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No naturally occurring blood group antibodies were detected in the crossmatching of 25 giant pandas

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The giant panda (*Ailuropoda melanoleuca*) is a vulnerable species of ursid, endemic to China. With over eight million years of evolution, it is considered a living fossil. Blood transfusion is an important means to treat giant pandas that are critically ill or severely injured, however the blood grouping of giant pandas, as the first element of blood transfusion, has not yet been clarified. Based on immunohematology, the saline-tube test, polybrene test and antigen test were carried out to detect IgM and IgG antibodies in the blood of giant pandas in this study. In parallel, human ABO typing reagent and lectin were used to identify whether the RBCs of giant pandas had a similar antigen as the human ABO blood group system. Here we found that there was no clinically significant agglutination in the crossmatching, indicating that giant pandas most likely lack naturally occurring blood group antibodies, unlike what is observed by incompatibility in humans. And due to the presence of interspecies antibodies, human blood typing reagents could not be used for the identification of giant panda blood types. This finding is of great significance for guiding the clinical blood transfusion of giant pandas. Although this study represents the largest cohort of giant panda blood typing to date, the sample size is still insufficient for species-level blood typing. New findings may emerge, especially as the genetic diversity of the sample increases (such as the introduction of wild populations). A major obstacle currently restricting the study of giant panda blood groupings is the preservation technology of giant panda blood. This requires extensive involvement of engineers and biologists in addition to hematologists.

Keywords Blood group, Giant panda, Immunohematology

The giant panda (*Ailuropoda melanoleuca*), with over eight million years of evolutionary history, is an obligate bamboo foraging species of ursid endemic to China and a beloved national treasure. As of November 2024, there were 757 captive giant pandas and nearly 1,900 wild giant pandas worldwide[1], making them still a vulnerable species[2]. Recent cases of disease within the captive population, including outbreaks of Canine Distemper Virus, have required blood transfusions as part of the treatment, however, this method is not yet well understood in the giant panda[3].

In 1989, Diao et al. reported for the first time a case of a giant panda that received a blood transfusion due to acute hemorrhagic necrotizing enteritis[4]. By 2025, a total of ten giant pandas were reported to have received blood transfusions in China[4–9]. Among them, nine were relieved of anemia after blood transfusion, and their primary disease symptoms improved, however, one of them died the next day due to non-availability of blood. Although some cases may not have been reported and this is likely an underestimated figure, overall, there have been few cases of giant panda blood transfusions. However, from these few reports, we found that blood transfusion was usually the last option for treating giant pandas. And almost every blood transfusion in giant pandas is faced with the same dilemma, there are few donors and the giant panda blood collection is difficult, often requiring veterinarians to anaesthetize and collect blood[4,6–9]. In addition, blood component preparation,

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blood preservation technology and giant panda blood banks have not been developed[10]. Finally, because of the rarity of giant pandas no blood group detection reagents have been developed[4–9].

The greatest significance of blood typing is to prevent hemolysis during blood transfusion. As the most serious complication of blood transfusion, hemolytic reaction can cause the recipient to suffer from physical discomfort, shock, acute renal failure and even death[11]. Undoubtedly, determining giant panda blood typing is the starting point for improving the application of blood transfusions in this species. Based on immunohematology, this is the largest cohort study to date to investigate blood groupings of giant pandas, in order to provide a basis for the treatment of giant pandas by blood transfusions.

Materials and methods

Collection of giant panda blood

All giant pandas used for this study were healthy captive adults, housed at the Chengdu Research Base of Giant Panda Breeding (CRBGPB), located in Chengdu, Sichuan Province, People's Republic of China. The breeding season of giant pandas is typically from March to May. During this period, general anesthesia is used at the CRBGPB to anesthetize giant pandas both for semen collection as well as artificial insemination, which is an ideal time to collect blood. During this period, the whole blood of the 25 giant pandas (#1–#25) was collected from the medial cephalic vein by experienced veterinarians (Table 1). These 25 giant pandas included eleven males and fourteen females, aged 3–20 years. The collection time was either in the morning or in the afternoon, each collection lasted about one minute, and the volume of blood collected was approximately 5 mL with EDTA-K2 anticoagulation. The anesthetic used was propofol (intravenous, 2.0–2.5 mg/kg) and isoflurane (inhalational, 1%–1.5%). In some cases, blood was collected without anesthesia from individuals that were trained by CRBGPB husbandry staff to participate in voluntary blood draws. Following blood collection, each sample was separated into red blood cell (RBC), plasma, and buffy coat by centrifugation (173 g, 10 min, 10 °C).

Crossmatching

The serologic tests included three tests, the saline-tube test, polybrene test and antigen test, which were all carried out at the same time. Among them, the saline-tube test and the polybrene test were used to detect IgM and IgG antibodies in the blood of giant pandas. Human ABO typing reagent (anti-serum & monoclonal antibody) and lectin (anti-H, anti-A1, anti-M, anti-N, and anti-P1) were used to identify whether the RBCs of giant pandas had a similar antigen as the human ABO blood group system. The detailed experimental methods are provided in Supplementary Protocols S1–S4.

Results

No clinically significant agglutination found in crossmatching

To explore the presence of naturally occurring antibodies, the most direct evidence is the crossmatching of RBC and plasma between allogeneic individuals. For this reason, we carried out a saline-tube test for IgM antibody screening and a polybrene test for IgG antibody screening. The results showed that some crossmatches in the test had transient agglutination, after gentle resuspension, all cell buttons dispersed, and no positive results were found (Fig. 1). Among them, transient agglutination appeared in 50 of the 235 saline-tube tests, with an incidence of 21.28% (Supplementary Table S1), and in 7 of the 235 polybrene tests, with an incidence of 2.989% (Supplementary Table S2). From the perspective of immunohematology, these findings suggest that giant pandas do not have clinically significant naturally occurring alloantibodies. This phenomenon also exists in the families Canidae, Mustelidae, Equidae, and Bovidae[12].

Due to the presence of interspecies antibodies, human blood typing reagents could not be used for the identification of giant panda blood types

In addition to crossmatching, we used human serologic typing reagents to test the RBC antigens of giant pandas. The results showed that the RBCs of giant pandas reacted strongly with human ABO antiserum (Fig. 2 A). Combined with the reaction results of giant panda plasma and human ABO typing cells in another study (all agglutination)[13], it can be seen that there are strict interspecific antibodies between giant pandas and humans, neither human RBC nor plasma can be transfused to giant pandas. At the same time, the monoclonal antibody test showed that giant panda RBCs did not agglutinate with anti-A, B, and H (Fig. 2B). This indicated that the human blood typing monoclonal antibody could not detect giant panda RBC antigen, and could not be used for the determination of giant panda blood type. Lectin reagents, are theoretically used for animal blood group identification without species specificity, and can be used as a good tool for identifying blood group antigens. However, we did not get any positive results from giant pandas (Fig. 2 C).

Discussion

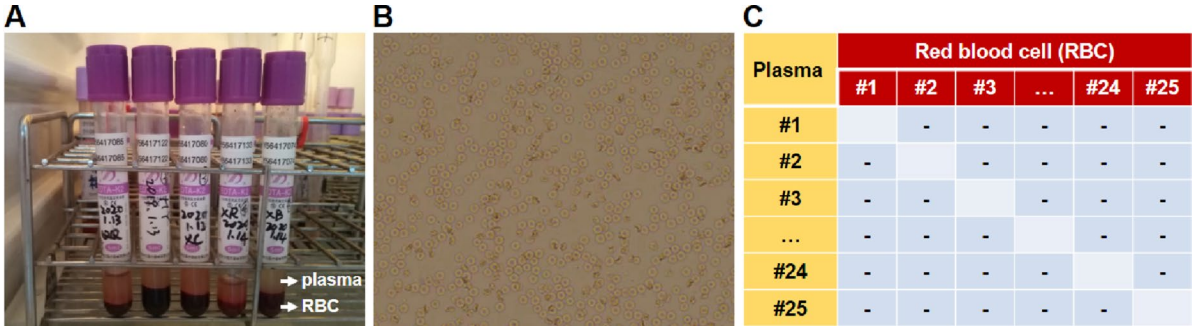
As a species vulnerable to extinction, the giant panda is the most famous flagship species for the conservation of wildlife and a beloved national treasure of China. Although the species is recovering in the wild, giant pandas are still at risk from both anthropogenic factors and disease outbreaks, especially in the captive population. Therefore, improving the veterinary care, including blood transfusion, is a necessary step to ensure the safety of the species. Given the lack of detectable naturally occurring blood group antibodies suggests that blood transfusions between allogeneic pandas may not have the severe adverse reactions caused by blood group incompatibility as seen in humans. However, crossmatching should not omitted. First, although this study is the largest cohort of giant panda blood typing experiments to date, the sample size is still insufficient for blood typing research on a species, and these samples mainly come from captive populations. As wild populations

no.		gender	age	pregnancy history	whole blood collection	
					volume (mL)	general anesthesia
#1		F	7	no	1.5	no
#2‡		F	4	no	2.0	no
#3‡		F	4	no	2.0	no
#4		F	12	yes	1.0	yes
#5		F	13	no	2.0	yes
#6		M	12	-	3.5	yes
#7		F	14	-	5	yes
#8		F	13	yes	5	yes
#9		F	14	no	3	yes
#10		M	20	-	5	yes
#11		F	16	yes	10	yes
#12		M	10	-	7	yes
#13		M	8	-	5	yes
#14		F	18	yes	5	yes
#15		M	3	-	7.4	no
#16		M	15	-	3.5	yes
#17		M	8	-	6	no
#18		F	4	no	5.5	no
#19		M	4	-	5.5	no
#20		M	13	-	5	yes
#21		F	5	no	5	yes
#22		M	12	-	5	yes
#23		F	10	no	4.5	yes
#24		M	5	-	5	no
#25§		F	-	yes	5	yes

Table 1. Baseline data of 25 giant pandas in this study.
F: female; M: male; -: not available; #2‡, #3‡: these two giant pandas had a history of transfusion; #25§: #25 was rescued from the wild, so her age could not be determined.

are introduced and sampling areas expand, new findings in blood types may emerge due to increased genetic diversity. Second, just as was found in humans and dogs, in which the RBC antigens exposed to the immune system after pregnancy or blood transfusion will induce the production of alloantibodies, and re-transfusion may trigger sever transfusion reactions[12,14,15]. Therefore, before we thoroughly figure out the blood group of giant pandas, we still recommend crossmatching before the allogeneic blood transfusion of giant pandas to avoid unknown adverse risks. In addition, Deng et al. also suggested that giving priority to donors who are healthy, adult, without blood transfusion history, and close relatives can increase the probability of successful matching[9].

At the same time, we should recognize that before breakthroughs are made in serological techniques and commercial reagents for giant panda blood typing, developing molecular typing is a beneficial supplement. Molecular blood typing relies on the identification of blood group antigen-coding genes and their polymorphisms. The release of the high-resolution genome of the giant panda provides a good opportunity to achieve this goal[16,17]. Learning from other species where blood group evidence has been found, and identifying blood type-



Plasma	Red blood cell (RBC)					
	#1	#2	#3	…	#24	#25
#1		-	-	-	-	-
#2	-		-	-	-	-
#3	-	-		-	-	-
…	-	-	-		-	-
#24	-	-	-	-		-
#25	-	-	-	-	-	

Fig. 1. Screening for IgM/IgG antibody in giant panda blood. (**A**) The whole blood of giant pandas. (**B**) Microscopy showed normal red blood cells morphology. (**C**) Serologic testing yielded no positive results. Crossmatching among #15–#19 included an additional 4 °C incubation to rule out cold-reactive antibodies; results remained negative. -: negative (compatible) crossmatch: a smooth suspension of red cells after re-suspension.

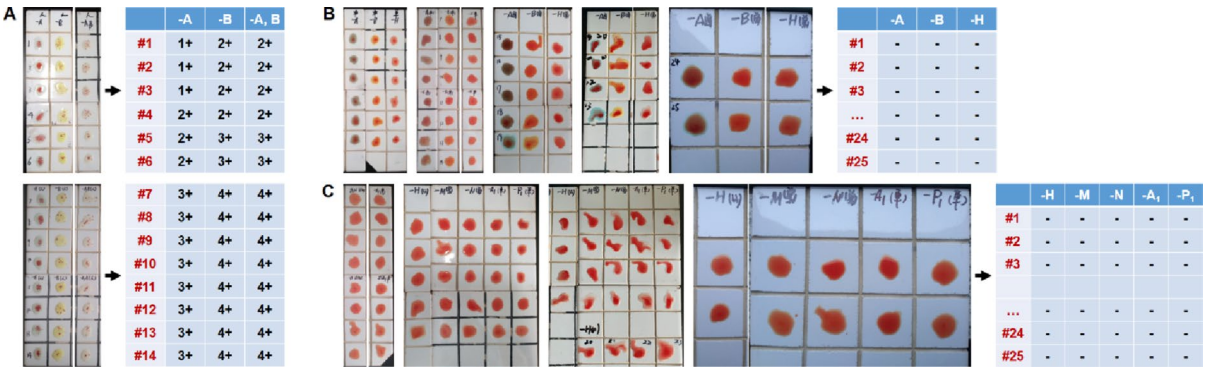


Fig. 2. Human serologic typing reagents applied to giant panda red blood cells. These findings indicate that, due to the presence of interspecies antibodies, human blood typing reagents (**A**) antiserum, (**B**) monoclonal antibody and (**C**) lectin could not be used for the identification of giant panda blood types.

related genes within the vast giant panda genome, will undoubtedly require extensive involvement of engineers and biologists in addition to hematologists.

The further study of giant panda blood typing requires one key step. We need to determine how to preserve giant panda blood. Collecting large amounts of giant panda blood is time consuming and difficult to arrange. Only when the anticoagulation and storage technology are mature can the blood group antigen maintain its immunogenicity, and then provide conditions for the study of blood groupings and production of specific reagents.

Finally, we should also keep in mind that a compatible crossmatch does not guarantee normal RBC survival, nor does it completely eliminate the risk of transfusion, and it is the responsibility of the veterinary team to remain up-to-date in transfusion practices to prevent transfusion-related complications.

Ethics approval and consent to participate

All methods and animal care procedures in this study were carried out in accordance with the ARRIVE guidelines (https://arriveguidelines.org), involved thorough review and received approval from the Institutional Animal Care and Use Committee of Chengdu Research Base of Giant Panda Breeding, with the assigned approval number 2019010. All methods were performed in accordance with the relevant guidelines and regulations of the People's Republic of China.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

Conceptualization: YY, XS, LT, NS, JW and HW; Investigation: YY, NS, XY, CY, SL, RH, LL, MY, YL, DZ, ZH, RZ, YX, XX and SX; Methodology: YY, LT, N.S and XS; Writing-Original Draft Preparation: YY; Writing-Review and Editing: YY, JEA and XS.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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