Comparative serological investigation between cat and tiger blood for transfusion

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ABSTRACT. Evidence suggests that non-domesticated felids inherited the same AB-erythrocyte antigens as domestic cats. To study the possible compatibility of tiger blood with that of other endangered felidae, blood samples from captive tigers and domestic cats were subjected to an in vitro study. The objectives of this study were to (1) identify whether the captive tigers had blood type AB and (2) determine the compatibility between the blood of captive tigers and that of domestic cats with a similar blood type. The anti-coagulated blood with ethylenediaminetetraacetic acid of 30 tigers was examined to determine blood type, and a crossmatching test was performed between tiger and cat blood. All 30 tigers had blood type A. Tube agglutination tests using tiger plasma with cat erythrocytes resulted in 100% agglutination (n=30) with type B cat erythrocytes and 76.7% agglutination (n=23) with type A cat erythrocytes. The 80% of major and 60% of minor compatibilities between blood from 10 tigers and 10 domestic cats with blood type A were found to pass compatibility tests. Interestingly, 3/10 of the tigers’ red blood cell samples were fully compatible with all cat plasmas, and 1/10 of the tiger plasma samples were fully compatible with the type A red cells of domestic cats. Although the result of present findings revealed type-A blood group in the surveyed tigers, the reaction of tiger plasma with Type-A red cell from cats suggested a possibility of other blood type in tigers.

KEY WORDS: agglutination, blood group, red blood cell, sera, tiger

Materials and Methods

Blood samples from Bengal tigers

All procedures with the captive Bengal tigers were approved by the Kasetsart University Animal Committee (ID number: ACKU59-VET-020). With clients’ informed consent, 30 captive Bengal tigers in the Sriracha-Tiger Zoo, Chonburi, Thailand, were enrolled in the study during their routine health examination. Of the 30 tigers, 10 were male, and 20 were female, with a median age of 5 years. The tigers were housed in individual enclosures and had access to shade and temperature-controlled environments.

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Determination of blood compatibility by crossmatching between Bengal tiger blood and type A blood from domestic shorthair cats

Blood samples from 10 captive Bengal tigers and 10 domestic shorthair cats with blood type A were collected for compatibility testing using major and minor crossmatching. The major and minor crossmatchings were adaptively performed as described elsewhere [17]. The major crossmatching test was performed by centrifuging 100 µl of Bengal tiger blood anti-coagulated with EDTA at 1,500 × g for 15 sec. The red blood cell pellet was collected and washed with PBS solution 3 times. Bengal tiger blood
with a 4% red blood cell concentration was mixed with 50 µl of plasma from cats with blood type A. The minor crossmatching test was performed by adding 50 µl of the tiger plasma to 4% red blood cells from cats with type A blood. The microtubes from both the major and minor crossmatching tests were incubated for 15 min. The agglutination results were observed microscopically at 40× magnification and recorded [17]. Agglutination indicates the presence of incompatibility between blood samples. The absence of agglutination indicates blood compatibility between captive Bengal tigers and domestic cats.

### Table 1. Slide agglutination reaction to reagent A (anti-A plasma), reagent B (anti-B plasma), reagent C (T. vulgaris lectin) and reagent D (PBS solution)

| Type of reagent | Number of agglutination reactions | Degree of agglutination |
|----------------|----------------------------------|-------------------------|
| Reagent A (anti-A serum) | 30 | 0 0 2 18 10 |
| Reagent B (anti-B serum) | 30 | 28 2 0 0 0 |
| Reagent C (T. vulgaris lectin) | 30 | 30 0 0 0 0 |
| Reagent D (PBS solution) | 30 | 30 0 0 0 0 |

### Table 2. Blood compatibility results between captive Bengal tigers and domestic cats with type A blood by individual tiger

| Tiger blood sample | Major crossmatching test | Minor crossmatching test |
|--------------------|--------------------------|--------------------------|
|                    | Negative | Positive | Negative | Positive |
| 1                  | 7        | 3        | 6        | 4        |
| 2                  | 9        | 1        | 7        | 1        |
| 3                  | 10       | 0        | 13       | 1        |
| 4                  | 6        | 4        | 6        | 4        |
| 5                  | 6        | 4        | 5        | 5        |
| 6                  | 10       | 0        | 10       | 0        |
| 7                  | 10       | 0        | 5        | 5        |
| 8                  | 6        | 4        | 4        | 6        |
| 9                  | 9        | 1        | 4        | 6        |
| 10                 | 7        | 3        | 3        | 7        |
| Total              | 80       | 20       | 60       | 40       |

### Table 3. Number of microscopic agglutination reactions from 100 crossmatching tests between captive Bengal tiger blood and blood from cats with type A blood

| Parameter | Minor crossmatching agglutination | Total |
|-----------|----------------------------------|-------|
| Major crossmatching agglutination | No     | 60   | 80   |
|                       | Yes    | 0    | 20   |
| Total                 |        | 60   | 100  |

Fisher’s exact test indicates a significant association between the agglutination results of major and minor crossmatching tests (P<0.001).

### Statistical analysis

Statistical analysis of the data was performed using the statistical software packages STATA12 (Stata Inc., College Station, TX, U.S.A.) and JMP Pro 10 (SAS Institute Inc., Cary, NC, U.S.A.). Blood typing prevalence was calculated as a percentage. The association of agglutination results for major and minor crossmatching tests between captive Bengal tigers and domestic cats was identified using Fisher’s exact test. P-values <0.05 were considered statistically significant.

### RESULTS

A survey of 30 blood samples from captive Bengal tigers was conducted using a slide agglutination test. The results indicated that all captive Bengal tigers in this study (n=30) had blood type A. Mild agglutination results were found in two blood samples from captive Bengal tigers with reagent A containing anti-A plasma (score 1); however, no agglutination was found in the samples with reagent C containing T. vulgaris lectin. Thus, our study indicated that all samples from the Bengal tigers were blood type A (Table 1).

Back typing was performed with red blood cells from type A and B blood from domestic cats and tiger plasma using a tube agglutination test. Using red blood cells from domestic cats with blood type A, 23 samples of captive Bengal tiger plasma (76.6%) induced agglutination reactions. Using red blood cells from domestic cats with blood type B, 30 samples of captive Bengal tiger plasma (100%) induced agglutination reactions.

A total of 100 blood compatibility tests were conducted between the blood from 10 captive Bengal tigers and 10 domestic cats with blood type A. Of the major compatibility tests between tiger red cells and cat serum, 80% had no agglutination. Of the minor compatibility tests between cat red cells and tiger serum, 60% had no agglutination. Interestingly, only 3 out of 10 tiger red blood cell samples (30%) were fully compatible with the plasma from 10 domestic cats. Only 1 out of 10 tiger plasma samples (10%) was fully compatible with type A red cells from 10 domestic cats (Table 2).

Twenty out of 100 crossmatching tests between captive Bengal tiger blood and blood type A from domestic cats with microscopic agglutination in the major crossmatching test also had microscopic agglutination in the minor crossmatching test.
crossmatching tests. With blood type A. The present study also identified a significant association between the agglutination results of major and minor crossmatching tests with sera from all cats with blood type A, and only 1 sample containing tiger plasma was fully compatible with sera from all cats with blood type A. The present study also identified a significant association between the agglutination results of major and minor crossmatching tests.

The RapidVet®-H Feline Blood Typing Agglutination Test Card has been used to evaluate the blood AB type in all cats in this study. The low sensitivity to identify blood type AB of card test has been reported in other studies [12, 14, 15]. This limitation should not affect the blood group interpretation in this study, because all cats in this study showed strong reaction with solution containing anti-A antibody and no reaction with solution containing anti-B solution.

Although, two tiger blood samples presented weak (+1) agglutination reaction to anti-B sera, both of these tiger blood samples had no reaction to anti-B lectin. In feline blood typing, the result of positive type-B antigen using anti-B sera and anti-B lectin induced 2+ to 3+ and 2+ to 4+ agglutination reactions, respectively [16]. Thus, it is unlikely that type-B antigen was identified in the present study. This study’s findings also confirmed previous research that identified only type A blood in tigers [8]. In the back-typing part of the present study, type-A plasma from captive Bengal tiger agglutinated 76.7% (n=23) and 100% (n=30) with type-A and type-B red cells from cats, respectively. This findings suggested that tiger may have other blood type in addition to the type AB group causing natural alloantibodies. It has been reported that naturally occurring alloantibodies can also be found in domestic cats [5].

To test the blood compatibility between captive Bengal tiger and domestic cats, blood crossmatching tests were conducted. In the major compatibility test, the cat plasma agglutinated 20% of the tiger red blood cell samples, whereas in the minor compatibility test, the tiger plasma agglutinated 40% of the cat red blood cell samples (Table 2). Our study also found that all samples that had microscopic agglutination in the major crossmatching test also had microscopic agglutination in the minor crossmatching test (Table 3). Twenty out of 80 samples (25%) that passed the major crossmatching test had microscopic agglutination in the minor crossmatching test (Table 3). The incompatible crossmatching results between cat and tiger blood with similar blood type A may suggest that additional blood type(s) exist [19]. A recent study indicated a novel Mik blood group may exist in addition to the AB blood group system in cats [19]. Identification of the Mik blood group in other felids has not yet been performed, and such analysis is limited in part due to the lack of a commercial test kit for the Mik blood group. Nevertheless, it should be noted that no blood typing device can replace crossmatching to identify red cell incompatibilities [10]. The limitation of this study is that the blood compatibility between tigers did not perform to evaluate the alloantigen and alloantibody among tigers. Further studies should be performed to evaluate the compatibility among tiger blood and post-transfusion reactions in tigers. Other limitation of this study is that only microscopic reaction was evaluated and recorded but macroscopic agglutination. However, this limitation should not limit the usefulness of the results in this study. Microscopic reaction can detect all occurring agglutination reactions.

Several studies had reported xenotransfusion in cats with dog blood [4, 6, 18]. The transfused canine RBC to cat was short-lived and causing intravascular hemolysis [4]. The sharing of the AB blood group system among the felid species [8] suggests a possible application of xenotransfusion. In this study, the crossmatching results indicated majority of tiger red cells are compatible to cat serum. These results suggested a possibility to use saline-washed red blood cells from tiger as a blood substitute for anemic cats in urgent need of a blood transfusion. With a large body weight, a unit of tiger blood can save life upto 10 small cats with anemia. Abundance of a tamed tiger in captivity together with a well training has been seen in various zoos in Thailand. The question remains whether it is possible to train tiger as a blood doner without general anesthesia. Moreover, the clinical usefulness and safety of xenotransfusion for cat patients using captive tiger blood remain unknown.

It is noteworthy that other blood components, including plasma proteins and white blood cells, may cause transfusion reactions that cannot be tested with crossmatching [10]. Since transfusion reactions between tiger plasma and cat red blood cells may occur, restricted red blood cell transfusions from tigers to other felid species may help prevent subsequent transfusion reactions [11]. Saline washing conducted three to four times [3] can help remove various blood components, such as plasma proteins, white blood cells, platelets and red blood cell metabolites. Incompatibility between blood type A from the different felids in this study suggests that the use of washed red blood cells could reduce transfusion reactions by removing undesirable plasma proteins [7]. Nonetheless, saline-washed red cells must be used within 24 hr of washing to mitigate the chance of bacterial infection and the reduction of red cell variability due to the removal of anti-coagulant-preservative solution. Moreover, xenotransfusion increases the potential for disease transmission among species [13]. Protocols to prevent transfusion-transmitted infections should be followed, and the proper selection and exclusion of prospective felid donors is crucial.

In summary, only blood type A was identified in the captive tigers enrolled in the present study. The information from this study may serve as a model in the application of blood from captive Bengal tigers to other small wild felids in need of a blood
transfusion. The results from this study indicated that tiger sera may react with type A red cells from domestic cats which can lead to undesirable transfusion reaction; however, majority of tiger red cells (80%) were not react with cat sera. These results suggested a possibility to use saline-washed red blood cells from tiger as a blood substitute for anemic cats in urgent need of a blood transfusion. Nonetheless, xenotransfusion of incompatible blood can lead to undesired transfusion reactions. More studies are required before attempting xenotransfusion among felid group.

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