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Absence of Bovine Leukocyte Adhesion Deficiency (BLAD) in Holstein Cattle from Mexico

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Abstract: Bovine leukocyte adhesion deficiency is an autosomal recessive genetic disease that produces in many cases the early death of Holstein calves, causing economic losses to milk producers and breeders. It is due to a point mutation in position 383 of cDNA of the CD18 gene, leading to a substitution of guanine for adenine and a change of glycine for aspartic acid (position 128). As a consequence, the normal CD11B/CD18 integrine is not functional. The affected animals show recurrent bacterial infections, delayed wound healing, stunted growth and early death. Many countries have followed a policy of detecting carrier bulls with a DNA test in order to eliminate them from Artificial Insemination (AI) programs with great success. In Mexico, no carrier detection program has been implanted, although, most of bulls used for AI comes from the USA, country that have done screening programs for the last 10 years. In order to determine the gene frequency of the BLAD mutation in Northeast Mexico, 61 Holstein animals were analyzed by PCR-RFLP. No evidence of the mutation was found, indicating that the use of USA, BLAD-free bulls in AI programs has probably eliminated the disease in Holstein population in Mexico.

Key words: BLAD, Holstein, PCR-RFLP, artificial insemination, bacterial infections, Mexico

INTRODUCTION

Bovine Leukocyte Adhesion Deficiency (BLAD) is a genetic autosomal recessive disease, characterized by recurrent pneumonia, ulcerative and granulomatous stomatitis, enteritis, periodontitis, delayed wound healing, neutrophilia and early death. These symptoms are due to a deficiency of a family of proteins on the surface of leukocytes, which allows them to leave the blood stream. It is caused by a point mutation in the gene encoding bovine CD18, producing a substitution of a guanine for adenine and of an aspartic acid to glycine at amino acid 128 (D128G) (Nagahata *et al*., 1997). This mutation has been traced to the bull Osbordale Ivanhoe, who had the largest genetic impact in the Holstein breed through artificial insemination programs, leading to frequencies of the mutant allele of 15% among bulls and 8% in cows (Nagahata, 2004). A similar condition has been studied in humans (leukocyte adhesion deficiency or LAD); it was determined that the disease results from the malfunctioning of the family of glycoproteins called the β_2 integrins, now termed CD11/CD18 family, which are vital to adhesion reactions. These molecules contain α and β subunits and the family share an identical β subunit (CD18), but can be distinguished by the α subunit. The mutation occurs in the β subunit (Nagahata *et al.* 1993; Nagahta, 2004).

BLAD can be diagnosed by a PCR-RFLP test to detect both affected and carrier animals (Tammen *et al*., 1996). Analysis of clinical symptoms has been correlated with the molecular diagnosis of BLAD, confirming that the mutation causes the disease (Nagahata *et al*., 1993). BLAD can also be diagnosed by radiography and non-isotopic Ligase Chain Reaction (LCR) (Batt *et al*., 1994), as well as by a PCR-SSCP (single strand conformation polymorphism) analysis (Li *et al*., 2007).

Some countries have conducted programs for the detection and eradication of the mutation. In Germany, the mutation rate declined from 11.6-9.9% (Tammen *et al*., 1996). No homozygotes with the mutation were found in Ukrania (Kostetskii *et al*., 1996). A frequency of 8.1% was reported for Holstein populations from Japan (Nagahata *et al*., 1997) and of 2.28% in Argentina (Poli *et al*., 1996).

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MATERIALS AND METHODS

In the present study, a total of 61 samples were analyzed from Holstein cows located in 5 different herds of the Northeast of Mexico (states of Nuevo Leon and Coahuila). DNA was extracted using the standard salting-out method. PCR primers and conditions used were according with the reported by Tammen *et al*. (1996) (Genbank accession number M81233). The expected product of amplification was of 101 base pairs (bp). It was used a MJ Research model PTC100 termocycler, with an initial denaturation step of 94°C for 5 min, 35 cycles of denaturation at 94°C, annealing at 56°C for 30 sec and elongation at 72°C for 30 sec and a final elongation step of 72°C for 5 min (Tammen *et al*., 1996). Reaction mix contained 5 μL of purified DNA at a concentration of 20 ng μL^{-1}, 2.5 μL of 10× PCR buffer with MgC1 at 1 and 0.2 mM of each dNTP, 0.4 mM each primer and 0.5 U of polymerase Taq in a total 25 μL mix with ultrapure sterile water. Visualization of amplification was performed by agarose gel electrophoresis, always using a molecular weight marker and negative control. Staining of DNA products was with ethidium bromide. An aliquot (10 μL) of the PCR product from each cow was digested with 5 U of restriction enzyme αTaq I, followed by incubation at 65°C for 1 h. Products of digestion were visualized by NuSieve agarose gel electrophoresis (4%), stained with ethidium bromide and compared with a molecular weight marker. The expected products of digestion are as follows: dominant homozygote (normal): three bands of 52, 32 and 17 bp; heterozygote (carrier): four bands of 84, 52, 32 and 17 bp; recessive homozygote (affected): two bands of 84 and 17 bp (Tammem *et al*., 1996).

RESULTS AND DISCUSSION

PCR amplification was successful in the 61 samples analyzed and the expected 101 bp band was obtained. In all of the 61 PCR products digested, only the bands of 52 and 32 bp were observed (Fig. 1). This indicates that all individuals had a normal BLAD genotype. Although in these individuals, it should be also observed the 17 pb band in this study, it was not possible its detection. However, this result does not constituted an unsolvable problem, since in the carrier individuals it should have been observed the 84 bp band and the affected individuals it should only the 84 bp band. Therefore, the 84 bp band was not present because the mutation was not present, whereas the 17 bp band was not detectable due to its small size.

There are at least three explanations for the results found in this study. First, it was only possible to sample

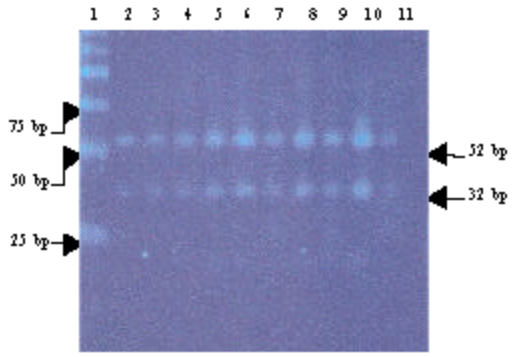

Fig. 1: Visualization of restriction fragments produced by the α*Taq*1 enzyme on the PCR products. Lane 1: Molecular weight marker. Lane 2-11: Bands of 52 and 32 bp can be observed.

cows grouped by their good health status, since the sick calves are kept by separate and are hidden from visitors. Therefore, it was very difficult to find animals with the disease. Second, it was not possible to analyze bulls, since in the herds analyzed there were only cows, which were serviced by AI, mostly by bulls kept in the USA and Canada. Third, the Holstein breeders are known to have one of the most rigorous record (both genealogical and production), as well as animal breeding programs, based in high quality AI bulls. Since, the USA had a detection program for BLAD carriers in Holstein animals, which allowed its frequency to drop to zero (Nagahata, 2004), it could be expected that the Mexican Holstein population, descendant of this animals, have also a very low or inexistent frequency of BLAD.

Similar results have been reported for countries such as Japan, where a frequency of 8.1% was found (Nagahata *et al*., 1997), whereas the overall frequency was 2.28% in Argentina (Poli *et al*., 1996). In Germany, the mutation rate declined from 11.6-9.9% (Tammen *et al*., 1996) and from 23-0% in the USA (Powell *et al*., 1996). No homozygotes with the mutation were found in Ukrania (Kostetskii *et al*., 1996). Also, other recent studies have found a very low or zero BLAD frequency in countries like Iran (Norouzy *et al*., 2005), Turkey (Akyuz and Ertugrul, 2006), India (Patel *et al*., 2007), Poland (Czarnik *et al*., 2007) and Germany (Schutz *et al*., 2008).

CONCLUSION

In agreement with other studies, the frequency found of BLAD in Northeast Mexico was very low in this case probably of zero. This result may indicate that because Mexican Holstein breeders use genetic material coming from the USA, country in which BLAD has been

eliminated, the disease has also been eradicated in Mexico. This led us to conclude that genetic improvements programs aimed to detect animals carrying prejudicial mutations have a large positive impact in the animal industry and must be implemented for other genetic diseases.

REFERENCES

Akyuz, B. and O. Ertugrul, 2006. Detection of Bovine Leukocyte Adhesion Deficiency (BLAD) in Turkish natives and Holstein cattle. Acta Vet. Hung., 54 (2): 173-178. PMID: 16841755.

Batt, C.A., P. Wagner, M. Wuedman, J. Luo and R. Gilbert, 1994. Detection of bovine leukocyte adhesion deficiency by nonisotopic ligase chain reaction. Anim. Genet., 25 (2): 95-98. PMID: 7912052.

Czarnik, U., G. Grzybowski, S. Kamiski, B. Prusak and T. Zabolewicz, 2007. Effectiveness of a program aimed at the elimination of BLAD-carrier bulls from Polish Holstein-Friesian cattle. J. Appl. Genet., 48 (4): 375-377. PMID: 17998595.

Kostetskii, I.E., S.M. Kirilenko, V.I. Glazko and A.A. Sozinov, 1996. A diagnostic analysis of a genetic mutation associated with a deficiency of leukocyte adhesion in cattle. Tsitol. Genet., 30 (2): 61-64. PMID: 9281202.

Li, Y.H., S.L. Zhang, G.W. Han, J.H. Xue and P. Zhao, 2007. A method for detecting bovine leukocyte adhesion deficiency in Holstein calves using polymerase chain reaction-single strand conformation polymorphism analysis. Yi Chuan, 29 (12): 1471-1474. PMID: 18065382.

Nagahata, H., H. Nochi, K. Tamoto, H. Taniyama, H. Noda, M. Morita, M. Kanamaki and G.J. Kociba, 1993. Bovine leukocyte adhesion deficiency in Holstein cattle. Can. J. Vet. Res., 57 (4): 255-261. PMID: 7903594.

Nagahata, H., T. Miura, K. Tagaki, M. Ohtake, H. Noda, T. Yasuda and K. Nioka, 1997. Prevalence and allele frequency estimation of Bovine Leukocyte Adhesión Deficiency (BLAD) in Holstein-Friesian cattle from Japan. J. Vet. Med. Sci., 59 (4): 233-238. PMID: 9152929.

Nagahata, H., 2004. Bovine Leukocyte Adhesion Deficiency (BLAD): A review. J. Vet. Med. Sci., 66 (12): 1475-1482. PMID: 15644595.

Norouzy, A., M.R. Nassiry, F. Eftekhari Shahrody, A. Javadmanesh, M.R. Mohammad Abadi and G.E. Sulimova, 2005. Identification of Bovine Leucocyte Adhesion Deficiency (BLAD) carriers in Holstein and Brown Swiss AI bulls in Iran. Genetica, 41 (12): 1697-1701. PMID: 16396457.

Patel, R.K., K.M. Sing, K.J. Soni, J.B. Chauhan and K.R. Sambasiva Rao, 2007. Low incidence of Bovine Leukocyte Adhesion Deficiency (BLAD) carriers in Indian cattle and buffalo breeds. J. Appl. Genet., 48 (2): 153-155. PMID: 17495349.

Poli, M.A., R. Dewey, L. Semorile, M.E. Lozano, C.G. Albariño, V. Romanowski and O. Grau, 1996. PCR screening for carriers of Bovine Leukocyte Adhesion Deficiency (BLAD) and Uridine Monophosphate Synthase (DUMPS) in Argentina Holstein cattle. Zentralbl Veterinarmed A., 43 (3): 163-168. PMID: 8693839.

Powell, R.L., H.D. Norman and C.M. Cowan, 1996. Relationship of bovine leukocyte adhesion deficiency with genetic merit for performance traits. J. Dairy Sci., 79 (5): 895-899. PMID: 8792288.

Schutz, E., M. Scharfenstein and B. Breing, 2008. Implication of complex vertebral malformation and bovine leukocyte adhesiohn deficiency DNA-based testing on disease frequency in the Holstein population. J. Dairy Sci., 91 (12): 4854-4859. PMID: 19038961.

Tammen, H., A. Kuczka and A. Trevieranus, 1996. An improved DNA test for bovine leukocyte adhesion deficiency. Res. Vet. Sci., 60: 218-221. PMID: 8735510.