Hereditary zinc deficiency syndrome in a calf

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SUMMARY

Bovine Hereditary Zinc Deficiency (BHZD) is an autosomal recessive disorder. A twin calf with signs consistent with BHZD. The coat was dull. Due to the typical skin lesions veterinary treatment with zinc was started. During treatment the skin lesions did not get worse but a recovery was not gained either. The lesions got bigger when the treatment was interrupted. The co-twin calf did not show any signs of BHZD. After amplifying a product of 294 base pair (bp) including the putatively causal SLC39A4 mutation the product was incubated with the restriction enzyme HpHI. A 148- and 147-bp product could be detected which was expected for the wildtype sequence. A 337-bp product including the putative PLD4 causal mutation was amplified. The G to A mutation (rs378824791) leads to the loss of the restriction site of the enzyme HpyCH4III. The putative causal mutation was not proven using this restriction fragment length polymorphism (RFLP). Further sequence analyses are needed to clarify the mutation causing BHZD in the present case. The present case also shows that there might be further mutations in Fleckvieh cattle responsible for BHZD.

KEY WORDS

BHZD; Cattle; PLD4; SLC39A4.

INTRODUCTION

Bovine Hereditary Zinc Deficiency (BHZD) is an autosomal recessive disorder. Due to this condition zinc is inadequately absorbed by the intestines. Impressive consequences are severe skin lesions in mainly mechanically stressed areas[1]. The disease is also known as hereditary parakeratosis, Adema disease or lethal trait A46[1]. Affected Holstein calves are born without clinical symptoms. Clinical abnormalities manifest at the age of four to eight weeks. These include loss of epidermis associated with desquamation and incrustation. At the beginning these lesions can be found especially around the muzzle, peri-ocular and auricular[1]. Furthermore, alopecia, growth retardation and insufficiency of the immune system can be seen[2]. At an advanced stage of the disease, the affected animals show decreased food intake and are dejected and dehydrated[1]. Histological examination reveals acantholysis in the stratum spinosum and persistent cell nuclei. Beside these findings, affected animals are emaciated and show hypotrophy of thymus, spleen and lymph nodes[1].

In Holstein cattle a causal mutation in SLC39A4 has been identified[3]. A G>A SNP (single nucleotide polymorphism) in intron 10 leads to a deletion of exon 10 of SLC39A4. BHZD in Holstein calves resulting from mutations in SLC39A4 can be ameliorated by highly-dosed oral zinc supplementation.

In contrast, affected Fleckvieh calves did not respond to oral zinc supplementation[4]. In Fleckvieh calves with signs similar to BHZD, Jung et al. (2014) found a putatively causal mutation (rs378824791) in the phospholipase D family member 4 encoding gene (PLD4) using genome-wide re-sequencing of the affected Fleckvieh calves. The mutation is predicted to lead to a premature stop codon with a shortened protein and disturbed protein activity. In addition, zinc deficiency-like (ZDL) syndrome is an inherited defect of Fleckvieh calves, with striking similarity to BHZD in a other study. However, the causative mutation in a phospholipase D4 encoding gene (PLD4) shows no connection to zinc metabolism[5].

MATERIALS AND METHODS

A twin calf with signs consistent with BHZD was submitted to the Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover. It descended from a Fleckvieh cow and a Blonde d'Aquitaine bull. The full pedigree of the affected calf could not be obtained. The Blonde d'Aquitaine bull was not known to have sired calves with hereditary diseases. The Fleckvieh cow had several calvings with normal calves.

At the beginning the twin calves developed equally. After four weeks one of the calves showed skin lesions, growth retardation and reduced feed intake. Its behaviour was very calm.

Genomic DNA was isolated using 500 µl EDTA blood by standard ethanol fraction. Precipitation was achieved by 6 M NaCl, 70% ethanol, and 100% ethanol (Carl Roth) in consecutive steps according to standard protocols. To identify the putatively causal mutations in PLD4 and SLC39A4 primer pairs based on the cow reference sequences of Ensembl and NCBI in UMD3.1 were designed.

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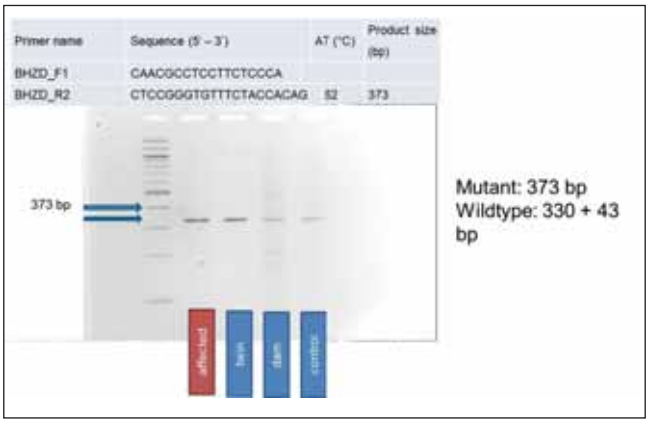

Table 1 - Primer sequences for amplification the BHZD-associated mutation within PLD4 using the restriction enzyme HpyCH4III.

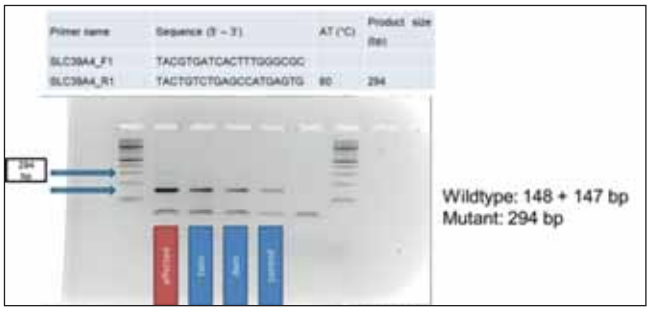

Table 2 - Primer sequences for amplification the BHZD-associated mutation within SLC39A4 using the restriction enzyme HpHI.

The amplified product of PLD4 had a size of 373 base pairs (bp). The product was incubated with the restriction enzyme HpyCH4III overnight at 37 °C. In the amplicon with the mutation (rs378824791) the enzyme will not produce any restriction fragment. In a PCR-product without the mutation, restriction fragments of 43 bp and 330 bp can be detected (Table 1).

The restriction digestion with HpyCH4III was analyzed on a 3% agarose gel stained with ethidium bromide. The amplified product of SLC39A4 had a size of 294 bp. This product was incubated with the restriction enzyme HpHI overnight at 37°C. In a PCR-product without the mutation, restriction fragments of 148 bp and 147 bp can be detected (Table 2). The mutation leads to a loss of the restriction site. The restriction digestion with HpHI was analyzed on a 2% agarose gel stained with ethidium bromide.

RESULTS

The calf showed a very calm behaviour (Figure 1). The coat was dull. Due to the typical skin lesions veterinary treatment with zinc was started. During treatment the skin lesions did not get worse but a recovery was not gained either. By owner information the lesions got bigger when the treatment was interrupted. The twin calf did not show any signs of BHZD. Blood samples of the affected animal, its twin and the dam were taken. After euthanasia the affected calf was examined in the Institute of Pathology. Respiratory (10 per minute) and heart rate (54 per minute) were slightly below the reference range. Mucous membranes were bright. The slightly sunken eyes indicated a light dehydrated condition. The lymph nodes (lnn.) subiliaci and cervicales superficiales were slightly enlarged. Caudal the left olecranon a 25 x 15 centimetres (cm) alopecic area with loss of epithelium was found. Cranially the shoulder joint a barky and scaly skin lesion with a diameter of 1 cm was found. In the inguinal region the skin was alopecic and scaly in a 30

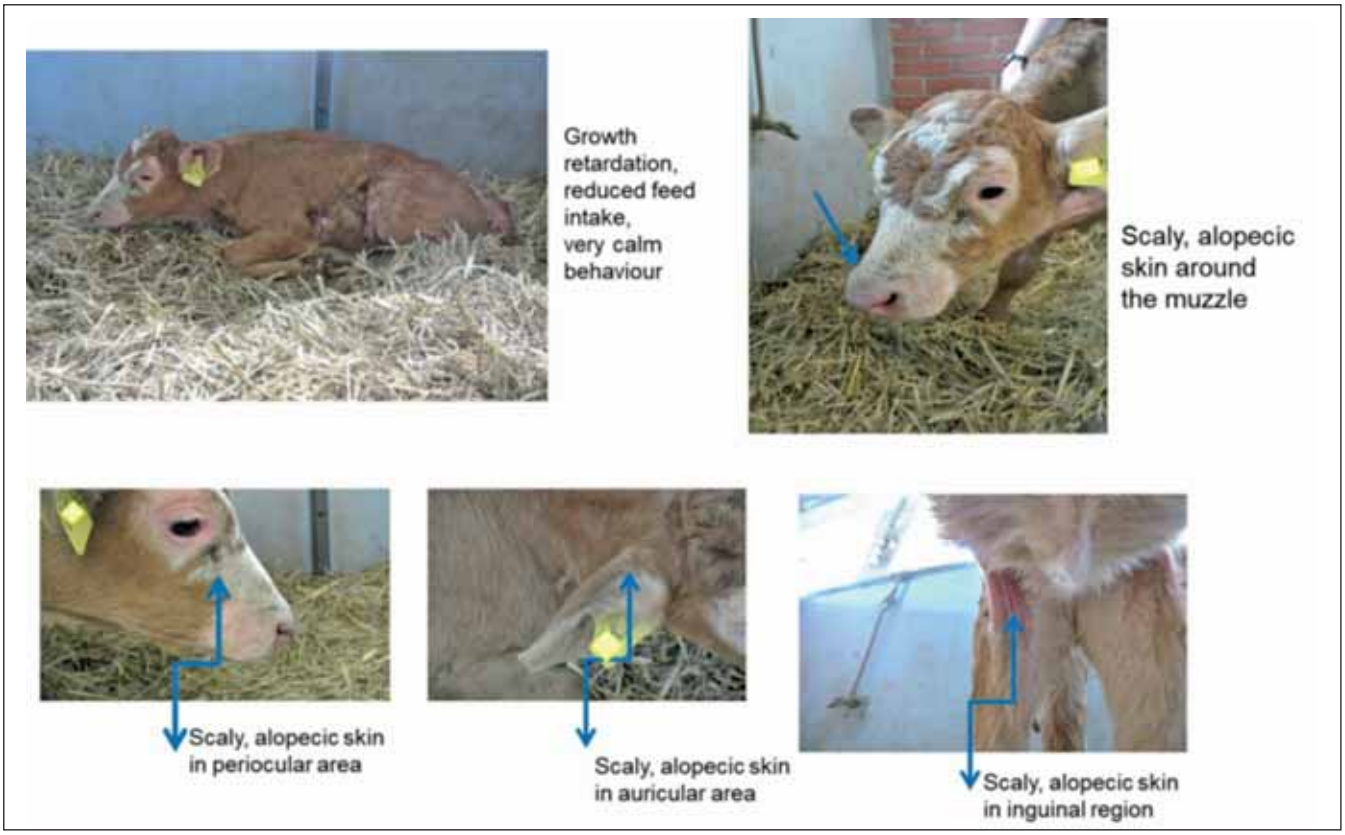

Figure 1 - A twin calf with signs consistent with BHZD.

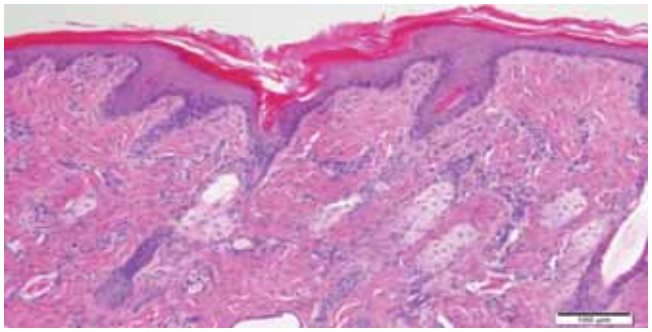

Figure 2 - Haematoxylin and eosin-stained skin section from the shoulder region of fore leg with hyperplastic epidermis and marked parakeratotic hyperkeratosis.

x 20 cm large area. Alopecic and scaly skin surrounded anus and vulva. The muzzle was surrounded by scaly and alopecic skin with a width of 0.5 cm (Figure 2). The skin over the entire body appeared scaly and exfoliated. It was fed with milk substitute. Hey, water and electrolytes were available ad libitum. The feed intake was very poor.

Laboratory analysis of blood showed a slight haemoconcentration. Although high doses of zinc were supplemented, the zinc level was at the lower limit of reference range. Because of the bad condition it was euthanized at the age of 61 days.

Histopathological examination of the present calf revealed infiltration of lnn. mesenterici, thymus, abomasum and small intestine with eosinophilic granulocytes. In addition, a purulent inflammation of the abomasum was found. The thymus had a size of 18 x 5 x 2 cm. All skin lesions were characterized by a moderate parakeratotic hyperkeratosis with spongiosis (Figure 2). Furthermore, a perivascular to diffuse dermatitis was present. Lymphocytes, macrophages, eosinophil granulocytes and plasma cells, in some cases with Russell bodies, were involved in this process. The epidermis was slightly hyperplastic (Figure 2). In the skin of the forelimb the excretory ducts were slightly dilated.

DISCUSSION

Affected calves in previous reports can be recomposed by permanent practice of complementary zinc given at high doses[2,6]. In our calf, due to the typical skin lesions veterinary treatment with zinc was started. During treatment the skin lesions did not get worse but a recovery was not gained either.

Lesions are mostly described by parakeratosis and dermatitis, and occur in areas of permanent skin flexion or in areas especially subjected to erosion. Hence, lesions are most extensive around the mouth, eyes, base of the ear, joints, and lower parts of the thorax, abdomen and limbs[7]. Similar to these symptoms, the table appears.

Parakeratosis and ulceration of the intestinal tract occurs and is usually meant clinically as stomatitis. Therefore, a decrease in eating capacity and growth retardation seen[1]. Similarly, growth retardation, reduced feed intake, very calm behaviour are the most obvious findings in this study.

Lymph nodes, thymus and gut-associated lymphoid tissue are hypoplastic, and affected animals are immunosuppressed due to impaired function of the immune system[7,8,9]. Animals often develop bronchopneumonia. Lesions are progressive and, if left untreated, calves die within 4 to 8 weeks after primary symptoms are observed[10]. In this study, due to typical skin lesions veterinary treatment with zinc was started. During treatment the skin lesions did not get worse but recovery was not gained either. The lesions became larger when the treatment was interrupted.

Mutations in SLC39A4 are known to cause defects resembling the phenotypic appearance of the present case in various species including cattle[3]. After amplifying a product of 294 base pair (bp) including the putatively causal mutation the product was incubated with the restriction enzyme HpHI. A 148- and 147-bp product could be detected which was expected for the wild-type sequence. The causal mutation for BHZD in Holstein cattle was not detected.

In Fleckvieh, a causative nonsense mutation in a PLD4 was identified[4]. A 337-bp product including the putative causal mutation was amplified. The G to A mutation (rs378824791) leads to the loss of the restriction site of the enzyme HpyCH4III. The putative causal mutation was not proven using this restriction fragment length polymorphism (RFLP). In addition, zinc deficiency-like (ZDL) syndrome is an inherited defect of Fleckvieh calves, with striking similarity to BHZD in a other study[5].

CONCLUSIONS

Further sequence analyses are needed to clarify the mutation causing BHZD in the present case. The present case also shows that there might be further mutations in Fleckvieh cattle responsible for BHZD.

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