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Abstract
The mutation responsible for Junctional Epidermolysis Bullosa (JEB) in Belgian draft horses in North America and other draft breeds in Europe has been identified. The mutation, a cytosine insertion (1368 insC) in the LAMC2 gene, results in absent expression of the laminin \( \gamma_2 \) polypeptide chain of laminin 5. JEB is inherited as an autosomal recessive trait. A polymerase chain reaction (PCR) test has been developed to identify carriers of the mutation using mane and tail hairs. Since October 2002, this test has been available to breeders of Belgian horses in the United States and Canada through their breed associations.

1. Introduction
Epidermolysis bullosa (EB) encompasses a group of otherwise heterogenous diseases of the skin and mucous membranes, which share the common feature of the formation of blisters and erosions in response to minor mechanical trauma [1,2]. In humans, most cases are inherited, and the clinical forms of hereditary EB are divided into three major types: EB simplex (EBS), junctional EB (JEB), and dystrophic EB (DEB). Each of these are typified by the level of skin separation within the dermal-epidermal basement membrane zone and by the proteins involved [2]. In JEB, blister formation takes place within the lamina lucida of the dermal-epidermal basement membrane. The JEB mutations in humans have been described in the three genes (LAMA3, LAMB3, and LAMC2) that encode the anchoring filament protein, laminin 5, and the two transmembrane components of the hemidesmosome (HD), collagen XVII, and integrin \( \alpha_6\beta_4 \). The Herlitz subtype of JEB (JEB-H) represents the most severe and the most frequent form of JEB in humans (> 50% of cases), and it is characterized by generalized blistering with erosions of the skin and mucous membranes. Extensive oral erosions are common, and enamel hypoplasia is present in JEB-H [3]. Ultrastructural and immunohistochemical observations show abnormalities in hemidesmosome anchoring filaments complexes. Immunostaining of the skin of patients affected by JEB-H reveals absence of laminin-5. The disease is lethal in early childhood, and the usual mode of transmission is an autosomal recessive trait [2].

In North America, the first reports of JEB in neonatal Belgian draft foals were published between 1988 and 1989 [4-6]. Based on the histopathological, ultrastructural, and immunohistochemical findings, this condition in Belgian draft horses corresponds to the severe (Herlitz) form of JEB in humans [7]. In the United States, JEB has also been reported in American Saddlebred horses since 1975 [8-10].

Recently, the mutation associated with the clinical signs of JEB in Belgian draft horses has been identified and is linked to the \( \gamma_2 \) subunit of the laminin-5 gene [7]. The mutation is a homozygous cytosine insertion in the genomic nucleic acid sequence of affected horses at position 1368 of the laminin \( \gamma_2 \) encoding polynucleotide, a frame shift, and a premature termination codon [7]. This results in an absent expression of the laminin \( \gamma_2 \) polypeptide. An autosomal recessive mode of inheritance of this mutation has been verified [7].

After the development of a commercial polymerase chain reaction (PCR) test to identify carriers of the LAMC2 mutation, this study was designed to conduct preliminary investigations on the presence and prevalence of LAMC2 carrier animals in Belgian draft horse, other draft horse, and American Saddlebred populations in North America and Europe.
Between October 2002 and May 2003, the LAMC2 mutation was present in European draft horses.

Germany, France, the United Kingdom, The Netherlands, and Sweden, and their mane hair samples were used to determine if the percentage of LAMC2 carriers identified in this program was 17.1% (56/328). Of the 252 samples submitted from male Belgian Draft Horses in North America, 34 (13.5%) were carriers, and of the 76 samples submitted from females, 22 (28.9%) were carriers.

Belgian Draft Horses in North America - Carriers of the LAMC2 mutation were identified on 11 of 12 farms (91.7%) sampled. The percentage of carriers in the population sampled was 32% (57/176), ranging from 8% (1/12) to 80% (4/5) on individual farms. Of the 12 farms sampled, 9 owned a stallion(s). The stallion was identified as a carrier on four of nine farms. The carrier rate on these four farms was 40.8% (31/76) compared with 20% (16/80) on the five farms where the resident stallion was not a carrier. Eight of the 12 farms sampled had previously had at least one JEB foal born in the past 5 yr. The carrier rate on these eight farms was 37% (44/119) compared with 22.8% (13/57) on the four farms where no JEB foal had been born.

Belgian Draft Horse Corporation and Canadian Belgian Horse Association Testing - Between October 2002 and May 2003, the percentage of LAMC2 carriers identified in this program was 17.1% (56/328). Of the 252 samples submitted from male Belgians, 34 (13.5%) were carriers, and of the 76 samples submitted from females, 22 (28.9%) were carriers.

3. Results

3.1 DNA Sample Collection

North America - Between May 2001 and February 2003, mane hair samples were collected from all registered Belgian draft horses (172 horses) located on 12 breeding farms. These farms were in Ontario (95 horses; 7 farms), Michigan (29 horses; 2 farms), Ohio (25 horses; 1 farm); Pennsylvania (18 horses; 1 farm), and Illinois (5 horses; 1 farm). Every registered Belgian draft horse present on the 12 farms at the time of sampling was tested. Eight of the 12 farms sampled had at least one JEB foal born on the farm in the previous 5 yr. From October 2002 to May 2003, the Belgian Draft Horse Corporation of America and Canadian Belgian Horse Association have forwarded mane hair samples from 328 registered Belgian draft horses (252 males; 76 females) to the Veterinary Genetics Laboratory, University of California Davis for the commercial PCR test for the LAMC2 mutation. This testing came after the introduction of mandatory testing for new breeding stallions since October 2002. Testing for mares has been on a voluntary basis.

Draft Horse Breeds in Europe - Between March 2001 and January 2003, 332 mane hair samples were collected from the following breeds of draft horses: Belgian Trekpaard (63 horses), Rheinisch Deutsches Kaltblut (63 horses), Breton (63 horses), Comtois (51 horses), Shire (45 horses), Belgische Koudbloed Flander (16 horses), Vlaams Paard (11 horses), Netherlands Trekpaard (10 horses), and Swedish Ardenner (10 horses). These horses were located on farms in Belgium, Germany, France, the United Kingdom, The Netherlands, and Sweden, and their mane hair samples were used to determine if the LAMC2 mutation was present in European draft horses.

American Saddlebred - Mane hair samples were randomly collected from 101 registered American Saddlebred horses on five farms in the United States and from 6 registered Saddlebred horses on one farm in South Africa. A JEB foal had been born on the farm in South Africa, and the sire and the dam of the JEB foal were included in the horses sampled.

3.2 Commercial PCR Test

PCR was performed on DNA samples with fluorescently labeled primers [11] designed to amplify the region containing the mutation. The mutation is a single base insertion, and thus, carriers have a PCR product that is one base longer than the normal allele. The single base difference is detected by analysis of the PCR products on an ABI 377 DNA sequencer [a].

3.3 DNA Sample Collection

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3.3.4 Belgian Draft Horses in North America

Belgian Draft Horses in North America - Carriers of the LAMC2 mutation were identified on 11 of 12 farms (91.7%) sampled. The percentage of carriers in the population sampled was 32% (57/176), ranging from 8% (1/12) to 80% (4/5) on individual farms. Of the 12 farms sampled, 9 owned a stallion(s). The stallion was identified as a carrier on four of nine farms. The carrier rate on these four farms was 40.8% (31/76) compared with 20% (16/80) on the five farms where the resident stallion was not a carrier. Eight of the 12 farms sampled had previously had at least one JEB foal born in the past 5 yr. The carrier rate on these eight farms was 37% (44/119) compared with 22.8% (13/57) on the four farms where no JEB foal had been born.

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American Saddlebred Horses in the United States and Saddlebred Horses in South Africa - No carriers of the LAMC2 mutation were detected in the 107 animals tested.

4. Discussion
Although the first reports of JEB in Belgian draft horses in North America were not published until 1988 [4-6], it is likely that most reported cases of EI were, in fact, foals with EB [12]. In retrospect, the first report that the authors believe described JEB in the horse was published in 1913 [13]. Skin lesions consistent with JEB were described in two foals examined in 1909 and 1910 at the Royal Veterinary and Agricultural University in Copenhagen, Denmark. The breed(s) affected was not stated [13].

In 1934, at the Veterinary College in Stockholm, Sweden, an Ardenner foal with a complete absence of epidermis and hair on the distal extremities from just above the carpus and tarsus was necropsied [14]. The temporary incisor teeth (I1, I2) as well as premolar teeth (P1 - P3) had broken out in all jaws. This condition was designated by the authors as "epitheliogenesis imperfecta neonatorum" (EIN). The mare's previous foal by the same stallion had been similarly affected with epidermal defects affecting three legs and the entire epiplum. The pedigree of the affected foals demonstrated that they were 13/16th Belgian ancestry [14].

In 1936, a brief report from The Netherlands [15] about an inherited skin disease was documented in seven foals with skin defects on the distal limbs, coronary band separation, and loss of a hoof (exungulation). All seven foals were sired by one stallion. No mention was made of the breed of the horse involved in these cases [15].

In 1937, a case report was published in Germany describing skin lesions in a newborn foal consistent with those previously noted for EIN [16]. In a thesis from the Tierärztliche Hochschule Hannover, Germany, Sonnek described the clinical findings and mode of inheritance of 28 cases of "epitheliogenesis imperfecta neonatorum equi" (EINE) that occurred between 1935 and 1944 in "cold-blooded" horses [17]. In the breeding analysis, nine stallions were found with affected offspring. Of the 28 cases, 19 were sired by sons of stallion "C," who was born in Belgium in 1921 and was exported to Germany in 1924. Between 1947 and 1957, five more EINE cases were documented in Germany [18]. It was concluded from both German studies that there was a recessive mode of inheritance, which was not gender associated [18]. Germany imported approximately 20,000 horses/yr from Belgium between 1901 and the beginning of World War I. Of this number, 140 - 180/yr were reproductive stallions.

The importation of draft horses from Belgium to North America commenced in 1885 and continued until 1916. Importations recommenced in 1920 and totally ceased on January 15, 1940. In the 1950s, there was a marked drop in Belgian horse numbers followed by a rapid expansion in the breed in the 1980s. The annual number of Belgian draft horses registered with the Belgian Draft Horse Corporation of America went from 3196 (1937) to 171 (1952) to 4065 (1982). The marked reduction in registered horses resulted in the breeding of consanguineous individuals and is believed to have been a major contributor to the occurrence of JEB in the Belgian draft horse in North America [7]. Throughout the world, most draft horse breeds went through a similar catastrophic drop in animal numbers in the 1950s and 1960s, which has been followed by a recent resurgence.

In France, the first cases of JEB in the horse were diagnosed in 1989 in five newborn foals in two French draft breeds. Three Breton and two Comtois newborn foals were affected [19]. The clinical, pathological, and ultrastructural findings [19,20] were identical to those observed in Belgian draft foals in North America [4-6]. Pedigree analysis indicated that JEB was inherited in an autosomal recessive manner [19,20]. It was suggested that JEB in the Breton and Comtois breeds may be caused by the same mutation responsible for JEB in Belgian horses [7]. Using primer sequences provided by one of the authors, it has been determined that the LAMC2 mutation detected in the Belgian horses is also responsible for JEB in these two French draft breeds [21]. The crossing of other draft horse breeds into these breeds, especially from Belgium, occurred during the late 18th and early 19th centuries in Europe.

Cases of EI have been documented in American Saddlebred foals since 1975 [8-10]. The clinical signs in American Saddlebred foals are very similar to those in Belgian draft horses: epidermis absent on the distal extremities and along the back and hindquarters, abnormal dentition, and lesions on the tongue, gum line, hard palate, and soft palate. Lesions may also be present on the anus and external genitalia. The erupting deciduous teeth are irregular and rough with jagged edges [9]. Pedigree analysis of 34 EI cases was consistent with an autosomal recessive mode of inheritance. All EI affected foals could
be traced to a single lineage, and it was consistent with a single individual being responsible for the spread of EI within Saddlebred horses [9]. The genotypic frequency of EI in the American Saddlebred horse population was estimated to be 4% [9]. When PCR testing was conducted on the DNA of the sire and dam of a JEB Saddlebred foal born in South Africa as well as over 100 American Saddlebred horse samples, there was no evidence that the LAMC2 mutation in the Belgian, Breton, and Comtois draft horse breeds is responsible for JEB in the American Saddlebred. Linkage disequilibrium analysis supported that a mutation in the LAM α3 was responsible for JEB in the American Saddlebred [9]. The LAMC2 gene has been mapped to equine chromosome 5 (ECA5) at 5p17-p16 [22], whereas the LAMA3 gene suspected of causing JEB in American Saddlebred foals [9] is located on equine chromosome 8 (ECA 8) at 8q14-q15 [22].

With the availability of a commercial PCR test for the LAMC2 mutation and with the appropriate genetic counseling, it is now possible for horse breeders to avoid the financial and genetic losses associated with the birth of JEB foals in the Belgian, Breton, and Comtois draft horse breeds. The potential exists to eliminate this disease from these breeds.

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Footnotes
[a] PE Applied Biosystems, Foster City, CA, 94404.

References

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