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Naturally occurring antibodies in cats against dog erythrocyte antigens and vice versa

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Abstract

Objectives The aim of this study was to investigate the presence of naturally occurring antibodies against canine erythrocyte antigens in cats and vice versa. The influence of canine and feline blood type on cross-match results was also studied.

Methods Blood samples from 34 cats and 42 dogs were used to perform test tube major and minor cross-match tests and blood typing. Blood from each cat was cross-matched with blood from 2–6 dogs, for a total of 111 cross-match tests. Haemolysis, macro- and microagglutination were considered markers of a positive cross-match.

Results Eighty-three overall major cross-match tests were positive at 37°C, 86 at room temperature and 90 at 4°C. The minor cross-match tests were positive in all but two cross-matches performed at 37°C, all tests performed at room temperature and all but one test performed at 4°C. No cats tested totally negative at both major and minor cross-matches performed with samples from any single dog. Prevalence of warm natural antibodies against canine erythrocyte antigens was lower in type B cats than in type A cats, regardless of the blood type of donor dogs.

Conclusions and relevance This study reveals a high prevalence of naturally occurring antibodies in cats against dog erythrocyte antigens and vice versa, and suggests that transfusion of cats with canine blood is not recommended as a routine procedure owing to the potential high risk of either acute severe or milder transfusion reactions.

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Introduction

Two feline blood group systems are known: AB (comprising types A, B and AB) and Mik (including types Mik positive and Mik negative).[1] Type A cats may have weak natural anti-B alloantibodies. In contrast, type B cats have strong natural anti-A alloantibodies, causing acute, severe haemolytic reactions against type A erythrocytes. Type AB cats do not have natural alloantibodies.[2] The Mik blood group system was recently identified in the USA.[3] Mik-negative cats can have naturally occurring anti-Mik alloantibodies that elicit acute haemolytic transfusion reactions.[3] Therefore, accurate identification of blood types is important in feline practice to reduce the possibility of potentially fatal transfusion reactions and obtain the best efficacy from blood transfusions.[4] While several feline AB typing kits are commercially available for clinical practice, typing of AB and B cats can still pose challenges because erroneous and discordant blood-typing results have been reported in cats.[4,5] Furthermore, they cannot account for antigens outside of the AB system (such as the Mik system) nor for alloantibodies present in the recipient.[6] The prevalence of non-AB blood types is unknown at present. Two recent studies, based on a limited number of cats, did not find evidence for non-AB blood type incompatibilities.[4,6] When possible, cross-match (XM) tests that detects recipient antibodies against donor erythrocytes (major XM) and donor antibodies against recipient erythrocytes

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(minor XM) should be performed prior to transfusion to increase patient safety.[2,6]

Blood transfusion in the feline species may be challenging. In fact, the small size of donors makes blood collection technically more difficult than in dogs, and sedation is usually required for bleeding donors. Moreover, the high prevalence of naturally occurring alloantibodies against feline red blood cell (RBC) antigens demands that blood typing is performed before any transfusion, and the need to use donors and recipients of the same blood type can make transfusions difficult in cats with rare blood types, such as B or AB.[1,2,7]

Despite xenotransfusions being abandoned in all other domestic species since the early 1900s, transfusion of canine blood to cats is still performed in veterinary practice as a life-saving procedure when haemoglobin-based oxygen carrier solutions are not available and a suitable feline donor cannot be found.[5,8–10]

Based on a limited number of cases reported in the veterinary literature, with most publications dating from 1960s, cats did not appear to have naturally occurring antibodies against canine RBC antigens.[8] However, a recent study reported significant incompatibilities detected by XM tests between feline and canine blood.[5] No severe acute adverse reactions have been described for cats receiving a single transfusion with canine blood.[5,8,9,11,12] Only mild transfusion reactions occasionally occurred during the transfusion or in the following week.[5,8] In most reports, cats transfused with canine blood improved clinically.[5,9,10,13] However, antibodies against canine RBCs were produced within 4–21 days of the transfusion, and any repeated transfusion with canine blood later than 6 days after the first one caused severe acute reactions which were frequently fatal.[8,11,12] Moreover, the lifespan of the transfused canine RBCs was very short (3–5 days).[5,14]

Because of the limited number of cases reported in the literature, more data are needed to evaluate the benefit and the risks of dog-to-cat xenotransfusions.

The purpose of this study was to assess the potential risk of adverse transfusion reactions in cats transfused with canine blood, by evaluating the occurrence of feline naturally occurring antibodies against canine RBC antigens and vice versa. The influence of blood types of cats and dogs on XM results was also investigated.

Materials and methods

Samples

Surplus material from diagnostic samples of 34 domestic shorthair cats and 42 dogs of 17 different breeds admitted to the Teaching Veterinary Hospital of University of Messina for elective surgery, an annual health check or health problems between February and November 2015 was used. Informed consent was obtained from owners and results from blood typing were offered to them free of charge. About 1 ml of K_2EDTA blood and, when available, up to 1 ml of blood serum were used to perform blood typing and XM tests. Haemolysed samples were excluded from the study. Blood was stored at 4°C until use and was brought to room temperature (RT) before testing. XM tests and canine blood typing were performed within 24 h of blood collection. Feline blood typing was performed within a week of blood collection.

Blood typing

The dog erythrocyte antigen (DEA) 1 system was typed using a commercial immunochromatographic test (Lab test DEA 1-Alvedia, Limonest, France) according to the manufacturer's instructions.

Blood typing of all cats was determined at the Veterinary Transfusion Research Laboratory (REVLab) Unit, Department of Veterinary Medicine, University of Milan, Italy, using a tube agglutination method and confirmed with a back-typing technique.[15] EDTA blood (150 µl) was centrifuged for 2 mins at 1000 *g* at RT. Plasma was removed and the RBC pellet was resuspended in 5 ml of saline solution (0.9% NaCl) and washed three times by repeating centrifugation, discharge of supernatant and addition of phosphate-buffered saline (PBS). Finally, 25 µl of a 5% RBC PBS suspension were put in three tubes and mixed, respectively, with 50 µl type B serum (anti-A reagent), 8 µg *Triticum vulgaris* lectin/ml in PBS solution (anti-B reagent) or saline solution (0.9% NaCl). These mixtures were incubated at RT for 15 mins before centrifugation for 15 s at 1000 *g*. Tubes were then gently shaken, checked for agglutination and considered positive if macroscopic agglutinates were observed. The cats were considered type A if agglutination was detected in the tube containing anti-A reagent, type B when agglutination was observed in the tube containing anti-B reagent and type AB if agglutination was seen in both tubes. Alloantibody testing was performed in all type B or AB samples to detect the presence or absence of alloantibodies. When a sample appeared to be AB or B, it was confirmed with the back-typing technique: washed 5% RBC suspension from the test sample, a known type A cat and a known type B cat were incubated with the plasma sample as described for tube agglutination to detect the presence (in type B cats vs type A RBCs) or absence (in type AB cats either vs type A and type B RBCs) of alloantibodies.

XM tests

XM procedures were always performed by the same experienced technicians, and checked by one of authors (MM).[16,17]

K_2EDTA tubes were centrifuged to separate RBCs from plasma, which were transferred to separate tubes. Cat (recipient) and dog (donor) RBCs were washed three times by adding about 1 ml of saline solution (0.9% NaCl),

Table 1 Results (agglutination and/or haemolysis) of major cross-match tests at the three temperatures of incubation

Type of result	4°C	RT	37°C
Negative for haemolysis and agglutination	14/104	18/104	28/111
Haemolysis positive and agglutination negative	2/104	5/104	12/111
Haemolysis negative and agglutination positive	85/104 (13)	77/104(12)	62/111 (18)
Positive for haemolysis and agglutination	3/104 (1)	4/104 (0)	9/111 (1)
Total	104 (14)	104 (12)	111 (19)

The number of agglutinations detected microscopically only is indicated in brackets
RT = room temperature

mixing gently and centrifuging at 1000 *g* for 1 min, then removing supernatant. Five percent donor and recipient RBC suspensions in saline solution were then prepared. When the amount of leftover samples was scant, priority was given to perform major XM testing, and to perform incubations at 37°C because both these evaluations are considered more relevant for predicting severe post-transfusion reactions in the recipient animal.[17] EDTA plasma was used when serum was insufficient or haemolytic.

Major XM testing

An equal amount of donor RBC suspension and recipient serum or plasma were placed in three tubes, mixed and incubated, respectively, at 4°C and RT for 30 mins, and at 37°C for 15 mins.[16] The tubes were then centrifuged at 115 *g* for 1 min and the supernatant was evaluated for haemolysis. Tubes were then shaken gently to resuspend cells and check for macroagglutination. If no obvious agglutination was observed in the tube, one drop of blood suspension was placed on a glass slide and examined for evidence of microagglutination. Haemolysis, macro- and/or microagglutination were considered markers of a positive XM.

Minor XM testing, donor and recipient controls

Minor XM, donor and recipient controls were performed, respectively, as described for major XM testing by mixing recipient RBC suspension and donor serum or plasma (minor XM), donor RBC suspension and donor serum or plasma (donor control), or recipient RBC suspension and recipient serum or plasma (recipient control). The controls were performed for all samples, apart from one cat, and only at RT.

Statistical analyses

Statistical analyses were performed using the GraphPad InStat v3.05 (GraphPad Software Inc, San Diego California, USA, 2000) statistic program for Windows 95. Fisher's exact test was used to determine whether there were statistical differences: (1) in frequency of haemolysis or agglutination according to temperature of incubation, both in the major XM and minor XM tests; (2) in frequency of positive results (haemolysis and/or agglutination) according to the recipient and donor blood type in the major XM test at the three temperatures of incubation. P values $\leqslant 0.05$ were considered significant.

Results

Blood typing

Fifteen dogs were DEA 1 negative, 12 were DEA 1 strong positive and 15 were DEA 1 weak positive.[18]

Twenty-seven cats were type A, three type B and four type AB. All type B and AB samples were confirmed by back-typing.

XM tests

Blood from each cat was cross-matched with blood from a variable number of dogs ranging from 2–6, for a total of 111 XMs. Ninety-seven complete XM tests, including major XM and minor XM, at the three different temperatures of incubation were obtained. Major XM testing was not performed in seven cases at both 4°C and RT, and minor XM testing was not undertaken in 10 cases at 4°C and RT and in four cases at 37°C. Eighty-three of 111 (74.8%) overall major XM tests proved positive at 37°C, 86/104 (82.6%) at RT and 90/104 (86.5%) at 4°C. Details about detection of haemolysis and/or agglutination are given in Table 1. The minor XM tests were positive in all but two XMs performed at 37°C (98.1%), all tests performed at RT (100%) and all but one test performed at 4°C (99%). Details about detection of haemolysis and/or agglutination are given in Table 2. No cats tested totally negative for both major XM and minor XM procedures performed using samples from any single matched dog. Major XM was negative at all three temperatures only in 2/104 (1.9%) tests, was negative at both 37°C and RT in 9/104 (8.6%) tests, and was negative at 37°C only in 28/111 (25.2%) tests. In major XM tests, haemolysis was significantly more frequent at 37°C (21/111; 18.9%) compared with RT (9/104; 8.6%) ($P = 0.032$) and 4°C (5/104; 4.8%) ($P = 0.0015$). Conversely, agglutination was significantly more frequent at 4°C (88/104; 84.6%) compared with 37°C (71/111; 63.9%) ($P = 0.0006$) and at RT (81/104; 77.9%) compared with 37°C ($P = 0.0354$). For minor XM tests, there was no significant difference in the

Table 2 Results (agglutination and/or haemolysis) of minor cross-match tests at the three temperatures of incubation

Type of result	4°C	RT	37°C
Negative for haemolysis and agglutination	1/101	0/101	2/107
Haemolysis positive and agglutination negative	1/95*	1/90*	0/96*
Haemolysis negative and agglutination positive	75/101 (4)	74/101 (1)	66/107 (2)
Positive for haemolysis and agglutination	18/101 (0)	15/101 (0)	28/107 (0)
Total	101 (4)	101 (1)	107 (2)

The number of agglutinations detected microscopically only is indicated in brackets
*This denominator is less than the total number reported in the column because in some cases all red blood cells were destroyed by haemolysis, and it was not possible to evaluate agglutination
RT = room temperature

Table 3 Results of major cross-match tests at the three temperatures of incubation according to feline blood type and dog erythrocyte antigen (DEA) classification of canine blood

Cat BT	Dog BT	4°C		RT		37°C	
		P	N	P	N	P	N
A	DEA 1 strong +	29 (90.6)	3	27 (84.4)	5	31 (86.1)	5
B	DEA 1 strong +	6 (100)	0	4 (80)	1	2 (33.3)	4
AB	DEA 1 strong +	6 (100)	0	6 (100)	0	5 (83.3)	1
A	DEA 1 weak +	17 (77.3)	5	18 (81.8)	4	18 (75)	6
B	DEA 1 weak +	2 (100)	0	2 (100)	0	2 (100)	0
AB	DEA 1 weak +	4 (80)	1	5 (100)	0	4 (80)	1
A	DEA 1 negative	20 (80)	5	21 (84)	4	18 (72)	7
B	DEA 1 negative	4 (100)	0	2 (50)	2	1 (25)	3
AB	DEA 1 negative	3 (100)	0	1 (33.3)	2	1 (33.3)	2

Data are n (%)
BT = blood type; RT = room temperature; P = positive haemolysis and/or agglutination; N = negative haemolysis and agglutination; (+) = positive

frequency of haemolysis or agglutination according to temperatures of incubation.

XM testing of each single cat showed different patterns of compatibility towards the 2–6 tested canine samples.

XM results based on feline and canine blood typing

Results of major XM tests based on canine and feline blood types are reported in Table 3. Significant differences were found only at 37°C for the two following combinations: (1) feline type A with canine DEA 1 strong positive (positive reactions: 31/36 [86.1%]) in comparison with feline type B with canine DEA 1 strong positive (positive reactions: 2/6 [33.3%]) ($P = 0.01$); (2) feline type A with canine DEA 1 strong positive (positive reactions: 31/36 [86.1%]) in comparison wih feline type B with canine DEA 1 negative (positive reactions: 1/4 [25%]) ($P = 0.02$).

Discussion

This study reveals a high prevalence of naturally occurring antibodies in cats against canine erythrocyte antigens and vice versa. In fact, no tested cat was totally negative for haemolysis and/or agglutination for both major and minor XM procedures performed at 4°C, RT and 37°C with samples from any single dog.

The presence of haemolysis or agglutination on major and minor XM testing implies that the recipient is not compatible, respectively, to the donor's RBCs or to the donor's plasma.[19] The presence of macroagglutination and haemolysis on major XM testing precludes the use of the donor's RBCs because it indicates that, in the recipient, a severe adverse acute transfusion reaction may occur.[11,20] Conversely, the presence of microagglutination may not necessarily indicate that the patient will have a severe adverse transfusion reaction.[15] It is commonly accepted that blood for transfusion ideally should be compatible at 37°C and RT, but major XM testing at 37°C is clinically the most important compatibility.[17] However, cold (4°C) incompatibilities can cause microthrombosis in acral capillary beds and therefore potentially ischaemic necrosis of the tip of ears, nose or tail during cold weather.[21]

In 57.6% (64/111) of major XM tests that we performed at 37°C, haemolysis and/or macroagglutination were found, suggestive of a high risk of severe acute transfusion reactions.[17] Moreover, feline haemolysins against dog RBCs were more prevalent at 37°C; conversely, haemoagglutinins were more prevalent at 4°C. A limitation of this study is the lack of controls at 4°C and at 37°C, owing to the restricted amount of available blood. Because of this, positive results at these incubation temperatures could have been overestimated. Furthermore, haemolytic reactions could have been underestimated when XM tests were performed using plasma obtained from EDTA blood. In fact, complement activation is responsible for in vitro haemolysis after anti-RBC antibodies reacted with RBC antigens, but it cannot occur when calcium and magnesium cations are chelated by EDTA.[22]

Further limitations of this study are that we did not test cats for the Mik system group, and we had the opportunity to test very few feline type B and AB samples because of their low prevalence in the feline population.[23,24] However, the prevalence of warm natural antibodies against canine RBCs was lower in type B cats than in type A only when matched with DEA 1 strong positive blood. We can therefore assume that type A cats more frequently have warm natural antibodies against DEA 1 strong positive RBCs and could have a higher risk for severe acute adverse reactions after xenotransfusion with DEA 1 strong positive donors.

Almost all minor XM tests in this study were positive, and mostly agglutination reactions were detected. When the volume of donor plasma transfused is small, antibodies in donor plasma become significantly diluted in the recipient blood stream, and therefore the results of the minor XM test may not be clinically relevant or may cause mild-to-moderate acute transfusion reactions.[17] However, transfusion of large amounts of canine whole blood containing antibodies against the recipient's RBCs may cause severe haemolysis and worsen a pre-existing anaemia.[8] This could occur as a result of repeated whole blood transfusions in subsequent days or of administration of large amounts of plasma.

Extensive data about pre-transfusion dog-to-cat XM tests are not available. In fact, published studies report information regarding XM tests in about 56 cases only.[5,8] Nineteen cats showed agglutination against canine RBCs on major XM tests, and in only two cases on minor XM tests.[8] Unfortunately, all these tests were performed at one temperature of incubation only: RT or 37°C. Moreover, minor XM tests, microagglutination or haemolysis were usually not evaluated.[5,8,11] Microagglutination and incompatibility reactions in major XM tests at RT or in minor XM tests can cause milder reactions and reduce the survival of transfused RBCs. This could be the reason why mild transfusion reactions have previously been reported occasionally during the transfusion or in the following week.[8,20] Furthermore, in some studies the lifespan of transfused canine RBCs was shortened to less than 4–5 days vs a 30 day half-life for compatible feline RBCs.[5,14,25]

Negative major and/or minor XM tests do not completely eliminate the risk associated with transfusions, and do not guarantee an expected lifespan of transfused erythrocytes, because delayed reactions can be caused by the production of antibodies against RBC antigens shortly after the transfusion.[26] Additionally, a negative RBC XM test does not predict an absence of reactions against leukocytes and plasma proteins.[26] Therefore, although XM tests are considered to be the standard test for assessing the risk of blood transfusion due to immunological reactions in practice, they are not fully predictive of the risk of transfusion reactions.[5]

This study, as also recently found by Euler et al,[5] consistently shows a high degree of incompatibility when dog and cat blood are cross-matched. Despite this, reports of acute transfusion reactions on first transfusion of dog blood to cats are rare, according to both publications dating from the 1960s and a few recent case reports.[5,9–13,25] The discrepancy between multiple reported safe dog-to-cat transfusions and consistent XM incompatibility could be owing to the fact that natural alloantibodies have changed over time, or that the prevalence of alloantibodies and feline blood types vary in different geographical areas, or that the older studies missed minor transfusion reactions. Finally, a low positive predictive value for adverse xenotransfusion reactions following an incompatible dog-to-cat XM cannot be excluded, but this positive predictive value cannot be explored in clinical settings, because blood is almost never transfused when a positive XM is obtained and, in emergency situations, cats are presumably transfused without performing XM testing with the donor dog.

Conclusions

Transfusion of cats with canine blood is not recommended as a routine procedure because the high prevalence of XM incompatibilities theoretically suggests an elevated risk of severe acute reactions or of milder reactions that make the xenotransfusion less beneficial than transfusion with matched feline whole blood. In exceptional circumstances where xenotransfusion is the only means available for the short-term stabilisation of a feline patient until obtaining compatible feline blood or bone marrow red-cell regeneration, XM tests should always be performed. A completely compatible canine blood might be extremely difficult to find and, in this case, dogs found to be negative at major XM tests (best at 37°C) would be preferred.

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