Original Article

Journal of Feline Medicine and Surgery
2022, Vol. 24(8) e194–e202
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DOI: 10.1177/1098612X221100082
journals.sagepub.com/home/jfm

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Lack of association between feline AB blood groups and retroviral status: a multicenter, multicountry 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 1). In addition, a sample of 33 cats with confirmed FIV infection was genotyped to determine blood groups (part 2).

Results In part 1, 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 ($P = 0.43$), between FeLV status and blood type ($P = 0.86$) or between FIV status and blood type ($P = 0.94$). There was no difference in the distribution of blood types between cats that were healthy and typed as possible blood donors vs sick cats that were typed prior to a possible transfusion ($P = 0.13$). In part 2, of the 33 FIV-infected cats, all blood group genotypes were identified, although this test did not discriminate type A from type AB.

Conclusions and relevance No relationship was 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.

Keywords: AB blood type system; retrovirus; feline leukemia; FeLV; feline immunodeficiency virus; FIV

Accepted: 21 April 2022

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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 and blood type have been found with West Nile virus,[5] hepatitis B virus[6] and norovirus infections.[7] There is also evidence to suggest 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 severe acute respiratory syndrome coronavirus 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 risks in animals. To our 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 (RHDV) infection in a virus strain-dependent manner, with some HBGAs facilitating infection. The results of this study suggest that polymorphism of expression of HBGAs might contribute to genetic resistance to RHDV.[17]

The major blood group system in cats is the AB system, which consists of three blood types: A, B and AB. Blood type is determined by three alleles, with A dominant over the rare AB, which is thought to be dominant over B.[18] Most clinical blood typing methods are phenotypic and rely on blood group antigen detection.[19] Genome-wide association studies have identified mutations in *CMAH*, which are believed to result in disrupted enzyme activity preventing the conversion of *N*-acetylneuraminic acid (NeuAc) to *N*-glycolyl-neuraminic acid (NeuGc) on the red blood cell surface.[18,20] Cats with blood type A have predominantly NeuGc, while cats with blood type B have NeuAc, and those with the rare type AB have both NeuGc and NeuAc expression on their red blood cells.[18] Several different mutations in *CMAH* have been identified, and some show selective expression in pure breeds.[20] The discovery of these *CMAH* polymorphisms associated with different blood types in cats has allowed the development of sequencing-based blood genotyping tests. However, genotyping schemes are restricted to include known polymorphisms, so do not 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, which 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 red blood cell destruction.[26] In a study comparing phenotypic and genotypic blood typing, there was 96% agreement (107/112 cats) but five 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 retrovirus–positive cats. In addition, we also suspected that we might see more type AB expression in cats presenting for critical care than in healthy cats.

Materials and methods

Study design

This study was conducted in two parts, which involved a retrospective multicenter study of cats with phenotypic blood type analysis and retroviral testing (part 1), and prospective blood group genotyping of a group of FIV-infected cats (part 2).

Part 1

Study population Study data were retrospectively collected from five veterinary facilities: one research laboratory in Italy, one veterinary teaching hospital (VTH) and one veterinary blood bank in the USA, and two VTHs with associated blood banks in Australia. Retrospective review periods correlated with 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 performed. All standard biosecurity measures and institutional safety procedures were followed when the authors originally collected and analyzed the feline blood samples. Additional ethical approval to retrospectively review case data was obtained at The University of Queensland (ANRFA 2022/AE000121).

Electronic medical records and cat blood donor records of the Veterinary Transfusion Research Laboratory (REVLab) at the University of Milan were available from January 2005 to December 2021. The Washington State University (WSU) VTH electronic medical record system was available from December 2010 to December 2021. The WSU 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 the University of Queensland's database was available from March 2017 to February 2022.

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 ⩽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 (FIV p15/p24/gp40: SNAP FIV/FeLV Combo Test [IDEXX Laboratories Italia]; FIV gp40: Anigen Rapid FIV Ab/FeLV Ag [BioNote]; FIV p15/p24: SNAP FIV/FeLV Combo Test [IDEXX Laboratories, USA]).

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]) were performed according to the manufacturer's instructions and as previously described.[32,33] A laboratory tube agglutination technique ('TUBE') using *Triticum vulgaris*,[24] a slide agglutination technique ('SLIDE') using *T vulgaris* and a gel typing kit ('GEL'; ID Gel-Test Micro Typing System [Diamed] – this test is no longer commercially available) were additional methods. At REVLab, all type B and AB results were confirmed by a back-typing technique as previously described.[32]

Part 2

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) in the field. Ethical approval for the study was obtained from the University of Sydney (N00/1-2012/3/5920 and 2017/1129).

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 commercially available FIV antibody PoC kits, as well as a positive FIV PCR result (FIV RealPCR; IDEXX Laboratories). Cats were concurrently screened for progressive FeLV infection as both PoC kits also detected FeLV p27 antigen (Anigen Rapid FIV/FeLV and Witness FeLV/FIV; Zoetis Animal Health). 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 h of blood collection using a commercially available kit (QIAamp DNA Mini Kit; Qiagen), as per the manufacturer's instructions, at the University of Sydney. The concentration and quality of extracted DNA was measured using a spectrophotometer (Nanodrop 1000; Thermo Fisher Scientific). 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 *CMAH* incorporating two single nucleotide polymorphisms 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 genomic DNA. Amplification was performed in a Bio-Rad T10096 well block cycler (Bio-Rad Labs) for 2 mins at 95°C followed by 37 cycles of 95°C for 15 s and 58°C for 30 s. Biotinylated PCR products were immobilized on streptavidin-coated Sepharose beads (GE Healthcare UK), purified and annealed with the sequencing primer (0.5 µM final concentration), as described in the PyroMark Gold Q96 reagents kit instruction manual

Table 1 Blood type listed by attributes (sex, reproductive status, breed, Hct, retroviral status, blood typing method and reason for typing) in a population of 709 cats tested for association between blood type and retroviral status

Variable	Level	Blood type		
		A (n = 621)	B (n = 68)	AB (n = 20)
Country (n = 709)	Italy	437	24	15
	USA	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
Breed (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
	Chinchilla	1	0	0
	Chinchilla 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
	Siamese	5	2	0
	Siamese mix	1	1	0
	Siberian Forest Cat	4	0	0
	Sphynx	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 (IQR)	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
	TUBE	283	19	8

Hct = hematocrit; IQR = interquartile range; FeLV = feline leukemia virus; FIV = feline immunodeficiency virus

(Qiagen). Pyrosequencing was performed in a PyroMark Q96 (Qiagen) 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).

Statistical analysis

The data were analyzed using standard descriptive statistics and reported as mean ± SD or median (range), based on their distribution. Univariate analysis of categorical data using the Fisher's exact test or χ^2 analysis was performed to determine possible associations between blood type and retroviral status, anemia and clinical status (healthy vs sick cats). Univariate analysis of categorical data using the Fisher's exact test or χ^2 analysis was also performed to determine possible associations between sex, blood type and retroviral status. A P value <0.05 was considered to be statistically significant. Data were analyzed using an open-source statistical software program (RStudio).

Results

Part 1

Population Baseline descriptive data for the included cats are 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 intact females, 157 spayed females, 137 intact males, 254 castrated males and 19 cats of unknown sex and neutering status due to incomplete information in the record. Most cats (n = 609; 85.9%) were non-pedigree or mixed breed varieties. There were 23 other cat breeds represented, with Maine Coon (n = 25) and Ragdoll (n = 17) cats the most common. The median age of the enrolled cats was 3 years (range 1 month–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 typing before mating to prevent feline isoerythrolysis in 33 (4.7%), preoperative 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 6.8% and SLIDE was used in 17 (2.4%). The GEL test was used in 14 cats (2.0%) 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 (84.1%) were type A, 10 (12.2%) were type B and three (3.6%) 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 statistically significant (P <0.001).

The 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 of blood type A,[36] two Siamese and one Siamese mix were found to have blood type B. The collected breed information was reported by the owner and was not verified by genetic tests.

Overall, 119 cats were retrovirus positive (Table 2). Of these, 47 were FeLV positive, 58 were FIV positive and 14 were positive for both FeLV and FIV. Of the retrovirus-positive cats, 36 were clinically ill and 22 were healthy and identified during preoperative or donor screening.

Table 2 Reason for testing blood type and retroviral status for each group in a population of 709 cats tested for association between blood type and retroviral status

Reason for testing	n	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 (20.5)	16 (9.1)	14 (8)	6 (3.4)
Mating	33	31 (93.9)	1 (3.0)	1 (3.0)	0	0	0	0
Preoperative	42	36 (85.7)	5 (11.9)	1 (2.4)	13 (31)	5 (12)	8 (19)	0
Unknown	2	2 (100)	0	0	0	0	0	0

Data are n (%)
FeLV = feline leukemia virus; FIV = feline immunodeficiency virus

Table 3 Statistical evaluation of potential association between retroviral status and blood type in a population of 709 cats

Retroviral status	All blood types (n)	*P* value	A blood type (n)	*P* value	B blood type (n)	*P* value	AB blood type (n)	*P* value
Any positive	119	0.43	109	0.17	8	0.31	2	0.55
All negative	590		512		60		18	
FeLV positive vs all retrovirus negative	47	0.86	43	0.49	3	0.61	1	1
FIV positive vs all retrovirus negative	68	0.94	52	0.68	5	1	1	1
FeLV/FIV both positive vs all retrovirus negative	14	0.59	14	0.23	0	0.38	0	1

FeLV = feline leukemia virus; FIV = feline immunodeficiency virus

The other 61 were typed during an epidemiological study and their health status was not recorded. Blood type relationship to retroviral illness could not be explored owing to the small number of cats and geographic variability, with more clinically ill cats recruited from Australia and more healthy cats from Italy. There was no relationship between overall retroviral status and blood type ($P = 0.43$). There was also no relationship specifically between FeLV status and blood type ($P = 0.86$), between FIV status and blood type ($P = 0.94$) or between FeLV/FIV coinfected status and blood type ($P = 0.59$) (Table 3). Male cats were significantly more likely to be FIV positive than female cats ($P < 0.001$). There was no sex predisposition with regard to FeLV positivity.

Clinical status

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

Clinically ill cats that were typed prior to a possible transfusion had a variety of diseases. Underlying primary disease processes included retroviral disease (n = 20), neoplasia (n = 19), immune-mediated disease (n = 17), *Mycoplasma haemofelis* infection (n = 10), kidney disease (n = 10), traumatic blood loss (n = 9), sepsis-related (n = 8), leukemia (n = 7), coagulopathy (n = 5), gastrointestinal blood loss (n = 4), feline infectious peritonitis (n = 3), other infectious disease (n = 3), unknown disease (n = 27), bone marrow toxicity (n = 1), hepatic lipidosis (n = 1) and diabetic ketoacidosis (n = 1). The underlying disease was undiagnosed in 27 cats. There was no difference in the distribution of blood types between cats that were healthy and typed as possible donors vs cats that 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 no difference in the distribution of blood types ($P = 1.0$). All cats infected with *M haemofelis* were from Australia. Of these 10 cats, eight (80%) were of blood type A and two (20%) were of type B.

Part 2

Population Thirty-three confirmed FIV-infected cats were included. One cat in this group was also FeLV positive. There were nine female spayed, one male intact and 23 male neutered cats. The median age was 7.3 years (range 3.4–16.1). There were 30 non-pedigree cats, one Bengal, one British Shorthair and one Himalayan. Of the 33 cats, 22 cats were of blood type A or AB, carrying *b*, seven were of type A or AB and not carrying *b*, and four were of 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, only a few veterinary studies 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 (odds ratio 0.1, 95% confidence interval 0–0.9).[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* species antigen was found. However, the small sample size of *Babesia* species antigen-positive dogs in that study could have confounded the association with DEA 1 blood type.[38] In the only feline study (available as an abstract), blood genotype *AA*, *Ab* or *bb* was determined in 263 cats from the UK that had previously tested positive (n = 131) or negative (n = 132) for *M haemofelis*, '*Candidatus* Mycoplasma haemominutum', and '*Candidatus* Mycoplasma turicensis' infection by quantitative PCR. The prevalence of each

individual haemoplasma species, single vs dual haemoplasma species infection and haemoplasma PCR copy number were compared between blood genotypes; no significant differences were found.[39]

In the current study, cats were tested for blood type and retroviral status for different reasons and by different methods. Some cats were healthy and the retrovirus was identified during screening, while in other cases cats were unwell and FIV/FeLV tested for possible retroviral-associated disease. In people with some types of leukemia, blood type antigen expression can vary with disease severity, so it is possible that a relationship could exist specifically with blood type and retroviral-related neoplasia but not with infection alone. It is also possible that retroviral status may have altered blood type expression, leading to discrepancies between phenotypic blood type test results and genotypic blood type. The present study did not include enough healthy retrovirus-positive cats or cats with FeLV-related leukemias or lymphomas to fully investigate this possibility. We could also not perform matched blood type phenotyping and genotyping on all cats. Further research is needed to investigate these factors.[15] Alternatively, blood type could be related to retroviral infection, but in the current study some retroviral infections may have been undetected by testing only for FeLV antigen and anti-FIV antibodies (part 1). Our group of FIV-infected cats confirmed with FIV PCR testing (part 2) makes this hypothesis less likely for this retrovirus. However, studies have shown that PCR provirus testing can identify regressive FeLV-infections that are missed with antigen testing alone.[27,40] We do not have information on the prevalence of FeLV regressive cats that are only FeLV proviral PCR-positive and p27 antigen-negative. Therefore, it would be interesting to evaluate blood type and retroviral status in a larger study specifically using FeLV PCR testing in addition to FeLV antigen detection, and looking at clinical manifestations of retroviral disease.

In this study, blood typing methods changed over time and were different between locations. Blood types B and AB classified phenotypically were back-typed for confirmation in many cats. While there were no clear notes of blood typing difficulties, this is hard to fully assess in a retrospective record review. Unlike previous studies, where discordant results were noted,[19,23–25] the majority of cats in the current study were typed by only 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 blood type B in cats is variable based on geography, with high numbers of cats with blood type B 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 haemotrophic mycoplasmas in cats from the UK did not reveal a relationship, further study looking at *Bartonella henselae* 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 multifactorial 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 targets for both HIV and FIV are T lymphocytes, HIV uses a different primary receptor (CD4) and at least seven coreceptors to the receptor and coreceptors 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 able to gain entry into cells only via CD134 and CXCR4, which are not expressed on red blood cells and instead expressed only on lymphocytes, macrophages, dendritic cells, microglia and astrocytes,[44,45] although further work is needed to confirm this.

Conclusions

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

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

Funding This study was supported, in part, by Piano di Sostegno alla Ricerca 2020, Linea 2, University of Milan, Italy. Funding for part 2 of this study was supported financially by the Australian Companion Animal Health Foundation (006/2013, 015/2017), and Boehringer Ingelheim Animal Health, Australia. IDEXX Laboratories, Zoetis Animal Health and BioNote donated FIV test kits for some of the animals tested. Mark Westman was supported by an Australian Postgraduate Award (APA), the Neil and Allie Lesue Scholarship and the Herbert Johnson Travel Grant Scholarship.

Ethical approval 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 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.

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