

Frequency of feline blood types in non-pedigree cats in France

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SUMMARY

Many studies have shown that the prevalence of feline blood types varies geographically as well as by breed. The most important blood group system in cats is the AB system (Types A, B and AB). Notably, all Type B cats possess strong, naturally occurring anti-A alloantibodies, which may be associated with life-threatening immune-mediated hemolytic transfusion reactions. The aim of this study was to determine the prevalence of feline blood groups in non-pedigree cats in France and calculate the relative risk of a life-threatening immune-mediated hemolytic transfusion reaction caused by unmatched transfusions in this population. Blood specimens collected from sick and healthy cats were obtained from a referral hospital (Auvergne Rhône Alpes region) and a veterinary clinical pathology laboratory which receives specimens from the entire French territory, with a majority from region of Auvergne Rhône Alpes. Blood typing was performed using immunochromatographic technology. Blood specimens from 231 non-pedigree cats were blood typed for the AB system. The prevalence of blood types was as follows: 89.6% were Type A, 10% were Type B, and 0.4% were Type AB. In this study, the prevalence of Type B exceeds that in numerous other countries, including the United States and Canada. The probability of a high-risk A-B mismatched transfusion was estimated to be 9%. The high prevalence of Type B non-pedigree cats in France highlights the importance of performing blood typing prior to any blood transfusion to avoid a potentially life-threatening hemolytic transfusion reaction.

Keywords: Blood groups, cat, erythrocyte antigen, transfusion

RESUME

Fréquence des groupes sanguins chez les chats européens en France

Plusieurs études ont mis en évidence que la prévalence des groupes sanguins félines varie géographiquement et selon la race. Le système de groupes sanguins le plus important chez les chats est le système AB (groupe A, B et AB). Notamment, tous les chats de groupe B possèdent naturellement de puissants alloanticorps anti-A pouvant être associés à des réactions transfusionnelles hémolytiques immunitaire possiblement mortelle. Le but de l'étude était de déterminer la prévalence des groupes sanguins félines chez les chats européens en France et de calculer le risque relatif de réaction transfusionnelle hémolytique immunitaire possiblement mortelle causée par une transfusion sans groupage (incompatibilité A-B) dans cette population. Les échantillons de sang ont été collectés prospectivement chez des chats malades ou en santé à un hôpital référé (région Auvergne Rhône Alpes) et un laboratoire vétérinaire de pathologie clinique dont les échantillons proviennent de toute la France, avec une majorité d'échantillon provenant de la région Auvergne Rhône Alpes. Les groupages pour le système AB ont été réalisés en utilisant une technologie immunochromatographique. Un total de 231 chats européens ont été testés et la prévalence des groupes sanguins était comme suit : 89,6% de groupe A, 10 % de groupe B et 0,4 % de groupe AB. La prévalence du groupe B dans cette étude excède celle de nombreux pays, notamment les Etats Unis et le Canada. La probabilité d'une transfusion à haut risque sans groupage au préalable a été estimée à 9%. La haute prévalence de groupe B chez les chats européens en France renforce l'importance de grouper tous les chats avant chaque transfusion pour éviter une potentielle réaction hémolytique transfusionnelle possiblement mortelle.

Mots clés : Groupes sanguins, chat, antigène érythrocytaire, transfusion

Introduction

Feline blood transfusion has become an indispensable therapeutic tool. The most common causes of anemia in cats requiring transfusion are hemorrhage, primary immune-mediated hemolytic anemia, and ineffective or absent erythropoiesis [3, 16]. The goal of transfusion medicine is to offer a safe and effective blood product to avoid transfusion reactions. To date, the feline blood group systems described include the well-defined feline AB blood group system (consisting of Types A, B and AB) [3, 12, 15, 16, 34] and the Mik group system (consisting of Mik-positive and Mik-negative) [3, 15, 34]. The most important blood group system in cats is the AB system, because all Type B cats possess strong naturally occurring anti-A alloantibodies, which have been associated with life-threatening immune-mediated hemolytic transfusion reactions [3, 12, 15, 16, 34]. One third of Type A cats have naturally occurring low-titer anti-B

antibody [17]. The antigens of the blood groups A and B are 2 gangliosides, which differ by the presence of N-acetylneuraminic acid (NeuAc) and N-glycylneuraminic acid (NeuGc), respectively. Type A cats express only NeuGc, and conversely, the Type-B antigen determinant is exclusively composed of NeuAc. Type AB red cells have equal amounts of NeuAc and NeuGc [3, 4, 12]. The A and B blood types are inherited as simple autosomal Mendelian traits, with blood Type A being dominant over B. Type AB is inherited as a third allele that is recessive to A and dominant to B [3, 4, 10, 12]. The feline AB blood group system is well described and is the first blood group, outside of non-human primates and humans, for which the genes have been identified [4].

The high titers of naturally occurring anti-A alloantibodies in a Type B cat result in rapid intravascular destruction of transfused Type A red blood cells. Fatal hemolytic transfusion reactions have been documented in

Type B cats receiving as little as 1 mL of incompatible Type A blood [17]. This process is thought to be mediated by IgM and the complement cascade. However, as the destruction of Type B blood cells transfused to a Type A cat is mostly an extravascular process mediated by IgG and IgM without complement activation, the transfusion reaction is typically less severe in this case [3, 10, 15, 20, 33]. Due to the risk of life-threatening reactions associated with the AB system, it is imperative to blood type cats prior to a transfusion [3, 10, 15, 16, 18, 20, 28, 33, 34]. Blood Type A has consistently been found to be the most common blood type among cats worldwide, whereas Type AB cats have been found to be consistently rare throughout the world [10, 12]. However, the prevalence of Type B cats in a given population varies considerably with respect to geographic location and breed [1, 2, 7, 8, 11, 13, 14, 19, 21-24, 26, 27, 29, 30].

The aim of this study was to assess the prevalence of blood types of non-pedigree cats in France and calculate the relative risk of a life-threatening immune-mediated hemolytic transfusion reaction caused by an unmatched transfusion in this population.

Materials and Methods

ANIMALS AND SPECIMENS

A total of 231 blood specimens from non-pedigree cats, i.e. either European Shorthair or Longhair cats, were blood typed; 131 specimens were obtained from the referral clinic in the Auvergne Rhône Alpes region (CHV St Martin), and 100 were obtained from the veterinary clinical pathology laboratory (Laboratoire Orbio). A signed consent form was obtained from all owners. The sampled population ranged in age from 3 months to 20 years (mean: 7.4 years) and included 107 females and 124 males. In addition to 39 specimens obtained for the screening of feline blood donors, 192 blood specimens were obtained from sick cats, including 18 anemic cats. No specimens were excluded because of in-saline autoagglutination.

This study was performed on leftover EDTA-containing blood specimens collected between January and October 2016. The blood specimens were obtained from the Centre Hospitalier Vétérinaire de St-Martin (CHV St-Martin, Bellevue, France), a referral center in the Auvergne Rhône Alpes region, and from the Laboratoire Orbio (Bron, France), a veterinary clinical pathology laboratory that receives specimens from throughout France. The age, gender, reason for visiting the hospital or reason for the blood test along with the address of the cat owner were recorded. All pedigree cats were excluded from this study. Cats were being sampled either to investigate a clinical disease or as part of a screening process for future blood donors.

ANALYTICAL METHODS

The EDTA blood specimens were stored at 4°C until they were blood typed, which occurred within 48 hours of collection. A microhematocrit analysis was performed on all specimens. If anemia was present, defined as a hematocrit less than 0,25 L/L, then autoagglutination was ruled out according to the following protocol: one drop of the blood was mixed with two drops of saline, and visual agglutination was assessed. When autoagglutination was present, typing was not performed.

Specimens were blood typed using an immunochromatographic technique (Quick Test A+B, Alvedia, Lyon, France) according to the manufacturer's recommendations. Briefly, the strip of the immunochromatographic cartridge was dipped in a solution containing 10 µL of EDTA blood and 3 drops of phosphate buffered saline and was visually inspected once the red cell suspension had diffused to the top of the strip (2-4 minutes). The cartridge was then placed in a holder and immediately read as follows: the presence of a red band at the position marked C (control) has to be present for result interpretation; the existence of a visible red band at the position marked A indicates the expression of the A antigen, and the presence of a red band at the B position indicates the expression of the B antigen on red blood cells. The accuracy of this blood typing kit has been evaluated previously [28], and two concordant readings were obtained before the specimen was determined to be Type AB. Retyping is recommended to confirm any AB cats [28].

STATISTICAL ANALYSES

Statistical analyses were conducted using the R software (R Foundation for Statistical Computing, Vienna, Austria). The association between the blood types and the origin of the specimens as well as the presence or absence of concomitant diseases were analyzed with Fisher's exact tests. Wilson confidence intervals (CI) set at 95% were estimated using the binconf procedure.

The probability of a high risk A-B mismatched transfusion (Type A or Type AB donor and Type B recipient) was calculated as the percentage of non-Type B cats multiplied by the percentage of Type B cats [14, 21].

Results

The overall prevalence of blood types in this non-pedigree feline population was 89.6% Type A (n=207, CI: 85.0–92.9); 10% Type B (n=23, CI: 6.7–14.5); and 0.4% Type AB (n=1; CI: 0.02–2.4). Of the 131 specimens from the referral clinic, 120 were Type A, and 11 were Type B, whereas of the 100 specimens of non-pedigree cats obtained from the veterinary clinical pathology laboratory, 87 were Type A, 12 were Type B, and 1 was Type AB. The prevalence did not vary significantly according to the origin of the specimens

between the referral center and the veterinary clinical pathology laboratory ($P=0.31$). Most of specimens ($n=175$ over $n=231$) come from region of Auvergne-Rhône-Alpes. The prevalence of blood types of Auvergne Rhône Alpes was 92 % Type A ($n=161$) ; 8% Type B ($n=14$) ; and 0 Type AB (Figure 1). In the rest of France, the prevalence of blood type was 82.1 % ($n= 46$) ; 16.1% Type B ($n=9$) and 1.8 % Type AB ($n=1$) (Figure 1). The prevalence varied significantly according to the origin of the specimens between Auvergne Rhône Alpes region and rest of France ($p= 0.028$).

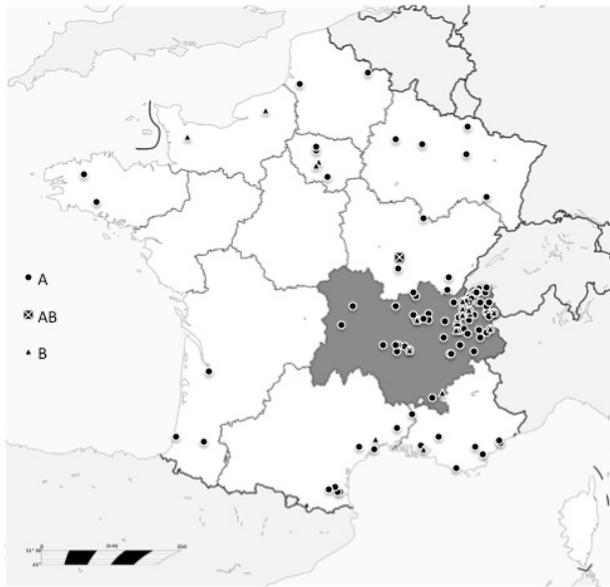


Figure 1: Map of blood type distribution for cats ($n=231$) between Auvergne Rhône Alpes region and other French regions.

The prevalence of blood types in sick cats ($n=192$) was 171 (89.1%) Type A, 20 (10.4%) Type B and one (0.5%) Type AB. The 18 specimens from anemic cats represented various conditions, including renal disease ($n=6$), infectious disease ($n=7$), digestive disease ($n=2$), neurological disease ($n=1$), neoplasia ($n=1$) and unknown ($n=2$). All 18 anemic cats were Type A. The healthy feline blood donors included 36 (92.3%) Type A and 3 (7.7%) Type B cats. The blood type prevalence did not vary significantly according to health status ($P=0.91$).

Based on the overall prevalence, the probability of a high risk A-B mismatched transfusion was estimated to be 9%.

Discussion

In the present study, Type A cats were dominant with an overall prevalence rate of 89.6% and 92% for only Auvergne Rhône Alpes region. Throughout the world type A is the most common, particularly European cats [3, 12, 15, 16, 34]. The overall prevalence of Type B in non-pedigree cats in France was 10.4%, and varied from 8 to 16.1% according to the geographical localisation, exceeds that in numerous other countries, including the United States and Canada (Table I) [8, 11]. Similar to our results, Eyquem *et al.* reported a blood

Type B prevalence rate of 14.9% in a population of 350 cats of unspecified breed in France in 1962 [6]. However, the prevalence of Type B blood has been reported to exceed 20% in several countries, including Australia, England, Greece and Turkey (Table I).

In this study, Type AB was rare, which is similar to other reports (Table I), with only one Type AB cat identified. A recent report recommended retyping after in-clinic typing with reference methods to confirm Type AB cats [28]. In the present study, two concordant readings were obtained before concluding that the cat was Type AB, but further investigation, such as backtyping (i.e., assessing for the presence/absence of alloantibodies), was not performed. Because of the rarity of Type AB blood, this type is unlikely to have influenced the overall prevalence of the blood types.

Marked geographical variations in the prevalence of blood types have previously been reported in non-pedigree domestic cats, and knowledge of blood type prevalence in a certain feline population may help assess the risk of transfusion reactions following unmatched transfusions as well as the risk of neonatal isoerythrolysis in kittens born to parents of unknown blood types.

The relatively high prevalence of Type B in France highlights the need for pre-transfusion blood typing in this feline population, and accordingly, in-house feline blood typing kits should be stored in every veterinary practice that is likely to perform blood transfusions. Ideally, healthy Types A and B feline blood donors will have been pre-identified to allow proper screening for transmissible infectious agents [9, 25, 32]. With a 9% probability of a life-threatening immune-mediated transfusion reaction in this population in France (based on our tested population), an unmatched feline blood transfusion is an unacceptably risky procedure. Indeed, the interaction of a Type B recipient's powerful naturally occurring anti-A alloantibodies with a Type A (or AB) donor's red cell antigen can activate complements and cytokines and result in a systemic inflammatory response [31]. While the severity of the response is directly related to the number of red cells destroyed, Type B cats may experience life-threatening events when administered as little as 1 mL of incompatible blood [17, 18, 31]. Furthermore, approximately 20% of Type A cats have weak anti-B alloantibodies. If Type B erythrocytes are transfused to Type A cats, the cells will only survive for a few days [3, 15]. As no universal feline donors exist, Types A and B cats should always receive type-compatible blood.

Because Type B feline blood donors may not be readily available, xenotransfusion has been advocated as an alternative by some veterinarians. It is important to remember that xenotransfusion of dog blood to cats is not recommended. In a recent report about 2 anemic cats, no severe acute adverse reactions were noted, presumably because cats do not have alloantibodies to dog erythrocyte antigens [5]. However, canine red cells could no longer be

Geographical region	Date	Number of cats	Blood group (%)		
			A	B	AB
France	Present study	231	89.6	10.0	0.4
Australia (Sydney) [21]	2005	187	62.0	36.0	1.6
Brazil (Rio de Janeiro) [23]	2008	172	94.8	2.9	2.3
Canada (Montreal) [8]	2014	178	94.4	5.0	0.6
Denmark (Copenhagen) [13]	1994	105	98.1	1.9	0.0
England (South east of England) [7]	2007	105	67.6	30.5	1.9
Greece [24]	2001	207	78.3	20.3	1.4
Hungary [2]	2001	81	100.0	0.0	0.0
Ireland (Dublin) [14]	2011	137	84.7	14.6	0.7
Italy (Northern Italy) [26]	2011	140	90.7	7.1	2.1
Portugal (Lisbon) [22]	2011	55	97.5	2.1	0.4
Portugal (Northern Portugal) [29]	2004	159	89.3	4.4	6.3
Spain (Barcelona) [27]	2004	100	94.0	5.0	1.0
Spain (Gran Canaria Island) [30]	2004	97	88.7	7.2	4.1
Turkey [1]	2006	301	73.1	24.6	2.3
UK [19]	1999	139	87.1	7.9	5.0
United States (Northeast) [11]	1991	1 450	99.7	0.3	0.0
United States (North Central and Rocky Mountain) [11]	1991	506	99.4	0.4	0.2
United States (Southeast) [11]	1991	534	98.5	1.5	0.0
United States (Southwest) [11]	1991	483	97.5	2.5	0.0
United States (West Coast) [11]	1991	812	94.8	4.7	0.5

TABLE 1 : Prevalence of AB blood groups in non-pedigree cats according to the geographical region [1, 2, 7, 8, 11, 13, 14, 19, 21-24, 26, 27, 29, 30].

detected as early as four days after the xenotransfusion [5, 15].

Blood typing does not replace cross-matching, as antibodies can form against antigens other than those associated with the AB system (Mik group) [3, 33, 34]. Therefore, hemolytic transfusion reactions can occur in previously naïve, AB-typed compatible cats. Moreover, reactions can be subclinical and result in a more rapid destruction of the donor blood cells. As Mik blood typing is not commercially available and because naturally occurring anti-Mik alloantibodies have been documented, cross-matching is recommended [34]. However, the prevalence of

Mik types has not been investigated outside of the United States, and limited information is available concerning its clinical significance. Furthermore, cross-matching should always be performed before a second transfusion if more than four days have elapsed since the first transfusion because new antibodies can form during this time. As expected, and consistent with previous reports [7, 21], no association was found between blood type and health status in the population tested.

This study confirms the relatively high prevalence of Type B blood in non-pedigree cats in France, and to the best of our knowledge, this is the first study conducted on the prevalence

of feline blood types in France since 1962. Determining the blood type of a cat is necessary before a blood transfusion due to the risk of a fatal transfusion reaction. For these reasons, the authors recommend that all potential donor cats be typed and screened for infectious diseases before they are used as blood donors. Due to a significant difference of feline blood type prevalence between Auvergne Rhône Alpes region and rest of France, similar prevalence studies should be conducted covering all the country. Because the prevalence of feline blood types varies greatly by breed, and to the best of our knowledge, no recent studies of blood types have been performed in pedigree cats in France, similar prevalence studies should be conducted on pedigree cats to obtain accurate information for this population.

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