jr. Comparative studies of vertebrate immunoglobulins. *J Immunol*. 1972;109:1272-1277.
33. van Vollenhoven RF. Sex differences in rheumatoid arthritis: more than meets the eye. *BMC Med*. 2009;7:12.
34. Rasch EK, Hirsch R, Paulose-Ram R, Hochberg MC. Prevalence of rheumatoid arthritis in persons 60 years of age and older in the United States: effect of different methods of case classification. *Arthritis Rheum*. 2003;48:917-926.
35. Nishimura K, Sugiyama D, Kogata Y, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann Intern Med*. 2007;146:797-808.
36. Andrysikova R, Kudlackova H, Toman M. Standardisation of ELISA for rheumatoid factor IgM detection in canine serum. *Acta Vet Brno*. 2009;78:129-136.
37. Bennett D, Nash AS. Feline immune-based polyarthritis – a study of 31 cases. *J Small Anim Pract*. 1988;29:501-523.
38. Hanna FY. Disease modifying treatment for feline rheumatoid arthritis. *Vet Comp Orthop Traumatol*. 2005;18:94-99.
39. Settipane GA, Pudupakkam RK, McGowan JH. Corticosteroid effect on immunoglobulins. *J Allergy Clin Immunol*. 1978;62:162-166.
40. Kawano T, Matsuse H, Obase Y, et al. Hypogammaglobulinemia in steroid-dependent asthmatics correlates with the daily dose of oral prednisolone. *Int Arch Allergy Immunol*. 2002;128:240-243.

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