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Blood Type Synchronization Using Dipstick Membrane in Domestic Cats (*Felis domestica*) on the Persian Cat (*Felis silvestris*) in Surabaya

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ABSTRACT

This research objective was to identify blood type polymorphism in domestic cats (*Felis domestica*) and Persian cats (*Felis silvestris*) using dipstick membrane and to know a blood cross match between domestic cats (*Felisdomestica*) and Persian cats (*Felissilvestris*). A total of 10 male domestic cats, 10 female domestic cats, 10 male Persian cats and 10 female Persian cats were studied. The cats were clinically healthy, one year of age and blood was collected intravenously. The result of blood type test showed that a total of 60% female domestic cats had blood type A and 40% blood type B. A total of 70% male domestic cats had blood type A and 30% blood type B. A total of 70% male Persian cats had blood type A and 30% blood type B. A total of 60% female Persian cats had blood type A and 40% blood type B. The Cross match blood test between male domestic cats and male Persian cats revealed 50% match and 50% did not match, while between female domestic cats and female Persian cats, 70% match and 30% did not match.
KEY WORDS: Blood type, domestic cats, Persian cats

INTRODUCTION

Anemia or a low number of red blood cells also occurs in cats due to several factors. Those factors are parasites; fleas (*Ctenocephalidesfelis*); worms (*Dipylidium caninum, Toxocaracati*); protozoa ((*Haemobartonela felis, Cytauxzoon felis*); virus such as Feline Leukemia Virus (FeLV) and Feline Immune Mediated Virus (FIV); poisons such as eating paracetamol and chocolate; PKD (Polycistic Kidney Disease); trauma such as fractures, wide incision wound; medical action such as surgery and nutrient imbalance, such as, rarely, iron deficiency. For an anemic cat or that with a low number of red blood cells, blood transfusion is the right choice to replace the blood loss. However, there is no animal blood banks, especially for cats, blood donors, blood typing test kit in Indonesia and there is also the lack of human resources who know about the process of blood transfusion.

Before doing a blood transfusion, we need to see the blood matches between the donors and the recipient [1]. According to Wardrop [2], cross match blood test should be used before performing blood transfusions in cats, although they have a history of blood transfusion before, because rejection will occur naturally from the antibodies in cats and cause serious effects if blood type A transfusion is given to cats with blood type B.

Blood is a liquid which composed of two parts, the blood plasma and blood cells. Blood cells were made up of three types, erythrocytes, leukocytes, and platelets [3]. Blood flows and circulates through the vascular system. Blood carries a variety of necessities of life to all cells in the body and brought the results of metabolic waste to secrete on the excretion organ [4].The main functions of blood in the circulatory system are as a transports medium, temperature regulator, and controlling liquid and bases balances. Blood volume of cats revolves between 4.7% - 9.65% of the cat's weight, and the factors that affect blood volume i.e. age, health status, diet, body size, activity, and the environment [5]. Erythrocytes (red blood cell) has the main functions to transport hemoglobine and to carries oxygen from lungs to the tissues [6]. Erythrocytes in domestic cats (*Felis domestica*) has biconcave disc-shape with diameter of 5.5 - 6.3 µm and they are in the circulation for around 120 days [4]. Total erythrocytes in cats is up to 7.3 million per mm^3 [5].

In blood transfusion, the first thing we need to do is to look for donors, then we perform the blood typing test. After the blood is available for transfusion, blood matching test is done between the blood of the donor and the recipient (cross matching blood test) to reduce and avoid the risk of allergic reaction and rejection in blood transfusions[2]. Not only in humans, cat also has blood type. There are three blood typesin a cat, A, B, and AB. There is no relationship of serologic antigens A and antigens B with antigens ABO in humans [7].

Almost 95% of cats has blood type A, including those in the United States [8]. On the blood type test of 139 non-pedigree cats and 2017 pedigree cats, it was found that 87.15% of non-pedigree cats had blood type A, 7.9%

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blood type B and 0.55% blood type AB. While in pedigree cats, 54.6% had blood type A, 40.1% of blood type B, and 1.6% blood type AB [9]. Domestic cat's (*Felisdomestica*) blood types from different countries indicate that the frequency of blood type A is more than B and AB [10,11].

Blood type antigen is characterized by specific proteins in erythrocytes membrane, i.e. NueGc-NueGc-Glucose-Galactose-Ceramide (NueGc as N-Glicolilneuraminic acid) as a major constituent of blood type A glycolipids (carried by gene A) and NueAc-NueAc-Glucose-Galactose-Ceramide as a constituent of blood type B (carried by gene B). Blood type AB is a form between blood type A and B[12,13].

The latest blood test on the cats and dogs using the technique of dipstick membrane (Alvedia ®, Villeurbanne, France). The work principle, by mixture the monoclonal antibodies type A and B on the cat, which can be seen on the membrane that has been mixed by buffer and blood. In a positive reaction,the red blood cells will be agglutinated by the antibodies, causing the appearance of a line on the membrane [9].

The best treatment for anemia is blood transfusion. To conduct the blood transfusions, we need blood identification and cross blood categorization. This research aims was to identify blood type polymorphism in domestic cats (*Felis domestica*) and Persian cats (*Felis silvestris*) using dipstick membrane and whether each blood type has a blood cross match between domestic cats (*Felis domestica*) and Persian cats (*Felis silvestris*).

MATERIALS AND METHODS

This was a descriptive study, in which the results were outlined in theory based on the results of the identification of blood type and cross-match between the blood of domestic cats (*Felis domestica*) and that of Persian cats (*Felis silvestris*).

Blood Type Test

To identify the blood type, used the quick test blood typing from Alvedia (Alice Veterinary Diagnostic). The cat name was written on the label of the quick test, then three drops of buffer was added on the holes provided on the package of the quick test. The blood collector strip was dipped intothe tube containing blood, and then to the hole containing the buffer. It was stirred evenly, then the membrane was placed into the hole, moved and stood until the blood suspension meet the membrane. The line of control became visible, and then the membrane was lifted to revealthe results of the blood test.

The Cross Match Blood Test

Blood sample as much as 2 ml,which remained in blood collection tube with EDTA (ethylenediamine tetraacetic acid), was subsequently used for cross match blood tests. The tube was put into the centrifuge machine, then it was centrifuged for 1 minute at 3000 rpm. Then, the blood plasma was taken and suspended in 2% red blood cells by mixing 0.1 ml red blood cells and 5 ml 0.9% solution. The suspension was subsequently mixed by centrifugationin 1 minute and the the supernatant was removed. It was resuspended in 5 ml of 0.9% saline solution, then centrifuged again and repeated three times.

Major cross match was done as follows: two drops of Persian cat's (*Felis silvestris*) plasma and two drops of red blood cells of the domestic cats (*Felis domestica*) were put on the tube. They were mixed with the whisk with the number of 8, and then incubated in room temperature for 30 minutes.

Minor cross match was as follows:Two drops of red blood cells of Persian cats (*Felis silvestris*) and two drops of domestic cats (*Felis domestica*) plasma was put in another tube. Mixed with the whisk with the number of 8, and then incubated in room temperature for 30 minutes.

For the first control, two drops of Persian cat(*Felis silvestris*) plasma and two drops of red blood cells of domestic cats (*Felis domestica*) were placed in the tube, and two drops of red blood cells of Persian cats (*Felis silvestris*) and two drops of domestic cats plasma (*Felisdomestica*) put in another tube, were centrifuged for one minute at 300 rpm.

For the second control,two drops of Persian cat (*Felis silvestris*) plasma and two drops of domestic cats (*Felis domestica*) whole blood were put into the tube, and two drops of Persian cat (*Felis silvestris*) whole blood and two drops of the domestic cats (*Felis domestica*) plasma were put in another tube, then being centrifuged for one minute at 300 rpm. Then, the agglutination and hemolysis were checked by puttinga drop of blood on the object glass, then the examination under a microscope was carried out.

RESULTS

Blood type test

The results of blood type test to 10 female-domestic-cats, 10 male-domestic-cat, 10 male-Persian-cats, 10 female-Persian-cats were shown in Table 1.

Table 1. Blood type test of male and female domestic-cats

No	Sample	Blood type	No	Sample	Blood type
1	Male-domestic-cat	A	1	Female-domestic-cat	A
2		A	2		A
3		B	3		A
4		A	4		B
5		A	5		A
6		B	6		A
7		A	7		A
8		A	8		B
9		B	9		A
10		B	10		B

The results of Blood Type Test of 10 male-Persian-cats and 10 female-Persian-cats were shown in Table 2.

Table 2. Blood type test of female and male Persian cats

No	Sample	Blood type	No	Sample	Blood type
1	Male-domestic-cat	B	1	Female-domestic-cat	A
2		B	2		A
3		A	3		A
4		A	4		B
5		A	5		B
6		A	6		A
7		A	7		A
8		A	8		B
9		B	9		B
10		A	10		A

Cross match blood test

The results of cross match blood test of male-domestic-cats and male-Persian-cats were shown in Table 3.

Table 3. Cross match blood test of male-domestic-cats and male-Persian-cats

NO	Sample	Blood type	NO	Sample	Blood type	Cross match blood test result
1	Male-domestic-cats	A	1	Male-Persian-cats	B	+
2		A	2		B	+
3		B	3		A	+
4		A	4		A	-
5		A	5		A	-
6		B	6		A	+
7		A	7		A	-
8		A	8		A	-
9		B	9		B	-
10		B	10		A	+

The results of cross match blood test of male-domestic-cats and male-Persian-cats were shown in Table 4.

Table 4. Cross match blood test of female-domestic-cats and female-Persian-cats

No	Sample	Blood type	No	Sample	Blood type	Cross match blood test result
1	Female-domestic-cats	A	1	Female-Persian-cats	A	-
2		A	2		A	-
3		A	3		A	-
4		B	4		B	-
5		A	5		B	+
6		A	6		A	-
7		A	7		A	-
8		B	8		B	-
9		A	9		B	+
10		B	10		A	+

Description:
- = cross match blood test results, there were no reaction of agglutination and hemolysis
+ = cross-match blood test results, there were agglutination and hemolysis

DISCUSSION

The formation of erythrocytes (erythropoiesis) is stimulated by the erythropoietin hormone, i.e. the glycoproteins, ninety percent were produced in interstitial cell of the peritubular renal and the other 10% in the liver. The formation of erythropoietin is stimulated by hypoxia or tissuestate changes in atmospheric pressure, reduced arterial blood oxygen levels, and reduced Hb concentrations. Erythropoietin induced erythropoiesis by increasing progenitor cell which bound to the erythrocytes formation process.

Based on the results, there was a little differencein the results of the reaction, i.e. at the level of its agglutination. Degree of agglutination were seen from blood clots on the tube after shaking until blood deposition in a control tube dissolved perfectly.

Agglutination rate which in the results of the reaction is not related to genotype composition in its erythrocyte, but resulting from biological differences existsin each individual. Blood type antigen is characterized by specific proteins on the membrane of the erythrocytes i.e. NeuGc-NeuGc-Glucose-Galactose Ceramide (NeuGcsebagai N-Glycolilneuraminicacid) as a major constituent of blood type A glycolipids (carried by gene A) and NeuAc-NeuAc Glucose-Galactose-Ceramide as a constituent of blood type B (carried by gene B) [14,15].

Gene A more is dominant than gene B [14, 15,16]. Thus,cats with blood A could be either homozygous A (AA), or heterozygous A (AB), so that the protein in its erythrocyte was a combination of gene A and gene Aor gene A and gene B.Cats that have blood type B, the genotype of the composition of blood type is only homozygous (BB).

Quick test of blood typing (dipstick membrane) is a mixture of monoclonal antibodies type A and B in cats that can be seen on the membrane that has been mixed by buffer and blood. Positive reaction of erythrocytesagglutination with secondary antibody appearson the dipstick membrane [2]. This is influenced by the level of sensitivity and superior specificity of monoclonal antibodies dipstick membrane itself.

The accuracy ofa sensitivity assay tool to detect or diagnose a disease or conditiondepends on monoclonal antibody dipstick membrane that detectsantigens from the tested blood.The specificity of the tools is the accuracy ofthe assay to detect negativeness or diagnose a disease and conditions. This monoclonal antibody belong to antigens membrane that can bind onlyto specific dipstick from cats.

The interaction of antigen and antibody has two categories. The primary category is the beginning of the reaction and antibody-antigen binding at the molecular level. The second category leads to precipitation or agglutination. The degree of agglutination in the reaction outcome is not related to the composition of the genotypes contained in its erythrocyte. Different rate of agglutination is because of biological differences existing in each individual [17].

The frequency of blood type A is more dominant than the blood type B, while blood type AB is very rare. Domestic cats from various countries have also shown that the frequency of blood type A is more than blood type B and AB. Cat blood AB classification system has three different phenotypes. The phenotype of A and B inheriting Madeline allele simple genetic. The alleles 'a' is more dominant than allele 'b', thus all blood type B is homozygous alleles 'b' (genotype b/b), whereas cats with blood type A mayhave both homozygous alleles of ' a ' (genotype a/a) and heterozygous allele (genotype a/b) [16, 17].

In this research, blood plasma of male and female local cats when given erythrocytes of male or females Persians with the same blood group A or B does not show agglutination and haemolysis, but when given erythrocytes from different blood groups A with B or B with A showed agglutination and haemolysis.

In cross-match blood test, if the precipitates dissolve again (hemolysis), result is stated as having negative reaction. Iif the precipitationdissolves into grains or not soluble at all, the reaction is expressed aspositive agglutination reaction. Before the transfusion, the main reason for conducting cross match blood test is to identify the hemolytic reactions in transfusion, providing optimal period of erythrocytes in transfusion, to prevent failure of subsequent blood transfusions and prevent neonatal isoerytrolysis [2].

CONCLUSION

The conclusion of this result were that a total of 60% female domestic cats had blood type A and 40% blood type B. A total of 70% male domestic cats had blood type A and 30% blood type B. A total of 70% male Persian cats had blood type A and 30% blood type B. A total of 60% female Persian cats had blood type A and 40% blood type B. The Cross match blood test between male domestic cats and male Persian cats revealed 50% match and 50% did not match, while between female domestic cats and female Persian cat, 70% match and 30% did not match.

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