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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

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Populations of wild felids are declining all over the world for various reasons, including illegal wildlife trade, hunting, poaching, habitat loss and loss of prey. Many big cats, such as Bengal tigers (*Panthera tigris tigris*), are scarce in Thai forests, but common in captivity. The AB blood group system has been identified as the major blood group system in the domestic cat [14] as well as in various wild felidae [8]. The cat AB antigens are different from human ABO antigens and are defined by specific carbohydrates on erythrocyte membranes [1]. Type A and type B cat erythrocytes have been found to have N-glycolyl- and N-acetyl-neuraminic acids on membrane glycolipids and glycoproteins, respectively [1]. In Thailand, the prevalence of domestic cats with blood type A, B and AB has been reported as 94.04, 5.96 and 0%, respectively, using an alloantibody test [16]. Previous research identified type A blood in 13 Bengal tigers [8]; however, the major blood group of Bengal tigers in captivity in Thailand remains unknown.

Common problems associated with surgical operations in wild felids include blood loss and hypotension [20]. In the veterinary field, blood transfusions, especially for critically ill patients, may be the only means of reviving anemic patients. Small endangered felids in captivity may also require blood transfusions, but the number of blood donors for small wild felids is extremely limited. Because it has been suggested that Bengal tigers carry the AB blood group [8], their blood may be useful as an alternative supply for transfusions across members of felid groups. The objectives of this study were to (1) survey blood groups of captive Bengal tigers and (2) determine the blood compatibility between captive Bengal tigers and domestic cats.

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

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age (range) of 8 (1–15) years old. Samples from ten of the tigers were selected to undergo a crossmatching test. Blood samples from captive Bengal tigers were drawn by physically restrain or application of general anesthesia using atropine sulfate (0.02 mg/kg, intramuscular; Atlantic Laboratories Corp., Ltd., Bangkok, Thailand) and tiletamine/zolazepam (2 mg/kg, intramuscular; Virbac Laboratories, Carros, France) as premedication and anesthesia induction, respectively. Five m*l* blood samples from all of the captive Bengal tigers were collected from the saphenous vein and immediately transferred into ethylenediaminetetraacetic-acid (EDTA) anti-coagulant tubes.

Blood samples from domestic cats

All of the blood samples from the 10 domestic cats enrolled in the study were collected according to the guidelines of the Kasetsart University Animal Committee (ID number: ACKU59-VET-020), and the owners signed an informed consent form. Prior to enrolling healthy cats to the study, a general physical examination and history taking were performed by veterinarian. All enrolled cats had not been receiving any whole blood or blood components and had been evaluated for blood types using commercially available blood typing test kit (RapidVet®-H Feline Blood Typing Agglutination Test Card; DMS Laboratories Inc., Flemington, NJ, U.S.A.). A five m*l* blood sample was collected from each cat and transferred immediately to EDTA anti-coagulant tubes. Of the 10 cats, 5 were male, and 5 were female. All cats were domestic shorthair cats with a median age (range) of 4 (1–6) years old.

Preparation of reagents A, B, C and D

Reagent A, or anti-A plasma, was prepared by collecting blood from cats with blood type B. Ten m*l* of blood from a cat with blood type B was added to 1.5 m*l* of citrate-phosphate-dextrose solution with adenine (CPDA; JMS Singapore Pte Ltd., Singapore). The mixture of blood and CPDA solution was centrifuged at 5,000 ×*g* for 5 min. The plasma was collected and used as reagent A.

Reagent B, or anti-B plasma, was prepared by obtaining blood from domestic cats with blood type A. Ten m*l* of blood sample from a cat with blood type A was mixed with 1.5 m*l* of CPDA solution. After centrifuging the mixture at 5,000 × *g* for 5 min, the plasma was collected and used as reagent B.

Reagent C was prepared using *Triticum vulgaris lectin* (Sigma Chemical Co., St. Louis, MO, U.S.A.). One hundred *μ*g of *T. vulgaris lectin* was prepared in 1 m*l* of phosphate-buffered saline (PBS) solution (Sigma Chemical Co.) [1].

PBS solution was used for Reagent D, which was a control solution. Reagent D was used to identify auto-agglutination of captive Bengal tiger blood.

AB blood type screening using slide agglutination assay

A slide agglutination assay to screen for blood type AB using reagents A, B, C and D was conducted immediately after the reagents were prepared [1, 8, 9, 15, 16]. Briefly, 50 *μl* of each reagent was dropped in four different locations on the glass slide. After adding 50 *μl* of captive Bengal tiger blood to each reagent, the samples were mixed gently using a clean needle tip. The agglutination results were recorded within 2 min of starting the reactions [1, 15]. Any agglutination reactions after 2 min were not included. The agglutination criteria were modified from a previous report [15] as follows: 0=no agglutination >120 sec, 1=minor agglutination occurred in 60–120 sec, 2=agglutination occurred within 30–60 sec, 3=obvious agglutination occurred in 10–30 sec, and 4 =agglutination <10 sec.

Blood was diagnosed as type A (positive type-A antigen), if blood agglutination occurred with reagent A (anti-A plasma). Blood was identified as type B (positive type-B antigen), if blood agglutination developed with reagent B (anti-B plasma) and reagent C (anti-B lectin). Blood was determined to be type AB (positive type-A and B antigen), if blood agglutination appeared with reagents A, B and C. Auto-agglutination was determined to have taken place when blood agglutinated with PBS solution [1].

Back typing between captive Bengal tiger plasma and red blood cells from domestic cats with type A and B blood

Back typing was performed to confirm blood type A and type B in the captive Bengal tigers. A tube agglutination test was performed for plasma back typing between the plasma of the captive Bengal tigers and the red blood cells of domestic cats with blood type A and B. Blood from cats with blood type A and B that had been anti-coagulated with EDTA was centrifuged at 5,000 ×*g* for 5 min. The red blood cell pellets were collected and mixed with PBS to obtain 4% hematocrit. Twenty-five *μl* of captive Bengal tiger plasma was added to three microtubes (600 *μl* microcentrifuge tube; Sigma-Aldrich) containing 25 *μl* of 4% red blood cells from cats with blood type A, 4% red blood cells from cats with blood type B and PBS solution, respectively [2]. After 15 min of incubation at room temperature, the mixtures in the three microtubes were centrifuged at 1,500 ×*g* for 15 sec. Tubes were gently agitated to suspend the non-agglutinating red cells before reading agglutination reaction [12]. Positive agglutination was determined by the presence of pellets of agglutination at the bottom of the microtubes, whereas a lack of agglutinated pellets was identified as negative agglutination.

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

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				
		0	1	2	3	4
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
		No	Yes	
Major crossmatching agglutination	No	60	20	80
	Yes	0	20	20
Total		60	40	100

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

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.

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

(Table 3). Twenty of eighty tests without microscopic agglutination in the major crossmatching test had agglutination in the minor crossmatching test (Table 3). There was a significant association between the agglutination results of major crossmatching and minor crossmatching tests ($P<0.001$).

DISCUSSION

Using the cat AB blood typing system, the present study found that all 30 captive Bengal tigers had blood type A. Neither the blood type B nor the blood type AB was identified in captive Bengal tigers. Back typing procedure using tiger plasma with type A and type B cat erythrocytes revealed 76.7% (n=23) and 100% (n=30) agglutination, respectively. Furthermore, the major and minor blood compatibilities between 10 captive Bengal tigers with blood type A and 10 domestic cats with blood type A were 80% and 60%, respectively. Interestingly, only 3 samples (30%) containing red blood cells from the tigers were fully compatible 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 compatilible 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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