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Red Blood Cell Surface Antigens Responsible for Neonatal Isoerythrolysis in Thoroughbred Horses of Jeju Island

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Abstract : This study was conducted to survey red blood cell (RBC) antigens Aa, Ca and Qa types, which are considered to be the most significantly associated with occurrence of neonatal isoerythrolysis, and the results are expected to provide valuable informations in organization of breeding plan, hence preventing the disease. Blood samples were collected from 262 Thoroughbred horses in Jeju island. Two percent cell suspension has been prepared from each sample and they were tested by indirect antiglobulin test. Of the 226 mare's samples, 9(3.98%) were Aa negative, 8(3.54%) were Ca negative, 17(7.52%) were Qa negative. Of the 36 Stallion's samples, 1(2.78%) was Aa negative, 3(8.33%) were Ca negative, 3(8.33%) were Qa negative. On the basis of these data, a database for breeding compatability could be set, and it would play an important role as a reference for arranging the mating partners.

Key words **:** Neonatal isoerythrolysis, Aa Ca Qa antigens, Indirect anti-globulin test, Thoroughbred.

Introduction

Neonatal isoerythrolysis (NI) or hemolytic anemia of the newborn, although not commonly encountered in equine practice, is an important immunologic disease of foals that often results in a fatal hemolytic crisis (15).

NI, an immunologic disorder causing red blood cell destruction, seen in newborn horse, mule foals and other species, infrequently occurs in the equine population (11,15,17, 18). It is preventable with an understanding of the pathogenesis of the disease, allowing the veterinarian and foal manager to modify breeding and management practices in order to decrease clinical significance of the disease (6,11,15,17,18).

Eight major blood group systems are present in horses; A, C, D, K, P, Q, T and U (1). Within each group system there are allelic factors responsible for inheritance and expression of red blood cell antigens (1). A foal is at risk for *neonatal isoerythrolysis (NI)* if the foal inherits an RBC factor from its sire for which the mare has pre-formed antibodies (15).

The mare's colostrum will contain antibodies against the factor the foal has inherited (2). If the foal consumes this colostrum, it will ingest antibodies capable of destroying the foal's own red blood cells (5).

Major NI risk factors are Aa, Ab, Ca, Da, Dc, Df, Ka, Pa, Ua, Qrs, Qb, Qc, Qa (9,18). In most reported cases of NI the Qa or Aa antigens are the cause of clinical illness as they are considered to be the most antigenic (17).

NI has not been associated with Ca antigen in the horse. Interestingly, it has been reported that mares lacking the Aa and Ca groups will spontaneously develop Ca antibody (1).

NI reportedly has a prevalence of 1% in Thoroughbred horses and a 2% prevalence in Standardbred horses. The percentage of Thoroughbred mares at risk for NI because they lack the Aa factor is 2%. Lack the Qa factor confers a greater risk of 16% (10).

Consumption and absorption of the colostrum leads to hemolysis of the foal's red blood cells (16). Extravascular hemolysis and removal of red blood cells via the reticuloendothelial system are the main means of red blood cell (RBC) destruction in the affected foal, intravascular hemolysis also occurs (16). Clinical signs are variable and may be peracute, acute, subacute, or subclinical (8). Usually foals are normal at birth and clinical signs do not develop until five hours to five days after birth (8). Clinical signs present in affected foals are due to anemia and are dependent upon the severity of that anemia (5,12,15). Two of the most important clinical signs of NI are icterus and hemoglobinuria (5,12). Foals with complete failure of passive transfer, for example, will not develop NI (8). Foals may demonstrate any or all of the following signs: weakness, lethargy, pallor, icterus, hemoglobinuria, tachycardia, tachypnia, fever and variable cardiovascular stability, some presenting in shock (3,8).

Neonatal isoerythrolysis is a highly preventable disease if several pre-foaling precautions are taken (8,10,15). The best way of disease prevention is to identify mares that are at risk of producing NI causing antibodies. Mares may be blood typed prior to breeding (8,10). Mares who lack the Qa or Aa blood group factor should be identified as mares at risk. The presence of the Ca antigen in the mare should also be evalu-

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ated (4,8).

Materials and Methods

Animals

In total, 262 Jeju Thoroughbred horses, which were 226 males and 36 stallions, were randomly selected for this study.

Sampling

RBC samples were extracted from whole blood samples. Twenty ml of blood was withdrawn from jugular vein and stored in EDTA-coated tubes and plain tube. RBC suspension (2%) was extracted from blood samples.

Aa, Ca antigen test

Agglutination reaction (Aa,Ca) was tested by serological procedures, and antierythrocyte antibody using 96 well microplate. Agglutination reaction interpreted by standard antibody (University of Queensland) 40 µl mixture 2% RBC suspension (3 times washing by 0.9% NaCl) for 45 minutes and 80 minutes in 37°C.

Qa antibody test

Hemolysis (Qa) was tested by serological procedures, and antierythrocyte antibody using 96 well microplate. Hemolysis reaction interpreted by standard antibody (University of Queensland) 40 µl mixture 20 µl complement (Absorption Rabbit serum) and 2% RBC suspension (3 times washing by 0.9% NaCl) for 120 minutes.

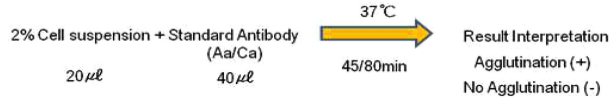

Fig 1. Aa, Ca antigen test; Agglutination reaction interpreted by standard antibody 40 µl mixture 2% RBC suspension for 45 minutes and 80 minutes in 37°C.

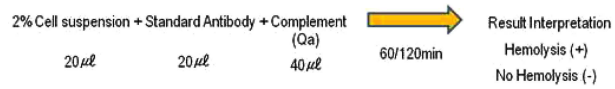

Fig 2. Qa antibody test ; Hemolysis reaction interpreted by standard antibody 40 µl mixture 20 µl complement and 2% RBC suspension for 120 minutes.

Results

Mare 226 heads and Stallion 36 heads which raised in Jeju island were carried out Aa, Ca antigen tests and Qa antibody test associated major blood type causing neonatal isoerythrolysis disease. This results showed in Table 1.

RBC antigens Aa, Ca, Qa negative mares showed Aa negative mares 9 heads (3.98%), Ca negative mares 8 heads (3.54%), Qa negative mares 17 heads (7.52%). RBC antigens Aa, Ca, Qa positive stallions was showed Aa positive stallions 35 heads (97.22%), Ca positive stallions 33 heads (91.67%), Qa positive stallions 33 heads (91.67%).

Discussion

In the Korean Thoughbred industry, there are around 2,500 mares and 90 stallions. These horses give birth to more than 1,300 foals every year and the number of foals are steadily increasing (4,7). Neonatal isoerythrolysis (NI) is the most common cause of hemolytic anemia and clinical icterus in neonatal foals (3,8). This potentially lethal disease may have a huge impact in the equine industry, and it, which is caused by incompatibility between dam's and offspring's blood group, therefore needs to be controlled and prevented.

The foals which in order to onset of Neonatal isoerythrolysis must have inherited Stallion's red blood cell antigen not mare's red blood cell antigen. This study evaluated blood groups associated with the outcomes of foals with Neonatal isoerythrolysis (13). For this, Aa and Qa antigens were mainly targeted and examined on as they are considered to be the most antigenic (1,15). Meanwhile, Ca antigen was also evaluated, although it does not cause the neonatal isoerythrolysis alone. The most common red blood cell antibody found in horses is the anti-Ca antibody, and is often a naturally occurring alloantibody. The mare, who is lacking both

Table 1. Frequencies of blood groups in 262 Thoroughbred mares and stallion in jeju island

		Total	Mare	Stallion
Aa	Positive	252heade(96.08%)	217heade(96.02%)	35heade(97.22%)
	Negative	10heade(3.92%)	9heade(3.98%)	1heade(2.78%)
Total		262heade(100%)	226heade(100%)	36heade(100%)
Ca	Positive	251heade(95.8%)	218heade(96.46%)	33heade(91.67%)
	Negative	11heade(4.20%)	8heade(3.54%)	3heade(8.33%)
Total		262heade(100%)	226heade(100%)	36heade(100%)
Qa	Positive	242heade(92.37%)	209heade(92.48%)	33heade(91.67%)
	Negative	20heade(7.63%)	17heade(7.52%)	3heade(8.33%)
Total		262heade(100%)	226heade(100%)	36heade(100%)

Aa and Ca antigens, might have developed Ca alloantibodies and lead to an antibody-mediated immunosuppression of immune response to the Aa blood group antigen (1). However, the samples showing Aa negative were all Ca positive in this experiment, and the correlation between Aa and Ca antigens has not been demonstrated unfortunately.

226 Mares and 36 Stallions selected in this study showed the following results: Aa, Qa and Ca positive percentage of the stallions were 97.22%, 91.67%, and 91.67%, respectively. On the other hand, the mares demonstrated Aa negative 9 heads (3.98%), Qa negative 17 heads (7.52%), and Ca negative (3.54%). From these results, there was no significant difference in the percentage of Aa negative samples, although the percentage of Qa negative animals was significantly lower than the results from previous studies, Aa negative 3% and Qa negative 17%, respectively (14). It may be attributed to geographical difference, although a further study with an increased number of samples is required to boost the power of the study.

Unfortunately, there was no specimen showing both Aa and Ca negative, therefore it was not possible to identify the effect of antibody-mediated immunosuppression (1). In addition, 5 maeres, which are suspicious of producing NI foals, were also examined, and only one mare showed Aa negative while the others were all positive to both Aa and Qa antigens. As a consequence, it was unattainable to reveal which antigenic factor has a stronger influence on clinical signs.

Although this study was designed to evaluate a limited number of antigenic factors causing neonatal isoerythrolysis, it is expected to provide a comprehensive data base when the stud farm makes a plan for breeding. In addition, it would be an important method of advanced preventive medicine using genetic informations of potential dam and sire. In order to achieve this, there must be a further effort to consolidate the results from this study using a larger number of samples. At the same time, the other minor antigenic factors known to cause NI should also be evaluated to complete the last puzzle.

Conclusions

In this study, we investigate throughbred mare 262 heads and stallion 36 heads for three blood type Aa, Ca, Qa which major causes the neonatal isoerythrolysis disease in Jeju island.

RBC antigens Aa, Ca, Qa positive stallions showed Aa positive stallions 35 heads (97.22%), Ca positive stallions 33 heads (91.67%), Qa positive stallions 33 heads (91.67%). RBC antigens Aa, Ca, Qa negative mares showed Aa negative mares 9 heads (3.98%), Ca negative mares 8 heads (3.54%), Qa negative mares 17 heads (7.52%).

Mares who lack the Qa or Aa blood group factor should be identified as mares at risk. The presence of the Ca antigen in the mare should also be evaluated. Mares who lack the Ca antigen often produce antibodies to that antigen, foals ingesting that antibody have not been shown to have clinical illness. Stallion blood types are often readily available from the breeder. Ideally stallions possessing the Qa or Aa blood group factor should not be bred to mares lacking either of those factors.

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제주도 더러브렛 종에서 신생마 적혈구용혈증을 유발하는 적혈구 표면 항원 조사

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요 약 : 본 연구의 목적은 제주도 내 더러브렛 경주마에서 신생마 동종면역성 적혈구 용혈증과 가장 밀접한 연관이 있다고 보고된 적혈구 항원 Aa, Ca, Qa의 발현 빈도를 확인하고자 하였다. 본 실험은 제주도 내에서 사육되고 있는 종빈마와 종모마 총 262두의 혈액에서 분리한 2% 적혈구 부유액이 사용되었으며, 간접 항체 검사을 통해 결과를 확인하였다. 226 두의 종빈마 중 Aa 음성이 9 두 (3.98%), Ca 음성이 8 두 (3.54%), Qa 음성이 17두 (7.52%)로 나타났으며, 36 두의 종모마 중 Aa 음성이 1두 (2.78%), Ca 음성이 3두 (8.33%), Qa 음성이 3두 (8.33%)로 확인되었다. 이들 결과를 토대로, 향후 말 번식에 있어서 종부 파트너 선택에 중요한 기초 자료를 제시하고 나아가 신생마 동종면역성 용혈성 빈혈 예방에 기여할 것으로 기대된다.

주요어 : 신생마 적혈구용혈증, Aa, Ca, Qa 항원, 간접 항체 검사, 더러브렛