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

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Keywords:	feline, dog erythrocyte antigen (DEA), xenotransfusion, blood type, cross-matching tests, cat erythrocyte antigen, canine
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 two to six dogs, for a total of 111 cross-match tests. Hemolysis, macro- and/or micro-agglutination 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 compared to A, 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 due to the potential high risk of either acute severe or milder transfusion reactions.

Replies to Reviewers' Comments to Author:

Reviewer: 2
Reviewers report for the author
Acceptable for publication with minor grammatical corrections only.

Minor corrections are written on scanned copy for use by authors and editor.
ANSWER: all corrections written on the scanned copy have been made.
We changed "cross-matching" to "cross-match" as suggested at lines 22 and 33 and also in the whole manuscript for consistency.

Other minor corrections:

Line 58: Say "(such as the Mik system)"
ANSWER: done

Line 282: Say "...does not predict an absence of reactions against leukocytes...."
ANSWER: done

Line 293: Say "....or that the prevalence of alloantibodies and feline blood types vary....."
ANSWER: done

Tables 1 and 2: The two tables are formatted differently. Table 1 would say, for example, 75, whereas Table 2 would say 75/101. Reformat one of the tables so that they both are formatted comparably.
ANSWER: we modified Table 1 as suggested

Reviewer: 1
Reviewers report for the author
The authors have addressed the comments by both reviewers very well and in an exceptionally clear way. The research is much clearer and reads very well to me.

Just a few very minor edits on the attached pdf.

ANSWER: all edits indicated on the attached pdf have been accepted.
As required at line 31, we checked the number of major XM positive at room temperature (# 86) and it is correct as well as at line 189 and in Table 1

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

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Key words: blood type, cross-matching tests, dog erythrocyte antigen (DEA), xenotransfusion, cat erythrocyte antigen, feline, canine

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 two to six dogs, for a total of 111 cross-match tests. Hemolysis, macro- and/or micro-agglutination 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 compared to A, 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 due to the potential high risk of either acute severe or milder transfusion reactions.

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 hemolytic reactions against type A erythrocytes. Type AB cats do not have natural alloantibodies.[2] The Mik blood group system was recently identified in USA.[3] Mik negative cats can have naturally occurring anti-Mik alloantibodies that elicit acute hemolytic 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) that detects recipient antibodies against donor erythrocytes (major XM) and donor antibodies against recipient erythrocytes (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 hemoglobin-based oxygen carrier solutions are not available and a suitable feline donor cannot be found.[5,8,9,10]

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 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, 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. Hemolyzed samples were excluded from the study. Blood was stored at 4°C until use and was brought to room temperature (RT) before testing. Cross-match and canine blood typing were performed within 24 hours after blood collection. Feline blood typing was performed within a week after blood collection.

Blood typing

The canine DEA 1 system was typed using a commercial immuno-chromatographic test (Lab test DEA1- Alvedia, Limonest, France) according to the manufacturer procedure.

Blood typing of all cats was determined at 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 1,000 x 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 PBS. Finally, 25 μL of a 5% RBCs PBS suspension were put in three tubes and mixed respectively with: 50 μL of type B serum (anti-A reagent), 8 μg of *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 1,000 × 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 versus type A RBCs) or absence (in type AB cats either versus type A and type B RBCs) of alloantibodies.

Cross-match tests

Cross-match 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), mixing gently and centrifuging at 1,000 x g for 1 min, then removing supernatant. Five

144 percent donor and recipient RBC suspensions in saline solution were then
145 prepared. When the amount of left over samples was scant, priority was given
146 to perform major XM testing, and to perform incubations at 37°C because both
147 these evaluations are considered more relevant for predicting severe post-
148 transfusion reactions in the recipient animal.[17] EDTA plasma was used when
149 serum was insufficient or hemolytic.

150 *Major cross-match*

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

160 *Minor cross-match, donor and recipient controls*

Minor XM, donor and recipient controls were respectively performed as described for major XM 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 statistic program (GraphPad Software Inc., San Diego California, USA, 2000) for Windows 95. The Fisher's exact test was used to determine whether there were statistical differences: a) in frequency of hemolysis or agglutination according to temperature of incubation, both in the major XM and minor XM tests; b) in frequency of positive results (hemolysis and/or agglutination) according to the recipient and donor blood type in the major XM test at the three temperatures of incubation. P values ≤ 0.05 were considered significant.

Results

Blood typing

Fifteen dogs were DEA1 negative, 12 were DEA1 strong positive and 15 were DEA1 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.

Cross-match tests

Blood from each cat was cross-matched with blood from a variable number of dogs ranging from two to six, for a total of 111 cross-matches. Ninety-seven complete XM tests including major XM and minor XM at the three different temperatures of incubation were obtained. Major XM was not performed in seven cases at both 4°C and RT, and minor XM was not done in 10 cases at 4°C and RT and in four cases at 37°C. Eighty-three/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 hemolysis 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 hemolysis 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, hemolysis was significantly more frequent at 37°C (21/111=18.9%) compared to 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 to 37°C (71/111=63.9%) (P= 0.0006), and at RT (81/104= 77.9%) compared to 37°C (P= 0.0354). For minor XM tests, there was no significant difference in frequency of hemolysis or agglutination according to temperatures of incubation.

Cross-match of each single cat showed different patterns of compatibility towards the two to six tested canine samples.

Cross-match results based on feline and canine blood type typing

Results of major XM 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: a) feline type A with canine DEA1 strong positive (positive reactions: 31/36=86.1%) in comparison to feline type B with canine DEA1 strong positive (positive reactions: 2/6=33.3%) (P=0.01); b) feline type A with canine DEA1 strong positive (positive reactions: 31/36=86.1%) in comparison to feline type B with canine DEA1 negative (positive reactions: 1/4= 25%) (P=0.02).

Discussion

This study reveals a high prevalence of naturally occurring antibodies in cats against dog erythrocyte antigens, and *vice versa*. In fact, no tested cat was totally negative for hemolysis 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 hemolysis 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 hemolysis on major XM 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 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 ischemic 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, hemolysis and/or macroagglutination were found, suggestive of a high risk of severe acute transfusion reactions.[17] Moreover, feline hemolysins against dog RBCs were more prevalent at 37°C, conversely hemoagglutinins were more prevalent at 4°C. A limitation of this study is the lack of controls at 4°C and at 37°C, due to the restricted amount of available blood. Because of this, positive results at these incubation temperatures could have been overestimated. Furthermore, hemolytic reactions could have been underestimated when XMs were performed using plasma obtained from EDTA blood. In fact, complement activation is responsible for in vitro hemolysis 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 compared to type A only when matched with DEA1 strong positive blood. We can therefore assume that type A cats more frequently have warm natural antibodies against DEA1 strong positive RBCs and could have a higher risk for severe acute adverse reactions after xenotransfusion with DEA1 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 hemolysis and worsen a pre-existing anemia.[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 red blood cells on major XM, and in only two cases on minor XM.[8] Unfortunately, all these tests were performed at one temperature of incubation only: RT or 37°C. Moreover, minor XM, microagglutination or hemolysis were usually not evaluated.[5,8,11] Microagglutination and incompatibility reactions in major XM at RT or in minor XM can cause milder reactions and reduce the survival of transfused red blood cells. 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 compared to a 30 days 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 (2016), 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.[5,9,10,11,12,13,25] The discrepancy between multiple reported safe dog-to-cat transfusions and consistent XM incompatibility could be due to the fact that natural alloantibodies have changed over time, or that the prevalence of alloantibodies and feline blood types vary in different geographic areas, or that the older studies missed minor transfusion reactions. Finally, a low positive predictive value for adverse xenotransfusion reactions following

incompatible dog-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 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 stabilization 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 negative at major XM (best at 37°C) would be preferred.

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Supplementary material

Table 1, table 2, table 3

Author note:

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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1 Tables

2 Table 1: Results (agglutination and/or hemolysis) of major XM test at the three temperatures of incubation.

3 The number of agglutinations detected microscopically only is indicated in brackets

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

4

5

Table 2: Results (agglutination and/or hemolysis) of minor XM at the three temperatures of incubation. 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 RBCs were destroyed by hemolysis, and it was not possible to evaluate agglutination

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

Table 3: Results of major XM tests at the three temperatures of incubation according to feline blood type and DEA classification of canine blood. P: positive hemolysis and/or agglutination; N: negative hemolysis and agglutination, BT: blood type

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