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Bovine Leukocyte Adhesion Deficiency in Danish Holstein-Friesian Cattle
II. Patho-Anatomical Description of Affected Calves

By *J. S. Agerholm, H. Houe, C. B. Jørgensen* and *A. Basse*

Department of Pathology and Epidemiology, National Veterinary Laboratory, Department of Clinical Studies, Division of Large Animal Medicine, Department of Animal Science and Animal Health, Division of Animal Genetics, and Department of Pharmacology and Pathobiology, Laboratory of Veterinary Pathology, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Agerholm, J.S., H. Houe, C.B. Jørgensen and A. Basse: Bovine leukocyte adhesion deficiency in Danish Holstein-Friesian cattle II. Patho-anatomical description of affected calves. Acta vet. scand. 1993, 34, 237-243. – The patho-anatomical findings in 3 Danish Holstein-Friesian calves affected with bovine leukocyte adhesion deficiency (BLAD) are described. The diagnosis was confirmed by genotyping for the BLAD mutagene by polymerase chain reaction technique. The main clinical symptoms were general unthriftyness and leukocytosis with a high proportion of neutrophils. None of the calves suffered from severe infections and the major pathological changes were different from those described in previously published cases. One calf had a fibro-granulomatous perilienitis and calcification of splenic stroma and pulmonic arteries. In the other cases only minor pathological changes were present.

BLAD; pathology; hereditary disease.

Introduction

Bovine leukocyte adhesion deficiency (BLAD) is a hereditary disease occurring in Holstein-Friesian cattle. The disease is due to a single base mutation in the gene coding for CD18 (*Shuster et al.* 1992). This results in a deficiency of the CD11b/CD18 glycoprotein complex located on the surface of neutrophil granulocytes (*Kehrli et al.* 1990). This complex is required for neutrophil migration through vascular endothelium into the extravascular tissue. Therefore, neutrophils with lack of CD11b/CD18 are unable to migrate to inflammatoric sites. Furthermore, CD11b/CD18 deficient neutrophils have shown other dysfunctions in several *in vitro* tests (*Kehrli et al.* 1990, *Nagahata et al.* 1987).

The clinical symptoms and pathological changes are related to the impaired neutrophil function and are mainly those of recurrent infections, reduced growth, and unthriftyness (*Takahashi et al.* 1987).

Cases of BLAD occur in a familiar pattern compatible with an autosomal recessive mode of inheritance (*Kehrli et al.* 1990, 1992). Several important American Holstein-Friesian bulls have been identified as carriers of the BLAD mutagene, and the mutagene has been spread to many countries, including Denmark through export of semen from these bulls (*Kehrli et al.* 1992, *Nielsen et al.* 1992).

This article describes the patho-anatomical findings in BLAD affected Holstein-Friesian calves in Denmark.

Materials and methods

Reports on unthrifty calves of the Danish Holstein-Friesian breed with a familiar relationship to known BLAD carrier bulls on both the maternal and paternal side were obtained from breeders. Blood samples were collected from calves, which were suspicious of having BLAD, and analyzed by polymerase chain reaction (PCR) technique to determine, if they were homozygous for the BLAD mutagene (*Jørgensen et al.* 1993). Homozygous calves were examined clinically and haematologically. Within 1 to 2 weeks after admission to the clinic, the calves were euthanized by intravenous injection of pentobarbital sodium. The calves were necropsied, and tissue samples from visceral organs and all macroscopic recognized pathological changes were taken. The tissue samples were fixed in 10% buffered neutral formalin, paraffine embedded, sliced 6µm, and haematoxylin-eosin stained. Additional stainings including Periodic Acid Schiff (PAS), May-Grunewald Giemsa, Grocott's Methenamine-Silver, and Kossa's method for calcium were applicated when pathological changes were present.

In connection to necropsy standard microbiological investigations were performed. These included 1) bacteriology on spleen, lung, intestinal lymph node, intestinal content, and tissues with pathological changes, 2) virological examination for bovine virus diarrhoea virus in lung and spleen, and bovine respiratory syncytial virus, parainfluenza-3 virus, and bovine adeno virus in the lung, 3) examination for mycoplasmas in the lung and 4) examination for intestinal parasites.

Results

Eight calves were found to be homozygous for the BLAD mutation by PCR analysis (*Jørgensen et al.* 1993). However, only 3 of these were available for clinical examination and necropsy. These calves, 2 eight-month-old male calves (case 1 and 2) and a one-month-

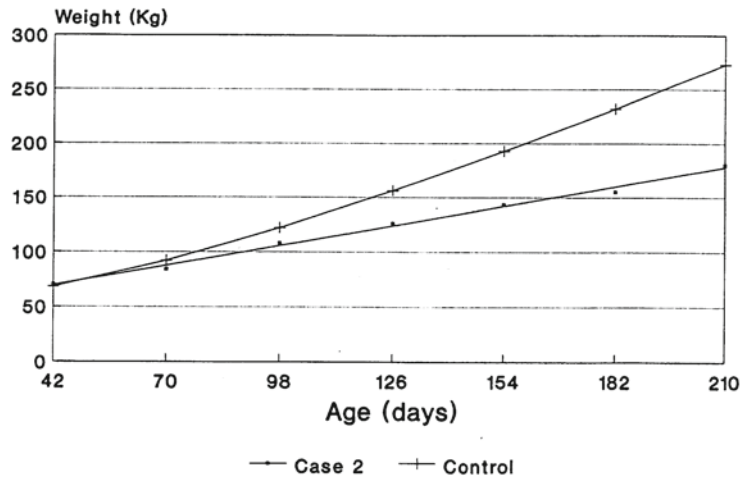

Figure 1. Development in body weight of a bovine leukocyte adhesion deficiency affected calf (case 2) compared to normal Danish Holstein-Friesian calves kept under the same management conditions. ■ affected calf, + control calves.

Table 1. Haematological findings in 3 calves affected with bovine leukocyte adhesion deficiency.

	Reference interval[1]	Case 1	Case 2	Case 3
Packed cell volume (%)	24-46	46	36	36
Total erythrocyte count (x 10^{12}/L)	5-10	7.46	5.57	5.60
Total leukocyte count (x 10^{9}/L)	4-12	61.60	66.00	54.20
Hemoglobin (mmol/L)	4.9 - 9.2	9.40	7.30	6.90
Mean erythrocyte cell volume (fl)	40 - 60	61.66	64.63	64.29
Lymphocytes (%)	45 - 75	21.0	11.0	-
Monocytes (%)	2 - 7	4.0	3.5	-
Neutrophils (%)	15 - 47	73.0	84.0	-
Eosinophils (%)	0 - 20	1.5	1.5	-
Basophils (%)	0 - 2	0.5	0	-
Serum protein concentration (G/L)	67.4 - 74.6[2]	85	93	-

1: According to *Jain,* 1986; 2: according to *Kaneko,* 1989.

old female (case 3), were reported due to unthriftyness or reduced growth rate. One of the calves (case 2) was obtained from a performance test station where it had been weighed monthly during 7 months. The growth rate compared to other Holstein-Friesian male calves (general average at the station) is shown in Fig. 1.

Blood samples from the calves were collected on arrival at the clinic and a routine haematological examination was performed (Table 1). On clinical examination all calves were found to be generally unthrifty with a more or less reduced physical development and in two cases (1 and 2) relatively large heads (Fig. 2). Necropsy was performed on all 3 calves. Case

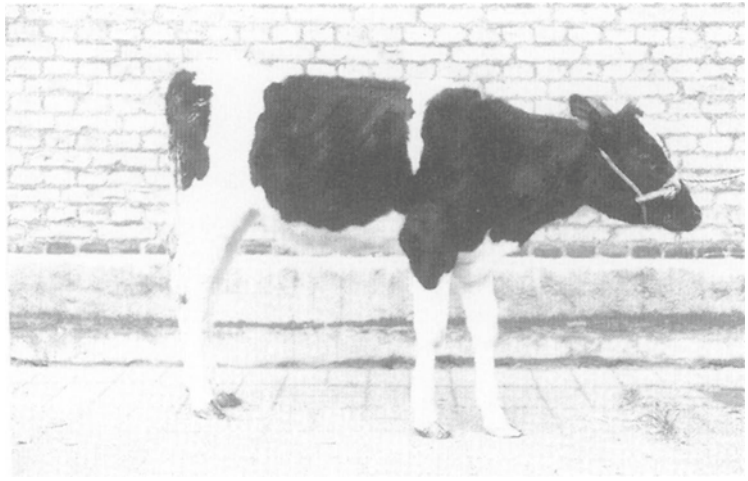

Figure 2. Eight month old male calf (case 1) affected with bovine leukocyte adhesion deficiency. Notice the unthrifty appearance and disproportional development.

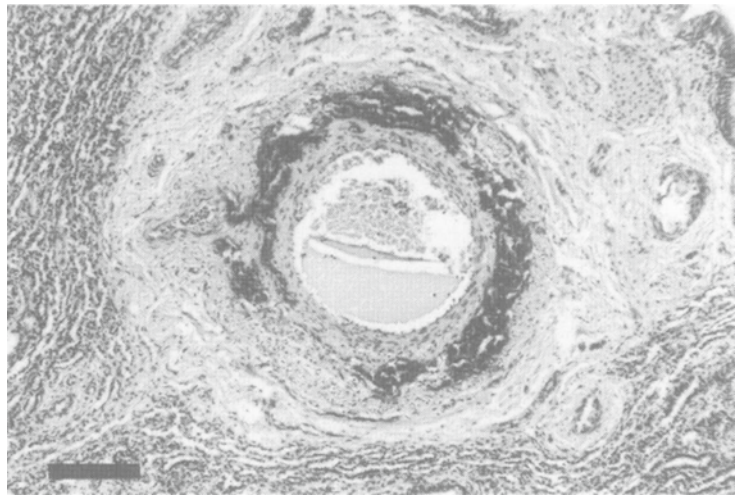

Figure 3. Calcification of pulmonic arteria in a calf with bovine leukocyte adhesion deficiency. Haematoxylin-eosin. Bar = 200 µm.

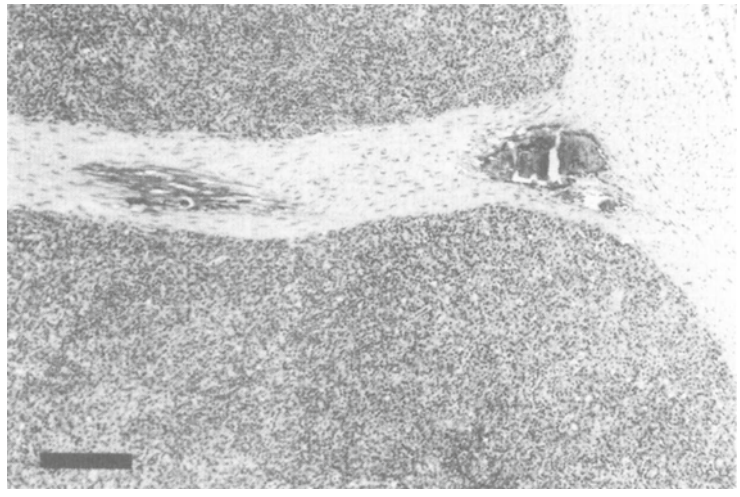

Figure 4. Calcification of splenic stroma in a calf with bovine leukocyte adhesion deficiency. Haematoxylin-eosin. Bar = 200 µm.

1 had multiple up to approx. 1 cm large areas of alopecia on the head composed of orthokeratotic hyperkeratosis and dermatitis with subepithelial and intraepidermal infiltration of lympho- and monocytes. Mycotic hyphae could not be demonstrated. At the sternal region a minor decubital ulcus was present. The ulcus had superficial necrosis with cellular in-

filtration and bleeding. Neutrophils were only present in areas of bleeding. In cavum nasi multiple necrotic ulcers with striking lack of neutrophil infiltration were present. In the lungs a minor area of bronchopneumonia and fibrotic pleuritis was present in the ventral parts of the cranial lobes. The changes consisted of pulmonic interstitial fibrosis, atelectasis, and proliferation of interstitial lymphatic nodules. Furthermore, arterial calcification involving both tunica media and externa was present (Fig. 3). Extensive calcification was also present throughout the splenic stroma (Fig. 4). In addition, a chronic fibrogranulomatous perilienitis with giant cells and macrophages containing hemosiderin was found. In the splenic red pulp many neutrophils could be observed. Additional minor changes were detected including superficial focal reticulitis, hepatic lymphocytic accumulations, and focal calcification in the thymus.

Case 2 showed small areas of dermatomycosis on the left shoulder region and ear rims. On histological examination these areas had extensive orthokeratotic hyperkeratosis with mycotic hyphae, hyperplasia, rete-ridge formation, and intraepidermal abscesses containing mainly lymphocytes. In the vascular structures many neutrophils could be observed. In the ventral parts of the cranial pulmonic lobes, a bronchopneumonia with fibrosis, atelectasis, and lymphocytic proliferation was present. At the right metatarsus a diffuse haemorrhagic-oedematous inflammation was found. Additional minor changes were present. These included multiple foci of abomasitis due to the presence of straw material in abomasal pits resulting in necrosis and lymphocytic reaction, neutrophil accumulation in splenic red pulp and hepatic sinusoides, focal pyelitis and multiple tubular foci of calcification in the kidneys, and paracortical hyperplasia of the intestinal lymph nodes.

In case 3 only minor lesions were found. On the head focal areas of orthokeratotic hyperkeratosis and hyperplasia were present. In the cranial pulmonic lobes, few lobuli with atelectasis were detected, and like the 2 other cases, many neutrophils could be observed in the splenic red pulp.

On bacteriological examination of the lung, spleen, intestinal lymph node, and intestinal content, these were either sterile or only with a normal bacterial flora. Inflamed tissue from the metatarsus (case 2) was sterile. *Actinomyces pyogenes, Pasteurella multocida,* and *Clostridium perfringens* type A were isolated from the nasal necrosis (case 1). Mycoplasmas or virus were not detected in any case. Intestinal parasites were only found in case 2 which had moderate subclinical infection with coccidia (500 oocysts/gram faeces).

Discussion

The general clinical appearance of the 3 BLAD affected calves was in some aspects similar to previously described cases. Unthriftyness which was present in all 3 cases seems to be a general unspecific finding in BLAD affected calves (*Stöber et al.* 1991, *Takahashi et al.* 1987). Fig. 1 shows that the reduced growth rate of case 2 already started at an age between 42 and 70 days, and that it decreased further during the first 210 days, so the difference between the body weight of the affected calf and the mean body weight of other Holstein-Friesian calves at the station was 93 kilo at the age of 210 days.

The severe leukocytosis in all 3 calves was due to an increased number of neutrophils. This is the primary haematological finding in BLAD affected calves, in which leukocytosis starts shortly after birth and can reach levels of more than 100×10^9 leukocytes /L (*Kehrli et al.* 1990, 1992).

All cases of previously described BLAD af-

fected calves suffered from severe bacterial infections including necrotic ileitis, in some cases with abscess formation, chronic pneumonia, ulceration and granulomatous inflammation of the oral cavity, periodontitis, and tooth loss (*Kehrli et al.* 1990, *Stöber et al.* 1991, *Takahashi et al.* 1987). These lesions are to some extent indicative for BLAD. However, none of these changes were present in the 3 cases described. Two calves had minor bronchopneumonic lesions, but only to a small extent without any clinical significance. It is therefore obvious that some calves can live for a longer period without developing severe infections, even under normal field conditions.

Extensive dermatomycosis has been described in a BLAD affected calf (*Stöber et al.* 1991). Dermatomycosis was also present in case 2, but only to an extent often seen in calves. However, the histopathological changes were to some extent different from normal findings, as there was a lack of neutrophil reaction, and as the epidermal microabscesses contained mainly lymphocytes.

The subcutaneous inflammation found on the right hind limb of case 2 was probably due to a trauma, even though major cutaneous lesions were absent. However, extensive subcutaneous inflammation has been described in other cases in connection to bone marrow biopsies (*Hagemoser et al.* 1983, *Stöber et al.* 1991). Therefore, BLAD affected calves seem predisposed for developing extensive subcutaneous inflammation, even in connection to minor lesions.

Soft tissue calcification occurred mainly in case 1, in which both pulmonic arteries and splenic stroma were affected. Calcification was also present in renal tubuli of case 2, while connective tissue calcification was not found in case 3. However, this could be due to the young age of this calf. Calcification of splenic stroma in BLAD affected calves has previously been described (*Stöber et al.* 1991, *Takahashi et al.* 1987), and seems to be a common histopathological finding. However, vascular, tubular, and thymal calcification has not been described previously. Especially vascular calcification is of importance as this condition may predispose for vascular rupture and eventually lethal internal haemorrhage. The specificity of vascular calcification is however uncertain, as this condition also occurs in connection to some general chronic debilitating diseases in cattle (*Jubb et al.* 1985).

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Sammendrag

Bovine leukocyte adhesion deficiency hos Sortbroget Dansk Malkerace.
II Pato-anatomisk beskrivelse af afficerede kalve.

Pato-anatomiske forandringer hos tre kalve af Sortbroget Dansk Malkerace afficeret af bovine leukocyte adhesion deficiency (BLAD) beskrives. De kliniske symptomer bestod generelt i utrivelighed og leukocytose med en høj andel af neutrofile granulocytter. Genotypning for BLAD mutagenet blev foretaget med polymerase chain reaction teknik. Ingen af kalvene havde alvorlige infektioner, og de patologiske forandringer var anderledes end beskrevet i tidligere diagnosticerede tilfælde. En kalv havde fibro-granulomatøs perilienitis samt forkalkning af miltens stroma og lungearterier. I de øvrige tilfælde fandtes kun lettere patologiske forandringer.

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Reprints may be requested from: J.S. Agerholm, Department of Pathology and Epidemiology, National Veterinary Laboratory, Bülowsvej 27, DK-1790 Copenhagen V, Denmark.