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ORIGINAL STUDY

Evaluation of the utility and accuracy of body fluids containing red blood cells to determine canine and feline blood types

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Presented in part at the European Veterinary Emergency and Critical Care Annual Congress 2021.

Abstract

Objective: To examine the accuracy of using body fluids macroscopically suspected to contain erythrocytes to determine the blood type in dogs and cats by use of an immunochromatographic cartridge (ICC), compared to systemic blood as the reference standard.

Design: Prospective study.

Setting: University teaching hospital.

Animals: Thirty client-owned dogs and 8 cats.

Interventions: Dogs and cats with a sanguineous or serosanguineous body fluid (SBF) that also required a blood sample were eligible for inclusion. PCV and blood type were determined in all blood and fluid samples. For body fluids with a low PCV and discordant blood type results compared to systemic blood, sample concentration and repeat blood typing from the fluid was performed when enough sample was available.

Measurement and Main Results: Body fluid samples consisted of 16 pleural (11 dogs; 5 cats), 12 peritoneal (10 dogs; 2 cats), and 4 canine pericardial effusions, 3 urine samples, and 1 each of feces and epistaxis from dogs and a seroma sample from a cat. Median (range) manual PCV of blood and fluid samples was 34% (14%–66%) and 6% (0.5%–70%) for dogs and 28% (14%–48%) and 14% (0.5%–19%) for cats, respectively. Dogs were correctly classified as being DEA 1 negative, DEA 1 positive, and DEA 1 weak positive when using body fluid for blood typing 13 of 14, 4 of 9, and 5 of 7, respectively. All reference blood type to fluid blood type (FBT) discordant results had a body fluid PCV equal to or below 2%. Subsequently concentrated body fluid samples had a PCV above 8% and repeat FBT matched reference blood type (RBT). All cats were classified as type A by all RBTs and FBTs.

Conclusions: Body fluids containing erythrocytes may be utilized to blood type dogs if sufficiently concentrated and type A cats.

KEYWORDS
blood typing, hemorrhagic effusions, immunochromatographic kits, transfusion medicine

1 | INTRODUCTION

Red blood cell transfusion can be lifesaving for critically ill patients with severe anemia. Although transfusion may provide great benefit, administration is not without risk. Dog erythrocyte antigen (DEA) 1 blood typing for recipient dogs and A/B typing for recipient cats are recommended prior to blood product administration in transfusion naïve patients and needed in patients that have previously received a blood transfusion in order to avoid acute hemolytic transfusion reactions and to optimize RBC survival.[1–3]

Blood groups are defined by inherited antigens found on the surface of RBCs. Serologic testing has identified 7 internationally recognized blood groups belonging to the dog DEA system, which includes DEA 1, 3, 4, 5, 6, 7, and 8.[1,4,7] Canine blood types outside the DEA system such as DAL[5] and Kai-1 and Kai-2[6] have also been described. DEA 1 is considered to be the most antigenic and clinically relevant blood type. Consequently, blood typing for the DEA 1 antigen is most widely used in a clinical setting.[1,2] The AB system is most commonly used to define blood type in cats, which includes type A, type B, and type AB.[1,8] Antigens outside of the AB system, such as Mik, have also been recognized[9] with a recent study identifying 5 different feline erythrocyte antigens by randomly cross-marching type A cats.[10]

Several methods are used to identify canine and feline blood types including laboratory-based techniques and commercially produced point-of-care assays. The immunochromatographic cartridge (ICC) is a point-of-care assay with high sensitivity and specificity when compared to other laboratory and point-of-care methods, with the benefit of being easy to perform and widely available.[11,12]

Only a small amount of blood is needed to blood type dogs and cats with the ICC method. However, in some situations, a sample obtained via venipuncture may not be readily available where a sanguineous or serosanguineous body fluid (SBF) might be available, for example, naturally excreted body fluids by free catch (including urine, feces, vomit, or nasal discharge) or surplus effusion from abdominocentesis, thoracentesis, and pericardiocentesis. Obtaining a patient's blood type from an SBF could be particularly useful in patients with severe anemia, risk of hemorrhage from sampling, those with low total blood volumes such as neonates or small size patients, intraoperatively hemorrhaging patients as well as in unstable patients where venipuncture can lead to stress and subsequent patient deterioration.

This study aimed to determine the feasibility of using body fluids macroscopically suspected to contain RBCs for identifying blood type in dogs and cats by use of an ICC, using systemic blood as the reference standard. A secondary aim was to ascertain whether there was a minimum PCV of the fluid required to obtain a reliable result. We hypothesized that DEA 1 and AB blood types as determined by use of SBF would have an excellent concordance with the patient's systemic blood, although the reliability would be influenced by the PCV of the SBF.

2 | MATERIAL AND METHODS

This was a prospective study performed between April 2020 and February 2021 at a small animal university teaching hospital. Full ethical approval from the institution's Ethical Review Committee was obtained prior to study enrollment.

2.1 | Patient selection and sample collection

Any dog or cat presenting with, or that developed, a sanguineous or serosanguineous peritoneal, pleural, or pericardial effusion requiring drainage for a therapeutic or diagnostic reason was considered for study enrollment. Patients with clinical evidence of blood loss into other voided body fluids (eg, hematuria, epistaxis, liquid hemorrhagic feces) were also eligible for inclusion. All samples included in the study were either surplus to clinical requirements or collected by noninvasive methods following excretion by the patient.

All patients included in the study had been blood typed using EDTA blood as part of their clinical management or had surplus EDTA blood available for blood typing. This blood type will be referred to as the reference blood type (RBT). Blood typing was performed by use of the relevant species-specific QuickTest[a,b] blood typing standard ICC as per manufacturer instructions.[11,12] DEA 1 weak positive dogs were defined when the red band at the DEA 1 mark was weaker than the control band. A valid test result required the presence of the control band on the cartridge.

A volume of 0.5 or 1 ml of SBF was collected and placed into a 0.5- or 1-ml EDTA tube filled up to the fill line. When the PCV of the SBF sample was suspected to be low, extra fluid was collected in an extra 1-ml EDTA tube or in a plain urine collection tube if enough volume was available.

2.2 | Data collection

Patient signalment, systemic blood PCV and total plasma protein, RBT, SBF PCV, and fluid blood type (FBT) alongside fluid etiology, collection method, and diagnostic category were assessed prospectively. Data were collected into a commercial spreadsheet application.[c]

2.3 | Reference blood type

A manual PCV and total plasma protein were performed on each EDTA systemic blood sample using a standard, previously described technique.[13] Blood typing was performed by use of the relevant species-specific QuickTest[a,b] blood typing standard ICC as per manufacturer instructions.

2.4 | Fluid blood type

A manual PCV was performed on each SBF sample using standard technique.[13] Blood typing was performed by use of the relevant species-specific QuickTest[a,b] blood typing standard ICC as before, instead substituting the recommended EDTA blood sample as advised by the manufacturer by the SBF stored in the EDTA tube. The blood type was read after 5 minutes to ensure sufficient time for complete fluid migration.

As canine ICC relies on the presence and intensity or absence of a red band at the DEA 1 mark of the cartridge, the same individual assessed all samples to limit subjectivity on blood type determination.

If an SBF sample with low PCV failed to determine a blood type result or the FBT was discordant with the RBT despite a control line present, a larger sample of the SBF was placed into a 1.5-ml Eppendorf tube and centrifuged at $13,500 \times g$ for 5 minutes to pellet RBCs when enough sample was available. Following this, the majority of the supernatant was removed and the cell pellet resuspended with few drops of the supernatant to achieve a concentrated sample. PCV and repeat FBT were then performed on this concentrated sample as previously described.

The blood and fluid samples from each patient were collected throughout the hospitalization length of the patient. Samples were either run immediately following collection or refrigerated for up to 7 days until blood type was performed.

2.5 | Statistical analysis

Data were collected into a commercial spreadsheet application[c] and analyzed using commercial statistical software.[d] Data were assessed for normality with visual assessment of Q–Q plots; body weight, age, blood PCV, and body fluid PCV were nonnormally distributed and reported as median and range. To assess the role of body fluid PCV on agreement between RBT and SBF, univariable binary logistic regression was performed. P-values < 0.05 were considered significant.

3 | RESULTS

A total of 38 paired blood and SBF samples were collected prospectively for the purpose of the study. Of these, 30 were from dogs and 8 from cats. Of the 30 dogs included in the study, there were 11 neutered females, 3 entire females, 8 neutered males, and 8 entire males. Median body weight of dogs was 28.2 kg (range: 3.2–61.9 kg) with a median age of 7.3 years (range: 3 months to 12 years). The cat group consisted of 3 neutered females, 2 neutered male, and 3 entire males. Median body weight of cats was 5.5 kg (range: 2.7–7 kg) and median age was 4 years (range: 8 months to 11 years).

The SBF samples consisted of 16 pleural effusions (11 dogs and 5 cats), 12 peritoneal effusions (10 dogs and 2 cats), 4 canine pericardial effusions, 3 canine urine samples, and 1 each of canine feces and epistaxis. One feline sample was obtained from an SC drain placed after surgical removal of an interscapular feline injection site sarcoma.
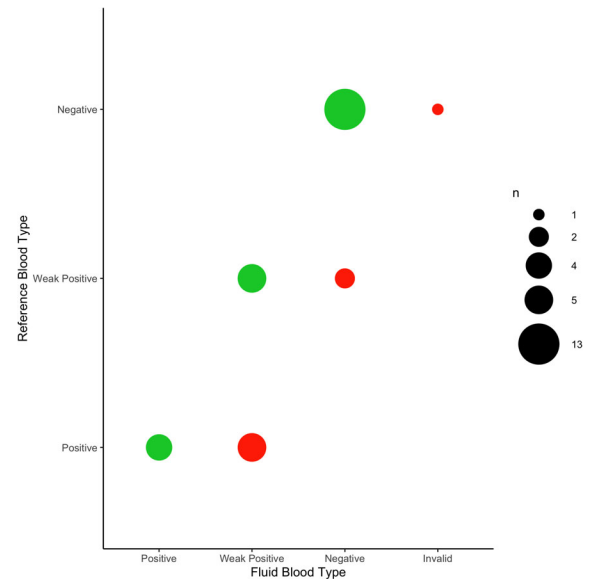
FIGURE 1 Agreement between reference blood type (RBT) and fluid blood type (FBT) in dogs. Green = matching results; Red = discordant results

When subclassified according to etiology, the majority of the SBF comprised 11 neoplastic effusions (10 dogs and 1 cat), 9 postoperative effusions collected from drains (6 dogs and 3 cats), 6 septic effusions (4 dogs and 2 cats), 4 samples from dogs that had suffered a trauma, and 3 dogs with postoperative abdominal effusion collected by abdominocentesis. In addition, there were 1 each of epistaxis and urine from 2 dogs with immune-mediated thrombocytopenia, and 1 each of idiopathic pericardial effusion in a dog, peritoneal fluid in a cat with disseminated intravascular coagulation, and a pleural effusion in a cat with chylothorax.

Median PCV was 34% (range: 14%–66%) and 28% (range: 14%–48%) for dogs and cats, respectively. Reference blood types for dogs were as follows: 14 of 30 DEA 1 negative, 9 of 30 DEA 1 positive, and 7 of 30 DEA 1 weak positive. All the cats were blood type A.

Median PCV of SBF was 6% (range: 0.5%–70%) and 9.5% (range: 0.5%–19%) for dogs and cats, respectively. FBT for dogs was as follows: 15 of 30 DEA 1 negative, 4 of 30 DEA 1 positive, 10 of 30 DEA 1 weak positive, and 1 of 30 invalid result. All cats were FBT A.

When FBT was compared to RBT in dogs, 13 of 14, 4 of 10, and 4 of 6 were correctly classified as DEA 1 negative, DEA 1 positive, and DEA 1 weak positive, respectively (Figure 1). Six DEA 1 positive dogs were incorrectly classified as DEA 1 weak positive by the FBT and 2 DEA 1 weak positive dogs were classified as being DEA 1 negative by the FBT (Figure 1). Only 1 DEA 1 negative dog on RBT had an invalid result on SBF (Figure 1); the SBF was a septic pleural effusion with a PCV of 1%. All SBFs with discordant RBT to FBT results had a PCV equal to or below 2% (Figure 2) and yielded a weak control band. All FBT that yielded a weak control band belonged to samples with a PCV equal to or below 3%. PCV was associated with agreement between RBT and FBT in dogs ($P = 0.0326$) (Figure 2).

All the cats in this study were blood type A and all FBT matched that of the RBT. Two fluid samples with PCVs of 0.5% and 5% failed to show
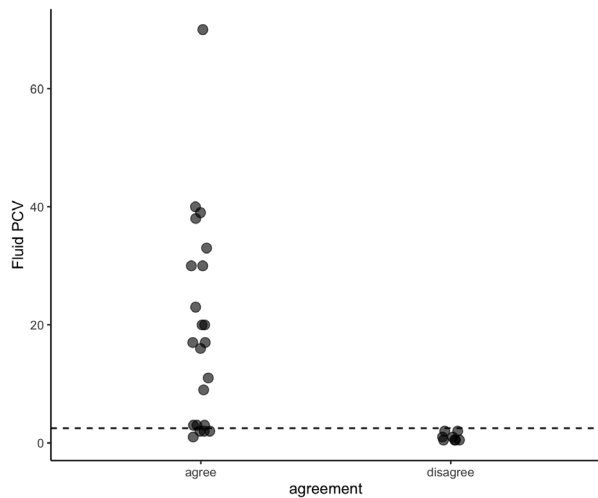
FIGURE 2 Agreement between reference blood type (RBT) and fluid blood type (FBT) according to PCV of the canine body fluid samples. Green = matching results; Red = discordant results
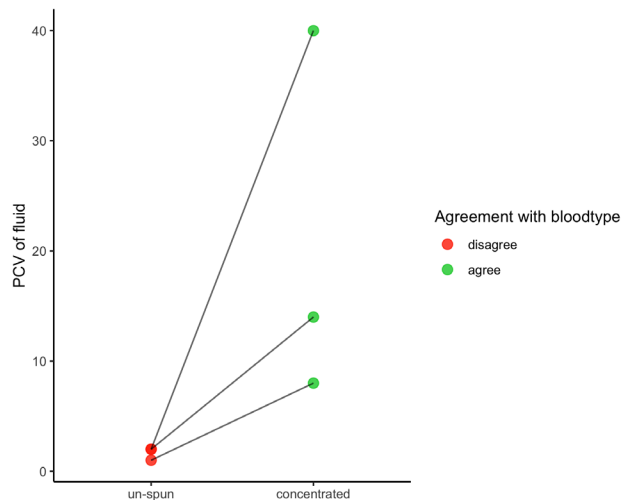
FIGURE 3 Agreement between reference blood type (RBT) and fluid blood type (FBT) in 3 unaltered versus concentrated canine body fluid samples

a control band on the typing cartridge but still yielded a visible red band on the A mark.

Three canine samples with low PCV and discordant results between RBT and FBTs were centrifuged, and a concentrated fluid sample was obtained. The PCVs obtained after concentrating the samples were 8%, 14%, and 40%. All concentrated SBFs were retyped and subsequently the FBT matched the RBT (Figure 3).

4 ⎪ DISCUSSION

In this study, body fluids containing RBCs were used successfully to blood type dogs and cats by use of a species-specific ICC. Although matching blood type results were obtained for all the cats independently of the SBF PCV, some canine fluid samples with a PCV equal

to or below 2% gave discordant results. However, concordant results were obtained in some of these samples when a concentrated sample was used to determine the FBT.

Although a small sample of blood is needed to blood type dogs and cats by using the ICC, there are some situations in which blood typing from a body fluid might be less invasive, quicker, or more convenient. For instance, factors such as a small patient size, the presence of anemia, severe dehydration, and hemostatic disorders can make venipuncture difficult or undesirable. Other settings in which the use of FBT might be indicated include patients that are actively bleeding (eg, intraoperatively, trauma patients) where a body fluid is readily available. To the authors' knowledge, attempts to determine the blood type in small animals and people by use of SBF have not been reported. In people, body fluids such as saliva or sweat have been used to determine individuals blood type A, B, AB, or O.[14] Although not all individuals secrete the RBC antigens in body fluids, approximately 80% of the population does.[15] It is unknown if such body fluids would be useful in determining blood type in dogs and cats.

Immunochromatographic methods to determine blood type have been shown to be accurate with PCV values of at least 10% and 4% in dogs and cats, respectively.[12,16,17] Despite the intensity of the DEA 1 band on ICC having been previously shown to fade with reductions in systemic blood PCV,[12] all fluid samples from cats and dogs with a PCV higher than 2% successfully identified the patient blood type by use of an ICC in our study. Interestingly, the feline ICC successfully determined the blood type in fluid samples with PCV values as low as 0.5%, suggesting possible increased sensitivity compared to the canine equivalent.

For the dogs included in the study, 9 of 30 had discordant blood type results when comparing blood and SBF. All discordant results were from fluid samples with a PCV equal to or below 2%. However, 3 fluid samples with PCV equal to or below 2% had matching results between RBT and SBF. When assessing the discordant results, 6 systemic DEA 1 positive blood types were associated with a DEA 1 weak positive when using fluid; 2 systemic DEA 1 weak positive blood types were associated with DEA 1 negative when using fluid; and 1 systemic DEA 1 negative blood type was associated with an invalid result when using an SBF. These discordant results are likely associated with decreased DEA 1 antigen concentration in low-PCV SBF as a negative systemic blood type never resulted in an SBF producing a positive or weak positive DEA 1 result. These results suggest that a DEA 1 positive result when using SBF to blood type is likely to be true, regardless of the SBF PCV. On the other hand, a DEA 1 negative or weak positive result when using an SBF with a PCV <3% will require hemoconcentration before being able to confidently determine the patient blood type. Regardless, these results are unlikely to have a significant implication from a clinical standpoint, whereas the opposite may do.

Three SBF samples that had discordant blood type results when compared to RBT were centrifuged and a concentrated fluid sample with a higher PCV was obtained. The FBT from the concentrated samples matched the RBT and showed a strong control band on the typing cartridge. Red blood cell concentration has previously been suggested as a technique to increase accuracy of blood type determination when

using an ICC and systemic blood to mitigate effects of anemia.[12,17] The results of this study support the use of RBC concentration of low-PCV SBF to increase the accuracy of FBT determination using an ICC. All samples with RBT and FBT discordant results yielded a weak control band on the typing cartridge. Although a previous study performed by Seth et al. showed that intensity of the control band was less affected than the DEA 1 band in anemic samples, the lowest PCV analyzed in that study was 10%.[12] In this study, samples with a PCV equal to or less than 2% had weak control bands.

Unlike the canine blood typing system, the feline system is easier to interpret as it relies on the presence of a red band on either the A or the B mark in A and B type cats, respectively, or a red band in each mark for AB cats.[11] All cats in this study were blood type A in all blood and SBF samples, independently of the sample PCV. Based on these results, it could be hypothesized that SBF will be equally useful in determining the blood type in B type and AB type cats. However, further studies with a larger sample size including type B and AB cats are needed to confirm this.

The most common source of SBF in this study was the pleural space followed by the peritoneal cavity in both dogs and cats. The samples were either obtained by thoracocentesis or abdominocentesis for clinical purposes or from thoracic and abdominal drains placed for therapeutic reasons or after a surgical procedure. Even though the sample size was small and this translated to a limited etiologic variety of SBF in cats, we hypothesize that other fluids such as urine and pericardial effusion will also be useful in determining cats blood types. Although a canine fecal sample was successfully used to determine a patient's blood type in this study, this was a very liquid sample; therefore, more solid feces might not be suitable to determine a patient's blood type. Sample processing to turn solid hemorrhagic feces into more liquid samples for the purpose of determining the blood type has not been investigated in this study.

The main limitations of this study are related to the low sample size of cats. No blood type B or AB cats were available for inclusion in the study during the study period, related to the lower prevalence of these blood types in the feline population. As already mentioned, another limitation is the low variety of fluid etiologies, especially in cats. Finally, only 3 of the canine samples with RBT to FBT discordant results were centrifuged and a concentrated sample was obtained for re-typing. However, the ones where sample concentration and repeat FBT was performed showed promising results.

This study shows that SBF can be used to blood type dogs and type A cats. Feline FBT showed great accuracy compared to RBT in type A cats. For dogs, all body fluids with a PCV equal to or lower than 2% should be concentrated before being used for typing in order to obtain a higher PCV and improve accuracy.

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ENDNOTES

[a] QuickTest DEA 1 blood group compatibility, Alvedia, Limonest, France.
[b] QuickTest A+B blood group compatibility, Alvedia, Limonest, France.
[c] Excel, Microsoft corporation, Redmond, WA.
[d] The R Project for Statistical Computing, The R Foundation, Vienna, Austria.

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