



CASE REPORT

Autosomal Cholesterol Deficiency in a Holstein Calf

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ABSTRACT

Cholesterol deficiency (CD) is an autosomal recessive defect in Holstein cattle caused by a mutation in the apolipoprotein B gene (*APOB*). This paper reports the clinical and pathological phenotype of a case of CD in a 5-months-old Holstein calf. Retarded growth, chronic, intermittent diarrhea, stomatitis, hypocholesterolemia and low blood triglycerides concentrations were the most important clinical and ancillary findings. Histopathologically, inflammation of the digestive organs was the most evident sign. Blood from the patient, the dam, sister1, sister2 and semen of the sire were tested for *APOB* mutation: the calf resulted homozygous, whereas the dam and the sire resulted heterozygous carriers. Both sisters were *APOB* mutation free. Cholesterol deficiency should be considered in the differential diagnosis of chronic diarrhea and failure to thrive in Holstein calves.

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INTRODUCTION

Cholesterol deficiency (CD) is an autosomal recessive genetic defect in Holstein cattle (Menzi *et al.*, 2016). The affected animals show unresponsive diarrhea, buccal lesions and retarded growth of unknown etiology and suffer hypocholesterolemia indicating a fat metabolism disorder (Mock *et al.*, 2016). Calves do not respond to symptomatic treatment and usually die within the first 6 months of their life (Kipp *et al.*, 2016). The causative mutation has been identified in exon 5 of the apolipoprotein B gene (*APOB*) (Charlier, 2016). The lack of *APOB* in homozygous mutant animals provokes a malabsorption of dietary fat and fat-soluble vitamins in the intestine and is assumed to impair cholesterol metabolism and transport in blood circulation and liver (Gross *et al.*, 2016). The genetic test allows the detection of animals with CD without pedigree information (Menzi *et al.*, 2016).

In humans, truncating mutations in *APOB* give rise to Human Familial Hypobetalipoproteinemia (FHBL) and homozygous show: steatorrhea, neurological dysfunction, vision problems, and non-alcoholic fatty liver (Welty, 2014).

CLINICAL CASE

History: A 5-months-old female Holstein calf was admitted to teaching hospital of the Department of Veterinary Medical Sciences of the University of Bologna

for in-depth clinical study due to a history of failure to thrive, intermittent diarrhea and progressive emaciation.

Clinical examination: The calf presented a reduced skeletal development if compared to cohort animals, was cachectic and showed muscular hypotonia. Difficulty with mastication was accompanied by sialorrhea and signs of buccal pain. The calf revealed gingival, sub-lingual and palatine lesions compatible with fibrinous-ulcerative stomatitis (Fig. 1). Body temperature was within the normal limits, whereas pulse and respiratory rates were slightly increased (100 bpm and 48 rpm, respectively). In addition, pulmonary auscultation revealed a moderate increase of the vesicular lung sound. A profuse foamy diarrhea increased in quantity and frequency; undigested material remained constantly present for the entire period of observation at the teaching hospital. Despite supportive treatment, the animal died 33 days after admission.

Ancillary diagnostic: Blood samples for cells count and clinical biochemistry were collected from the calf, the dam and two maternal half-sisters. Severe hypocholesterolemia (2.0 mg/dl; reference range: 80-120 mg/dl) and hypotriglyceridemia (1.0 mg/dl; reference range: 12-31 mg/dl) were the most important findings in the calf. Although not so extreme, also the dam showed hypocholesterolemia (62 mg/dl; reference range: 80-120 mg/dl), whereas the two half-sisters had blood cholesterol values within the normal range.



Fig. 1: Fibrinous-ulcerative gingivitis. Note the hemorrhagic background partially covered by an adherent and detachable yellowish to pink membrane. *Candida albicans* was isolated from the buccal swab.



Fig. 2: Esophagitis. Note the ulceration of the cranial third of the esophageal mucosa with exposing of the submucosa and a marked tissue retraction.



Fig. 3: Segmental enteritis of the small intestine. Note the generalized congestion and hyperemia.

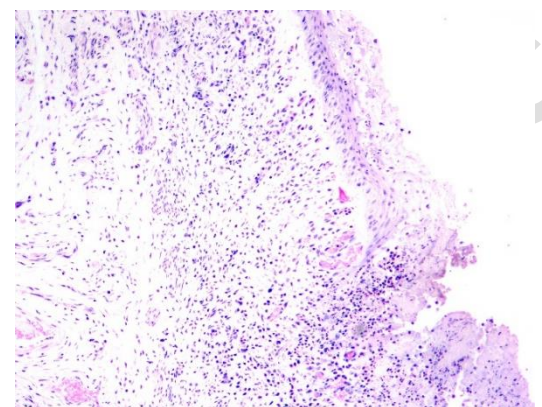


Fig. 4: Severe subacute lymphoplasmacytic esophagitis. Note the lack of the epithelium (ulcer). H&E 10X.

The calf showed also a slight anemia (hemoglobin 7.2 gr/dl) and hypoproteinemia (total protein 5.61 g/dl, with albumin corresponding to 1.96 g/dl). Calf's blood resulted negative for BVDV antigens and antibodies. In addition, calf's fecal sample resulted negative to *Eimeria* spp., *Cryptosporidium* spp. and to BVDV (RT-PCR). Diarrhea caused by dietetic management was excluded by clinical history.

Furthermore, a buccal swab and an endoscopy of the nasal cavity and larynx were performed in the patient: swab samples were positive to *Candida albicans*, whereas the endoscopy revealed a slight rhinolaryngitis associated to a severe tracheitis.

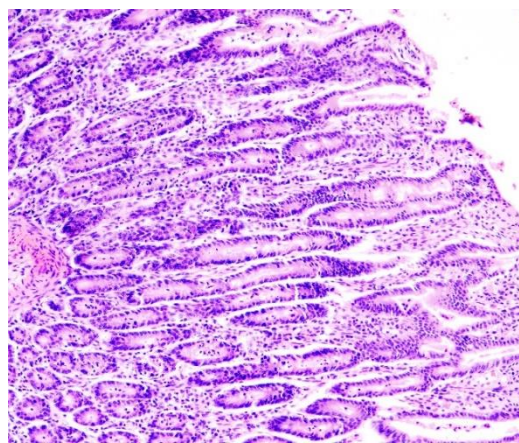


Fig. 5: Moderate acute duodenal enteritis. Note the cellular infiltration constituted mainly by lymphoplasmacytic cells but also by some neutrophil granulocytes. H&E 10X.

Postmortem findings: At gross examination, the animal showed a poor corporal condition (1.5/5), exhibiting scarce subcutaneous adipose tissue. The perineal region and the pelvic limbs were defiled with feces. The buccal cavity showed a fibrinous-ulcerative stomatitis. A profound segmental ulceration of the cranial third of the esophageal mucosa with exposed submucosa and a marked tissue retraction was present (Fig. 2). The ulcerative areas were covered by fibrin. Furthermore, the intestine revealed a moderate amount of bright yellow, partially foamy to fatty content and presented a segmental enteritis (Fig. 3). A congestion of the serosa covering the colon was also present, as well as a slight perihepatitis. A laryngitis and a presence of slight deposits of greenish exudate at the level of the first rings of the trachea were observed. The brain presented hyperemia of meningeal vessels (arachnoid and pia mater). A slight cerebral edema was also noticed. Samples of the esophagus and intestine were fixed and processed for histology. In both organs the most remarkable findings indicated a lymphoplasmacytic inflammation of different grade of severity (Fig. 4 and 5).

Genetic analysis: Genomic DNA extracted from the blood samples of the affected calf, the dam, the two half-sisters, as well as from semen straw of the sire obtained by retail dealer was screened for the mutation of the *APOB*. The calf resulted homozygous for the *APOB* mutation, the dam and the sire heterozygous carrier, the half-sister homozygous wild type.

DISCUSSION

Although diarrheic syndrome may be considered quite a common condition in calves, the same cannot be said when it acquires a chronic course in the absence of evidence of parasitic infestations or inappropriate nutritional management. In these cases, CD, at least in the Holstein breed, should be included in the differential diagnosis. Moreover, the suspect does increase if the patient additionally presents stomatitis and failure to thrive.

Although of moderate intensity, the intermittent diarrhea, the retarded growth, the progressive emaciation and the buccal lesions presented by the calf of this paper, were in compliance with what described in patients affected by CD by Mock *et al.* (2016). Cachexia, muscular atrophy,

signs of diarrhea, intestine diffusely filled with a moderate amount of bright yellow, partially foamy to fatty content and enteritis were also described in other cases of CD by Kipp *et al.* (2016). The latter author reported also respiratory findings as in the here described animal.

Enteritis, failure to thrive, hypocholesterolemia and low TG concentration, are the characteristic signs of the human FHBL (Welty, 2014). In FHBL the malabsorption of lipid-soluble vitamins (A, D, E, K), leads to retinal degeneration, neuropathy, and coagulopathy (Lee and Hegele, 2014). Neurologic disorders are therefore consequences of cerebellar dysfunction and demyelination of the central and peripheral nervous systems (Lee and Hegele, 2014; Welty, 2014). Although the reported calf did not show any neurological signs, hyperemia of meningeal vessels and a slight cerebral edema were present supporting similar mechanisms.

In addition, also the presence of esophagitis and perihepatitis of the calf reminds of the FHBL (Lee and Hegele, 2014). Despite the absence of histological signs of fatty liver, the calf showed increased alkaline phosphatase and total bilirubin (both components, direct and indirect).

Kipp *et al.* (2016) reported that homozygous CD calves had markedly decreased concentrations of LDL-C consisting of cholesterol bound to APOB. Apolipoproteins form the structural proteins of lipoproteins allowing to transport lipophilic cholesterol and triacylglycerol in hydrophilic blood (Kipp *et al.*, 2016). APOB is the protein that binds cholesterol to form LDL-C and VLDL-C (Kipp *et al.*, 2016). In general, APOB-containing lipoproteins carry lipids from site of synthesis and site of absorption to various sites of utilization for energy production, storage, membrane assembly, or steroid hormone production (Marcovina and Packard, 2006). Unfortunately, in the described patient no data are available in respect to the values of LDL-C and VLDL-C.

In this study, total cholesterol was below reference values also in the dam: nevertheless, she did not present any clinical signs of malabsorption. Also Gross *et al.* (2016) observed no clinical signs of maldigestion in heterozygous carriers of the APOB mutation, both in calves and adult animals. This may conclude that despite lower plasma concentrations of TG and total cholesterol, heterozygous animals are apparently able to maintain cholesterol and lipoprotein homeostasis adequate for, e.g., steroid hormone biosynthesis and cell membrane function (Gross *et al.*, 2016). However, it might also be speculated that these effects might not be fully overt resulting in possible unspecific signs of reduced fertility, growth, and health (Gross *et al.*, 2016).

As cholesterol is an essential component of the reticulocyte membrane, red blood cells of affected animals might be more fragile than normal, explaining the low RBC, Hb, and Ht observed in the described case as well as by Inokuma *et al.* (2017) and Mock *et al.* (2016).

A protein-losing enteropathy might be the explanation for the observed hypoproteinemia and hypoalbuminemia, as also reported by Kipp *et al.* (2016).

In respect to the isolation of *Candida albicans* from the buccal swab and the slight rhino-laryngitis associated to the severe tracheitis, a secondary infection as a consequence of vitamins induced increased susceptibility may be postulated. Hypovitaminosis was shown to

decrease the resistance to infections in cattle (Xiuyuan *et al.*, 2012).

Unspecific clinical signs of diarrhea and failure to thrive in young calves are the main challenges for the diagnosis of CD in cattle. However, CD must be considered in the differential diagnosis in all case of unresponsiveness chronic course. The total cholesterol and TG evaluation is the first step for the diagnosis of CD that, however, can be definitely confirmed as inherited disease only by the genetic test. An early diagnosis of CD may prevent an ineffective utilization of antibiotics and eventually also an unnecessary and hopeless suffering of the diseased animal.

Conclusions: Despite the generality of the single clinical findings, a clinical picture including unresponsive chronic diarrhea, retarded growth and buccal lesions in Holstein calves should always address the clinical suspect also to the CD. The determination of blood cholesterol is decisive for the diagnosis, that may be etiologically confirmed by the genetic test for APOB mutation. If CD will be considered in the differential diagnosis of such a clinical pictures further cases of the defect may be expected in the future. Therefore, awareness of this fact is urged among breeders and veterinarians in order to identify possible carriers, thus preventing unnoticed spread of the anomaly in the Holstein populations.

Authors contribution: Conceptualization: AG, JJ. Formal analysis: AG, GM. Investigation: MB, GM, JJ, CD. Project administration: AG. Supervision: CD, AG. Validation: CD, AG. Writing – original draft: AG, JJ. Writing – review & editing: AG, GM. All authors critically revised the manuscript for important intellectual contents and approved the final version.

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