TESTING BLOOD PRE-TRANSFUSION COMPATIBILITY IN A GROUP OF CATS

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Summary
Unlike in dogs, the feline erythrocyte antigenic system is represented by 3 blood types (A, B and AB), composed of only 2 agglutinogens, against which there are naturally plasmatic anti-erythrocyte antibodies (anti-A or anti-B), with clinical importance. This fact increases considerably the risk of adverse transfusion reactions, at the first transfusion.

Despite these risks, the use of transfusion therapy in feline patients with various severe diseases may be particularly effective if blood products with a high degree of compatibility and appropriate to the disease status are available. For this purpose we intended to assess the efficacy of the tests used for feline blood compatibility evaluation, RapidVet-H Feline (DMS Laboratories) and Rapid DME VET A+B (Alvedia), available in our country. In association with blood typing, we also resorted to assessment of blood compatibility through cross-match tests (slide and tubes methods), while identifying the potential difficulties in interpreting the results.

According to the results obtained by blood typing tests, 10 mixed breed cats revealed the presence of the A erythrocyte antigen. Cross-match tests proved to be more variable and the results showed 3 negative reactions (absence of agglutination/haemolysis), one pseudo-agglutination reaction (erythrocyte rouleaux) and a positive agglutination reaction. The microscopic examination of the erythrocyte rouleaux (pseudo-agglutination) and clarification of the reactions by adding saline, allowed the correct assessment of the compatibility between the blood donor and the recipient.

Based on the analysis of overall results achieved by blood typing and Crossmatch tests, with an average degree of difficulty, we consider that these tests significantly decrease the risks of adverse reactions and encourage the implementation of blood transfusion therapy in cats.

Keywords: blood typing, Crossmatch, blood transfusion, cats.

Introduction
Currently, small animal medicine recognizes an important development, with extensive diagnostic and therapeutic possibilities. The complexity of various diseases in cats led to the development and implementation of new therapeutic procedures, which also imply blood transfusion therapy.

The feline A-B antigenic system is different from dogs and similar to the human ABO blood group system. The sphingoglycolipid structure, which determines blood type in cats, has been identified as a sialic acid residue on a ceramide dihexose backbone. The sialic acid residues are composed of N-glycolyneuraminicacid and N-acetylneuraminic acid for type A and B respectively, and both for the type AB (Hohenhaus, 2004). A new erythrocyte antigen, called ‘’Mik’’ was recently discovered in domestic shorthaired cats (Weinstein, 2007). Due to the presence of plasmatic anti-erythrocyte isoantibodies (anti-A and anti-B), the adverse reactions produced by incompatible transfusions in cats from different blood type donors, may be severe or even fatal. The incompatibilities may express through agglutination or hemolysis, a phenomenon that underlies the immunohematologic diagnostic tests.
In cats, pre-transfusion testing of the blood type and Crossmatch to determine blood compatibility is essential, because of the risk of adverse transfusion reactions (Klaser et al., 2005), even at the first transfusion.

**Material and methods**

Implementation of transfusion therapy in a group of critically ill feline patients, involved prior testing of blood compatibility between donor and receiver by blood typing and/or Crossmatch. For early detection of adverse reactions during transfusion, the patients were carefully monitored throughout the blood administration, using general methods of semiology (auscultation, thermometry) and special methods (pulse oximetry, electrocardiography).

**Animals.** The evaluation of blood compatibility using blood typing tests was performed in 10 domestic cats (6 donors and 4 receivers). In addition, Crossmatch tests were performed before every blood transfusion. The age of the patients included in this study ranged from 4 months to 13 years, including 6 females and 4 males of mixed breed.

Blood typing was performed using RapidVet-H Feline (DMS Laboratories) and Rapid DME VET A+B (Alvedia) kits, which may typify all blood groups standardized in cats (A, B and AB).

*The card test (RapidVet-H)* is based on the use of two reagents: *Triticum vulgaris* lectins, for the detection of blood group B and monoclonal anti-A antibodies, for the detection of the blood type A. This test reveals agglutination reactions on the card between erythrocytes with antigens A, B or AB and lyophilized antiseraums, specific to one of the antigens. The agglutination, read macroscopically, has a distinct intensity in type A in comparison with type B, due to the different nature of the antiseraum used. However, cats whose RBCs react with both reagents can be reviewed in a reference laboratory to confirm the blood type AB which occurs very rarely.

*The quick test DME VET A+B (Alvedia)* is based on the migration of red blood cells on a membrane previously treated with specific monoclonal antibodies (anti-A and anti-B), under the influence of a buffer flux moving along due to capillary action. In these conditions, positive erythrocytes bounded by specific antibodies are highlighted as a red line on the membrane. To confirm the accuracy of the migration line there is also a specific control line for comparison (C), required to be identified in each valid test.

The general interpretation of blood type results is based on the presence of agglutination, which indicates a positive result for the investigated blood type. The highest level of blood compatibility is given by the identification of the same blood type in both the donor and the recipient. Each test includes materials for testing procedures and a small guide with the testing stages, also described extensively by other authors (Ognean, 2010).

The blood cross-match test, performed by tube and slide methods, has been made in order to detect the incompatibility reactions, expressed by agglutination or hemolysis. The absence of these results during the first 3 minutes means very good blood compatibility. The procedure includes 4 steps: major, minor and auto agglutination control in both partners. In case of false positive agglutination reactions (the occurrence of erythrocyte rouleaux), the clarification was performed by addition of saline to reagents and microscopic examination.

*Rapid slide Crossmatch test* was performed according to a simplified version cited by Ognean (2009), in order to detect blood incompatibilities, manifested through agglutination. The test procedure consisted in: collection and labeling of EDTA blood samples and their centrifugation (2500 rpm, 4 minutes); preparation and labeling of microscope slides for major Crossmatch (donor RBCs + donor plasma), minor
Crossmatch (receiver RBCs + donor plasma) and auto-agglutination receiver and donor control (RBCs+plasma from the same cat); reagents are deposited with a micropipette on each slide (3 µL RBCs and 9 µL plasma) and mixed; the results are read (macro- and microscopically) in 3 minutes, at room temperature.

Tube test Crossmatch represents a variant adapted after Abrams-Ogg (2000) with the following stages: collection and labeling of 0,5-1 ml EDTA blood samples; centrifugation (at 1000-1500 g, 5-10 minutes); plasma separation from RBCs sediment; preparation of an erythrocyte suspension from donor and recipient (0,2 ml RBC+ 4,8 ml normal saline); labeling 3 sets of 4 micro tubes with the stage of Crossmatch: major and minor crossmatch, donor and receiver auto-agglutination control; testing major Crossmatch by putting in contact two drops of receiver plasma with one drop of RBCs donor suspension; testing the minor Crossmatch by mixing two drops of donor plasma with one drop of RBCs receiver suspension; control of auto-agglutination by mixing one drop of RBCs suspension with two drops of plasma from the same cat (donor and receiver); incubation of the tubes at 3 different temperatures (22-25°C, 37°C and at 4°C); tube centrifugation (at a low speed, for 15-30 seconds); macroscopic and microscopic reading of the results (hemolysis or agglutination).

Results

Data obtained following the investigation of blood type in a group of blood donor and receiver cats (10), lead to the exclusive (100%) identification of the erythrocyte antigen A (blood type A).

Table1. Pre-transfusion testing of blood compatibility in a group of cats (6 donors and 5 receives)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Blood type</th>
<th>Crossmatch test</th>
<th>Compatibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donors</td>
<td>Receivers</td>
<td>Major</td>
</tr>
<tr>
<td>1</td>
<td>A A</td>
<td>0 0</td>
<td>✓</td>
</tr>
<tr>
<td>2</td>
<td>A -</td>
<td>+ +</td>
<td>✓</td>
</tr>
<tr>
<td>3</td>
<td>A A</td>
<td>0 0</td>
<td>✓</td>
</tr>
<tr>
<td>4</td>
<td>A A</td>
<td>0 0</td>
<td>✓</td>
</tr>
<tr>
<td>5</td>
<td>A, A A</td>
<td>0* 0*</td>
<td>✓</td>
</tr>
</tbody>
</table>

0 = Absence of agglutination or hemolysis; *=pseudo-agglutination (erythrocyte rouleaux); - =no test performed; ✓=assured compatibility; + =agglutination due to autoantibodies.

We consider that the use of RapidVet-H Feline and Rapid DME VET A+B blood typing tests in cats allowed the facile identification of the blood group A, with clear enough positive agglutination reactions on the card or strip (fig. 1). Blood type results obtained on the card were not enlightening every time, regarding the intensity of agglutination reactions between erythrocyte antigen A and the specific monoclonal antibodies.

The summary of Crossmatch test results showed 3 negative results (the absence of agglutination/hemolysis), a pseudo-agglutination result (erythrocyte rouleaux) and a positive result (agglutination) in association with the patient’s blood auto-agglutination.

Microscopic examination of the erythrocyte rouleaux (fig. 2) and the clarification of pseudo-agglutination reactions by addition of saline, allowed the correct assessment of blood compatibility between donor and receiver.
Whole blood transfusion in a group of 5 feline patients did not result in any adverse effects during administration (maxim 4 hours). At 24 hours after transfusion, 4 patients (80%) presented a positive evolution of the general status, while one patient with severe multiple trauma and active bleeding died during blood transfusion.

Generally, the patients tolerated blood administration relatively well, excepting one 4 months old patient, with cranial trauma and signs of hyperexcitability, in which blood administration was difficult and it had to be rushed.

The use of blood transfusion therapy in a group of 5 feline patients revealed many therapeutic benefits, especially when blood compatibility was established and blood administration was performed correctly.

Discussion

Blood typing of feline blood donors and receivers in this study, shows the predominance of group A (100%), the most common blood group seen in cats (between 94-100%) from most geographical regions (Sparkes and Gruffydd-Jones, 2000). A high degree of compatibility was met in this study because blood transfusion partners belonged to the same blood type.

In contrast with dogs, cats have naturally occurring antibodies against erythrocytes of the opposite blood type. All type-B cats had strong hemagglutinins and hemolysins mostly of the IgM class, responsible for the clinical incompatibility reactions, as neonatal isoerythrolysis or transfusion reactions (Bucheler and Giger, 1993). Testing blood type using the card method in a feline patient from this study (female in the late stage of gestation, with immune mediated hemolytic anemia) couldn’t be performed because of patient blood auto-agglutination. In these conditions, only the donor’s (the patient’s mother) blood group could be tested. Type-A donors had weak agglutinins of the IgM class and weak hemolysins, consisting of approximately equal parts of IgG and IgM class, which shorten the survival of transfused group B RBCs, but they don’t cause neonatal isoerythrolysis (Bucheler and Giger, 1993). In this case, due to the presence of auto-antibodies in the receiver plasma, the Crossmatch result showed incompatibility. In this situation, use of an erythrocyte washing process would exclude false agglutinations, but the process is time consuming. However, the patient showed no intra-transfusion adverse reaction and was subsequently positive.

In this species knowledge of blood groups is important for arranged mating and better understanding of the neonatal hemolytic anemia, a disease which appears in kittens as a consequence of incompatibility between maternal and fetal blood. Cats get one gene for blood type from their mother (in the egg) and one from their father (in the sperm). The genes for blood type A are dominant to the genes for blood type B. The fetus may inherit a certain antigenic structure from the father (which is
missing in the mother) and when newborn kittens with type A blood drink colostrum from a mother with type B blood the antibodies contained in the colostrum are absorbed, then bind to the kitten's RBCs and destroy them (Giger and Casal, 1997). By blood typing the queen and the tom before mating, neonatal isoerythrolysis may be prevented.

None of the cases subjected to blood transfusion showed real positive (incompatibility) results at Crossmatch test, which shows the serologic compatibility between donor and receiver (Giger, 2000). In a previous study 8 major Crossmatch incompatibilities were detected from117 testings, but 7 of these cats were previously transfused (Weingart et al., 2004).

Performing blood Crossmatch test is recommended before every transfusion, especially in patients diagnosed with cancer. In literature, various Crossmatch methods are cited, but in most situations, rapid slide techniques may be as relevant as tube methods (Ognean et al., 2009); taking this into consideration, the majority of the tests were performed on slides. Although the sensitivity of the new Crossmatch tests increased significantly, most researchers in the field consider that the blood typing test is essential to establish blood transfusion compatibility in cats and to prevent any risk of adverse transfusion reaction (Giger et al., 2005). However in the absence of blood typing kits, the Crossmatch test can be extremely useful. The negative results obtained in this study also confirm that cats belonged to the same blood group.

Cases with multiple trauma included in this study (40%) represented the major indication for whole blood transfusion, a situation also cited by Klaserat et al. (2005) in 126 transfused cats: hemorrhage 52%, hemolytic anemia 10% and 38% bone marrow disease. Generally, in trauma patients often present with active bleeding and hypovolemic shock, which requires an emergency medical intervention for reestablishing hemostasis and replace the lost blood. In this regard, blood typing of any cat is strongly recommended, before the advent of an accident, to avoid the delay of emergency therapy.

Conclusions

The homogeneity of the erythrocyte antigenic system in the group of tested cats (n = 10), proved by the exclusive presence of blood group A, increased the frequency of transfusion compatibility between patients and blood donors;

Performing the Crossmatch test using various procedures, completes blood compatibility testing, it is cheap and may prevent serious adverse acute intra-transfusional reactions;

The absence of adverse transfusion reactions in all patients supports the efficacy of pre-transfusion testing and encourages the implementation of blood transfusion therapy in feline medicine.

References


