NOTE *Internal Medicine*

Clinical and Pathological Aspects of Hemophilia A in Japanese Brown Cattle

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ABSTRACT. A coagulopathy with subcutaneous bleeding and muscular or peritracheal/periesophageal bleeding occurred in two male Japanese Brown calves of the same dam. One of the affected calves died three days after the onset of bleeding and the other survived normally until being slaughtered despite once suffering from subcutaneous hematoma. Hemostatic tests of the latter case showed prolonged activated partial thromboplastin time (APTT), and severely reduced factor VIII activity. In addition, von Willebrand factor activity, determined by the human platelet aggregation test, was within the normal range; therefore, the calf was diagnosed with hemophilia A. These are the first bovine cases of hemophilia A definitely diagnosed clinicopathologically.
KEY WORDS: factor VIII, hemophilia A, Japanese Brown cattle.

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Hemophilia is a hereditary hemorrhagic disease whose existence has long been known in humans, and is a general term for factor VIII deficiency (hemophilia A) and factor IX deficiency (hemophilia B). The genes for coagulation factors VIII and IX are located on the X chromosome. Hemophilia is the only known X-linked recessive disorder among the hereditary coagulation factor deficiencies, most of which are autosomal recessive inherited diseases. In animals, factor IX deficiency has been reported only in a few species including dogs and cats [1, 16]; however, factor VIII deficiency has been reported in a larger number of species including dogs [3], cats [10], horses [6], and sheep [13]. Dogs in particular have been used as a human-disease model, helping reveal the relationship between the causal mutation and the symptoms and pathophysiology [12] as well as for gene therapies [15]. In cattle, hemophilia A has been reported only in Herefords [5], and in that case a pedigree study was not conducted and the mode of inheritance was unknown. Furthermore, despite the low factor VIII activities, hemophilia A was not definitely diagnosed in the Herefords case, leaving the possibility of von Willebrand disease type III.

Recently, we found two bleeding cases in a pure and a mixed-breed Japanese Brown calves in the Kumamoto Prefecture of Japan. The animals had subcutaneous bleeding and muscular or peritracheal/periesophageal bleeding. These are the first definite cases of hemophilia A in cattle diagnosed clinicopathologically and genetically, and this paper reports on the clinical courses of the cases, and the results of hemostatic tests and dissection.

The subjects were two male calves of the same Japanese Brown cattle dam mated with two different sires. One had a Japanese Black cattle sire (Case 1), and the other was pedigreed, with a Japanese Brown cattle sire (Case 2). These calves had no other siblings, and maternal close relatives of the dam were not alive. Case 1 grew without signs of medical problems after birth, and received an open castration procedure at 4.5 months with no obvious bleeding. However, at 7 months, a palm-sized subcutaneous mass was formed between the 7th and the 11th ribs, which gradually grew to the size of a human child's head a week later. The mass was a hematoma containing unclotted blood. After the removal of the contents, it grew back to the original size within a few hours. The clotting of the venous blood took more than 60 min in a plain plastic tube. The general physical conditions of the animal, such as vigor and appetite, were good, and the hematoma was self-absorbed in approximately 15 days. The animal then grew without further problems and was slaughtered at 26 months. During this time, the dam was crossed with a Japanese Brown cattle sire and gave birth to a male calf (Case 2). This calf grew without signs of medical problems after birth, but a swelling suddenly developed between the lower jaw and the chest at 4 months, and unclotted blood in the swelling was confirmed by paracentesis. The calf had difficulty in eructation due to esophageal obstruction caused by the swelling, which resulted in the ruminal distension with gas. The symptom continued to worsen and the animal soon became unable to walk. The animal died three days after the onset of the symptom, despite the elimination of the rumen gas via a catheter and treatment with steroids and antibiotics. It was unlikely that these two animals ingested poisonous plants or rodenticides, which can cause vitamin K deficiency; therefore, hereditary bleeding disorder was suspected.

We collected blood samples from Case 1 and the dam, and performed hemostaic screening tests including platelet

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Table 1. Results of hemostatic screening tests and coagulation factor assays of Case 1 and the Dam

Parameters	Dam	Case 1	Normal range[d)]
A. Hemostatic screening[a)]			
Platelet ($10^4/\mu$L)	319	216	310 ± 126
Fibrinogen(mg/dL)	–[c)]	373	217 ± 71
PT (sec)	33.3	27.8	30.2 ± 4.3
APTT (sec)	43.4	72.7	39.7 ± 6.6
B.Coagulation Factors (%)[b)]			
Intrinsic pathway			
Factor VIII	30.8	5.8	} 100
IX	55.2	36.8	
XI	37.5	23.6	
XII	61.8	94.3	
Extrinsic and common pathways			
Factor II	65.5	74.7	} 100
V	42.8	65.1	
VII	85.7	115.2	
X	61.8	94.3	

a) Bleeding time and FDP (fibrinogen/fibrin degradation product) were not examined.
b) Factor XIII was not examined.
c) –: not examined.
d) Mean ± SD from pooled normal bovine plasma (n=325, Japanese Black cattle).

count, fibrinogen, prothrombin time (PT), and activated partial thromboplastin time (APTT). In Case 1, the platelet count, fibrinogen and PT were within the normal ranges, but APTT was prolonged (Table 1). We therefore suspected that the bleeding manifestations were caused by an abnormality within the intrinsic coagulation factors.

Measurement of each specific coagulation factor activities in Case 1 and the dam revealed that only the factor VIII activity, among the intrinsic coagulation factors, was severely decreased in Case 1 (5.8% of normal) and moderately deceased in the dam (30.8% of normal) (Table 1). Although the factor XI activity was also moderately decreased in Case 1 (23.9% of normal), it was not compatible with factor XI deficiency reported in the Japanese Black cattle which shows marked reduction of the factor XI activity to < 10% of normal [9]. Furthermore, genotyping of the factor XI gene revealed normal homozygous genotype in Case 1. The maximum human-platelet aggregation of the bovine plasma in the absence of ristocetin was 72.9% in Case 1 and 79.2% in the dam (control value: 85.2%). Since the bovine von Willebrand factor aggregates human platelet in the absence of ristocetin [4], the presence of active von Willebrand factors were confirmed in case 1 and the dam thus excluding the possibility of von Willebrand disease Type III. The vitamin K-dependent coagulation factors (II, VII, IX and X) were not significantly reduced excluding the possibilities of acquired coagulopathies. Based on these findings, Case 1 was diagnosed as hemophilia A, and the dam as an obligate carrier.

An autopsy of Case 2 was performed. The area between the lower jaw and the chest was swelled (Fig. 1), the abdominal circumference was remarkably distended, and the visible mucous membrane was pale-colored. After removing the skin, intermuscular and intramuscular blood spots of various sizes were confirmed, mainly between the neck and the chest. Massive bleeding was confirmed in the peritracheal and periesophageal areas and extended to the thoracic cavity. The bleeding caused constriction of the esophagus and the trachea. Although some of those blood was coagulated , the majority of the hemorrhage remained unclotted (Fig. 2). Bleeding was confirmed in the anterior part of the thoracic cavity through to the area near the aortic bifurcation of the heart. Blood clots of various sizes were scattered on the surface of the serosa of the digestive tract. The parenchymatous organs such as the liver and the kidney were remarkably discolored, indicating that the animal was suffering from anemia. Histological observations showed increased giant cells in the marrow, but did not confirm extramedullary hematopoiesis in the spleen and the liver. Since we could not collect fresh blood samples, hemostatic tests were not performed in Case 2. Genotyping of the factor XI gene in Case 2 revealed heterozygous genotype.

In human hemophilia, the earliest bleeding manifestations do not appear during the neonatal period but rather in early childhood. The levels of symptoms are classified as mild, moderate, or severe according to the plasma level of the factor VIII activity, which usually correlates with the disease severity. Mild cases are typified by prolonged bleeding only after surgery or trauma, and no other problems. However, severe cases are associated with apparently spontaneous bleeding episodes affecting joints, muscles, internal organs, and the brain. Joint bleeding (hemarthrosis) is the most characteristic feature of severe hemophilia, and chronic arthropathy with loss of joint movement, fixed flexion contracture and severe muscle wasting are caused by repeated bleeding [2].

In dogs, horses and sheep, as in humans, multiple hematomas and spontaneous bleeding occur in muscular and soft tissues and the central nervous system, with the severity of the symptoms depending on the levels of the factor VIII activity [1, 8, 11, 14]. The intraarticular hemorrhage, a typical symptom in severe cases, also occurs in dogs, horses, and sheep [1]. Large dog breeds, which have high joint loads, are more prone to the condition than smaller dog breeds [1]. While deaths due to umbilical cord bleeding during the neonatal period have been reported in sheep [14], spontaneous bleeding occurs more frequently during infancy. Cats rarely show spontaneous bleeding. Although there are cases of prolonged bleeding after castration, with some cases resulting in death, symptoms in cats have been reported to be milder than in other species [10]. In the cases with coagulopathy reported in Herefords [5], spontaneous (subcutaneous bleeding and hemoperitoneum) and post-castration bleedings lead to deaths. However, hemarthrosis which is a common symptom in the human condition, was not observed in the Herefored cattle.

In Case 1, spontaneous bleeding was not fatal since the subcutaneous hematoma was self-absorbed and other significant bleeding episodes were not confirmed. In Case 2, it

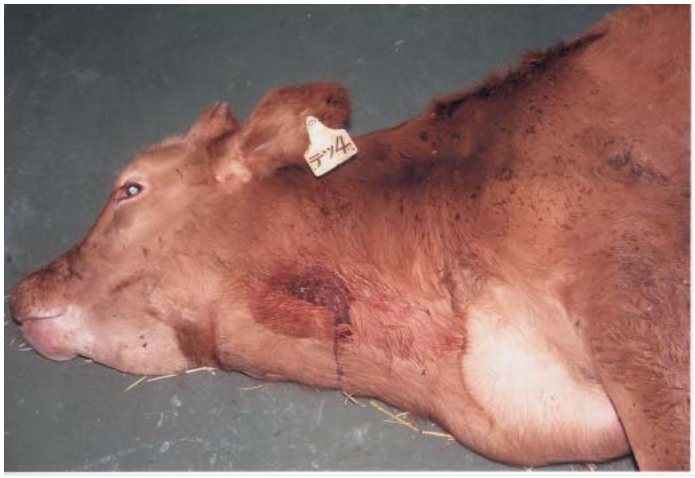

Fig. 1. Subcutaneous swelling of the neck (Case 2)

Fig. 2. Peritrachea/periesophageal bleeding (Case 2)

appeared that bleeding occurred in small vessels near the heart, and then unclotted blood spread extensively in the peritracheal/periesophageal areas between the neck and the chest, leading to death due to the acute ruminal tympany caused by esophageal obstruction. It was ,therefore, considered that these symptoms were related to the site of spontaneous bleeding rather than the bleeding tendency. Although sudden fatal bleeding did occur in Case 2, due to the fact that spontaneous bleeding such as hemarthrosis and ecchymoma were not observed, these cases were not considered as severe types of hemophilia reported in humans and other animals. The relation between the heterozygous status of factor XI deficiency in Case 2 and the relatively severe bleeding symptom is unclear.

One nucleotide substitution in the factor VIII gene has been found in the affected calves and the dam was heterozygote of the substitution [7]. The factor VIII gene has been localized on the X chromosome in all mammalian species including cattle and human. Although the relationship between this substitution and the bleeding disorder has not yet been confirmed, the following evidences indicate X-linked recessive inheritance of the disease. First, the two male calves which showed the hemorrhagic symptoms were born from the same dam and the sires of each calf were genetically unrelated. Second, the dam of the affected calves showed no clinical symptoms but moderately reduced factor VIII activity. Together with the significant reduction of the factor VIII activity, these genetic evidences showing X-linked inheritance also suggested that the bleeding disorder is hemophilia A.

As only two cases have so far been reported, and low frequency of the substitution have been observed in the Japanese Brown cattle population [7], Hemophilia A is considered not to cause a serious problem in the breeding and production of Japanese Brown cattle.

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