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Monitoring the positive conversion of anti-erythrocyte antibodies in blood transfusion donor horses

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To confirm the positive conversion of antibodies against erythrocyte antigens in horses, possible blood transfusion donor horses selected from draft horse populations were periodically monitored with an indirect antiglobulin (Coombs) test for approximately 3 years. In this study, 19 horses (16 females and 3 males) were investigated, and five mares showed alloantibodies during the monitoring period. Four mares were typically pregnant when positive conversion was detected, whereas no particular cause of conversion could be observed for one mare based on its clinical records. In the analyzed horses, most positive conversions were possibly due to pregnancy, as conversion occurred more often during this period than after parturition. Pregnancy is considered a key event for positive conversion. Additionally, in cases in which unknown causative sensitization is confirmed, continuous monitoring with a test to detect antibodies should be performed, even if the possible donor is selected and maintained.

Key words: *alloantibody, blood transfusion, donor, horse*

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Blood transfusion can be used as a therapeutic treatment for hemorrhage and anemia, such as neonatal isoerythrolysis in horses. During transfusion, any blood type incompatibility that might induce a significant reaction in the recipient should be avoided as much as possible. However, it is difficult to identify blood donors that match all potential recipients completely. Crossmatch testing prior to transfusion is often available to ensure safe transfusion [8, 13, 14]; however, the entire crossmatch procedure requires a few hours. A more appropriate pre-transfusion test may be the crude method of transfusing a small volume of donor blood into the recipient and monitoring the recipient's vital signs before proceeding further [4]. However, both of these procedures are unrealistic during emergencies. In every scenario, a pre-prepared blood donor horse without the risk of incompatibility would be more useful. An appropriate donor without particular risks is often chosen based on the blood type and the absence of alloantibodies against erythrocyte antigens. Generally, horses lacking Aa and Qa erythrocyte antigens can be regarded as donor candidates, and ideally, no anti-erythrocyte antibodies should be found in horses [6, 8, 15].

Approximately 10% of horses have naturally occurring anti-erythrocyte antibodies [7, 14], but they usually have low titers [11]. For instance, anti-Ca, which comprises the majority of naturally occurring antibodies, has been studied in detail [2, 5, 9, 10], although Ca incompatibility has a minimal clinical effect [1, 14]. However, previously sensitized individuals are more likely to carry pathogenic anti-erythrocyte antibodies; thus, horses with a history of pregnancy, parturition, or transfusion should be avoided as blood donors [4]. Similar to these cases, antibodies acquired due to sensitization may depress selected donor horses. Confirmation of various cases of positive conversion due to the acquisition of and/or an increase in anti-erythrocyte antibodies is important to ensure the safety of donors. In this study, possible blood transfusion donor horses with no anti-erythrocyte antibodies were monitored for the detection of antibodies at different time points, and the causes of positive conversion according to their clinical records were investigated.

In a previous study, we identified donor horses lacking

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Aa and Qa erythrocyte antigens and anti-erythrocyte antibodies from draft horse populations [6]. Nineteen horses (16 females and 3 males) from the Breton and Percheron populations at the National Livestock Breeding Center Tokachi Station were investigated in this study. Whole blood samples were obtained from these horses five times over a period of three years (Table 1). Blood was collected according to a sampling protocol approved by the Committee for Animal Research and Welfare of the Laboratory of Racing Chemistry (20-04). Clinical records pertaining to internal medicine, surgery, pregnancy, parturition, and transfusion were provided by the Department of Animal Health and Medicine of National Livestock Breeding Center Tokachi Station.

To detect antibodies against erythrocyte antigens in plasma, an indirect antiglobulin test (Coombs test) was performed using a polyclonal anti-horse γ-globulin antibody as previously described [6, 16]. A panel of blood cells was obtained from three horses that were typed, involving Aa, Ca, Db, Dc, Dd, De, Dg, Dh, Df, Dk, Dl, Dn, Pa, Pb, Qa, Qb, Qc, and Ua antigens. In this analysis, a titer of 1:4 or higher was regarded as positive for an incompatible reaction.

Table 1 shows the results for the antibodies detected during the monitoring period. Positive titers (1:4–1:8) were observed in five females but not in the other 11 females or three males. For the males, further investigations were omitted because no noteworthy clinical records were found. Clinical records of pregnancy, parturition, abortion, endometritis, and laceration (small head lacerations) before the detection of antibodies were found for the five females. In four of them, although positive conversion of anti-erythrocyte antibodies was found after endometritis, laceration, or parturition, the antibodies were typically detected during the gestation period. It is reasonable to assume that pregnancy was the main cause of the acquisition or increase in antibodies.

This study confirmed that positive conversion occurred more often during the gestation period than after parturition. In contrast, 11 other mares with no detectable antibodies also experienced pregnancy during the monitoring period. There was a total of 38 records in the reproductive histories of the 16 mares in this analysis, and seven positive conversions (18.4%) were observed (Table 1). Prevalence may depend on sensitization due to the combination of dam and foal blood types. However, it is considered that pregnancy is one of the key events for positive conversion, as described previously [4], although the small number of analyzed samples and few data for non-pregnant mares were limitations in this study. In addition, nothing was reported in the clinical records for one mare (horse ID, P) before the detection of the anti-erythrocyte antibodies (Table 1). This indicates that an unknown causative sensitization occurs due to factors other than pregnancy, parturition, and transfusion, which have been described previously [4], or

Table 1. Results of monitoring clinical records and positive titers of anti-erythrocyte antibodies in 19 donor horses

Horse ID	Breed	Sex	Previous pregnancy experience (Y/N)	Sampling date (month and year), clinical records, and positive titers of anti-erythrocyte antibodies									
				Aug 2019		Jun 2020		Dec 2020		Jun 2021		Dec 2021	
				Record	Titer	Record	Titer	Record	Titer	Record	Titer	Record	Titer
A	Breton	F	Y	-		PRG		PRG		PAR, PRG		PRG	
B	Breton	F	Y	PRG		PAR, PRG		ABO		PRG		PRG	
C	Breton	F	Y	ABO		PRG		PRG		PAR, PRG		ABO	
D	Breton	F	Y	PRG		PAR		-		PRG		PRG	
E	Breton	F	N	-		PRG		PRG		PAR, PRG		PRG	
F	Breton	F	N	-		-		-		PRG		PRG	
G	Breton	F	N	PRG		PAR, PRG		**PRG**	**1:4**	PAR, PRG		**PRG**	**1:4**
H	Percheron	F	Y	PRG		PAR, PRG		PRG		PAR, PRG		PRG	
I	Percheron	F	Y	PRG		PAR, PRG		PRG		PAR, PRG		PRG	
J	Percheron	F	Y	PRG		PAR, PRG		PRG		PAR, PRG		PRG	
K	Percheron	F	Y	PRG		PAR		-		**PRG, endometritis**	**1:8**	PRG	1:8
L	Percheron	F	Y	ABO		**PRG**	**1:4**	ABO		**PRG**	**1:4**	PRG	1:4
M	Percheron	F	N	PRG		PAR		-		PRG		PRG	
N	Percheron	F	N	-		PRG		PRG		PAR, PRG		PRG	
O	Percheron	F	N	PRG		**PAR, PRG**	**1:4**	PRG		PAR, PRG		**PRG, laceration**	**1:4**
P	Percheron	F	N	-		-		**-**	**1:4**	PRG	1:4	ABO	1:4
Q	Breton	M		-		-		-		-		-	
R	Breton	M		-		-		-		-		-	
S	Percheron	M		-		-		-		-		-	

The data in bold were confirmed before positive conversion occurred. PRG, pregnancy; PAR, parturition; ABO, abortion; -, no records; F, female; M, male; Y, yes; N, no.

the visible clinical symptoms.

It was unclear whether all the antibodies detected in this analysis were harmful, and the observed titers were relatively weak (<1:16) and generally positive in the antibody tests (e.g., https://www.vet.cornell.edu/animal-health-diagnostic-center/testing/protocols/immunology/crossmatch). Based on the agglutinin reaction against a panel of blood cells in the Coombs test (data not shown), they were not antibodies against Aa and Qa antigens, which are the most immunogenic and often responsible for neonatal isoerythrolysis [3, 12, 17], and not anti-Ca antigen, which is the majority of naturally occurring alloantibodies [2, 5]. In addition, all but one of the mares (horse ID, K), were free of anti-Ca because they had a Ca-positive blood type. The antibodies detected during this period may be inconsequential during a transfusion reaction. However, since blood donor candidates, such as the analyzed horses, generally lack Aa and Qa antigens, there is always a risk of acquiring anti-Aa and Qa. Therefore, it is important to continuously monitor antibodies even if a possible donor is selected and maintained as a possible donor, for instance, by yearly screening, as pointed out previously [8].

In conclusion, positive conversion of anti-erythrocyte antibodies is induced not only by well-known causes such as pregnancy, parturition, and transfusion, but also by unknown causative sensitization. Hematolytic antibodies, such as anti-Aa and Qa, are likely to be acquired constantly, as possible donor candidates lack Aa and Qa antigens. Periodic monitoring of anti-erythrocyte antibodies is necessary to ensure donor safety.

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