Lack of association between feline AB blood groups and retroviral status: A multicenter, multi-country study

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Abstract:	Objectives: The relationship between blood group antigens and disease has been studied in humans. Blood types have been associated with both decreased and increased rates of various infections. In addition, blood group expression has been shown to vary with some cancers and gastrointestinal diseases. The objective of this study was to explore whether there is a relationship between blood type and retroviral infections in cats. Methods: Case records from a veterinary research laboratory, veterinary teaching hospitals, and veterinary blood banks were retrospectively searched for cats where both blood type and retroviral status [feline leukemia (FeLV), feline immunodeficiency virus (FIV), or both] were listed (part one). In addition, a sample of 33 cats with confirmed FIV infection were genotyped to determine blood groups (part two). Results: In part one, a total of 709 cats were identified, 119 of which were positive for retroviral infection. Among all cases, 621 were type A (87.6%), 68 were type B (9.6%), and 20 were type AB (2.8%). There was no relationship between overall retroviral status (positive/negative) and blood type (p=0.43), FeLV status and blood type (p=0.86), nor between FIV status and blood type (p=0.94). There was no difference in the distribution of blood types between cats who were healthy and typed as possible blood donors versus sick cats who were typed prior to a possible transfusion (p=0.13). In part two, of the 33 FIV infected cats, all blood group genotypes, were identified, though this test did not discriminate type A from type AB. Conclusions and relevance: There was no relationship identified between feline retroviral status and blood type in this study. The relationship

	between blood type and other disease states requires further study in veterinary patients.

Lack of association between feline AB blood groups and retroviral status: A multicenter, multi-country study

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19 **Keywords:** AB blood type system; retrovirus; feline leukemia; FIV

20 **Running title**: AB Blood Type system and Retroviral Status

Abstract

Objectives: The relationship between blood group antigens and disease has been studied in humans. Blood types have been associated with both decreased and increased rates of various infections. In addition, blood group expression has been shown to vary with some cancers and gastrointestinal diseases. The objective of this study was to explore whether there is a relationship between blood type and retroviral infections in cats.

Methods: Case records from a veterinary research laboratory, veterinary teaching hospitals, and veterinary blood banks were retrospectively searched for cats where both blood type and retroviral status [feline leukemia (FeLV), feline immunodeficiency virus (FIV), or both] were listed (part one). In addition, a sample of 33 cats with confirmed FIV infection were genotyped to determine blood groups (part two).

Results: In part one, a total of 709 cats were identified, 119 of which were positive for retroviral infection. Among all cases, 621 were type A (87.6%), 68 were type B (9.6%), and 20 were type AB (2.8%). There was no relationship between overall retroviral status

(positive/negative) and blood type (p=0.43), FeLV status and blood type (p=0.86), nor between FIV status and blood type (p=0.94). There was no difference in the distribution of blood types between cats who were healthy and typed as possible blood donors versus sick cats who were typed prior to a possible transfusion (p=0.13). In part two, of the 33 FIV infected cats, all blood group genotypes, were identified, though this test did not discriminate type A from type AB.

Conclusions and relevance: There was no relationship identified between feline retroviral status and blood type in this study. The relationship between blood type and other disease states requires further study in veterinary patients.

Introduction

In humans, blood group antigens were originally identified as important for transfusion compatibility. These structures are now known to serve many purposes and can vary in expression and geographic distribution secondary to selection pressure from endemic diseases. Blood group antigens can act as receptors for pathogens.(1) Studies have shown that people with different ABO blood types have different susceptibility to certain diseases.(2–4) For example, blood group O provides a selective advantage against severe malaria but may predispose to more severe signs with cholera. (2) Associations between viral disease risk or severity of symptoms with blood type have been found with West Nile virus,(5) hepatitis B virus,(6) and norovirus infections.(7) There is also evidence suggesting that blood group antigens and red blood cells could play a role in the susceptibility or protection against human immunodeficiency virus (HIV) infection and also in susceptibility to SARS-CoV-2.(8,9) These selective pressures may be linked to the geographic distribution of different blood types.(2)

Blood type association with increased susceptibility to certain infections may also increase the risk of related cancers such as an increased risk of gastric cancer related to *Helicobacter pylori* in individuals with type A blood.(10)

Blood group antigen expression can also change with disease. These phenomena have been recognized with both neoplasia and gastrointestinal disorders in people.(11–16) In hematopoietic diseases, such as acute myeloid leukemia, loss or weakening of antigen expression can be seen. This change may be due to disruptions of the enzymes that are involved in production of ABO antigens. In some case reports, ABO expression returning to normal is a sign that the leukemia is in remission.(15)

While there is growing evidence that blood groups affect host susceptibility to some infections in humans, little is known about blood group associated infectious disease risk in animals. To the authors' knowledge, there is a single experimental study in rabbits, in which histo-blood group antigens (HBGAs) were found to act as attachment factors for rabbit hemorrhagic disease virus infection (RHDV) in a virus strain-dependent manner, with some HBGAs facilitating infection. The results of this study suggest that

77 polymorphism of expression of HBGAs might contribute to genetic resistance to
78 RHDV.(17)

79 The major blood group system in cats is the AB system which consists of 3 blood
80 types: A, B, and AB. Blood type is determined by 3 alleles with A dominant over the rare
81 AB, which is thought to be dominant over B.(18) Most clinical blood typing methods are
82 phenotypic and rely on blood group antigen detection.(19) Genome wide association
83 studies have identified mutations in the cytidine monophospho-N-acetylneuraminic acid
84 hydroxylase (CMAH) gene that are believed to result in disrupted enzyme activity
85 preventing conversion of N-acetylneuraminic acid (NeuAc) to N-glycolyl-neuraminic acid
86 hydroxylase (NeuGC) on the red blood cell surface.(18,20) Cats with blood type A have
87 predominantly NeuGc while blood type B have NeuAc and those with the rare type AB
88 have both NeuGc and NeuAc expression on their red blood cells.(18) Several different
89 mutations in *CMAH* have been identified and some show selective expression in pure
90 breeds.(20) The discovery of these *CMAH* polymorphisms associated with different blood
91 types in cats has allowed the development of sequencing-based blood genotyping tests.
92 However, genotyping schemes are restricted to include known polymorphisms, so do not
93 yet form a replacement for phenotypic typing in transfusion settings. (19) Additionally,

some of the erythrocyte antigens that determine blood type can also be expressed on other cell types. The blood group A antigen is expressed on lymphocytes of blood type A cats, but the B antigen does not appear to be expressed on the lymphocytes of type B cats.(21)

Mistyping and difficulty typing have been reported in cats.(19,22–25) In one study, discordant results between phenotypic typing methods were seen specifically in cats with feline leukemia virus (FeLV) infection.(23) In another study, a severely anemic kitten showed agglutination that initially indicated an AB blood type but on repeat typing when the kitten was healthy, it was found to be blood type A.(22) In addition, in one study of feline blood types in a referral population, type AB was seen more frequently in cats with infectious disease, neoplasia, and RBC destruction.(26) In a study comparing phenotypic and genotypic blood typing, there was 96% agreement (107 of 112 cats) but 5 discordant results, all involving type B phenotype suggesting there are additional unknown polymorphisms in *CMAH* linked to blood type B. (19)

FeLV and feline immunodeficiency virus (FIV) are retroviral agents that cause a variety of systemic clinical signs in infected cats with anemia being a common feature of

both.(27,28) The blood mistyping seen in some FeLV infected cats could suggest that FeLV utilizes feline AB blood type antigen in pathogenesis or that the virus changes blood group expression.(23) In addition, as the A red cell antigen is also present on the lymphocytes of blood type A cats and FIV and FeLV have feline lymphocytes as their target cells for pathogenicity, these viruses might interact with blood group lymphocyte antigens as receptors.(29,30) Given these suspicions and reports showing a relationship between blood types and disease in human medicine, the objective of this study was to explore whether there is a relationship between blood type and retroviral infections in cats.

Given the differential expression of blood group A on lymphocytes, we hypothesized that there would be a higher incidence of type A and AB in retroviral positive cats. In addition, we also suspected that we might see more AB expression in cats presenting for critical care than in healthy cats.

Materials and methods

2.1 Study design

This study was conducted in two parts which involved: (i) a retrospective multicenter study of cats with phenotypic blood type analysis and retroviral testing, and (ii) prospective blood group genotyping of a group of FIV infected cats.

Part One:

2.2 Study population

Study data was retrospectively collected from five veterinary facilities: one research laboratory in Italy, one veterinary teaching hospital (VTH) and one veterinary blood bank in the United States, and two VTHs with associated blood banks in Australia. Retrospective review periods correlated to the length of time each facility had electronic medical records available. Cats were included if they had both blood typing and retroviral testing for FeLV and FIV. All standard biosecurity measures and institutional safety procedures were followed when the authors originally collected and analyzed the feline blood samples. Additional ethics approval was obtained at The University of Queensland to retrospectively review case data (ANRFA 2022/AE000121).

Electronic medical records and cat blood donor's records of the Veterinary Transfusion Research Laboratory (REVLab) at the University of Milan, were available from

January 2005 to December 2021. Washington State University Veterinary Teaching Hospital (WSU) electronic medical record system was available from December 2010 to December 2021. The Washington State University Blood Bank cat donor database was available from January 2008 to January 2022. Murdoch University's database was available from December 2014 to February 2022 and University of Queensland's database was available from March 2017 to February 2022.

2.3 Data collection and methodology

Data collected included, when available, age, sex, breed (as reported by the owner and attending veterinarian), blood type, blood type methodology, retroviral testing results, hematocrit (Hct), reason for testing, and diagnosis. Cats with a Hct value less than or equal to 26% were considered to be anemic.(31)

Retroviral testing was performed via commercially available point-of-care (PoC) test kits detecting antibody to FIV target antigens p15,p24 or gp40 and detecting FeLV p27 antigen (SNAP FIV/FeLV Combo Test, IDEXX Laboratories Italia Srl, Milan, Italy (FIV p15/p24/gp40); Anigen Rapid FIV Ab/FeLV Ag, BioNote, Gyeonggi-do, Korea (FIV gp40);

and SNAP FIV/FeLV Combo Test, IDEXX Laboratories, Westbrook, Maine, USA (FIV p15/p24)).

Phenotypic blood typing was done by one of five methods (Table 1). A commercial in clinic card agglutination (CARD (RapidVet-H, DMS Laboratories) and immunochromatographic technique (STRIP, Feline Lab and Quick test, A+B, Alvedia, Limonest, France) were performed according to manufacturer's instructions and as described.(32,33) A laboratory tube agglutination technique (TUBE) using *Triticum vulgaris* (24), slide agglutination technique (SLIDE) using *Triticum vulgaris,* and a gel typing kit (GEL, ID Gel-Test Micro Typing System, Diamed AG, 1785 Cressier sur Morat, Switzerland- this test is no longer commercially available) were additional methods. At REVLab, all type B and AB results were confirmed by back-typing technique as described.(32)

Part two

2.4 Study population

To further investigate the interaction between retroviral status and blood type, DNA banked from 33 FIV infected cats acquired as part of a previous study was used for genetic blood typing.(34) These were healthy client-owned cats with outdoor access that were recruited from five Australian states/territories to evaluate the effectiveness of a commercially available FIV vaccine (Fel-O-Vax FIV, Boehringer Ingelheim, Fort Dodge, IA, USA) in the field. Animal ethics approval for the study was obtained from University of Sydney (N00/1-2012/3/5920 and 2017/1129).

2.5 Retroviral testing and blood genotyping

Blood was collected from conscious cats using jugular or cephalic venipuncture, immediately aliquoted into EDTA tubes and stored at 4°C. Cats were confirmed to have FIV infection through positivity on two different FIV commercially available antibody PoC kits as well as a positive FIV PCR result (FIV RealPCR, IDEXX Laboratories East Brisbane, Queensland, Australia). Cats were concurrently screened for progressive FeLV infection since both PoC kits also detected FeLV p27 antigen (Anigen Rapid FIV/FeLV and Witness FeLV/FIV, Zoetis Animal Health, Lyon, France). Additional FeLV testing was performed including proviral PCR testing and neutralizing antibody testing, for confirmation and to assist with assigning presumptive progressive or regressive infection status.(35)

Genomic DNA extraction was performed within 24 hours of blood collection using a commercially available kit (QIAamp DNA Mini Kit; Qiagen, Valencia, CA, USA), as per the manufacturer's instructions, at the University of Sydney, Sydney, NSW, Australia. The concentration and quality of extracted DNA was measured using a spectrophotometer (Nanodrop 1000; Thermo Fisher Scientific, Waltham, MA, USA). DNA was stored at −80°C until transferred to Langford Vets, Diagnostic laboratories for testing.(34) Blood group genotyping was carried out by running a PCR to amplify a fragment of the CMAH gene incorporating two single nucleotide polymorphisms (SNPs) associated with B blood type.(19) The PCR comprised 10 µL 2 x GoTaq Master Mix (Promega), 0.8 µL 5 µM each forward and reverse primers (19), 5.2 µL water and 4 µL gDNA. Amplification was performed in a Bio-Rad T10096 well block cycler (Bio-Rad Labs. Ltd.) for 2 minutes at 95°C followed by 37 cycles of 95°C for 15 seconds and 58°C for 30 seconds. Biotinylated PCR products were immobilized on streptavidin coated Sepharose beads (GE Healthcare UK Ltd.), purified and annealed with the sequencing primer (0.5µM final concentration) as described in the PyroMark Gold Q96 reagents kit instruction manual (Qiagen Inc.). Pyrosequencing was performed in a PyroMark Q96 (Qiagen Inc.) automated 96-well pyrosequencer according to the manufacturer's instructions with a nucleotide

dispensation order of GCTAGTCGATCTG. Pyrosequencing data were evaluated using PyroMark Q96 v2.5.10 software (Qiagen Inc.).

2.6 Statistical analysis

The data was analyzed using standard descriptive statistics and reported as mean ± standard deviation (SD) or median and range, based on their distribution. Univariate analysis of categorical data using the Fisher's exact test or χ2 analysis was performed to determine possible associations between blood type and retroviral status, anemia, and clinical status (healthy versus sick cats). Univariate analysis of categorical data using the Fisher's exact test or χ2 analysis was also performed to determine possible associations between sex, blood type, and retroviral status. Associations were described using a probability (*p*) value < 0.05 as statistically significant. Data was analyzed using an open-source statistical software program (RStudio).

Results Part One:

3.1 Population

Baseline descriptive data from the included cats is listed in Table 1. A total of 709 cases met inclusion criteria, with 476 (67.1%) from REVLab, 132 (18.6%) from Murdoch

University, 82 (11.6%) from WSU and the WSU Blood Bank, and 19 (2.7%) from the University of Queensland. The study population included 141 female intact, 157 female spayed, 137 male intact, 254 male castrated, and 19 cats of unknown gender and neutering status due to incomplete information in the record. Most cats (609, 85.9%) were non-pedigree or mixed breed varieties. There were 15 other cat breeds represented with Maine Coon (n=25) and Ragdoll (n=17) cats the most common. The median age of enrolled cats was 3 years with a range of one month to 18.4 years.

Blood typing and retroviral testing were performed for various purposes: part of an epidemiological study in 247 (34.8%), blood donor screening in 210 (29.6%), prior to possible blood transfusion in clinically anemic cats in 175 (24.7%), blood type before mating to prevent feline isoerythrolysis in 33 (4.7%), pre-operative diagnostics prior to a surgical procedure in 42 (5.9%), and for unknown reasons in two cats (0.3%) (Table 2).

The STRIP blood typing test was used in 320 cats (45.1%), TUBE was used in 310 (43.7%), CARD was used in 48 cats (6.8%), and SLIDE was used in 17 (2.5%). The GEL test was used in 14 (1.9%) and used in an additional 15 cats to confirm CARD results.

Among the 709 cases, 621 (87.6%) cats were type A, 68 (9.6%) were type B, and 20 (2.8%) were type AB (Table 1). Of cats from REVLab, 437 (91.8%) were type A, 24 (5.0%) were type B, and 15 (3.2%) were AB. Of cats from WSU, 69 (84.2%) were type A, 10 (12.1%) were type B, and three (3.7%) were type AB. Of cats from Australia (Western Australia and Queensland), 115 (76.2%) were type A, 34 (22.5%) were type B, and two (1.3%) were AB. This difference in blood type distribution between countries, with a higher incidence of type B cats in the western USA than in Italy, and an even higher incidence in Australia, was highly statistically significant ($p < 0.001$).

Blood types for each breed are listed in Table 1. Similar to previous reports, type AB was only identified in Ragdolls and non-pedigree cats.(18) In contrast to previous reports that Siamese cats are only blood type A,(36) two Siamese and one Siamese mix were found to be blood type B. The collected breed information was reported by the owner and was not verified by genetic tests.

Overall, 119 cats were retroviral positive (Table 2). Of those, 47 were FeLV positive, 58 were FIV positive, and 14 were positive for both FeLV and FIV. Of the retroviral positive cats, 36 were clinically ill cats and 22 were healthy and identified during pre-operative or donor screening. The other 61 were typed during an epidemiological

252 study and their health status was not recorded. Blood type relationship to retroviral
253 illness could not be explored due to the small number of cats and geographic variability
254 with more clinically ill cats recruited from Australia and more healthy cats from Italy.
255 There was no relationship between overall retroviral status and blood type (p=0.43).
256 There was also no relationship specifically between FeLV status and blood type (p=0.86),
257 between FIV status and blood type (p=0.94) nor between FeLV/FIV co infected status and
258 blood type (p=0.59) (Table 3). Male cats were significantly more likely to be FIV positive
259 than female cats (p<0.001). There was no sex predisposition to FeLV positivity.

260 **3.2 Clinical Status**

261 In 624 cats in which a Hct was measured, 208 were anemic (33.3%). Hematocrit
262 ranged from 3.9% to 58% with a median of 30.2%. There was no relationship between
263 blood type and the presence of anemia (p=0.11).

264 Clinically ill cats who were typed prior to a possible transfusion had a variety of
265 diseases. Underlying primary disease processes included retroviral disease (20), neoplasia
266 (19), immune mediated disease (17), *Mycoplasma haemofelis* infection (10), kidney
267 disease (10), traumatic blood loss (9), related to sepsis (8), leukemia (7) coagulopathy (5),

gastrointestinal blood loss (4), Feline Infectious Peritonitis (3), other infectious disease (3), unknown disease (27) and one each of bone marrow toxicity, hepatic lipidosis, and diabetic ketoacidosis. The underlying disease was undiagnosed in 27 cats. There was no difference in the distribution of blood types between cats who were healthy and typed as possible donors versus cats who were clinically anemic and typed prior to possible transfusion (p=0.13). When this question was examined specifically for Australian cats (100 clinical cases, 50 donors), there was also no difference in the distribution of blood types (p=1). All the cats infected with *Mycoplasma haemofelis* were from Australia. Of these 10 cats, 8 (80%) were blood type A and two were type B (20%).

Part Two:

3.3 Population

Thirty-three confirmed FIV infected cats were included. One cat in this group was also FeLV positive. There were 9 female spayed, 1 male intact, and 23 male neutered cats. The mean age was 7.7 years with a range from 3.4 to 16.1 years. There were 30 non-pedigree cats, 1 Bengal, 1 British Shorthair, and 1 Himalayan. Of the 33 cats, 22 cats were blood type A or AB, carrying *b*, 7 cats were type A or AB and not carrying *b,* and 4 cats

were type B (homozygous for *b*). The one FeLV positive/FIV positive (presumably progressively FeLV-infected) cat in this group was homozygous for *b*.

Discussion

While there is growing evidence that blood groups affect host susceptibility to certain infections in humans, there are only a few veterinary studies that have investigated the possible relationship between blood groups and disease prevalence. There are two previous veterinary studies in dogs that examine this question. One study suggested that Cocker Spaniels with blood group antigen DEA 7 were at decreased risk for immune mediated hemolytic anemia, with an odds ratio of 0.1 (95% CI, 0 to 0.9).(37) Possible mechanisms discussed included changes in red cell membrane structure or expression of a unique autoantigen.(37) In another study, no significant relationship between DEA 1 blood type and the presence of *Babesia spp.* antigen was found. However, the small sample size of *Babesia* spp. antigen-positive dogs in that study could have cofounded the association with DEA 1 blood type. (38) In the only feline study (available only as an abstract), blood genotype *AA*, *Ab* or *bb* was determined in 263 cats from UK that had previously tested positive (n=131) or negative (n=132) for *Mycoplasma*

300 *haemofelis*, 'Candidatus Mycoplasma haemominutum', and 'Candidatus Mycoplasma
301 turicensis' infection by quantitative PCR. The prevalence of each individual haemoplasma
302 species, single versus dual haemoplasma species infection, and haemoplasma PCR copy
303 number, were compared between blood genotypes and no significant differences were
304 found.(39)

305 In the current study, cats were tested for blood type and retroviral status for
306 different reasons and by different methods. Some cats were healthy and the retrovirus
307 was identified during screening, while in other cases, cats were unwell and FIV/FeLV
308 tested for possible retroviral-associated disease. In people with some types of leukemia,
309 blood type antigen expression can vary with disease severity so it is possible that a
310 relationship could exist specifically with blood type and retroviral related neoplasia but
311 not with infection alone. It is also possible that retroviral status may have altered blood
312 type expression, leading to discrepancies between phenotypic blood type test results and
313 genotypic blood type. We did not have enough healthy retroviral positive cats nor cats
314 with FeLV related leukemias or lymphomas to fully investigate this possibility nor could
315 we perform matched blood type phenotyping and genotyping on all cats. Further
316 research is needed to investigate these factors.(15) Alternatively, blood type could be

317 related to retroviral infection but in the current study some retroviral infections may have
318 been undetected by only testing for FeLV antigen and anti-FIV antibodies (part one). Our
319 group of FIV infected cats confirmed with FIV PCR testing (part 2) makes this hypothesis
320 less likely for this retrovirus. However, studies have shown that PCR provirus testing can
321 identify regressive FeLV infections that are missed with antigen testing alone.(27,40) We
322 do not have information on the prevalence of FeLV regressive cats that are only FeLV
323 proviral PCR-positive and p27 antigen-negative. Therefore, it would be interesting to
324 evaluate blood type and retroviral status in a larger study specifically using FeLV PCR
325 testing, in addition to FeLV antigen detection, and looking at clinical manifestations of
326 retroviral disease.

327 In this study, blood typing methods changed over time and were different
328 between locations. Blood type B and AB classified phenotypically were back-typed for
329 confirmation in many cats. While there were no clear notes of blood typing difficulties,
330 this is hard to fully assess in a retrospective record review. Unlike previous studies where
331 discordant results were noted,(19,23–25) the majority of cats in the current study were
332 only typed by a single methodology. In future studies, a common methodology of blood

typing with back typing confirmation for all B and AB cats should be used with clear notes of any discordant results.(19,23)

Human studies have revealed that some of the geographic differences in blood type distributions around the world may occur in part due to different geographic disease distributions. The incidence of B blood type in cats is variable based on geography, with high numbers of B cats found in Australia.(41) The distribution of blood types in our study agreed with previously reported geographic differences in blood type distribution, with a higher incidence of type B cats in Washington state than in northern Italy and an even higher incidence in Australia.(41,42) It would be interesting in future studies to assess for correlations between blood type and susceptibility to infectious agents that vary geographically. While the study of blood type distribution and hemotrophic mycoplasmas in cats from the United Kingdom did not reveal a relationship, further study looking at *Bartonella hensalae* and *Cytauxzoon felis* should be considered. It is possible that decreased susceptibility to these or other putative agents is conferred by the presence of one or more A alleles, thereby counterbalancing selection against this allele.(41)

Our study did not reveal any significant correlations between feline blood type and retroviral infection. Our original hypothesis was that feline retroviruses could interact

with type A lymphocyte antigens as receptors.(29,30) Studies investigating HIV have demonstrated that the pathogenesis of retroviral infections is multi-factorial and the role of blood group antigens is likely complex. Further genetic sequencing and examination of blood group polymorphisms is likely needed for further understanding.(9) While the main target for both HIV and FIV are T lymphocytes, HIV uses a different primary receptor (CD4) and at least seven co-receptors to the receptor and co-receptor used by FIV to gain entry to cells (CD134 and CXCR4, respectively).(43) It has been suggested that polymorphic blood group antigens expressed on the surfaces of other cells including red blood cells and platelets may also be utilized as attachment receptors by HIV.(9) We were unable to confirm a possible similar mechanism with blood group antigens in cats for FIV attachment after evaluating a group of cats with confirmed FIV infection. This finding suggests that FIV is only able to gain entry into cells via CD134 and CXCR4, which are not expressed on red blood cells and instead only expressed on lymphocytes, macrophages, dendritic cells, microglia and astrocytes.(44,45), although further work is needed to confirm this.

Conclusions

366 This is the first study to assess the association between feline blood types and
367 retroviral status. We found no statistically significant association, although larger studies
368 are needed to confirm these results. There is a paucity of studies in veterinary medicine
369 that investigate the possible association between blood type and disease prevalence.
370 Further studies in this area will help our understanding of disease mechanisms and
371 improve our ability to diagnose and treat these diseases in clinical patients.

372 **Footnotes**

373 *RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA*
374 *URL http://www.rstudio.com/.*

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376 None

377 **Conflict of Interest**

378 The authors declared no potential conflicts of interest with respect to the research,
379 authorship, and/or publication of this article.

380

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Ethical Approval and Informed Consent

The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in JFMS. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

Data

The raw data and the dataset generated for this study are available upon request to the corresponding authors.

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524

525 **Tables**

526 Table 1. Blood type listed by attributes (sex, reproductive status, breed, Hct, retroviral
527 status, blood typing method, and reason for typing) in a population of 709 cats tested for
528 association between blood type and retrovirus status. DSH=Domestic Shorthair,
529 DMH=Domestic Medium Hair, DLH=Domestic Longhair, FeLV = Feline Leukemia Virus, FIV
530 = Feline Immunodeficiency Virus.

Variable	Level	Blood type		
		A n=621	B n=68	AB n=20
Country N=709	Italy	437	24	15
	United States	69	10	3
	Australia	115	34	2
Sex n =690	Male	332	50	10
	Female	270	18	10
Reproductive status n = 690	Intact	253	21	5
	Neutered	349	47	15
Breeds n = 709	Non-pedigree	516	60	17
	Abyssinian	2	0	0
	Bengal	2	0	0
	Birman	4	0	0
	British shorthair	6	0	0
	Burmese	4	0	0
	Burmilla	1	0	0
	Chartreux	3	0	0
	Chartreux Mix	1	0	0
	Chincilla	1	0	0

	Chincilla Mix	1	0	0
	Exotic Shorthair	2	0	0
	Himalayan	0	1	0
	Maine Coon	25	0	0
	Manx	2	0	0
	Norwegian Forest cat	5	0	0
	Persian	3	0	0
	Ragdoll	17	2	2
	Ragdoll Mix	1	0	0
	Russian Blue	3	1	0
	Scottish Fold	1	1	0
	Selkirk Rex	1	0	0
	Siberian Forest Cat	4	0	0
	Siamese	5	2	0
	Siamese Mix	1	1	0
	Siberian Forest cat	4	0	0
	Sphinx	6	0	0
	Thai Mix	1	0	0
	Tonkinese	2	0	0
	Turkish Van	1	0	0
Hct (%) n = 624	**Median**	30.3	28.1	30.6
	Range (min-max)	3.9-58	4-49.6	15-42.5
Anemia n = 624	**Present (Hct ≤26%)**	173	29	6
	Absent (Hct > 26%)	368	35	13
Retrovirus infection status n = 709	**Negative**	512	60	18
	Positive	109	8	2
Retrovirus n = 119	**FeLV+**	43	3	1
	FIV+	52	5	1
	FIV+FeLV	14	0	0
Blood typing test n= 709	**CARD**	44	3	1
	GEL	13	0	1
	SLIDE	13	4	0
	STRIP	268	42	10

AB Blood Type and Retroviral Status

	TUBE	283	19	8

531

533 Table 2. Reason for testing blood type and retroviral status for each group in a population

534 of 709 cats tested for association between blood type and retrovirus status

Reason for testing	Number	A Blood type	B Blood type	AB Blood type	Retrovirus positive	FELV positive	FIV positive	FELV/FIV positive
All Cases	709	621 (87.6%)	68 (9.6%)	20 (2.8%)	119 (16.8%)	47 (6.6%)	58 (8.2%)	14 (2.0%)
Epidemiologic Study	247	228 (92.3%)	13 (5.3%)	6 (2.4%)	61 (24.7%)	25 (10.1%)	30 (12.1%)	6 (2.4%)
Donor	210	183 (87.2%)	20 (9.5%)	7 (3.3%)	9 (4.3%)	1 (0.5%)	6 (2.9%)	2 (0.9%)
Prior to Blood Transfusion	175	141 (80.6%)	29 (16.6%)	5 (2.8%)	36 (20%)	16 (8.9%)	14 (7.8%)	6 (3.3%)
Mating	33	31 (93.9%)	1 (3.0%)	1 (3.0%)	0	0	0	0
Pre-operative	42	36 (85.7%)	5 (11.9%)	1 (2.4%)	13 (32.5%)	5 (12.5%)	8 (20%)	0
Unknown	2	2 (100%)	0	0	0	0	0	0

535

537

538 Table 3. Statistical evaluation of potential association between retroviral status and blood

539 type in a population of 709 cats

Retroviral Status	All Blood Types	P value	A Blood Type	P value	B Blood Type	P value	AB Blood Type	P value
Any Positive	119	*0.43*	109	*0.17*	8	*0.31*	2	*0.55*
All Negative	590		512		60		18	
FeLV Positive versus all retrovirus negative	47	*0.86*	43	*0.49*	3	*0.61*	1	*1*
FIV Positive versus all retrovirus negative	68	*0.94*	52	*0.68*	5	*1*	1	*1*
FeLV/FIV both positive versus all retrovirus negative	14	*0.59*	14	*0.23*	0	*0.38*	0	*1*

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