Received: 12 May 2022 | Revised: 29 August 2022 | Accepted: 21 September 2022

DOI: 10.1002/vetr.2282

Vet Record

ORIGINAL RESEARCH

Evaluating the association between blood genotype or phenotype and haemoplasma infection in UK and Italian cats

Eva Spada[1] | **Paola Galluzzo**[2] | **Alessandra Torina**[2] | **Guido R. Loria**[2] | **Roberta Perego**[1] | **Francesca Grippi**[2] | **Valeria Blanda**[2] | **Luciana Baggiani**[1] | **Alessia D'Amico**[1] | **Maria G. Pennisi**[3] | **Chris R. Helps**[4] | **Richard Malik**[5,6] | **Mark Westman**[7] | **Barbara Gandolfi**[8] | **Sarah Spencer**[9,10] | **Daniela Proverbio**[1] | **Séverine Tasker**[10,11]

[1]Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi, Italy

[2]Istituto Zooprofilattico Sperimentale della Sicilia 'Adelmo Mirri', Palermo, Italy

[3]Department of Veterinary Sciences, University of Messina, Messina, Italy

[4]Langford Vets, Bristol Veterinary School, University of Bristol, Langford, UK

[5]Centre for Veterinary Education, University of Sydney, Sydney, New South Wales, Australia

[6]School of Veterinary and Animal Science, Charles Sturt University, Wagga Wagga, New South Wales, Australia

[7]Sydney School of Veterinary Science, University of Sydney, Sydney, New South Wales, Australia

[8]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, Missouri, USA

[9]Comparative Biological Sciences, Royal Veterinary College, London, UK

[10]Bristol Veterinary School, University of Bristol, Bristol, UK

[11]Linnaeus Veterinary, Shirley, UK

Correspondence
Eva Spada, Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi, Italy.
Email: eva.spada@unimi.it

Funding information
Piano di Sostegno alla Ricerca 2020, Linea 2, University of Milan, Italy; Clinical Research Grant Funding from Langford Vets

Abstract

Background: In humans, blood groups are associated with varying prevalence of infections. The aim of this study was to determine if associations exist between the feline AB blood group system and haemoplasma infection.

Methods: Data from two studies were combined. In the first study, DNA samples from 131 haemoplasma-infected and 132 haemoplasma-uninfected UK cats underwent pyrosequencing to determine their blood genotype as *AA*, *Ab* or *bb*. In the second study, blood samples from 160 Italian cats of known blood phenotype A, B or AB underwent PCR testing for feline haemoplasma species DNA.

Results: Haemoplasma infection was demonstrated in cats of all phenotypes and genotypes. A significantly higher number of *Ab* genotype cats tested positive for overall haemoplasma infection status ($p = 0.04$) and for *Mycoplasma haemofelis* infection ($p = 0.03$).

Limitations: Haemoplasma-infected Italian cats were few, possibly increasing the chance of type II error, and the presence of purebred cats in the sample population may have had a confounding effect.

Conclusions: Feline haemoplasmas do not appear to preferentially use either blood type A or B antigens as attachment sites for erythrocyte colonisation. Further investigations in a larger number of haemoplasma-infected cats of known blood phenotype are warranted to explain the association between genotype *Ab* and haemoplasma infection.

KEYWORDS
AB group system, blood type, feline, haemotropic mycoplasma, infections

INTRODUCTION

Erythrocytes have a complex cell surface consisting of the cell membrane surrounded by a glycocalyx – a set of polysaccharides, glycolipids and glycoproteins that are linked covalently to the cell membrane. The glycocalyx of the erythrocyte membrane contains particular gangliosides characterised by one or more residues of neuraminic acid (sialic acid).[1] In the cat, the neuraminic acid form of these gangliosides determines the blood group antigen(s) present and thus blood type.[2]

The major blood group system in cats is the AB system, which consists of three blood phenotypes: type A, the most common in feline populations, especially in non-pedigree cats; type B, more common in certain breeds of cats and in non-pedigree cats in some countries; and type AB (also known as type C), the rarest blood type in the feline population.[3] The erythrocyte membrane of type A cats is characterised by the presence of N-glycolylneuraminic acid (NeuGc) as the predominant residue, but lesser amounts of N-acetylneuraminic acid (NeuAc) are also present. Type B cats are characterised by the presence of only NeuAc, while type AB cats have similar amounts of NeuGc and NeuAc on the surface of their erythrocytes.[2,4]

The A, B and AB blood types are genetically determined, and the genetic basis of the feline AB blood group system has been investigated, with blood group being determined by variants in the cytidine monophospho-N-acetylneuraminic acid hydroxylase (*CMAH*) gene.[5] The CMAH enzyme determines the type of neuraminic acid on erythrocytes and is active in type A cats, converting NeuAc to NeuGc, while non-functional in type B cats.[5] Since both neuraminic acids are present on the erythrocytes in type AB cats,[4] CMAH must convert some NeuAc to NeuGc in these cats. Genetic variants associated with the B blood type in most cats have been identified.[5] More recently, the multiple causative variants leading to phenotype AB in domestic cats,[6] in purpose-bred cats[7] and for blood phenotype AB specific to Ragdolls[8] have been identified. In a study comparing phenotypic and genotypic blood typing, there was 96% agreement (107 of 112 cats), with the remaining 4% comprising five discordant results that all involved type B phenotype; this suggested that additional unknown polymorphisms existed in *CMAH* linked to blood phenotype B,[9] which have since been identified.[6,7]

Feline blood types are inherited according to Mendelian genetics[10] and determined by three alleles, *A*, *b* and *ab*, with *A* being dominant over the rare *ab* allele, which in turn is dominant over *b*.[7,11] Thus, cats with blood type A are genetically *A*/*A*, *A*/*ab* or *A*/*b*. Cats with type AB are *ab*/*ab* or *ab*/*b*, while cats with type B are always *b*/*b*.[7] Currently, genetic testing for feline blood genotypes identifies the recessive *b* allele that is associated with type B blood. Cats with two copies of the *b* allele (*b*/*b*) have phenotype B blood. Cats with only one copy of the *b* allele can be phenotype A (*A*/*b*) or the rare blood phenotype AB (*ab*/*b*). Therefore, genetic testing currently cannot distinguish between phenotype A and the rare AB phenotype.[5]

Blood group antigens are known to be important for transfusion compatibility, but over time it has been recognised that these structures can also serve other functions. Neuraminic acids are ubiquitous on mammalian cell membranes and possess physiological functions in synaptogenesis, synaptic transmission and cellular signalling,[12] as well as roles in oncogenesis and the pathogenesis of some infectious diseases in people.[13] Blood group antigens can also have a direct role in pathogenic infections as they can act as cell surface receptors for bacteria (e.g., *Mycoplasma pneumoniae*, *Yersinia pestis*, *Vibrio cholerae*), viruses (e.g., human immunodeficiency virus, norovirus, parvovirus) and protozoa (e.g., *Plasmodium* spp.), facilitating host colonisation and/or allowing pathogens to evade the innate or adaptive immune responses.[14-17]

Little is known about possible associations between blood group antigens and pathogens in veterinary medicine.[18-20] Genetic factors may affect susceptibility and/or tolerance to certain infections, and different blood types may exert either protective or predisposing effects for haemoplasma infection.[21] Haemoplasmas are haemotropic mycoplasmas, bacteria without rigid cell walls, that parasitise erythrocytes. Three feline haemoplasma species are known to exist: *Mycoplasma haemofelis* (Mhf), '*Candidatus* Mycoplasma haemominutum' (CMhm) and '*Candidatus* Mycoplasma turicensis' (CMt). Infection is common worldwide and can cause significant anaemia, sometimes with concurrent erythrocyte autoantibodies.[21] Epidemiological studies have shown variations in haemoplasma prevalence among different feline populations in various geographical regions, and among individuals living in the same geographical area due to factors such as age, lifestyle and habitat (e.g., client-owned vs. stray).[22-33]

To date, no data are available on the possible associations between different blood types and haemoplasma prevalence in cats. As erythrocyte pathogens, haemoplasmas may feasibly use glycolipids, including those that determine blood group antigens, to interact with the erythrocyte membrane.[2] Indeed, in people, neuraminic acid-containing receptors have been implicated in the adherence of *M. pneumoniae*, an important bacterial pathogen responsible for human respiratory tract diseases, to erythrocytes and other cell types, causing a transient autoimmune disorder characterised by the presence of high-titre erythrocyte autoantibodies.[17,35,36]

Previously published data have suggested that feline haemoplasmas induce cleavage of NeuAc or disruption of glycopeptides or glycolipids, either cross-reacting with or uncovering cryptic antigenic determinants on the cell surface, rendering them immunogenic.[36] We hypothesise that there might be an association between feline blood type and haemoplasma infection, as NeuAc or NeuGc present on feline erythrocytes could serve as attachment sites

for haemopathogens. The aim of this observational study was to evaluate any association between feline haemoplasma infection status and either blood genotype in cats from the UK (study 1) or blood phenotype in cats from Italy (study 2).

MATERIALS AND METHODS

Study 1—UK samples

Archived DNA samples from 263 client-owned UK cats that had previously tested positive (n = 131) or negative (n = 132) for Mhf, CMhm and/or CMt infection by quantitative PCR (qPCR, methodology as in Peters et al.[26]) at Langford Vets, University of Bristol, UK, were used in study 1. These DNA samples had been previously extracted from whole-blood samples submitted to Langford Vets by referring veterinarians between 2014 and 2015 for haemoplasma PCR testing for diagnostic purposes. Positive qPCR results obtained from the 131 haemoplasma-infected cats comprised 19 Mhf, 112 CMhm and nine CMt results, including 122 cats with single species infections and nine cats with dual species infections. No triple haemoplasma species infections were detected in this population. Haemoplasma qPCR testing had been requested by the submitting veterinarian based on a clinical need for infection diagnosis, but no further signalment or clinical data on the cats from which the archived DNA samples were derived were available.

Feline blood genotyping, to determine cats as genotype *AA* (corresponding to phenotype A or AB), *Ab* (corresponding to phenotype A or AB) or *bb* (corresponding to phenotype B), was performed on the archived DNA samples by pyrosequencing for two variants in exon 2 of *CMAH* as described by Tasker et al.[9] The PCR primers used to amplify an 80 bp region of *CMAH* (exon 2) were: forward 5′-Biotin-GAAGACCGGCAAAGATTTCAT-3′ and reverse 5′-CTCCTTGATGCTTGCACAC-3′. The pyrosequencing primer was 5′-ACACGTTCTTGCAC-GCCCTCA-3′. Biotinylated PCR products were immobilised on streptavidin-coated sepharose beads (GE Healthcare UK), purified and annealed as per the kit instruction manual (Pyro-Mark Gold Q24, Qiagen). Pyrosequencing was performed in a PyroMark Q24 (Qiagen) automated 24-well pyrosequencer, according to the manufacturer's instructions, with a nucleotide dispensation order of GCTGTCGATCT. Pyrosequencing data were evaluated using PyroMark Q24 v2.0.6 software (Qiagen). Samples found to be c.142G>A homozygous, c.139C>T homozygous or c.142G>A and c.139C>T compound heterozygous were classified as blood genotype B (*bb*).

Study 2—Italian samples

Archived frozen ethylenediamine tetra-acetic acid (EDTA)-anticoagulated whole-blood samples from Italian cats, most of which were previously tested in an epidemiological study of AB blood group phenotype, were used in this study.[37] A total of 448 samples were typed in the previous study, which aimed to evaluate the prevalence and differences in blood phenotype distribution between feline populations in northern versus southern Italy. Of these, 378 were blood type A, 38 were type B and 32 were type AB. These blood sample were collected for clinical reasons at the Universities of Milan (northern Italy) and Messina (southern Italy) between November 2014 and December 2015.

Three different groups of Italian cats were sampled: (i) client-owned cats presented for routine check-ups before neutering or for diagnostic purposes in the case of sick cats, (ii) shelter cats evaluated prior to adoption or neutering, and (iii) stray colony cats before neutering and for preventative health reasons. Data on signalment (breed, sex, reproductive status and age [young adults 3 months to 2 years; adults 3–10 years; seniors older than 11 years]), habitat (owned, shelter or stray) and origin (northern vs. southern Italy) were available for most cats. Blood phenotype was determined using a tube agglutination method, using a back typing technique to confirm all type B and AB cats, as previously described,[37] at the Veterinary Transfusion Research Laboratory (REVLab) of the University of Milan, Italy. Being rarer blood types, all available frozen blood type B and AB blood samples were included in the Italian study population. For each type B and AB sample, a type A sample with similar signalment, habitat and origin was chosen for inclusion. If more than one matched type A samples was available, all were included. The final population comprised 160 feline blood samples: 86 (53.7%) type A, 38 (23.7%) type B and 36 (22.5%) type AB.

DNA was extracted from the archived EDTA whole-blood samples using the commercial DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol, and qPCR testing was performed to detect haemoplasma infection using previously reported primers[27] and the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). DNA (5 μl) was added to a 20 μl mix containing 1× of SSo advanced Universal Probes Supermix (Bio-Rad) and 0.5 μl of each primer (10 μM) and TaqMan probe (10 μM). The instrument programme consisted of an initial denaturation of 3 minutes at 95°C followed by 40 cycles of amplification, which included a denaturation step at 95°C for 10 seconds and an annealing/extension step at 60°C for 30 seconds. All runs included field positive samples and a negative control (DNAase/RNAase free water). PCR testing was performed at Istituto Zooprofilattico Sperimentale (IZS) of Sicily, Italy.

The 'Strengthening the Reporting of Observational Studies in Epidemiology' recommendations were followed to describe the study methods.[38]

TABLE 1 Characteristics of the two studies that aimed to determine if a possible association existed between feline blood type and haemoplasma status

	Study 1: UK feline samples	Study 2: Italian feline samples
Type of study	Retrospective	Retrospective matched case control
Selection of population	By haemoplasma infection status	By blood type (phenotype) status
Total no. of samples evaluated	263	160
No. of haemoplasma-infected cats	131 (49.8%) (19 Mhf [7.2%], 112 CMhm [42.6%], 9 CMt [3.4%])	28 (17.5%) (16 Mhf [10.0%], 16 CMhm [10.0%], 1 CMt [0.6%])
No. of haemoplasma-uninfected cats	132 (50.2%)	132 (82.5%)
Blood type categorisation method	By genotype (122 *AA* [46.4%], 104 *Ab* [39.5%], 37 *bb* [14.1%])	By phenotype (86 A [53.8%], 38 B [23.8%], 36 AB [22.5%])

Abbreviations: CMhm, '*Candidatus* Mycoplasma haemominutum'; CMt, '*Candidatus* Mycoplasma turicensis'; Mhf, *Mycoplasma haemofelis*.

TABLE 2 Blood group genotyping results and statistical analysis of haemoplasma infection status and the blood genotypes *AA*, *Ab* and *bb* in 263 UK cats

		Blood genotype			
PCR result		***AA* (*n* = 122), *n* (%)**	***Ab* (*n* = 104), *n* (%)**	***bb* (*n* = 37), *n* (%)**	**χ^2; *p*-value**
Any haemoplasma	Positive (*n* = 131)	56 (21.3)	60 (22.8)	15 (5.7)	$\chi^2 = 4.602$; $p = 0.10$
	Negative (*n* = 132)	66 (25.1)	44 (16.7)	22 (8.4)	
Mhf	Positive (*n* = 19)	6 (2.3)	12 (4.6)	1 (0.4)	$\chi^2 = 4.985$; $p = 0.08$
	Negative (*n* = 244)	116 (44.1)	92 (35.0)	36 (13.7)	
CMhm	Positive (*n* = 112)	49 (18.6)	49 (18.6)	14 (5.3)	$\chi^2 = 1.507$; $p = 0.47$
	Negative (*n* = 151)	73 (27.8)	55 (20.9)	23 (8.7)	
CMt	Positive (*n* = 9)	5 (1.9)	4 (1.5)	0 (0.0)	$\chi^2 = 1.536$; $p = 0.46$
	Negative (*n* = 254)	117 (44.5)	100 (38.0)	37 (14.1)	
Single versus dual infection	Single (*n* = 122)	52 (39.7)	55 (42.0)	15 (11.5)	$\chi^2 = 1.314$; $p = 0.52$
	Dual (*n* = 9)	4 (3.1)	5 (3.8)	0 (0.0)	

Note: Statistical significance was $p < 0.05$.
Abbreviations: CMhm, '*Candidatus* Mycoplasma haemominutum'; CMt, '*Candidatus* Mycoplasma turicensis'; Mhf, *Mycoplasma haemofelis*.

Statistical methods

Possible associations between blood group genotype, phenotype and haemoplasma infection status (overall haemoplasma infection prevalence, individual haemoplasma species prevalence, single vs. multiple haemoplasma infection) were investigated by chi-squared analysis or the Fisher's exact test, as appropriate. Data from study 1 of UK cats using blood genotype and from study 2 of Italian cats using blood phenotype were also combined to test for any association between cats of blood type B (i.e., cats defined as genotype *bb* or phenotype B) and blood type non-B (i.e., cats defined as genotype *AA* or *Ab*, or phenotype A or AB) and overall haemoplasma, Mhf, CMhm, CMt and single versus multiple infection status. This analysis was not performed for cats with blood type A and AB because phenotype/genotype classifications for these blood types cannot be merged (genotype results *AA* and *Ab* do not differentiate between phenotype A or phenotype AB). Significance was set at $p < 0.05$. Odds ratios (OR) with a 95% confidence interval (CI) were calculated when a statistically significant association was identified. Statistical analysis was conducted using commercially available software (MedCalc Statistical Software, version 20.023, MedCalc Software, Ostend, Belgium).

RESULTS

The characteristics of the two studies are summarised in Table 1.

Study 1—UK samples

Blood group genotyping of the 263 cats revealed 122 (46.4%) *AA*, 104 (39.5%) *Ab* and 37 (14.1%) *bb* cats. Of the 131 cats that tested positive for haemoplasma species, 56 (43%) were *AA*, 60 (46%) were *Ab* and 15 (11%) were *bb*. Of the 132 that tested haemoplasma negative, 66 (50%) were *AA*, 44 (33%) were *Ab* and 22 (17%) were *bb*. No significant difference in the prevalence of haemoplasma infection between the three blood genotypes was found (Table 2).

When cats with an *Ab* genotype were compared to cats with a non-*Ab* genotype (*AA* and *bb*), they were more likely to be haemoplasma PCR positive ($p = 0.04$, OR = 1.7, 95% CI = 1.0–2.8). This was due to a higher

number of *Ab* genotype cats being infected with Mhf compared to cats with non-*Ab* genotype ($p = 0.03$, OR = 2.8, 95% CI = 1.1–7.5) (Table 3). No other significant associations were found (see Table 3).

Study 2—Italian samples

Of 160 selected feline blood samples, 70 (43.7%) were from owned cats, 35 (21.9%) from shelter cats and 55 (34.4%) from stray colony cats; 103 (64.4%) were from northern Italy and 57 (35.6%) were from southern Italy. A total of 136 (85%) were domestic shorthair cats and 24 (15%) were purebred cats (15 Ragdolls, two Persians, two Bengals, two Scottish Folds, one Siberian, one Maine Coon and one British shorthair); 82 (51.2%) were males and 78 (48.7%) were females. Age was recorded for 133 cats; 81 (50.6%) were young adults aged less than 2 years, 59 (39.8%) were adult cats (3–10 years) and 14 (9.5%) were senior (older than 11 years). Median age was 2 years (range: 3 months to 16 years).

Analysis by qPCR revealed 28 (17.5%) haemoplasma-positive samples; Mhf and CMhm were detected in 16 samples each and CMt in one sample. Of the infected cats, 23 had a single species infection and five had dual species infections (four Mhf and CMhm; one CMhm and CMt). No triple haemoplasma species infections were found.

Statistical analysis revealed no significant association between blood phenotype and haemoplasma infection (Tables 4 and 5).

Combined studies 1 and 2—UK and Italian samples

Combining studies 1 and 2, blood type B cats (i.e., cats with genotype *bb* or phenotype B) represented 75 out of 423 (17.7%) samples. Of these type B cats, 21 out of 75 (28.0%) were haemoplasma-infected versus 138 of 348 (39.7%) for non-blood group B cats. Haemoplasma prevalence did not differ significantly between the two groups ($\chi^2 = 3.564$, $p = 0.06$). Four of 75 blood group B cats were Mhf-infected versus 32 of 348 non-type B-infected cats ($p = 0.36$, Fisher's exact test), while 18 out of 75 were CMhm-positive versus 110 of 348 non-type B CMhm-positive cats ($\chi^2 = 1.689$, $p = 0.19$) and no type B cats were CMt-infected compared to 11 of 348 non-type B-infected cats ($p = 0.22$, Fisher's exact test). Of 159 haemoplasma-infected cats, 20 type B cats had a single infection compared to 124 non-type B cats with a single infection, while dual infection was recorded in only one type B cat versus 14 non-type B cats ($p = 0.70$, Fisher's exact test).

DISCUSSION

We investigated potential associations between feline blood type and haemoplasma infection because many infectious diseases in human patients are linked to

TABLE 3 Comparison of haemoplasma infection status among the different blood genotypes *AA*, *Ab* or *bb* in 263 UK cats

	PCR haemoplasma status								
Blood genotype	**Any haemoplasma-infected cats ($n = 131$)**	**Any haemoplasma-uninfected cats ($n = 132$)**	**Mhf-infected cats ($n = 19$)**	**Mhf-uninfected cats ($n = 244$)**	**CMhm-infected cats ($n = 112$)**	**CMhm-uninfected cats ($n = 151$)**	**CMt-infected cats ($n = 9$)**	**CMt-uninfected cats ($n = 254$)**	**Single infection ($n = 122$)**	**Dual infection ($n = 9$)**
AA	56	66	6	116	49	73	5	117	52	4
Non-*AA*	75	66	13	128	63	78	4	137	70	5
χ^2; *p*-value	$\chi^2 = 1.385$; $p = 0.24$		$\chi^2 = 1.799$; $p = 0.18$		$\chi^2 = 0.544$; $p = 0.46$		$p = 0.74$[a]		$p = 1.00$[a]	
Ab	60	44	12	92	49	55	4	100	55	5
Non-*Ab*	71	88	7	152	63	96	5	154	67	4
χ^2; *p*-value	$\chi^2 = 4.259$; $p = \mathbf{0.04}$ OR = 1.7 (95% CI = 1.0–2.8; $p = 0.04$)		$\chi^2 = 4.759$; $p = \mathbf{0.03}$ OR = 2.8 (95% CI = 1.1–7.5; $p = 0.04$)		$\chi^2 = 1.438$; $p = 0.23$		$p = 0.74$[a]		$p = 0.73$[a]	
bb	15	22	1	36	14	23	0	37	15	0
Non-*bb*	116	110	18	208	98	128	9	217	107	9
χ^2; *p*-value	$\chi^2 = 1.474$; $p = 0.23$		$p = 0.50$[a]		$\chi^2 = 0.395$; $p = 0.53$		$p = 0.37$[a]		$p = 0.60$[a]	

Note: Statistically significant results ($p < 0.05$) are in bold.
Abbreviations: CI, confidence interval; CMhm, '*Candidatus* Mycoplasma haemominutum'; CMt, '*Candidatus* Mycoplasma turicensis'; Mhf, *Mycoplasma haemofelis*; OR, odds ratio.
[a]*p*-Values from Fisher's exact test.

TABLE 4 Blood phenotyping results and statistical analysis of haemoplasma infection status and blood phenotypes (A, B and AB) in 160 Italian cats

PCR result		Blood phenotype			
		A ($n = 86$)	B ($n = 38$)	AB ($n = 36$)	χ^2; p-value
Any haemoplasma	Positive ($n = 28$)	16	6	6	$\chi^2 = 0.167$; $p = 0.92$
	Negative ($n = 152$)	70	32	30	
Mhf	Positive ($n = 16$)	9	3	4	$\chi^2 = 0.257$; $p = 0.88$
	Negative ($n = 144$)	77	35	32	
CMhm	Positive ($n = 16$)	8	4	4	$\chi^2 = 0.108$; $p = 0.95$
	Negative ($n = 144$)	78	34	32	
CMt	Positive ($n = 1$)	1	0	0	$\chi^2 = 0.866$; $p = 0.65$
	Negative ($n = 159$)	85	38	36	
Single versus dual infection	Single ($n = 23$)	14	5	4	$\chi^2 = 1.299$; $p = 0.52$
	Dual ($n = 5$)	2	1	2	

Note: Statistical significance was $p < 0.05$.
Abbreviations: CMhm, '*Candidatus* Mycoplasma haemominutum'; CMt, '*Candidatus* Mycoplasma turicensis'; Mhf, *Mycoplasma haemofelis*.

blood group.[14-17] Furthermore, feline haemoplasmas may induce cleavage of NeuAc, disrupting erythrocyte glycopeptides or glycolipids, and cross-react with, or uncover, cryptic antigenic determinants on the cell surface, rendering them immunogenic.[36]

In human patients, initiation of invasion by malaria merozoites is mediated through specific interactions between protozoan receptors and ligand molecules on the erythrocyte membrane. Duffy blood group antigenic determinants on the erythrocyte surface are required for invasion of erythrocytes by *Plasmodium vivax* merozoites. There is a complete absence of the molecule carrying the Duffy blood group antigens on erythrocytes in almost all West African people, and this absence provides protection from *P. vivax*.[17]

While there is growing evidence in people that blood group can affect host susceptibility to infectious agents,[14-17] little is known about the effect of blood type on infectious diseases in animals. An experimental study in rabbits demonstrated that histo-blood group antigens (HBGAs) act as attachment factors for rabbit haemorrhagic disease calicivirus, with certain HBGAs facilitating infection.[19] A study of apparently healthy mixed-breed dogs from Zimbabwe infected by *Babesia* spp. found no association between canine erythrocyte antigen 1.1 blood type and *Babesia* spp. infection.[18] The results of the current study add to the limited veterinary literature in this area. Recent published research identified no relationship between feline retroviral status and AB blood group system types in a multicentre, multicountry study.[20] To the authors' knowledge, this is the first study investigating the possible relationship between blood type and haemoplasma status in cats. The hypothesis that haemoplasma infection status could be associated with blood phenotype was not supported in the populations investigated in the current study.

When comparing cats with *Ab* genotype versus non-*Ab* genotype in the UK population, the former were 1.7 times more likely to test positive for any haemoplasma species infection. On an individual haemoplasma species level, cats with an *Ab* genotype were 2.8 times more likely to be Mhf-positive compared to cats with a non-*Ab* genotype. We have not found a plausible biological explanation for this association. The current genetic test for feline blood genotype cannot distinguish between blood phenotype A and type AB.[5] Therefore, a genotype *Ab* cat could be either a phenotype A or AB cat. Phenotype A and AB cats both have NeuGc on the surface of their erythrocytes, while type B cats are characterised by the presence of only NeuAc.[2,4] The fact that 21 type B cats were haemoplasma infected, with four of these being Mhf infected, excludes the notion that NeuGc could serve as an attachment site on host erythrocytes for feline haemoplasmas in general and for Mhf in particular. One aspect we were not able to explore in the UK study was the breeds of the feline population investigated. Certain breeds have an higher prevalence of type A and type AB blood phenotype, such as Maine Coons[39] and Ragdolls,[5,40] respectively. It is possible that breed affects both genotype and predisposition to haemoplasma infection. In addition to genetic predisposition, environmental/husbandry effects in some breeds (e.g., group housing, intensive breeding) may predispose to transmission of haemoplasma infection, although the natural route of haemoplasma transmission in the field is not known.[41] In the absence of data on the breeds in the feline UK population, unfortunately this effect cannot be explored.

Additional information regarding both blood genotype and haemoplasma infection prevalence was gleaned from this study. The blood genotype prevalences determined in study 1 of the UK cats (46.4% *AA*, 39.5% *Ab* and 14.1% *Ab*) were comparable with previous published data from the UK, where 112 cats were genotyped as 52.7% *AA*, 35.8% *Ab* and 12.5% *Ab*.[9]

The prevalence of haemoplasma infection of 17.5% in the Italian population was consistent with previous worldwide and Italian epidemiological studies. In Italy, epidemiological studies have reported haemoplasma prevalences ranging from 11.6% in 958 owned cats[25] to 33.1% in 260 stray colony cats from northern

TABLE 5 Comparison of haemoplasma infection status among the different blood phenotypes A, B or AB in 160 Italian cats

Blood phenotype	PCR haemoplasma status									
	Any haemoplasma-infected cats ($n = 28$)	Any haemoplasma-uninfected cats ($n = 132$)	Mhf-infected cats ($n = 16$)	Mhf-uninfected cats ($n = 144$)	CMhm-infected cats ($n = 16$)	CMhm-uninfected cats ($n = 144$)	CMt-infected cats ($n = 1$)	CMt-uninfected cats ($n = 159$)	Single infection ($n = 23$)	Dual infection ($n = 5$)
A	16	70	9	77	8	78	1	85	14	2
Non-A	12	62	7	9	8	66	0	74	9	3
χ^2; *p*-value	$\chi^2 = 0.156$; $p = 0.69$		$\chi^2 = 0.044$; $p = 0.83$		$\chi^2 = 0.100$; $p = 0.75$		$p = 1.00$[a]		$p = 0.66$[a]	
B	6	32	3	35	4	34	0	38	5	1
Non-B	22	100	13	109	12	110	1	121	18	4
χ^2; *p*-value	$\chi^2 = 0.100$; $p = 0.75$		$p = 0.76$[a]		$p = 1.00$[a]		$p = 1.00$[a]		$p = 1.00$[a]	
AB	6	30	4	32	4	32	0	36	4	2
Non-AB	22	102	12	112	12	112	1	123	19	3
χ^2; *p*-value	$\chi^2 = 0.022$; $p = 0.88$		$p = 0.76$[a]		$p = 0.76$[a]		$p = 1.00$[a]		$p = 0.29$[a]	

Note: Statistical significance was $p < 0.05$.
Abbreviations: CMhm, '*Candidatus* Mycoplasma haemominutum'; CMt, '*Candidatus* Mycoplasma turicensis'; Mhf, *Mycoplasma haemofelis*.
[a]*p*-Values from Fisher's exact test.

Italy.[30] While CMhm was the most commonly detected species in previous worldwide and Italian studies,[22-33] the prevalence of CMhm was the same as Mhf (10% for each) in this Italian population. Selecting samples based on blood type and the larger number of young adults (50.7%), compared to adult (39.8%) and senior cats (9.5%), may have led to this result. CMhm is usually more prevalent in older cats, presumably because cats have an increasing risk of acquiring chronic subclinical infection over their lifetime.[22,23] In agreement with previous studies,[23-26,28] CMt was the least commonly identified haemoplasma species, with only one Italian cat infected. This cat was from southern Italy, where infection with CMt has previously been reported in owned cats.[24] This cat had a dual CMt and CMhm infection, again in agreement with studies showing that CMt infections often occur with other haemoplasma co-infections, especially CMhm.[23,26,28]

Limitations to this study could have led to the lack of association between blood group and haemoplasma infection. First, all samples analysed had been convenience samples collected retrospectively, rather than being part of a randomised prospective study. For the Italian study, samples were selected based on known blood phenotype, so that all three blood phenotypes were represented (including the rarer type B and AB blood types), and the number of haemoplasma-infected cats in this population was therefore relatively small, possibly increasing the chance of type II error. Similarly, the UK samples selected were chosen based on haemoplasma infection status, so that approximately equal numbers of haemoplasma-infected and non-infected cats were included. Additionally, the presence of purebred cats in the feline sample population may have had a confounding effect in both studies. This is because some breeds may be more prone to developing certain infections (e.g., feline infectious peritonitis[42]), and as discussed above, could also have a higher frequency of certain blood types, for example, type AB in Ragdolls[5,40] or type B in British Shorthairs.[43] This could also explain the low, although not statistically significant, *p*-value of 0.06 for haemoplasma-infected blood type B cats (i.e., cats with genotype *bb* or phenotype B) versus non-B cats in the combined studies 1 and 2. However, the low number of purebred cats in the Italian study (24 of 160 cats), and the lack of knowledge of feline breeds in the UK study, precluded a powered statical evaluation of the effect of feline breeds on our results. Further investigation into the possible effect of breed is therefore warranted.

In summary, haemoplasma infection was recorded in cats of all blood phenotypes and genotypes. This suggests that feline haemoplasmas do not preferentially use either NeuAc or NeuGc as attachment sites for erythrocyte colonisation. Our finding that cats with *Ab* genotype were more likely to be infected with haemoplasmas (particularly Mhf) than non-*Ab* genotype cats warrants further investigation. Future studies examining a greater number of haemoplasma-infected cats with known blood phenotype are war-

ranted to explain our results and expand our knowledge on possible associations between feline blood antigen type and haemoplasma infection.

AUTHOR CONTRIBUTIONS

Conceptualisation, supervision, writing—original draft and editing: Eva Spada, Séverine Tasker and Daniela Proverbio. *Methodology and writing—original draft*: Paola Galluzzo, Alessandra Torina, Guido R. Loria, Roberta Perego, Francesca Grippi, Valeria Blanda, Luciana Baggiani, Alessia D'Amico, Sarah Spencer and Maria G. Pennisi. *Investigation, writing—review and editing*: Chris R. Helps, Richard Malik, Mark Westman and Barbara Gandolfi.

ACKNOWLEDGEMENTS

The authors would like to thank Vito Priolo and Cyndi Mangano for their help in obtaining the feline samples at University of Messina. Part of this study was already presented as an oral presentation and published as abstract as follows: Spencer S, Helps C, Malik R, Gandolfi B, Tasker S. Lack of an association between blood genotype and haemoplasma infection in UK cats. In: Proceedings of BSAVA Congress, Birmingham, UK. 2016. Open access funding was provided by Università degli Studi di Milano within the CRUI-CARE agreement. The authors would also like to thank Dave Morris of the Molecular Diagnostic Unit at Langford Vets and Chelsea Hicks (previously of Bristol Veterinary School, University of Bristol and Langford Vets) for provision of Clinical Research Grant Funding used to perform study 1 in UK cats. Study 2 of this research was supported, in part, by Piano di Sostegno alla Ricerca 2020, Linea 2, University of Milan, Italy.

Open Access Funding provided by Universita degli Studi di Milano within the CRUI-CARE Agreement.

CONFLICTS OF INTEREST

Chris R. Helps holds an honorary position at the University of Bristol and previously worked at the Diagnostic Labs, Langford Vets, University of Bristol, which undertakes commercial genetic testing of cats, including blood genotyping. None of the other authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

DATA AVAILABILITY STATEMENT

Data that support the finding of this study are available on request from the corresponding author.

ETHICS STATEMENT

Blood sampling in all cats (UK and Italy) was originally performed by veterinarians solely for the patient's benefit and for diagnostic purposes. Only surplus blood was used for this study. Based on the University of Milan's, the University of Messina's and the University of Bristol's animal use regulations at the time, formal ethical approval was not needed as cats were sampled for diagnostic purposes and informed consent was given by the owners, the director of the shelters, vets and the legal representative of the feline colonies for storage and use of surplus blood samples and data for scientific purposes.

ORCID

Eva Spada https://orcid.org/0000-0003-3898-6955
Paola Galluzzo https://orcid.org/0000-0003-1766-5327
Alessandra Torina https://orcid.org/0000-0003-1555-8309
Francesca Grippi https://orcid.org/0000-0003-1470-6496
Valeria Blanda https://orcid.org/0000-0003-4657-407X
Chris R. Helps https://orcid.org/0000-0003-3405-4843
Mark Westman https://orcid.org/0000-0001-7400-1960
Daniela Proverbio https://orcid.org/0000-0003-3922-6069
Séverine Tasker https://orcid.org/0000-0002-4059-1402

REFERENCES

1. Smith JE. Erythrocyte membrane: structure, function, and pathophysiology. Vet Pathol. 1987;24:471–6.
2. Andrews GA, Chavey PS, Smith JE, Rich L. N-glycolylneuraminic acid and N-acetylneuraminic acid define feline blood group A and B antigens. Blood. 1992;79(9):2485–91.
3. Gavazza A, Rossi G, Antognoni MT, Cerquetella M, Miglio A, Mangiaterra S. Feline blood groups: a systematic review of phylogenetic and geographical origin. Animals. 2021;11:3339.
4. Griot-Wenk M, Pahlsson P, Chisholm-Chait A, Spitalnik PF, Spitalnik SL, Giger U. Biochemical characterization of the feline AB blood group system. Anim Genet. 1993;24:401–7.
5. Bighignoli B, Niini T, Grahn RA, Pedersen NC, Millon LV, Polli M, et al. Cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) mutations associated with the domestic cat AB blood group. BMC Genet. 2007;8:27.
6. Kehl A, Heimberger K, Langbein-Detsch I, Boehmer S, Raj K, Mueller E, et al. Molecular characterization of blood type A, B, and C (AB) in domestic cats and a CMAH genotyping scheme. PLoS One. 2018;13(9):e0204287.
7. Kehl A, Mueller E, Giger U. CMAH genotyping survey for blood types A, B and C (AB) in purpose-bred cats. Anim Genet. 2019;50(3):303–6.
8. Gandolfi B, Grahn RA, Gustafson NA, Proverbio D, Spada E, Adhikari B, et al. A novel variant in CMAH is associated with blood type AB in Ragdoll cats. PLoS One. 2016;11(5):e0154973.
9. Tasker S, Barker EN, Day MJ, Helps CR. Feline blood genotyping versus phenotyping, and detection of non-AB blood type incompatibilities in UK cats. J Small Anim Pract. 2014;55:185–9.
10. Auer L, Bell K. The AB blood group system of cats. Anim Blood Groups Biochem Genet. 1981;12:287–97.
11. Giger U, Bucheler J, Patterson DF. Frequency and inheritance of A and B blood types in feline breeds of the United States. J Hered. 1991;82:15–20.
12. Schauer R. Chemistry, metabolism, and biological functions of sialic acids. Adv Carbohydr Chem Biochem. 1982;40:131–234.
13. Bardor M, Nguyen DH, Diaz S, Varki A. Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells. J Biol Chem. 2005;280(6):4228–37.
14. Anstee DJ. The relationship between blood groups and disease. Blood. 2010;115(23):4635–43.
15. Cooling L. Blood groups in infection and host susceptibility. Clin Microbiol Infect. 2015;28(3):801–70.

16. Loomes LM, Uemura K, Childs RA, Paulson JC, Rogers GN, Scudder PR, et al. Erythrocyte receptors for *Mycoplasma pneumoniae* are sialylated oligosaccharides of I antigen type. Nature. 1984;307(5951):560–3.
17. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmidium vivax* in blacks. N Engl J Med. 1976;5:302–4.
18. Dhliwayo S, Makonese TA, Whittall B, Chikerema SM, Pfukenyi DM, Tivapasi MT. A study on the prevalence of dog erythrocyte antigen 1.1 and detection of canine *Babesia* by polymerase chain reaction from apparently healthy dogs in a selected rural community in. J S Afr Vet Assoc. 2016;87(1):a1409.
19. Nyström K, Le Gall-Reculé G, Grassi P, Abrantes J, Ruvoën-Clouet N, Le Moullac-Vaidye B, et al. Histo-blood group antigens act as attachment factors of rabbit hemorrhagic disease virus infection in a virus strain-dependent manner. PLoS Pathog. 2011;7(8):e1002188.
20. Spada E, Jung H, Proverbio D, Perego R, Baggiani L, Ciuti S, et al. Lack of association between feline AB blood groups and retroviral status: a multicenter, multi-country study. J Feline Med Surg. 2022;24(8):e194–202.
21. Tasker S, Hofmann-Lehmann R, Belák S, Frymus T, Addie DD, Pennisi MG, et al. Haemoplasmosis in cats: European guidelines from the ABCD on prevention and management. J Feline Med Surg. 2018;20(3):256–61.
22. Tasker S, Binns SH, Day MJ, Gruffydd-Jones TJ, Harbour DA, Helps CR, et al. Use of a PCR assay to assess the prevalence and risk factors for *Mycoplasma haemofelis* and "*Candidatus* Mycoplasma haemominutum" in cats in the United Kingdom. Vet Rec. 2003;152(7):193–8.
23. Willi B, Boretti FS, Baumgartner C, Tasker S, Wenger B, Cattori V, et al. Prevalence, risk factor analysis, and follow-up of infections caused by three feline hemoplasma species in cats in Switzerland. J Clin Microbiol. 2006;44(3):961–9.
24. Persichetti MF, Pennisi MG, Vullo A, Masucci M, Migliazzo A, Solano-Gallego L. Clinical evaluation of outdoor cats exposed to ectoparasites and associated risk for vector-borne infections in southern Italy. Parasites Vectors. 2018;11(1):136.
25. Latrofa MS, Iatta R, Toniolo F, Furlanello T, Ravagnan S, Capelli G, et al. A molecular survey of vector-borne pathogens and haemoplasmas in owned cats across Italy. Parasites Vectors. 2020;13:116.
26. Peters IR, Helps CR, Willi B, Hofmann-Lehmann R, Tasker S. The prevalence of three species of feline haemoplasmas in samples submitted to a diagnostics service as determined by three novel real-time duplex PCR assays. Vet Microbiol. 2008;126(1–3):142–50.
27. Sykes JE, Terry JC, Lindsay LL, Owens SD. Prevalences of various hemoplasma species among cats in the United States with possible hemoplasmosis. J Am Vet Med Assoc. 2008;232(3):372–9.
28. Gentilini F, Novacco M, Turba ME, Willi B, Bacci ML, Hofmann-Lehmann R. Use of combined conventional and real-time PCR to determine the epidemiology of feline haemoplasma infections in northern Italy. J Feline Med Surg. 2009;11(4):277–85.
29. Lobetti R, Lappin MR. Prevalence of Toxoplasma gondii, Bartonella species and haemoplasma infection in cats in South Africa. J Feline Med Surg. 2012;14(12):857–62.
30. Spada E, Proverbio D, Galluzzo P, Della Pepa A, Bagnagatti De Giorgi G, Perego R, et al. Prevalence of haemoplasma infections in stray cats in northern Italy. ISRN Microbiol. 2014;2014:298352.
31. Ravagnan S, Carli E, Piseddu E, Da Rold G, Porcellato E, Zanardello C, et al. Prevalence and molecular characterization of canine and feline hemotropic mycoplasmas (hemoplasmas) in northern Italy. Parasites Vectors. 2017;10(1):1–7.
32. Bergmann M, Englert T, Stuetzer B, Hawley JR, Lappin MR, Hartmann K. Risk factors of different hemoplasma species infections in cats. BMC Vet Res. 2017;13:52.
33. Marenzoni ML, Lauzi S, Miglio A, Coletti M, Arbia A, Paltrinieri S, et al. Comparison of three blood transfusion guidelines applied to 31 feline donors to minimise the risk of transfusion-transmissible infections. J Feline Med Surg. 2018;20(8):663–73.
34. Razin S, Kahane I, Banai M, Bredt W. Adhesion of mycoplasmas to eukaryotic cells. Ciba Found Symp. 1981;80:98–118.
35. Kahane I, Banai M, Razin S, Feldner J. Attachment of mycoplasmas to host cell membranes. Rev Infect Dis. 1982;4(suppl):S185–92.
36. Zulty JC, Kociba GJ. Cold agglutinins in cats with haemobartonellosis. J Am Vet Med Assoc. 1990;196(6):907–10.
37. Spada E, Perego R, Baggiani L, Salatino E, Priolo V, Mangano C, et al. Prevalence of blood types and alloantibodies of the AB blood group system in non-pedigree cats from northern (Lombardy) and southern (Sicily) Italy. Animals. 2020;10:1129.
38. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 2007;4(10):1623–7.
39. Spada E, Antognoni MT, Proverbio D, Ferro E, Mangili V, Miglio A. Haematological and biochemical reference intervals in adult Maine Coon cat blood donors. J Feline Med Surg. 2015;17(12):1020–7.
40. Proverbio D, Spada E, Perego R, Della Pepa A, Bagnagatti De Giorgi G, Baggiani L. Assessment of blood types of Ragdoll cats for transfusion purposes. Vet Clin Pathol. 2013;42(2):157–62.
41. Barker EN. Update on feline hemoplasmosis. Vet Clin North Am Small Anim Pract. 2019;49(4):733–43.
42. Pesteanu-Somogyi LD, Radzai C, Pressler BM. Prevalence of feline infectious peritonitis in specific cat breeds. J Feline Med Surg. 2006;8(1):1–5.
43. Knottenbelt CM, Addie DD, Day MJ, Mackin AJ. Determination of the prevalence of feline blood types in the UK. J Small Anim Pract. 1999;40(3):115–8.

How to cite this article: Spada E, Galluzzo P, Torina A, Loria GR, Perego R, Grippi F, et al. Evaluating the association between blood genotype or phenotype and haemoplasma infection in UK and Italian cats. Vet Rec. 2022;e2282. https://doi.org/10.1002/vetr.2282