

Evaluation of the incompatible prevalence by blood crossmatching tests in dogs

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| ARTICLE INFO                                                                                                                                                                                                                                                                                                                          | ABSTRACT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
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| <p><b>Research Paper</b></p> <p>Received: November 18, 2024<br/>Revised: January 04, 2025<br/>Accepted: January 08, 2025</p> <p><b>Keywords</b></p> <p>Crossmatching<br/>Dogs<br/>Ho Chi Minh City<br/>Incompatibility</p> <p><b>*Corresponding author</b></p> <p>Nguyen Thi Thuong<br/>Email:<br/>thuong.nguyenthi@hcmuaf.edu.vn</p> | <p>Crossmatching is one of the most important tests before a transfusion in small animal practice, helping to reduce the risk of post-transfusion reaction in dogs with unknown or experienced blood transfusion history. In this study, crossmatching assessments were performed after washing and resuspending the collected blood. Two types of crossmatching methods was conducted: major crossmatching and minor crossmatching. Based on an agglutination reaction, crossmatching allows the detection of naturally occurring alloantibodies or alloantibodies produced as a consequence of sensitization. In this study, there were 10 incompatible cases in total of 26 cases of dogs taken to the veterinary hospital and Greyhounds (incompatible rate 38.46%). The results showed that higher proportions of incompatible cases were recorded in group of dogs from 1 to 5 years old (26.92%), body weight from 5 - 10 kg (19.23%), female (23.08%), foreign breeds (34.62%), commercial feed (34.62%) and in-house (23.08%). The dogs in the same house or neighborhood, there were 38 incompatible cases in the total of 134 cases (28.36%), in which the highest proportion was found in dogs from 1 - 5 years old had (13.43%), female (15.67%), and foreign breeds (15.67%). In the management groups, in-house dogs had a lowerproportion of incompatibility with each other than cage-free dogs, with 2.24% and 26.12% respectively. Although the results indicated that age, body weight, gender, and breed did not affect the pre-transfusion crossmatching results, it is necessary to perform the crossmatching test before a transfusion to reduce the risk of hemolytic transfusion reactions in dogs.</p> |

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## 1. Introduction

Blood transfusion is being applied widely in dogs and cats in veterinary practice in Vietnam. Crossmatching is one of the most important steps before a transfusion in dogs. The main reason of performing crossmatching is to provide safe blood to the right dog at the right time. Crossmatching determines the serological compatibility between the patient and donor blood, based on an agglutination reaction, and allows detection of naturally occurring alloantibodies or alloantibodies produced as a consequence of sensitization (Vidushi et al., 2020). Cross-matching is indicated when the transfusion history is unknown or the dog has been transfused more than 4 days ago (Davidow et al., 2021). Even though crossmatching is performed before the transfusion, it is still possible that the patient can experience post-transfusion reactions. There are two assessments of crossmatching: major cross-match and minor cross-match (Day & Kohn, 2012). Although transfusion is widely applied in veterinary practice, there are almost no studies about factors such as age, body weight, gender, breed, feed and management, which can affect the prevalence of incompatible crossmatching results. Even though crossmatching reduces the risk of hemolytic transfusion reactions, it does not completely eliminate the risk of transfusion reactions in dog (Tocci & Ewing, 2009). Appropriate RBC survival and/or complete elimination of transfusion reactions are not guaranteed, because low titers of anti-RBC antibodies may not cause enough agglutination for detection, even with a compatible crossmatch test (Zaremba et al., 2019).

Factors, such as age, breed, gender, feed and management, have not been studied for any

association with the result of crossmatching in dogs. Greyhound is a good candidate for being a source of blood donor, due to the big size of this dog breed (Gibson & Abrams-Ogg, 2012), however, there are not many researches about using Greyhound's blood for transfusion in dogs in Ho Chi Minh City. Therefore, this study was carried out to evaluate the prevalence of the incompatibility in crossmatching between the blood of Greyhound and dogs that were taken to the vet hospital, and between dogs living in the same house or neighbor. Furthermore, the study was also to evaluate if factors such as age, gender, breed, feed, and management had affected the prevalence of incompatible results.

## 2. Materials and Methods

### 2.1. Blood sampling

A total of 26 blood samples were collected from dogs from October 2022 to October 2023 at Nong Lam University Veterinary Hospital and the Veterinary Clinic in Ho Chi Minh City.

### 2.2. Crossmatching assay procedure

The crossmatching assessments were major cross-match and minor cross-match. The protocol based on the protocol of (Lanevski & Wardrop, 2001). Blood collected from the donors and patients are stored in EDTA (ethylenediaminetetraacetic acid) tubes. The EDTA tubes containing blood are centrifuged with 3.400 rpm. Washing red blood cells (RBCs): resuspend 0.25 mL of RBCs in 2 - 4 mL of saline, then centrifuge in 1 min, and remove the supernatant. Repeat the procedure twice, and finally remove the supernatant. Resuspend 0.1 - 0.2 mL of RBCs in approximately 4.8 mL saline in order to obtain a 2 - 4% RBC solution. In 3 tubes identified "Major", "Minor" and "Control" add

the following Table 1. Incubate tubes in 15 min at 37°C. Reading results: Note serum color and record any hemolysis. Then resuspend the red cell button into the overlying serum layer, noting the presence of agglutinating clumps. Place a drop of resuspended RBCs on a slide, apply coverslip, and read at 100X and 400X microscope. If the crossmatch is compatible, the RBCs should be individually distributed. Hemolysis

(compared to control) or agglutination is seen with an incompatible crossmatch Rouleaux, a physiological plasma-related phenomenon, may sometimes be observed. In order to distinguish this from agglutination, centrifuge the tubes again for 15 sec, remove serum, and add 2 drops of saline; then centrifuge the tubes once more and reexamine the RBC suspensions.

**Table 1.** Crossmatching composition in the major, minor and control tubes

|         | “Major” crossmatch     | “Minor” crossmatch     | Control                                                      |
|---------|------------------------|------------------------|--------------------------------------------------------------|
| Patient | 2 drops of serum       | 1 drop of RBC solution | 1 drop of patient RBC solution<br>& 2 drops of patient serum |
| Donor   | 1 drop of RBC solution | 2 drops of serum       | 1 drop of donor RBC solution<br>& 2 drops of donor serum     |

These assessments were performed in Nong Lam Veterinary Hospital’s Laboratory. In the first experiment, the blood of Greyhound dogs from the previous study of Nguyen et al. (2024) was collected for cross-matching with other patients. All dogs were fully vaccinated within the last 12 months and received prophylactic flea treatment regularly. Dogs did not receive any raw meat diet and were completely healthy and relaxed. The dogs were fed with commercial dry feed (France) (900 gr). All dogs were checked physically and all clinical signs, including body temperature, heart and respiratory rate, and appetite were normal. Blood from dogs taken to the veterinary hospital, was also collected in 1 mL EDTA tubes, and then crossmatching was immediately performed with Greyhounds’ blood.

In the second experiment, dog blood living in the same house or neighborhood, was collected in 1 mL EDTA tubes, and then crossmatching was performed immediately between them. The information on age, gender, breed, feed, and management was also collected.

**2.3. Statistical analysis**

The collected data was analyzed with Microsoft Excel 2016 and test with Minitab 17.

**3. Results and Discussion**

**3.1. Prevalence of incompatibility crossmatching between Greyhounds and other patient dogs**

A total of 26 dogs were tested for compatibility with Greyhound dogs from the previous study of Nguyen et al. (2024) to investigate the prevalence of incompatibility crossmatching and evaluate factors that could be relevant to the crossmatching results. In 26 cases in the veterinary clinic, there were 10 dogs whose blood was incompatible with the Greyhounds’ blood (38.46%). The data are summerized in Table 2.

**Table 2.** The prevalence of incompatibility crossmatching between dogs taken to the veterinary clinic and Greyhounds

|             | Factor            | Total cases | Incompatibility |                |
|-------------|-------------------|-------------|-----------------|----------------|
|             |                   |             | Cases           | Prevalence (%) |
| Age         | Below 1 year old  | 0           | 0               | 0.00           |
|             | 1 - 5 years old   | 21          | 7               | 26.92          |
|             | Above 5 years old | 5           | 3               | 11.54          |
| Body weight | Below 5 kg        | 11          | 2               | 7.69           |
|             | 5 - 10 kg         | 8           | 5               | 19.23          |
|             | Above 10 kg       | 7           | 3               | 11.54          |
| Gender      | Male              | 13          | 4               | 15.38          |
|             | Female            | 13          | 6               | 23.08          |
| Breed       | Domestic          | 3           | 1               | 3.85           |
|             | Foreign           | 23          | 9               | 34.62          |
|             | Mix               | 0           | 0               | 0.00           |
| Feed        | Rice              | 0           | 0               | 0.00           |
|             | Commercial feed   | 21          | 9               | 34.62          |
|             | Mix               | 5           | 1               | 3.85           |
| Management  | In-house          | 10          | 6               | 23.08          |
|             | Cage-free         | 16          | 4               | 15.38          |
|             | Total             | 26          | 10              | 38.46          |

In the first experiment, the prevalence of incompatible crossmatching of the group of 1 - 5 years old, which was the highest in three age groups, followed by the group of above 5 years old. In the below 1 year old group, there were no cases of incompatible crossmatching between Greyhounds and dogs taken to the veterinary hospital. The blood of the Greyhounds was tested compatibility with 11 dogs below 5 kg, 8 dogs from 5 - 10 kg, and 7 dogs above 10 kg. The compatible cases were 2, 5 and 3 in dogs of below 5 kg, 5 - 10 kg, and above 10 kg, respectively. In gender groups, the prevalence of male dogs' blood compatible with Greyhounds' blood was 15.38% (4 cases), which was less than the prevalence in the female group (23.08%, 6 cases). In breed groups, the prevalence of positive results in foreign breeds accounted for 34.62% of

total compatible cases, which was much higher than in the domestic group (with 3.85%). There were 9 incompatible cases in a total of 21 dogs fed with commercial feed, while there was only 1 incompatible case in the mixed feed group. In the management group, the cases of in-house dogs whose blood was incompatible with Greyhounds' blood accounted for 23.08%, while cage-free dogs accounted for 15.38%.

Retired Greyhounds are universal blood donors whose blood can be given to most dogs. The main reasons for Greyhounds being ideal blood donors are they have an appropriate size (> 25 kg), a gentle temperament, a high hematocrit, large and easy-to-access jugular veins (Couto, 2005). Previous reports indicated that Greyhounds might have lower levels of some coagulation factors (Lara-García et al., 2008;

Vilar et al., 2008) CBC, platelet count, OSPT, APTT, platelet function with PFA-100. When compared with other breeds, Greyhound's blood had significantly lower concentrations of von Willebrand factor (vWF), coagulation factor X, and fibrinogen. However, they are still suitable for use as plasma donors (Walton et al., 2014). It has been reported that Greyhounds have a larger blood volume (about 110 to 120 mL/kg B.W) than other dog breeds with a similar size (80 to 100 mL/kg B.W) (Lisciandro et al., 2009). The result indicated that the compatible results were not associated with age, body weight, gender, breed, feed and management between dogs taken to the veterinary hospital and the Greyhounds ( $P > 0.05$ ).

### 3.2. Prevalence of incompatibility crossmatching between dogs in the same house

In many cases requiring the blood transfusion to be performed as soon as possible, a Greyhound as a blood donor or stored blood was usually unavailable. Even though having different hematologic and hemostatic profiles compared to Greyhounds, non-Greyhounds' blood can be used to aid in anemia situations (Chang et al., 2021), and these non-Greyhounds can be other dogs from the same house. Donor dogs should be from 1 to 8 years of age, greater than 23 kg, and provide 450 mL per donation, screening for blood-borne pathogens, up-to-date with vaccinations and dewormed (Wardrop et al., 2016; Kuo & McMichael, 2020). Donation volumes of 15 to 20 mL/kg from dogs (Lanevski & Wardrop, 2001). In the second experiment, the blood of dogs in the same house or neighborhood was compatible with each other. The data were showed in Table 3.

**Table 3.** The prevalence of incompatibility crossmatching between dogs in the same house or neighbor

| Factor      |                   | Total cases | Incompatibility |                |
|-------------|-------------------|-------------|-----------------|----------------|
|             |                   |             | Cases           | Prevalence (%) |
| Age         | Below 1 year old  | 65          | 16              | 11.94          |
|             | 1-5 years old     | 59          | 18              | 13.43          |
|             | Above 5 years old | 10          | 4               | 2.99           |
| Body weight | Below 5 kg        | 58          | 17              | 12.69          |
|             | 5-10 kg           | 54          | 16              | 11.94          |
|             | Above 10 kg       | 22          | 5               | 3.73           |
| Gender      | Male              | 75          | 17              | 12.69          |
|             | Female            | 59          | 21              | 15.67          |
| Breed       | Domestic          | 67          | 16              | 11.94          |
|             | Foreign           | 62          | 21              | 15.67          |
|             | Mix               | 5           | 1               | 0.75           |
| Feed        | Rice              | 89          | 25              | 18.66          |
|             | Commercial feed   | 25          | 7               | 5.22           |
|             | Mix               | 20          | 0               | 0.00           |
| Management  | In-house          | 31          | 3               | 2.24           |
|             | Cage-free         | 103         | 35              | 26.12          |
| Total       |                   | 134         | 38              | 28.36          |

In the age groups, the highest proportion of incompatibility was in the group of 1 - 5 years old (13.43%), followed by the below 1-year-old group (11.94%) and above 5 years old (2.99%). The same goes with body weight groups: dogs in the below 5 kg group had a higher percentage of incompatibility (12.69%) than dogs in 5 - 10 kg group (11.94%) and in above 10 kg group (3.73%). The incompatible results of female dogs accounted for 15.67%, which was higher than the male dogs (12.69%). The foreign breed had the highest proportion of positive results (15.67%). The domestic breed's incompatible proportion was 11.94%. There was only 1 incompatible result in the mixed-breed group. Dogs fed with rice had the most cases of incompatible results, with 25 cases (accounting for 18.66%). The commercial feed accounted for 5.22%, with 7 cases. There was no incompatible case in the mixed feed group. In management groups, dogs with cage-free living had more cases than those in-house (35 and 3 dogs, respectively). Even though Greyhounds are great blood donors, they are not always available in many cases. In contrast, dogs from the same house or neighborhood are usually available. Age, body weight, gender and breed did not affect the compatible results ( $P > 0.05$ ).

#### 4. Conclusions

In the studies, age, body weight, gender, and breed were not associated with the prevalence of incompatible crossmatching results between dogs taken to the veterinary hospital and the Greyhounds, or dogs in the same house or neighborhood. Greyhounds are the ideal blood donor due to their size, large jugular veins, and temperament. Dogs in the same house or neighborhood are available blood source in some emergencies. The prevalence of incompatibility crossmatching in dogs in this study was 28.36

- 38.46%. Even though the results indicated that age, body weight, gender, and breed did not affect the pre-transfusion crossmatching results, it is still recommended to perform the crossmatching test before a transfusion in order to reduce the risk of post-transfusion hemolytic reactions. However, more tests should be carefully performed before the transfusion, such as complete blood count, biochemistry profile, infectious diseases scanning and blood typing.

#### Conflicts of interest

The authors have no conflicts of interest to declare.

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