TAKE HOME MESSAGE

- Factor XI deficiency, an inherited condition that affects Holstein cattle worldwide, leads to excess bleeding, reduced reproductive performance and increased susceptibility to disease.

- Current testing methods do not always discriminate between normal and carrier animals but have been used to eliminate carrier animals from Canadian sires destined for export.

- Collaborative research involving scientists from Illinois, Canada, and Australia is seeking to establish the molecular basis for the condition so that a more reliable test can be developed.

INHERITANCE AND DISTRIBUTION OF FACTOR XI DEFICIENCY

Factor XI is one of more than a dozen proteins involved in blood clotting. An inherited deficiency of Factor XI results in a bleeding disorder that has been documented in humans, dogs, and cattle. The bovine form of the disease was first discovered in Holstein cattle in Ohio in 1969. It was later observed among Holstein-Friesians in Canada, England, and Australia. Like DUMPS and BLAD, it is inherited in an autosomal recessive manner. Accordingly, carriers (heterozygotes) of the defective gene are outwardly normal, while affected animals (homozygotes) have a mild hemophilia-like disorder; 25 percent of offspring of mating a carrier bull to a carrier cow will be affected with Factor XI deficiency.

In 1975, a herd of Factor XI deficient animals was established at the University of Guelph (Canada) by identifying heifers that were carriers and breeding them to a carrier sire. This herd has proven to be invaluable in studying the genetics of the condition and its consequences for health and reproduction. Selective mating established its mode of transmission. Affected animals have less than 10 percent of normal biological activity of plasma Factor XI, with most having less than 1 percent. Carrier animals have from 20 to 60 percent of normal levels, with a mean value of 38 ± 10 percent. While affected animals can survive for years with no overt clinical signs, they do appear to have, as a group, higher mortality and morbidity. They are often referred to as "poor doers".

CONSEQUENCES OF FACTOR XI DEFICIENCY

Unlike many other blood clotting disorders, Factor XI deficiency may or may not be accompanied by spontaneous or induced bleeding episodes. Continued bleeding from the umbilical cord is sometimes seen in affected calves. Prolonged oozing of blood following dehorning and castration may also be observed. Bleeding episodes may be catastrophic; for example, one affected cow died after calving as a result of hemorrhaging into her lungs. Affected cows frequently have pink colored colostrum. Blood in milk led to the identification of the condition in a British dairy herd.
Maintaining the small research herd of Factor XI deficient animals in Guelph has proven to be a challenging experience. Some animals have been lost to excessive bleeding. Affected cows have also been difficult to breed and offspring of planned matings have suffered higher pre- and postnatal mortality, compared to normal offspring in the same herd. Furthermore, affected animals appear more susceptible to diseases such as pneumonia, mastitis and metritis.

Because affected cows have a 50 percent higher frequency of being repeat breeders than normal herd mates, ovarian function has been examined. Daily blood sampling showed that plasma progesterone concentrations were slower to decline at the end of the estrous cycle in affected cows. The average estrous cycle length for affected cows was longer, 24.7 + 2.1 days compared to 22.9 + 3.0 days for the control cows. Ultrasound monitoring was used to assess ovulatory follicular development. As shown in Figure 1, follicular diameter was smaller in affected cows, which may be due to lower peak levels of plasma estradiol measured around ovulation.

**Figure. 1.** Follicle size in normal and Factor XI affected cows. Ultrasound scanning was used to monitor the size of follicles in 6 normal and 6 affected cows after treatment with cloprostenol to induce ovulation. Mean sizes of follicles in affected animals are substantially smaller up to and including the time of ovulation. [From Veterinary Research Communications (1995)].

**CURRENT TESTING METHODS FOR FACTOR XI DEFICIENCY**

Because of the deleterious effects of this disorder to the Holstein population and the requirement that semen imported into Britain be tested free of the condition, a screening program was initiated in 1987 for all Canadian Holstein sires destined for export. In the first 5 years of the program, almost nine hundred bulls were tested. No affected animals were detected but 29 bulls were identified as carriers and culled. The screening program has resulted in the elimination of the disorder from all Canadian bulls destined for export.
One problem that has arisen during the testing program is that it is not always possible to classify a carrier animal on the basis of results obtained from the laboratory analysis of a single blood sample. The testing is based on the analysis of the coagulation activity of Factor XI in a blood sample. Poor blood collection technique or the inadequate or inappropriate anti-coagulation of the blood sample can alter the results. Current practice is to confirm results for any potential carrier on a second blood sampling. However, test results for some animals do not permit their status to be correctly determined. This is because of overlap in values for some normal and carrier animals, as illustrated in Figure 2. The values for affected animals are very low and readily distinguished. The means of carriers and normals are different, as are most individuals in these categories. However, some carrier and normal animals fall into a "gray area" between the two. For such animals, expensive and time-consuming progeny testing is needed to confirm their status. A more reliable laboratory test is needed. Because Factor XI deficiency is a genetic condition, it should be amenable to a DNA-based test that would be very accurate and reliable. Such a test has been developed for the comparable human condition. A collaborative research program involving scientists from Illinois, Canada, and Australia has been initiated to develop such a test for the bovine disorder.

Figure 2. Distribution of plasma Factor XI activity for affected animals. Affected animals have little to no activity, carrier animals have intermediate activity and normal animals have full activity. Because of a range of values for each category, an overlap exists between values for carrier and normal animals; animals with values between 50 and 60 percent cannot be accurately diagnosed solely by this test. [From Canadian Journal of Veterinary Research (1994)].

CURRENT RESEARCH ON FACTOR XI DEFICIENCY

In order to develop a DNA-based test for bovine Factor XI deficiency, the gene and the mutation(s) that cause the disorder must first be characterized. The first step towards this
goal is to determine the coding sequence for the gene. In animal species, a gene is a linear sequence of nucleotides that include segments called exons (which will dictate the order of amino acids to be incorporated into the protein product of the gene) and segments called introns (which are spacers that don't dictate the amino acid code of the protein and whose function is not well understood.) The gene for Factor XI in humans has been well characterized and has been shown to contain 15 exons and 14 introns. The gene in cattle will have a similar organization, but the exact sequence of nucleotides will differ slightly. So far, we have isolated and characterized 3 exons and 2 introns for bovine Factor XI. We know the exact sequence of nucleotides that make up these exons and introns. We also have measured the full length of the coding segment. Once we have sequenced the coding segment of Factor XI for normal animals, we will determine the change in that nucleotide sequence that results in the disease. Based on that result, we will design a DNA-based test for it.

PROSPECTS OF EVENTUAL CONTROL

While biochemical genetic diseases in dairy cattle transmitted as autosomal recessive traits can cause significant economic loses to the industry, they also have the potential of being eliminated by selective breeding. Since carrier animals, like normal animals, usually exhibit no clinical symptoms of the disease, their identification and elimination from breeding stock is essential to prevent its dissemination. Factor XI deficiency is an example of a biochemical genetic defect that will be amenable to accurate, rapid and inexpensive testing. Improvement in laboratory tests to clearly identify carriers and normals for this condition will eventually lead to its control and to the further improvement of the Holstein breed. This will benefit dairy producers and dairy consumers by increasing the efficiency of milk production.

A FINAL WORD

Dairy producers can help with the eventual control of Factor XI deficiency by reporting to us any instances of bleeding disorders among their animals. If you observe prolonged bleeding from the umbilical cord of a newborn calf, or from dehorning or castration, or pink colostrum, please contact us so that we can follow up on your observations.