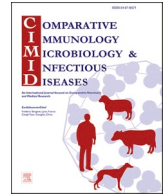




Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Full length article

Susceptibility or resistance to *Bartonella* spp. infection in cats: Does the phenotype-B blood matter?

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ARTICLE INFO

Keywords:

cat blood groups
Bartonella henselae
AB group system
NeuAc
erythrocyte receptors
zoonosis
Italy

ABSTRACT

Blood types can influence host susceptibility to infectious diseases by modifying erythrocyte surface antigens that act as pathogen receptors. In cats, AB blood group system polymorphisms determine the expression of distinct sialic acids, with the N-glycolylneuraminic acid (NeuGc) primarily expressed in type A, the N-acetylneuraminic acid (NeuAc) in type B, and both in type AB erythrocytes. This study investigated whether feline blood type influences susceptibility to *Bartonella henselae*, the agent of cat-scratch disease. A total of 454 blood and serum samples from stray colony, shelter, and owned cats in northern (Milan, Lombardy) and southern (Palermo, Sicily) was analyzed. Blood phenotyping was performed using tube agglutination, and back-typing, and immunochromatography were used to confirm type B and AB samples. *B. henselae* infection was determined by indirect immunofluorescence antibody test (IFAT) and real-time PCR. Univariate and multivariable logistic regression analyses evaluated associations between blood type, demographic factors, and infection markers. Overall, *B. henselae* infection prevalence was 19.4% (95% CI:15.8–23.3). Seropositivity was 33.3% among IFAT-tested cats, while PCR positivity was 8.8%. Geographic origin and lifestyle were strong predictors of infection: cats from southern Italy and stray colony or shelter cats were at significantly higher risk, while owned cats were protected. Blood type B was independently associated with PCR positivity (OR=2.6, 95% CI:1.1–5.6, $P = 0.017$). This association may reflect differences in NeuAc-mediated bacterial adhesion, but causality cannot be inferred from these data. These findings support a potential role of erythrocyte glycan composition in feline–*Bartonella* interactions but warrant further molecular confirmation.

Significance statement: This study provides preliminary evidence of an association between feline AB blood group system phenotypes and *Bartonella henselae* infection markers. By integrating serological and molecular diagnostics across a large, geographically diverse cat population, we observed that type B cats—characterized by NeuAc-rich erythrocyte membranes—had an approximately 2.6-fold greater probability of *B. henselae* DNA positivity. These findings support the hypothesis of a potential receptor-mediated mechanism underlying pathogen–erythrocyte interactions in cats, analogous to human blood group-dependent infectious processes. The work highlights how naturally occurring variation in erythrocyte glycan composition may shape host–pathogen dynamics, advancing our understanding of the evolutionary immunology of vector-borne diseases in companion animals.

Abbreviations: NeuAc, N-acetylneuraminic acid; NeuGc, N-glycolylneuraminic acid; OR, Odds ratio; RBCs, red blood cells; PCR, polymerase chain reaction; X2, chi-square test.

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<https://doi.org/10.1016/j.cimid.2026.102493>

Available online 1 June 2026

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1. Introduction

Blood group antigens are cell-surface molecules with diverse biological roles that extend far beyond transfusion compatibility. In humans, numerous blood group systems have been associated with variable susceptibility to infectious diseases, reflecting how pathogens exploit these antigenic structures as receptors or co-receptors for cell entry [1,2]. For example, individuals with blood group O exhibit partial protection against severe *Plasmodium falciparum* malaria but may be more prone to severe cholera [3,4]. Such associations illustrate that selective pressures exerted by pathogens at least partly drive the evolutionary maintenance of blood group polymorphisms. Comparable evidence in veterinary species, however, remains limited. The only experimental animal study that has implicated erythrocyte surface carbohydrates—including histo-blood group antigens (HBGAs)—as determinants of pathogen adhesion and infection severity is HBGAs mediate strain-dependent attachment of rabbit hemorrhagic disease virus [5].

In cats, blood types are inherited according to Mendelian law, with A being dominant over B. According to the combination of dominant and recessive alleles, feline blood groups include three phenotypes: A, B, and AB [6]. The biochemical distinction among these phenotypes lies in the terminal sialic acid residues on erythrocyte membrane glycolipids and glycoproteins. Type A erythrocytes express primarily N-glycolylneuraminic acid (NeuGc), type B erythrocytes express only N-acetylneuraminic acid (NeuAc), and type AB erythrocytes express both in similar proportions [7]. Mutations within the *CMAH* gene, which encodes cytidine monophospho-N-acetylneuraminic acid hydroxylase, are responsible for the production of NeuAc and the type B phenotype [8]. Although blood typing is routinely performed for transfusion compatibility, the potential influence of these sialic-acid-based differences on host–pathogen interactions has yet to be systematically investigated, and only in recent years have some studies been performed. In particular, no evidence of a relationship was found between AB blood group systems types and *Toxoplasma gondii* [9], retroviral (feline immunodeficiency virus - FIV- and feline leukemia virus - FeLV) [10], and feline coronavirus [11] infections. The only significant associations were with feline phenotype AB and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [12] and with Ab genotype and *Mycoplasma haemofelis* infection [13]. However, the mechanisms underlying these associations remain unexplored.

Bartonella species are fastidious, facultative intracellular bacteria able to infect mammalian erythrocytes and endothelial cells [14,15]. Domestic cats (*Felis catus*) are the principal reservoir hosts for *Bartonella henselae*, the causative agent of cat-scratch disease in humans [16]. Infection in cats is typically asymptomatic but can result in prolonged bacteremia, facilitating transmission via the cat flea *Ctenocephalides felis* [17,18]. Reported prevalence of *Bartonella* infection in cats varies widely between and within countries, influenced by climate, flea burden, and animal lifestyle [19–21]. Recent Italian studies have demonstrated marked geographic differences, with southern populations showing substantially higher prevalence [22,23] than northern ones [24,25].

Genetic and biochemical factors may also shape susceptibility and tolerance to *Bartonella* spp. infection. In humans, *B. bacilliformis*, the agent of Carrion's disease, invades erythrocytes through interactions with glycophorins A/B and band 3 proteins, which are closely related to blood group loci of the MNS, Diego, and Duffy systems [26]. However, a previous study showed no significant statistical differences between allele frequencies and disease patterns of Carrion's disease in blood groups MNS, Diego, and Duffy in Amazonas, Peru [27]. To date, no data are available exploring whether feline blood phenotypes influence susceptibility to *Bartonella* infection.

Because *Bartonella* spp. target erythrocyte membrane glycoproteins during invasion [28], and feline blood types are defined by specific sialic acid residues, it is plausible that differences in sialic acid composition

could alter bacterial adhesion efficiency. Based on these biochemical and epidemiological premises, the present study aimed to evaluate the possible relationship between feline AB blood group system phenotypes and *B. henselae* infection in distinct feline populations from northern and southern Italy. Our objective was to determine whether blood phenotype is associated with *B. henselae* infection markers, while accounting for epidemiological variables such as origin, lifestyle, and reproductive status. Clarifying whether blood type antigens are associated with *Bartonella* spp. infection markers may offer novel insights into host–pathogen interactions and the evolutionary interplay between erythrocyte glycobiology and vector-borne bacterial infections in companion animals.

2. Study design

2.1. Study population

This observational, cross-sectional study included surplus blood samples from cats living in northern and southern Italy. The northern cohort comprised animals from Milan metropolitan area (Lombardy region), whereas the southern group consisted of cats from Palermo (Sicily region). Samples originated from three defined populations: stray colony cats captured for trap–neuter–release (TNR) programs, shelter cats awaiting adoption, and owned cats presented for routine health screening or diagnostic testing. For stray and shelter cats, sampling was performed during general anesthesia for neutering as part of national zoonosis surveillance and population control programs (Italian Law 281/1991). All samples represented residual material collected for clinical purposes, with no additional sampling performed for this research.

For each cat, data recorded included geographic origin, lifestyle category (colony, shelter, owned), sex, neuter status, breed, and age (estimated from clinical records or dentition). Only animals with sufficient volume of EDTA blood and serum were enrolled. Because this study used residual clinical samples, paired EDTA blood and serum were not available for every cat; therefore, serological and molecular testing performed according to the residual volume of the sample type.

2.2. Sample and analyses

EDTA-anticoagulated whole blood and corresponding sera from southern Italy were processed immediately for serological and molecular investigations. Then the whole blood was shipped to Transfusion Veterinary Research Laboratory (REVLab) in northern Italy for blood typing. Conversely, northern samples were phenotyped locally and subsequently stored frozen until shipped to Experimental Zooprophy-lactic Institute of Sicily (IZS) in southern Italy for *Bartonella henselae* testing.

Blood phenotype (types A, B, AB) was determined by tube agglutination using *Triticum vulgaris* lectin as anti-B reagent and type-B plasma as anti-A reagent [29,30]. All type B and AB results were confirmed by back-typing and an immunochromatographic strip test (LabTest BT A + B, Alvedia, France) [30,31].

Antibodies to *B. henselae* were detected using the commercial test MegaFLUO *Bartonella henselae* IFA IgG (Megacor Diagnostik GmbH, Horbranz, Austria) according to the manufacturer's instructions and as previously described [22].

The *B. henselae* DNA was extracted using the commercial kit Pure-Link™ Genomic DNA Mini Kit (Thermo Fisher Scientific, Monza, Italy), following the manufacturer's protocol, and stored at −20 °C. All blood samples were screened using real-time PCR to check for the presence of *Bartonella* spp. DNA as previously described [32]. Real-time PCR-positive samples were submitted to end-point PCR for sequencing of the 16–23S ribosomal RNA intergenic spacer when residual DNA volume and amplification quality were adequate. The obtained sequences were analyzed for nucleotide sequence identity by comparing them with

reference strains in the GenBank database using the Basic Local Alignment Search Tool (BLAST, available online at https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LC=blasthome).

2.3. Sample size calculation

Power analysis was conducted using previously reported feline *Bartonella* prevalences: in previous studies in cats living in the metropolitan area of Milan (Lombardy region, North of Italy) and in the Palermo area (Sicily region, South of Italy), the blood prevalence (PCR or blood culture) of *Bartonella* spp. was 5% [33] and 35% [22], respectively, while the seropositivity at IFAT was 17% [24] and 47% [22], respectively. A previous study on blood group in cats indicated a prevalence of type A, B, and AB groups from northern Italy of 91%, 5%, and 4%, respectively, compared to 72% 12% and 11% from southern Italy [30]. At a 95% confidence level, a power of 90% was achieved by analyzing a total sample of at least 339 cats. Ultimately, 454 cats were included, providing robust analytical power to detect associations between blood type and infection status.

2.4. Statistical analysis

Data were managed using MedCalc Statistical Software (MedCalc Statistical Software version 22.013, MedCalc Software Ltd, Ostend, Belgium). Descriptive statistics for continuous data, such as age, were tested for normality using the Shapiro–Wilk test and expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) as appropriate. Categorical variables were summarized as absolute numbers and percentages.

Associations between *B. henselae* infection status and potential risk factors—including geographic origin (north vs south Italy), lifestyle (colony, shelter, owned), sex, neuter status, breed, age class (young ≤ 1 year; adult 1–10 years; senior > 10 years), and AB blood phenotype (A, B, AB)—were initially explored by univariate analysis using the chi-square (χ^2) or Fisher’s exact test, as appropriate.

Variables with $P < 0.10$ in univariate testing were subsequently entered into a multivariate binary logistic regression model to identify independent predictors of infection. Independent models were constructed separately for overall infection status (combined IFAT + PCR), for IFAT seropositivity, and for PCR positivity. A stepwise backward elimination procedure was applied, retaining variables with $P < 0.05$ in the final models. The Hosmer–Lemeshow goodness-of-fit statistic was used to evaluate model adequacy, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to quantify associations. All statistical tests were two-tailed, and results were considered significant at $P < 0.05$.

3. Results

3.1. Overall infection prevalence and population characteristics

A total of 454 samples were included: 174 serum samples tested with IFAT, 444 tested with PCR, and 164 tested with both methods. Overall, 88/454 cats were classified as infected with *B. henselae* (19.4%; 95% CI: 15.8–23.3). Among these 88 infected cats, 49 (55.7%) were IFAT-positive, 30 (34.1%) were PCR-positive, and 9 (10.2%) were positive by both methods. Sequences obtained from 10 and 1 PCR-positive samples that yielded amplicons of adequate quality shared 100% and 99% nucleotide sequence identity, respectively, with *B. henselae* sequences previously reported in GenBank (CP020742.1 and MT095053.1).

The characteristics of the feline population evaluated and the results of univariate analysis of factors associated with the infection are reported in Table 1. Exact ages were available for 286 cats, with a median of 2 years (range 2 months–16 years; IQR 1–5 years). Breed was

Table 1
Characteristics of an Italian feline population of 454 cats investigated for *Bartonella henselae* by IFAT and PCR and analyzed for factors associated with infection at univariate analysis (Chi-square test). In bold, statistically significant P value < 0.05.

Parameter	Variable	<i>Bartonella henselae</i> overall infection (IFAT+ real time PCR)			
		Infected n = 88	Uninfected n = 366	Chi-squared, P value	OR (95% CI), P value
Origin n = 354	North Italy n = 234	44	190	0.104	-
	South Italy n = 220	44	176	0.7475	-
	Habitat n = 408				
Habitat n = 408	Stray colony cats n = 234	55	179	3.274	-
	Shelter cats n = 75	20	55	0.0704	-
	Owned cats n = 99	8	91	2.262	-
	DSH n = 300	70	230	0.1326	0.2 (0.1–0.5) 0.0010
Breed n = 324	Purebred n = 24	0	24	7.121	14.9 (0.8–249.6) 0.0592 (0.00–1.11) 0.0592
	Male n = 216	45	171	0.0076	-
	Female n = 189	37	152	0.098	-
Reproductive Status n = 405	Neutered n = 405	11	68	0.7538	-
	Intact n = 405	71	255	2.424	-
Age class n = 383	Young (0–1 yrs) n = 72	14	58	0.1195	-
	Adult (1–10 yrs) n = 281	57	224	0.001	-
	Senior (>11 yrs) n = 31	5	26	0.9739	-
	Blood phenotype n = 454			0.330	-
Blood phenotype n = 454	A n = 328	70	258	0.5658	-
	B n = 71	15	56	0.255	-
	AB n = 55	3	52	0.6137	-
				2.894	-
Blood phenotype n = 454				0.0889	-
				0.163	-
				0.6861	-
				7.753	0.2 (0.0–0.6) 0.0108

available for 324 cats, with most being domestic shorthair (DSH) or longhair (DLH) cats, while the 24 purebred cats were: 15 Ragdolls, 2 Scottish Folds, 2 Bengals, 2 Persians, 1 Siamese, 1 British Shorthair, and 1 Maine Coon.

In univariate analysis, the risk factors for overall *B. henselae* infection were being a blood type AB cat and a DSH/DLH cat, whereas being an owned cat and a purebred cat were protective (Table 1). However, multivariate logistic regression analysis of significant factors associated with overall infection, based on the univariate analysis, identified only being an owned cat as a protective factor for overall infective status (OR=0.2, 95%CI: 0.0–0.5, $P = 0.0008$). Being a purebred cat or a cat with AB blood phenotype was not an independent factor significantly associated with overall *B. henselae* infection.

3.2. Serological results

Among the 174 cats tested by IFAT, 58 (33.3%) were seropositive

(titre $\geq 1:64$) and 116 (66.7%) seronegative. Eleven cats had titres of 1:64, and 46 had titres $\geq 1:128$. Five cats with titres of 1:32 were classified as seronegative. Table 2 reports the characteristics of the feline population analyzed by IFAT and the results of univariate analysis of factors associated with seropositivity.

Univariate analysis showed no significant association between AB blood group system phenotypes and seropositive status. Seropositivity was significantly associated with origin from southern Italy and with shelter habitat, whereas origin from northern Italy and being a colony cat were protective (Table 2). However, in multivariate analysis, only being a shelter cat (OR = 4.3; 95% CI: 1.8–10.1; P = 0.0008) and origin from southern Italy (OR = 5.1; 95% CI: 2.2–11.6; P = 0.0001) remained independent risk factors for *B. henselae* seropositivity.

3.3. Molecular results

Of 444 cats tested by PCR, 39 (8.8%) were positive for *B. henselae* DNA. Univariate analysis identified blood phenotype B, southern origin, stray colony lifestyle, and intact reproductive status as factors associated with PCR positivity. In contrast, northern origin, owned status, and neutered status were protective (Table 3).

In multivariate logistic regression, only stray colony lifestyle (OR =

Table 2

Characteristics of an Italian feline population of 174 cats investigated for *Bartonella henselae* and analyzed for factors associated with seropositivity for specific *B. henselae* IgG at univariate analysis (Chi-square test). In bold, statistically significant P value < 0.05.

Parameter	Variable	<i>Bartonella henselae</i> IgG IFAT			
		Positive n = 58	Negative n = 116	Chi-squared, P value	OR (95% CI), P value
Origin n = 174	North Italy n = 124	34	90	6.753 0.0094	0.4 (0.2–0.8) 0.0101
	South Italy n = 50	24	26		2.4 (1.2–4.8) 0.0101
Habitat n = 158	Stray colony cats n = 114	32	82	6.744 0.0094	0.3 (0.1–0.8) 0.0103
	Shelter cats n = 33	18	15	7.644 0.0057	2.9 (1.3–6.5) 0.0068
	Owned cats n = 11	4	7	0.025 0.8745	-
	DSH/DLH n = 148	47	101	0.461 0.4973	-
Breed n = 149	Purebred n = 1	0	1		
	Male n = 85	30	55	0.251 0.6163	-
Gender n = 158	Female n = 73	23	50		
	Neutered n = 27	10	17	0.177 0.6739	-
Reproductive Status n = 158	Intact n = 131	43	88		
	Young (0–1 yrs) n = 25	6	19	0.969 0.3248	-
Age class n = 149	Adult (1–10 yrs) n = 116	38	78	0.026 0.8722	-
	Senior (>11 yrs) n = 8	5	3	3.465 0.0627	-
	Blood phenotype n = 174				
Blood phenotype n = 174	A n = 155	53	102	0.470 0.4930	-
	B n = 15	4	11	0.326 0.5678	-
	AB n = 4	1	3	0.127 0.7213	-

Table 3

Characteristics of an Italian feline population of 444 cats investigated for *Bartonella henselae* and analyzed for factors associated with parasite DNA at univariate analysis (Chi-square test). In bold, statistically significant P value < 0.05.

Parameter	Variable	<i>Bartonella henselae</i> real-time PCR			
		Positive n = 39	Negative n = 401	Chi-squared, P value	OR (95% CI), P value
Origin n = 444	North Italy n = 224	12	212	6.610 0.0101	0.4 (0.1–0.8) 0.0122
	South Italy n = 220	27	193		2.4 (1.2–5.0) 0.0122
Habitat n = 398	Stray colony cats n = 232	30	202	8.591 0.0034	3.3 (1.4–7.8) 0.0052
	Shelter cats n = 67	3	64	2.213 0.1369	-
	Owned cats n = 398	4	95	4.307 0.0380	0.3 (0.1–0.9) 0.0465
	DSH/DLH n = 290	26	264	2.339 0.1262	-
Breed n = 314	Purebred n = 24	0	24		
	Male n = 212	18	194	0.077 0.7808	-
Gender n = 395	Female n = 183	17	166		
	Neutered n = 74	1	73	6.342 0.0118	0.1 (0.0–0.8) 0.0350
Reproductive Status n = 395	Intact n = 321	34	287		8.6 (1.1–64.2) 0.0350
Age class n = 375	Young (0–1 yrs) n = 69	8	61	1.012 0.3144	-
	Adult (1–10 yrs) n = 277	24	253	0.023 0.8789	-
	Senior (>11 yrs) n = 30	0	30	3.034 0.0815	-
	Blood phenotype n = 444				
Blood phenotype n = 444	A n = 319	25	294	1.265 0.2607	-
	B n = 70	12	58	7.231 0.0072	2.6 (1.2–5.5) 0.0091
	AB n = 55	2	53	2.071 0.1501	-

3.0; 95% CI: 1.1–7.6; P = 0.0192) and blood phenotype B (OR = 2.6; 95% CI: 1.1–5.6; P = 0.0170) were retained as independent factors associated with PCR-confirmed *B. henselae* detection. Neutered status and geographic origin did not remain significant in the final model.

4. Discussion

This study represents the first investigation examining whether feline AB blood group phenotypes are associated with *B. henselae* infection markers. Our results show that cats with blood phenotype B were significantly more likely to be PCR-positive for *B. henselae* DNA compared with phenotype A or AB cats, which is compatible with the hypothesis that erythrocyte surface composition may modulate bacterial adhesion or persistence. As expected, lifestyle and geographic origin also played major roles: stray colony cats from southern Italy exhibited markedly higher infection prevalence than owned cats or northern populations, consistent with the influence of vector exposure and environmental factors on *Bartonella* epidemiology [22, 23]. Younger age or sex did not appear to be associated with infection, differing from previous reports that identified age under 18 months as a risk factor [23].

Overall infection prevalence (19.4%) was consistent with previous Italian studies reporting a broad range from 2.5% to 46% of cats positive by culture or PCR, depending on the type of population investigated [22, 23,33–36]. In previous studies, cats from southern Italy showed higher infection rates than those from the north [24], probably due to regional climate differences that favor an ectoparasite burden. It is well-established, in fact, the role of *Ctenocephalides felis* in transmission and bacterial maintenance [19–21,37]. In our multivariate analysis, lifestyle emerged as the strongest risk factor, with colony cats showing approximately threefold higher odds of infection compared with owned cats, reflecting increased parasite exposure in free-roaming populations.

The observation of higher *Bartonella* DNA detection in phenotype-B cats (OR = 2.6) suggests a possible association with infection markers. The biochemical difference among feline blood types is in the terminal sialic acids on erythrocyte glycolipids: type A cells express NeuGc primarily, whereas type B cells display only NeuAc [7]. NeuAc is a critical recognition determinant for several pathogens in humans, including influenza viruses [38] and *Escherichia coli* [39], and contributes to bacterial evasion of host immunity [40]. Erythrocyte surface NeuAc could therefore hypothetically provide binding sites facilitating *Bartonella* attachment and invasion.

Bartonella spp. are known to interact with membrane glycoproteins such as glycophorins A/B and Band 3 [26,28]. Because glycophorins carry sialylated portions, variations in NeuAc expression—as seen in phenotype B feline erythrocytes—could strengthen bacterial adhesion. This hypothesis is consistent with human studies showing that antigens of the MNS, Diego, and Duffy systems act as erythrocyte receptors for *Bartonella bacilliformis* [26]. Enhanced binding could lead to more efficient bacterial entry and prolonged intra-erythrocytic survival, which would explain the higher probability of PCR detection in type-B cats. Furthermore, sialic-acid patterning can modulate complement activation and recognition by sialic-acid-binding immunoglobulin-like lectins (Siglecs), allowing pathogens and infected cells to evade immune surveillance, reduce complement-mediated clearance, and prolong bacteremia or parasitemia [41].

Interestingly, no association was observed between blood type and antibody seropositivity. This suggests that the blood group effect may influence early stages of infection or bacterial persistence within erythrocytes rather than the overall antibody response. Not all *B. henselae* infections result in detectable seroconversion, and PCR/IFAT discordance may reflect the timing of infection, intermittent bacteremia, immune tolerance, or intrinsic limitations of serological assays. Moreover, PCR positivity indicates detection of *Bartonella* DNA at the time of sampling and should not be interpreted as definitive evidence of increased susceptibility at the population level [14,15,20].

Despite this intriguing association, the results should be interpreted considering important limitations. First, the proportion of phenotype B cats was limited, reflecting their natural distribution [30], and the cross-sectional design of the study precludes causal inference and does not allow infection dynamics to be reconstructed. Additionally, lifestyle and environmental exposure are major confounding variables that could mask or mimic genetic susceptibility effects. Although multivariable analyses were performed, residual confounding by unmeasured or incompletely measured variables, particularly flea burden, duration of ectoparasite exposure, and colony or shelter-level clustering, cannot be excluded. Therefore, blood phenotype B should be interpreted as a risk marker associated with PCR positivity rather than as a demonstrated mechanistic determinant of infection. Importantly, a direct interaction between *B. henselae* and feline sialic acids has not yet been demonstrated. Experimental studies assessing bacterial adhesion to NeuAc- and NeuGc-expressing erythrocytes, as well as glycomic characterization of feline RBC membranes, are necessary to confirm this mechanism. Nevertheless, our findings align with evidence that blood group antigens can act as pathogen receptors and influence infection risk, as documented in human malaria, cholera, and viral diseases [1,2]. Confirming whether and how feline erythrocyte glycosylation patterns are

associated with vector-borne bacteria, such as *Bartonella*, may help guide the implementation of both diagnostic strategies and comparative models of zoonotic infection.

5. Conclusions

This study provides evidence of a possible association between feline blood phenotype B and increased *B. henselae* PCR positivity. NeuAc, which is expressed on type B erythrocyte surface, is hypothesized to facilitate bacterial adhesion and colonization, although behavioral and environmental factors, particularly lifestyle and geographic location, must be considered when interpreting infection markers. Understanding the potential interactions between blood group antigens and pathogen invasion mechanisms could advance both feline disease prevention and comparative infectious disease research. Future studies should include experimental binding assays or glycomic profiling to validate whether *Bartonella* species preferentially adhere to NeuAc-dominant erythrocytes.

CRediT authorship contribution statement

Rosalia D'Agostino: Writing – review & editing, Visualization, Investigation, Formal analysis. **Carmela Sciacca:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Valeria Blanda:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Francesca Grippi:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Eva Spada:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Daniela Proverbio:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Roberta Peregò:** Writing – review & editing, Validation, Methodology, Formal analysis. **Luciana Baggiani:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Marta Meropiali:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Paola Galluzzo:** Writing – review & editing, Investigation, Formal analysis, Data curation.

Funding sources

None.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

References

- [1] D.J. Anstee, The relationship between blood groups and disease, *Blood* 115 (2010) 4635–4643.
- [2] L. Cooling, Blood groups in infection and host susceptibility, *Clin. Microbiol. Rev.* 28 (3) (2015) 801–870.
- [3] J.A. Rowe, I.G. Handel, M.A. Thera, A.M. Deans, K.E. Lyke, A. Koné, et al., Blood group O protects against severe *Plasmodium falciparum* malaria through the mechanism of reduced rosetting, *PNAS* 104 (44) (2007) 17471–17476.
- [4] J.B. Harris, A.I. Khan, R.C. LaRocque, D.J. Dorer, F. Chowdhury, A.S.G. Faruque, et al., Blood group, immunity, and risk of infection with *Vibrio cholerae* in an area of endemicity, *Infect. Immun.* 73 (11) (2005) 7422–7427.
- [5] K. Nyström, G. Le Gall-Reculé, P. Grassi, J. Abrantes, N. Ruvoën-Clouet, B. Le Moullac-Vaidye, et al., Histo-blood group antigens act as attachment factors of rabbit hemorrhagic disease virus infection in a virus strain-dependent manner, *PLoS. Pathog.* 7 (8) (2011) e1002188.

- [6] L. Auer, K. Bell, The AB blood group system of cats, *Anim. Blood Groups Biochem. Genet.* 12 (1981) 287–297.
- [7] G.A. Andrews, P.S. Chavey, J.E. Smith, L. Rich, N-glycolylneuraminic acid and N-acetylneuraminic acid define feline blood group A and B antigens, *Blood* 79 (9) (1992) 2485–2491.
- [8] B. Bighignoli, T. Niini, R.A. Grahm, N.C. Pedersen, L.V. Millon, M. Polli, et al., Cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) mutations associated with the domestic cat AB blood group, *BMC Genet.* 8 (2007) 27.
- [9] E. Spada, G. Tattarletti, D. Proverbio, R. Perego, L. Baggiani, G. Donato, et al., The AB blood group system phenotype does not play a role in *Toxoplasma gondii* infection in cats, *Pathogens* 14 (2025) 1227.
- [10] E. Spada, H. Jung, D. Proverbio, R. Perego, L. Baggiani, S. Ciuti, et al., Lack of association between feline AB blood groups and retroviral status: a multicenter, multicountry study, *J. Feline Med. Surg.* 24 (8) (2022) e194–e202.
- [11] E. Spada, A. Carrera Nulla, R. Perego, L. Baggiani, D. Proverbio, Evaluation of association between blood phenotypes A, B and AB and feline coronavirus infection in cats, *Pathogens* 11 (2022) 917.
- [12] E. Spada, F. Bruno, G. Castelli, F. Vitale, S. Reale, V. Biondi, et al., Do blood phenotypes of feline AB blood group system affect the SARS-CoV-2 antibody serostatus in cats? *Viruses* 14 (2022) 2691.
- [13] E. Spada, P. Galluzzo, A. Torina, G.R. Loria, R. Perego, F. Grippi, et al., Evaluating the association between blood genotype or phenotype and haemoplasma infection in UK and Italian cats, *Vet. Rec.* 192 (12) (2023).
- [14] E.B. Breitschwerdt, R.G. Maggi, B.B. Chomel, M.R. Lappin, Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings, *J. Vet. Emerg. Crit. Care* 20 (2010) 8–30.
- [15] J. Brunt, L. Guptill, D.L. Kordick, S. Kudrak, M.R. Lappin, American Association of Feline Practitioners 2006 Panel report on diagnosis, treatment, and prevention of *Bartonella* spp. infections, *J. Feline Med. Surg.* 8 (4) (2006) 213–226.
- [16] B.B. Chomel, H.J. Boulouis, E.B. Breitschwerdt, Cat scratch disease and other zoonotic *Bartonella* infections, *J. Am. Vet. Med. Assoc.* 224 (8) (2004) 1270–1279.
- [17] B.B. Chomel, R.C. Abbott, R.W. Kasten, K.A. Floyd-Hawkins, P.H. Kass, C.A. Glaser, et al., *Bartonella henselae* prevalence in domestic cats in california-risk-factors and association between bacteremia and antibody-titers, *J. Clin. Microbiol.* 33 (9) (1995) 2445–2450.
- [18] B.B. Chomel, R.W. Kasten, J.B. Henn, S. Molia, *Bartonella* infection in domestic cats and wild felids, *Ann. N. Y. Acad. Sci.* 1078 (2006) 410–415.
- [19] B.B. Chomel, E.T. Carlos, R.W. Kasten, K. Yamamoto, C.C. Chang, R.S. Carlos, et al., *Bartonella henselae* and *Bartonella clarridgeiae* infection in domestic cats from the Philippines, *Am. J. Trop. Med. Hyg.* 60 (4) (1999) 593–597.
- [20] L. Guptill, C.C. Wu, H. HogenEsch, L.N. Slater, N. Glickman, A. Dunham, et al., Prevalence, risk factors, and genetic diversity of *Bartonella henselae* infections in pet cats in four regions of the United States, *J. Clin. Microbiol.* 42 (2) (2004) 652–659.
- [21] M.R. Lappin, J. Hawley, Presence of *Bartonella* species and *Rickettsia* species DNA in the blood, oral cavity, skin and claw beds of cats in the United States, *Vet. Dermatol.* 20 (5–6) (2009) 509–514.
- [22] F. Grippi, P. Galluzzo, A. Guercio, V. Blanda, F. Santangelo, S. Sciortino, et al., Serological and molecular evidence of *Bartonella henselae* in stray cats from southern Italy, *Microorganisms* 9 (2021) 979.
- [23] M.S. Latrofa, R. Iatta, F. Toniolo, T. Furlanello, S. Ravagnan, G. Capelli, et al., A molecular survey of vector-borne pathogens and haemoplasmas in owned cats across Italy, *Parasites Vectors* 13 (2020) 116.
- [24] E. Spada, I. Canzi, L. Baggiani, R. Perego, F. Vitale, A. Migliazzo, et al., Prevalence of *Leishmania infantum* and co-infections in stray cats in northern Italy, *Comp. Immunol. Microbiol. Infect. Dis.* 45 (2016) 53–58.
- [25] M. Fabbri, N. Vicari, M. Tranquillo, C. Pozzi, P. Prati, D. De Meneghi, et al., Prevalence of *Bartonella henselae* in stray and domestic cats in different Italian areas: evaluation of the potential risk of transmission of *Bartonella* to humans, *Parassitologia* 46 (1–2) (2004) 127–129.
- [26] E.L. Buckles, E. McGinnis Hill, Interaction of *Bartonella bacilliformis* with human erythrocyte membrane proteins, *Microb. Pathog.* 29 (2000) 165–174.
- [27] O. Acosta, L. Solano, J. Escobar, M. Fernandez, C. Solano, R. Fujita, Frequencies of blood group systems MNS, diego, and duffy and clinical phases of carrion's disease in Amazonas, Peru, *Interdiscip. Perspect. Infect. Dis.* 8 (2014) 576107.
- [28] H. Deng, Q. Pang, B. Zhao, M. Vayssier-Taussat, Molecular mechanisms of *Bartonella* and mammalian erythrocyte interactions: a review, *Front. Cell. Infect. Microbiol.* 8 (431) (2018).
- [29] K. Stieger, H. Palos, U. Giger, Comparison of various blood-typing methods for the feline AB blood group system, *Am. J. Vet. Res.* 66 (8) (2005) 1393–1399.
- [30] E. Spada, R. Perego, L. Baggiani, E. Salatino, V. Priolo, C. Mangano, 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* 10 (2020) 1129.
- [31] E. Spada, D. Proverbio, L. Baggiani, G. Bagnagatti De Giorgi, R. Perego, E. Ferro, Evaluation of an immunochromatographic test for feline AB system blood typing, *J. Vet. Emerg. Crit. Care* 26 (1) (2016) 137–141.
- [32] M. Alamán Valtierra, C. Simón Valencia, H. Fuertes Negro, A. Unzueta Galarza, B. Flores Somarriba, N. Halaihel Kassab, Molecular epidemiology of *Bartonella henselae* in stray and sheltered cats of Zaragoza, Spain, *Rev. Esp. Salud Publica* 90 (2016) e1–e11.
- [33] M. Fabbri, L. De Giuli, M. Tranquillo, R. Bragoni, M. Casiraghi, C. Genchi, Prevalence of *Bartonella henselae* in Italian stray cats: evaluation of serology to assess the risk of transmission of *Bartonella* to humans, *J. Clin. Microbiol.* 42 (1) (2004) 264–268.
- [34] V.V. Ebani, F. Bertelloni, F. Fratini, Occurrence of *Bartonella henselae* types I and II in Central Italian domestic cats, *Res. Vet. Sci.* 93 (1) (2012) 63–66.
- [35] V.V. Ebani, L. Guardone, F. Marra, I. Altomonte, S. Nardoni, F. Mancianti, Arthropod-borne pathogens in stray cats from northern Italy: a serological and molecular survey, *Animals* 10 (12) (2020) 1–16.
- [36] M.F. Persichetti, M.G. Pennisi, A. Vullo, M. Masucci, A. Migliazzo, L. Solano-Gallego, Clinical evaluation of outdoor cats exposed to ectoparasites and associated risk for vector-borne infections in southern Italy, *Parasites Vectors* 11 (1) (2018) 136.
- [37] M.R. Lappin, B. Griffin, J. Brunt, A. Riley, D. Burney, J. Hawley, et al., Prevalence of *Bartonella* species, haemoplasma species, *Ehrlichia* species, *Anaplasma phagocytophilum*, and *Neorickettsia risticii* DNA in the blood of cats and their fleas in the United States, *J. Feline Med. Surg.* 8 (2) (2006) 85–90.
- [38] F. Broszeit, N. Tzarum, X. Zhu, N. Nemanichvili, D. Eggink, T. Leenders, et al., N-Glycolylneuraminic acid as a receptor for Influenza A viruses, *Cell. Rep.* 27 (11) (2019) 3284–3294.
- [39] M. Bardor, D.H. Nguyen, S. Diaz, A. Varki, Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells, *J. Biol. Chem.* 280 (6) (2005) 4228–4237.
- [40] B. Dudek, J. Rybka, G. Bugla-Płoskońska, A. Korzeniowska-Kowal, B. Futoma-Kołoch, A. Pawlak, et al., Biological functions of sialic acid as a component of bacterial endotoxin, *Front. Microbiol.* 13 (2022) 1028796.
- [41] H. Läubli, A. Varki, Sialic acid-binding immunoglobulin-like lectins (Siglecs) detect self-associated molecular patterns to regulate immune responses, *Cell. Mol. Life Sci.* 77 (4) (2020) 593–605.