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Investigation of erythrocyte antigen frequencies in draft horse populations in Japan to assess blood donor suitability

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Erythrocyte alloantigen frequencies of draft horses in Japan were investigated to assess blood donor suitability for transfusion. Here, 148 Japanese draft, 69 Percheron, and 65 Breton horses were blood-typed and subjected to an indirect antiglobulin test. Regarding the major immunogenic factors, the rates of Aa- and Qa-negative horses ranged from 0.35 to 0.49 and from 0.82 to 1.00, respectively. The rate of alloantibody-positive horses ranged from 0.12 to 0.35. Although the prevalence of alloantibodies in these horses was higher than that expected naturally, the rates of Aa- and Qa-negative horses were higher than those of some breeds reported previously. The current draft horse population could provide potential candidates for donors, and the obtained information may contribute to the selection of a safe donor for transfusion.

Key words: *blood type, draft horse, transfusion*

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Blood transfusion is a clinical treatment performed in cases of hemorrhage and anemia. Generally, it is important to avoid blood type incompatibility, which would induce a significant reaction in the recipient; thus, blood type is checked by cross-match testing and/or a computer cross-match of blood types between the donor and recipient. However, as such cross-match testing is time-consuming and not realistic in an emergency, a pre-prepared herd of blood donor horses must be available.

In reality, however, it would be difficult to find blood donors that match all potential recipients because a horse's blood type is determined by combinations of multiple factors of seven blood group systems, A, C, D, K, P, Q, and U. Therefore, it is reasonable to choose a potential donor horse who potentially has a compatible blood type to avoid the risk of incompatibility in blood transfusion; such a horse is called a "universal donor horse". In Japan, the demand for securing blood donors tends to increase in many clinical scenarios.

Among the equine erythrocyte antigenic factors, Aa and Qa are the most immunogenic. Equine neonatal isoerythrolysis (NI) is caused by an incompatibility of blood group alloantigens between a mare and foal, and the antigenic factors Aa and Qa are most often responsible [3, 9, 14]. The transfusion of blood from a donor positive with these antigens will result in the development of a high alloantibody titer in the recipient that can cause severe hemolysis upon subsequent exposure [10]. Additionally, although horses infrequently develop naturally occurring alloantibodies, the majority are antibodies against Ca and Aa factors [7, 11]. Anti-Aa is capable of causing acute hemolytic reactions after incompatible transfusion [7]. Aa and Qa negativity is essential for a potential donor, and the selection of a horse whose blood type has no such risk factor is important for blood donation. Furthermore, in transfusion, whole blood should be from an Aa-negative/Qa-negative blood donor that is free of antibodies against erythrocyte antigens to ensure that further incompatibilities do not exist and additional hemolysis does not ensue [12].

Frequencies of particular alloantigens differ depending on the horse breed. Aa and Qa are highly prevalent among light breed horses [10]. The frequencies of Aa (0.98) and Qa (0.85) in the Thoroughbred and those of Aa (0.97) and Qa (0.37) in the Arabian have been estimated according to

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the reported allele/phenogroup frequencies [1]. However, the Standardbred, Morgan, Quarter horse, Paso Fino, and Peruvian Paso show lower estimated frequencies of Aa (0.74–0.82) and Qa (0.01–0.32) than light breed horses [1]. In Japan, it is well known in the veterinarian community that Haflinger horses have low frequencies of Aa and Qa. However, as the number of horses of these breeds, except for light breed horses, is relatively small in Japan, potential donor candidates are currently limited.

After light breed horses, draft horses are the next most common horses in Japan; in 2017, 41,959 light breed horses and 5,115 draft horses were raised in Japan, according to a report published by the Japan Equine Affairs Association (https://www.bajikyo.or.jp/). Nevertheless, the distribution of blood types in the current draft horse populations has not been investigated. In this study, we aimed to investigate the frequencies of erythrocyte antigens in draft horse breeds in Japan to assess their suitability as potential blood donors.

Whole blood samples were obtained from 148 Japanese draft horses (JDs) that were mainly used for Ban'ei horse racing and were originally crossbred from the Percheron (PR), Breton (BR), and Belgian breeds. With the approval of the Ban'ei Horse Racing Promotion Section in the Agricultural Affairs Department of Obihiro City Hall, we used the remaining blood samples from post-race doping tests performed at the Drug Analysis Department in the Laboratory of Racing Chemistry on blood samples collected after horseraces. Additionally, whole blood samples were obtained from 69 PRs and 65 BRs, both groups of which were purebred horses that belonged to the National Livestock Breeding Center Tokachi Station. Blood collection was carried out according to the sampling protocol approved by the Committee for Animal Research and Welfare of the Laboratory of Racing Chemistry (20-04).

Blood typing for seven blood groups, A, C, D, K, P, Q, and U, was performed using agglutinating anti-serum reagents against Ca, Da, Db, Dc, Dd, De, Df, Dg, Dh, Di, Dk, Dl, Dn, and Ka and hemolytic anti-serum reagents against Aa, Ab, Ac, Pa, Pb, Pd, Qa, Qb, Qc, and Ua factors with complement. Additionally, an indirect antiglobulin test (Coombs test) to detect alloantibodies against erythrocyte antigens in the plasma was carried out using a polyclonal anti-horse γ-goblin antibody, as described by a previous method [13]. A panel of blood cells was obtained from three horses that were typed, which involved Aa, Ca, Db, Dc, Dd, De, Dg, Dh, Df, Dk, Dl, Dn, Pa, Pb, Qa, Qb, Qc, and Ua antigens. These examinations were performed using a standard protocol that is used for blood typing by the Genetic Analysis Department in the Laboratory of Racing Chemistry.

Table 1 shows the frequencies of erythrocyte antigens in the studied populations. This study did not focus on the allele/genogroup frequency of each blood group. According to the frequency data, in the JD, PR, and BR, the rates of Aa-negative horses were 0.49, 0.39, and 0.35, respectively. These values were higher than those of the light breed horses and other reported breeds (0.02–0.26) [1]. The rates of Qa-negative horses in the JD, PR, and BR populations were 1.00, 1.00, and 0.82, respectively, which are similar to those of Standardbred, Morgan, Paso Fino, and Peruvian

Table 1. Frequencies of 24 erythrocyte alloantigens in three draft horse populations in Japan

Population	n	Frequencies of erythrocyte antigens (blood group factors)													
		Agglutinating													
		Ca	Da	Db	Dc	Dd	De	Df	Dg	Dh	Di	Dk	Dl	Dn	Ka
JD	148	0.62	0.30	0.04	0.47	0.90	0.43	0.01	0.75	0.44	0.01	0.04	0.69	0.01	0.00
		(0.38)	(0.70)	(0.96)	(0.53)	(0.10)	(0.57)	(0.99)	(0.25)	(0.56)	(0.99)	(0.96)	(0.31)	(0.99)	(1.00)
PR	69	0.51	0.36	0.00	0.46	0.93	0.41	0.00	0.78	0.41	0.00	0.19	0.80	0.00	0.00
		(0.49)	(0.64)	(1.00)	(0.54)	(0.07)	(0.59)	(1.00)	(0.22)	(0.59)	(1.00)	(0.81)	(0.20)	(1.00)	(1.00)
BR	65	0.55	0.48	0.00	0.38	0.97	0.15	0.00	0.72	0.42	0.00	0.38	0.85	0.03	0.00
		(0.45)	(0.52)	(1.00)	(0.62)	(0.03)	(0.85)	(1.00)	(0.28)	(0.58)	(1.00)	(0.62)	(0.15)	(0.97)	(1.00)

Population	n	Frequencies of erythrocyte antigens (blood group factors)									
		Hemolytic									
		Aa	Ab	Ac	Pa	Pb	Pd	Qa	Qb	Qc	Ua
JD	148	0.51	0.49	0.39	0.73	0.24	0.00	0.00	0.09	0.44	0.54
		(0.49)	(0.51)	(0.61)	(0.27)	(0.76)	(1.00)	(1.00)	(0.91)	(0.56)	(0.46)
PR	69	0.61	0.28	0.04	0.71	0.57	0.00	0.00	0.03	0.32	0.49
		(0.39)	(0.72)	(0.96)	(0.29)	(0.43)	(1.00)	(1.00)	(0.97)	(0.68)	(0.51)
BR	65	0.65	0.48	0.03	1.00	0.00	0.00	0.18	0.18	0.18	0.58
		(0.35)	(0.52)	(0.97)	(0.00)	(1.00)	(1.00)	(0.82)	(0.82)	(0.82)	(0.42)

The values in parentheses indicate the negative frequency for each antigen. JD, Japanese draft horse; PR, Percheron; BR, Breton.

Paso horses (0.85–0.99) [1]. The rates of both Aa- and Qa-negative horses in the JD, PR, and BR populations were 0.49, 0.39, and 0.35, respectively.

Using the results of the indirect antiglobulin test, the proportion of individuals that possessed antibodies against erythrocyte antigens within each population could be estimated. Those of the JD, PR, and BR populations were 0.12, 0.35, and 0.25, respectively. Approximately 10% of horses have naturally occurring alloantibodies [11]; thus, each rate within the PR and BR populations is higher. Although the JD population consisted of active racehorses, both the PR and BR populations included broodmares that had experienced pregnancy. The prevalence of acquired antibodies observed in this study may have been influenced by such a difference among the studied populations; however, further investigation will be needed. The rates of both Aa- and Qa-negative horses with no detection of antibodies against erythrocyte antigens in the JD, PR, and BR populations were 0.37, 0.19, and 0.25, respectively.

The present results included 22 blood antigens, except for Aa and Qa. Both Qc and Ua antigens could be considered a primary cause of NI, in addition to Aa and Qa, based on previous data [3, 9]. Furthermore, Ab, Ac, Db, Pa, Pb, and Qb antigens are involved in NI cases [6, 12, 14]. Additionally, of the approximately 10% of horses that have natural alloantibodies, Ca antibodies are present most often, and although the Ca antigen has a minimal clinical effect [11], Ca incompatibility has been studied [2, 4, 5, 8, 11]. These antigens have the potential to exert a significant reaction caused by incompatibility in transfusion, and exposure to them might result in a high alloantibody titer, which would cause severe hemolysis; therefore, it is important to check if they are present and determine their frequencies. Overall, the present blood typing data for the draft horse population will contribute to the selection of safer donors for transfusion.

In conclusion, the current population of draft horses in Japan shows comparatively low frequencies of immunogenic antigens, Aa and Qa, and therefore contains potential candidate blood donors.

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