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Assessment of risks of feline mismatched transfusion and neonatal isoerythrolysis in the Lyon (France) area

Alexandra Nectoux[1], Maryline Guidetti[2], Anthony Barthélemy[1], Céline Pouzot-Nevoret[1], Guillaume L Hoareau[3] and Isabelle Goy-Thollot[1]

Abstract

Objectives The aims of this study were to update the prevalence of different feline blood types in the Lyon (France) area, as well as to determine the risk of mismatched transfusion (MT) and neonatal isoerythrolysis (NI) in kittens with parents of unknown blood type.

Methods Blood samples were obtained from blood donor cats and cats admitted to an intensive care unit in Lyon. AB blood typing was performed using an immunochromatographic strip. The risk of MT was estimated by adding the risk of a major transfusion reaction and the risk of a minor transfusion reaction. The risk of NI was estimated according the equation $(p^2)(q^2) + 2pq(q^2)$, with q being the b allele frequency and $p = 1 - q$. The results were analysed by absolute and relative frequency analysis and multivariate analysis.

Results The cohort study population included 320 non-pedigree cats and 37 pedigree cats. The prevalence of blood types A, B and AB was 84.3%, 14.0% and 1.7%, respectively. Considering non-pedigree cats, the prevalence of types A, B and AB was 83.7%, 14.4% and 1.9%, respectively. There were no significant differences of blood type distribution by sex ($P = 0.73$) or by breed ($P = 0.90$). Based on these percentages, the risks of MT and NI in non-pedigree cats were 24.3% and 12.3%, respectively.

Conclusions and relevance The prevalence of type B cats is high in the Lyon area and associated with high risks of MT and NI. These results confirm the importance of performing blood typing prior to any blood transfusion or mating.

Keywords: Haemolysis; blood typing; blood type systems; erythrocyte antigen

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Introduction

Feline blood types are described using the AB system, defined by the existence of types A, B and AB blood groups based on the inheritance of three alleles (a, which is dominant, a^{ab} and b), which is different from the human ABO system. It has been established that type B cats have strong naturally occurring anti-A alloantibodies and type A cats have weak naturally occurring anti-B alloantibodies.[1–3] The A and B red blood cell (RBC) antigens are sialic *N*-glycolyl- and *N*-acetyl-neuraminic acids, respectively.[4] Several genetic mutations have been identified in the cytidine monophosphate-N-acetylneuramic acid hydrolase (*CMAH*) gene associated with types A, B and AB, but the precise functional effects of these variants have not been determined, and genotyping assays have not been completely accurate until recently.[4–6]

[1]Intensive Care Unit (SIAMU), University of Lyon, VetAgro Sup, Marcy l'Etoile, France
[2]Dianov Laboratories, Limonest, France
[3]Clinical Investigation Facility, David Grant USAF Medical Center, Travis AFB, CA, USA

Corresponding author:
Alexandra Nectoux DVM, MSc, Intensive Care Unit (SIAMU), University of Lyon, VetAgro Sup, 1 Avenue Bourgelat, F-69280 Marcy l'Etoile, France
Email: alexandra.nectoux@vetagro-sup.fr

From a clinical standpoint, strong anti-A alloantibodies are responsible for an acute haemolytic reaction, whereas weak anti-B alloantibodies produce milder reactions such as shortened survival of transfused RBCs.[2] Absorption of maternal colostrum with anti-A alloantibodies from a type B queen by a type A or a type AB kitten within the first hours of life may be associated with a high mortality rate due to an acute haemolytic reaction, namely neonatal isoerythrolysis (NI).[7]

While the AB system is the most important feline blood system, incompatibilities in cross-matching,[8–11] and acute haemolytic transfusion reactions despite correct AB system matching, have been described.[12] According to these observations, other feline blood types were suspected.[6,13] For instance, naturally occurring anti-Mik alloantibodies were described in 2007.[8] Alloantibodies against these non-AB blood antigens could be naturally occurring,[9–11] or could be produced after a blood product transfusion.[14] While the literature on feline non-AB blood type is growing, the prevalence of these blood types has rarely been described.[8]

While significant breed and geographical variations have been described, type A is the most frequent feline blood type in the world.[15–34] Because of life-threatening transfusion reactions in cases of incompatibility, current recommendations for veterinary transfusion advocate for blood type determination of both donor and recipient in the AB system prior to the first transfusion.[6,35] If AB typing is not available, or in previously transfused cats, cross-matching is necessary. Moreover, it is now recommended that cross-matching is introduced into routine pre-transfusion testing protocols, even the first transfusion.[14] Risks of transfusion reaction and NI depend on the proportion of both anti-A and anti-B alloantibodies and therefore on the prevalence of blood types in one region. Blood type distribution varies widely across countries and was first described in France in 1962.[36]

The first aim of this study was to update the prevalence of feline blood types in France, specifically in the Lyon area. The second aim was to assess the risks of mismatched transfusion (MT) in the AB system and NI in France. We hypothesised that blood type B prevalence is high in Lyon and that the risks of MT and NI are high.

Materials and methods

This retrospective study was conducted between January 2011 and January 2017. The studied cohort included cats presented to the intensive care unit (ICU, SIAMU) of VetAgro Sup, University of Lyon, for blood donation or medical care requiring blood type determination (immune-mediated haemolytic anaemia, severe bleeding or surgical procedures, etc). For all cats, age, breed and sex were recorded.

Blood was collected in tubes containing EDTA with a 1:9 volume ratio of anticoagulant to blood and analysed immediately or stored at 4°C for less than a week for subsequent blood typing. Blood type was determined with a commercially available immunochromatographic strip kit (CHROM Method, Lab Test A+B; Alvedia). Blood typing procedures were performed according to the manufacturer's recommendations. The existence of a visible red band at the position marked A or B indicated the expression of the respective antigen on the RBC membrane (Figure 1).

Furthermore, if the available blood was sufficient to perform additional tests, blood type was established via flow cytometry and analysis was performed using the FACS Calibur analyser (Becton Dickinson). Data were collected for 10,000 events through a gated region from each sample (CellQuest Pro software; Becton Dickinson) and the mean fluorescence intensity (MFI) was obtained. The A and B antigen RBC surface expression was designated as negative for an MFI <10 and positive for any MFI $\geqslant 10$. Incubation time, reagent and blood sample volumes were decided based upon laboratory experience and the manufacturer's instructions.[1,37]

Results were analysed by absolute and relative frequency analysis. Distribution of blood types were compared using multivariate analysis by one-way ANOVA. All analyses were performed with statistical software (R version 3.4.3) and significance was set at $P<0.05$.

As previously described, the risk of a life-threatening transfusion reaction is defined as the risk of an unmatched transfusion between a type A or AB donor

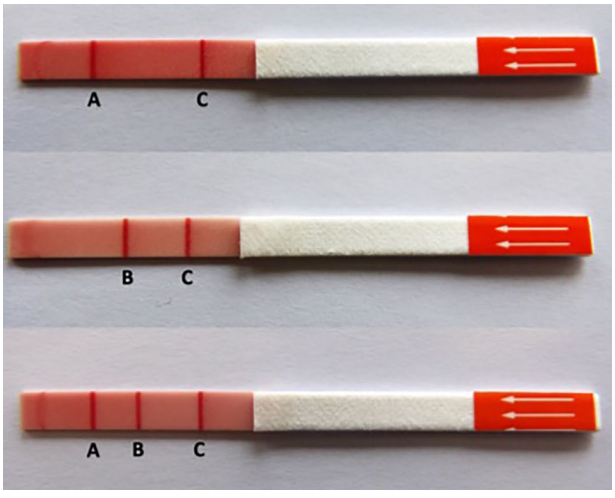

Figure 1 Feline blood type results on an immunochromatographic strip kit (CHROM Method, Lab Test A+B; Alvedia). The existence of a visible red band at the position marked A or B indicated the expression of the antigen on red blood cell membranes. A cat was considered type A if only a clear red band at the A level was present, type B if the band was at the B level and AB if both bands were present. The C band represents the positive control

and a type B recipient.[15,19] This risk, called major transfusion reaction (MTR), was calculated as the percentage of type B cats multiplied by the percentage of non-type B cats (type A and type AB cats). The risk of a minor transfusion reaction (mTR) that can reduce the RBC life span (type B donor and type A recipient) was calculated as the percentage of type B cats multiplied by the percentage of type A cats. The risk of an MT owing to incompatibility in the AB system is the addition of MTR and mTR. Finally, the estimated mating risk for NI was calculated as previously described.[2] The b allele frequency (q) was first calculated assuming a Hardy–Weinberg equilibrium, $p^2 + 2pq + q^2 = 1$ with $p = 1 - q$; q^2 = proportion of type B cats. The proportion of mating risk is $(p^2)(q^2) + 2pq(q^2)$. These formulae do not include type AB cats and the a^{ab} allele. As our data include AB cats, they are not in an equilibrium. Consequently, type AB cats were ignored when using the Hardy–Weinberg formula.

Results

A total of 357 cats from the Lyon area were included in the study. The cohort population ranged in age from 5 months to 17 years, with a median age of 3.2 years. There were 320 non-pedigree cats (170 males and 150 females). There were 37 pedigree cats (21 male and 16 females) of various breeds (eight Siamese, seven Birman, five Persian, four Bengal, three Chartreux, three Maine Coon, two Ragdoll, one Angora, one Abyssinian, one Norwegian Forest Cat, one Thai and one British Shorthair).

Of the 357 cats, blood type was obtained with flow cytometry and immunochromatographic strips in 38 cats. In all cases, both techniques showed concordant results ($r = 1$, $P < 0.005$).

In the overall population of 357 cats, 301 (84.3%) were type A, 50 (14.0%) were type B and six (1.7%) were type AB (Table 1). Of the 37 pedigree cats, 33 (89.2%) were type A, four (10.8%) were type B and none were type AB. Of the 320 non-pedigree cats, 268 (83.7%) were type A, 46 (14.4%) were type B and six (1.9%) were type AB (Figure 2, Table 1). Blood type A was significantly more present than blood type B in pedigree and non-pedigree cats ($P = 0.002$). There were no significant differences of blood type distribution by sex ($P = 0.73$) or by breed ($P = 0.90$).

Based on our non-pedigree cohort population of 320 cats, the risk of MTR was 12.3% and the risk mTR was

Table 1 Prevalence of blood types A, B and AB in non-pedigree and pedigree cats

	Total no. of cats	No. (%) of type A cats	No. (%) of type B cats	No. (%) of type AB cats
Non-pedigree	320	268 (83.8)	46 (14.4)	6 (1.9)
Males	170	142 (83.5)	24 (14.1)	4 (2.4)
Females	150	126 (84.0)	22 (14.7)	2 (1.3)
Pedigree	37	33 (89.2)	4 (10.8)	0
Males	21	20 (95.2)	1 (4.8)	0
Females	16	13 (81.3)	3 (18.7)	0
Total	357	297 (83.2)	49 (13.7)	6 (1.7)

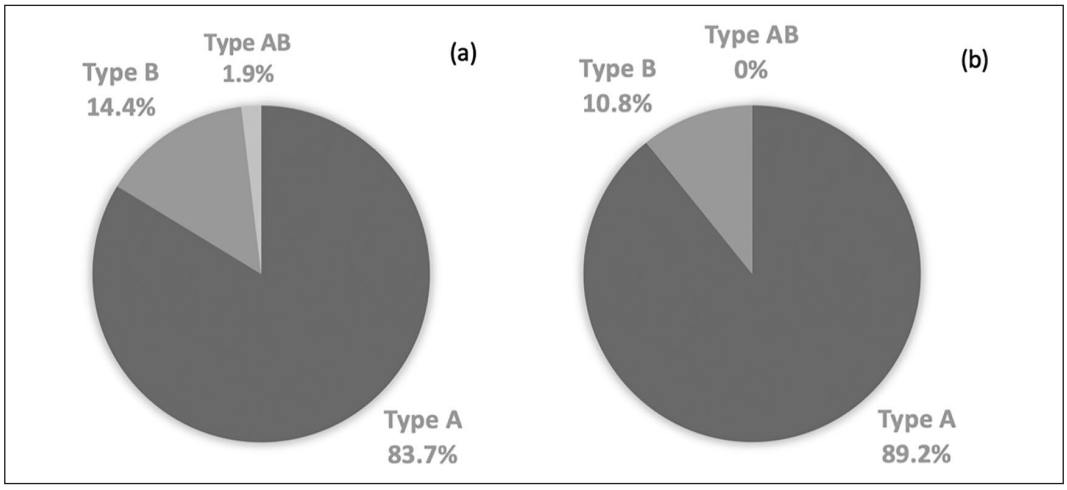

Figure 2 Prevalence of blood types A, B and AB in (a) non-pedigree and (b) pedigree cats

12.0%. The risk of AB system MT was 24.3%. The proportion of mating risk for NI was 12.3%.

Discussion

While type A remains the most predominant feline blood type in the Lyon area, type B prevalence is also important. In France, blood type distribution was described in a 1962 survey of 350 cats in Paris; 85% were type A and 15% were type B, with no pedigree status reported.[36] Recently, blood type was determined in 231 non-pedigree cats in France, and the prevalence of type A, B and AB were 89.6%, 10% and 0.4%, respectively.[38] The reported prevalence of MTR in non-pedigree cats was estimated to be 9%,[38] which is lower than that reported in our population (12.3%). This difference can be explained by a larger number of regional type B cats or a larger number of cats in our population.

Several feline blood typing kits are now available in routine practice, including agglutination cards, immunochromatographic strips and cartridge techniques.[39–41] Recent studies showed that the immunochromatographic strip method has a higher sensitivity and specificity for A and B antigen detection.[40,41] Flow cytometry is currently used in laboratories to settle discordant blood typing results obtained by other techniques.[1] As previously described,[42] our study showed agreement between flow cytometry and the immunochromatographic strip kit. These two techniques use the same monoclonal anti-A and anti-B antibodies.

To our knowledge, no previous studies have highlighted a significant relationship between sex and blood type, which is consistent with our results. To avoid bias owing to particular breeds with a high blood type prevalence (ie, Siamese), risks were only calculated in non-pedigree cats. Geographical variations in the prevalence of blood types have been reported in cats from different countries (Table 2).[15–34] Risks of MTR, mTR, MT and mating risk for NI were also calculated in those countries using the same calculation as in the present study (Table 2). For these, we used previously published prevalences in the AB system.[15–33] The calculation of these risks in different countries using previously published data has never been performed until now. The risk of MT ranged from 0% (in Croatia and Hungary) to 45.3% (in Australia). The risk of NI ranged from 0% (in Croatia and Hungary) to 23% (in Australia). The five countries where the risks of MT and NI were the most elevated were Australia, the UK, Turkey, Greece and Ireland. Based on our data, France appears to be the sixth country most at risk of MT and NI. These calculated risks appeared to be directly related to the prevalence of type B (36%, 30.5%, 24.6%, 20.3%, 14.6% and 14.4% for Australia, UK, Turkey, Greece and Ireland, respectively, and 14.4% in our study in France).

Risks of MTR and mTR only consider acute and delayed immunological transfusion reactions caused by antigen–antibody reactions in the AB system. It is well known that most type B cats have high titres of high-affinity anti-A alloantibodies. In contrast, approximately

Table 2 Prevalence of blood types A, B and AB in non-pedigree cats in several countries

Country (city/region)	No. of domestic cats	Type A (%)	Type B (%)	Type AB (%)	MTR (%)	mTR (%)	MT (%)	Mating risk for NI (%)
Australia (Sydney)[15]	187	62	36	1.6	22.9	22.3	45.3	23
UK (south east)[16]	105	67.6	30.5	1.9	21.2	20.6	41.8	21.2
Turkey[17]	301	73.1	24.6	2.3	18.5	18	36.5	18.5
Greece[18]	207	78.3	20.3	1.4	16.2	15.9	32.1	16.2
Ireland (Dublin)[19]	137	84.7	14.6	0.7	12.5	12.4	24.9	12.5
France (this study)	320	83.8	14.4	1.9	12.3	12	24.3	12.3
China (Bejing)[20]	262	88.2	11.4	0.4	10.1	10.1	20.2	10.1
Spain (Gran Canaria)[21]	97	88.7	7.2	4	6.7	6.4	13.1	6.7
Italy (north)[22]	140	90.7	7.1	2.1	6.6	6.4	13	6.6
Germany[23]	Not reported	93.9	5.4	0.7	5.1	5.1	10.2	5.1
Canada (Montreal)[24]	178	94.4	5	0.6	4.8	4.7	9.5	4.8
Spain (Barcelona)[25]	100	94	5	1	4.8	4.7	9.5	4.8
Portugal (north)[26]	159	89.3	4.4	6.3	4.2	3.9	8.1	4.2
Brazil (Rio de Janero)[27]	172	94.8	2.9	2.3	2.8	2.7	5.5	2.8
Portugal (Lisbon)[28]	55	97.5	2.1	0.4	2.1	2	4.1	2.1
Denmark (Copenhagen)[29]	105	98.1	1.9	0	1.9	1.9	3.8	1.9
USA[30]	3785	98.1	1.7	0.1	1.7	1.7	3.4	1.7
Switzerland[31]	1014	99.6	0.4	0	0.4	0.4	0.8	0.4
Croatia (Zagreb)[32]	30	96.7	0.0	3.3	0.0	0.0	0.0	0.0
Hungary[33]	73	100	0.0	0.0	0.0	0.0	0.0	0.0

Calculation of risks for major transfusion reaction (MTR), minor transfusion reaction (mTR), mismatched transfusion (MT) and neonatal isoerythrolysis (NI)

16.4–100% of type A cats have low-affinity anti-B alloantibodies.[3,43] The percentage of type A cats with anti-B alloantibodies varies with geographical location and methodology. A recent study reported the existence of a type B cat with no anti-A alloantibodies and an absence of anti-B alloantibodies in 10 tested type A cats.[42] Recent research focused on naturally occurring alloantibodies highlighted the existence of non-AB alloantibodies.[11,42] Calculations to evaluate the risks of transfusion reaction only based on the AB system type prevalence can therefore underestimate clinical risks. Performing a cross-match test prior to the first transfusion is necessary to optimally prevent immunological transfusion reaction.[11]

As previously described, the mating risk of NI can be estimated using the Hardy–Weinberg equation, but only if excluding type AB cats.[2] In our study, as described earlier, data were not in Hardy–Weinberg equilibrium, but AB cats were in a very few proportion.[17] To be rigorous, a novel equation including AB cats must be found to estimate the mating risk of NI.

This study carries some limitations. In our population study, only 37 cats were pedigree. This could be due to a selective bias owing to the retrospective nature of the study, or owing to a low prevalence of pedigree cats in the Lyon region. Because of the small number of pedigree cats in each breed, the risks of MTR, mTR, MT and NI were not calculated. Future studies are needed to interpret breed-specific risks. Geographical variations of AB blood type prevalence have been shown in the same country.[26,28] Here, only the Lyon area was investigated, so our population may not be a representative sample of the feline population in France. A future study in cats from different regions in France might be interesting to assess the geographical impact on AB blood type prevalence in France and to confirm the prevalence found in our study.

Conclusions

Feline blood type B prevalence is high in the Lyon area and associated with high risks of MT and NI. France represents one of the countries with the highest risks of MT and NI. According to these results, establishing blood type prior to any blood transfusion or mating is necessary.

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Conflict of interest M Guidetti was employed by, and I Goy-Thollot has been a scientific advisor to, Dianov. Reagents for this study were donated by Alvedia, which is a commercial supplier of blood typing and cross-match kits. However, the study design and execution, as well as data analysis and manuscript writing, were performed independently.

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Ethical approval and informed consent Due to the retrospective nature of this study, ethical approval and informed consent were not required.

ORCID iD Alexandra Nectoux https://orcid.org/0000-0002-5563-8509

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