AUTOSOMAL RECESSIVE CHOLESTEROL DEFICIENCY IN A HOLSTEIN CALF

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To study the phenomena of disease without books is to sail an uncharted sea,

While to study books without patients is not to go to sea at all.

SIR WILLIAM OSLER
To: Mum, Dad, Roxy, Bispo, Quick, Branca, Nuno and Pipa for their love and support.
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Abstract

Cholesterol deficiency (CD), a newly identified autosomal recessive inherited genetic defect in Holstein cattle, has been reported to have unresponsive diarrhea as a clinical sign, failure to thrive, hypocholesterolemia and the animals usually die within the first weeks or months of life. CD is caused by a mutation of the bovine apolipoprotein B gene (APOB). The objective of the present report is to describe the clinical and pathological phenotype, understand the steps needed to perform a correct diagnosis and execute a treatment of the affected Holstein calf homozygous for the APOB mutation. One Holstein calf with clinical history of intermittent diarrhea and erosions in the buccal cavity was admitted to the Clinic for Ruminants of Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy. Furthermore, there was blood collected from 3 related healthy cows (mother, sister 1, sister 2) and semen from the father. This case report included a full clinical description of the clinical phenotype and pathological phenotype, blood hematological and biochemical analysis, and measurements of cholesterol and triglycerides (TG). The animal suffered a natural death 33 days after the admission to the clinic. A genetic test was performed as described by Menzi et al. (2016) using blood for sampling (affected calf, mother, sister 1, sister 2) and semen (father) to determine the APOB genotype. The calf was confirmed homozygous for the APOB mutation. The father and the mother, as expected, were heterozygous carriers of the APOB mutation and the sisters were free of the APOB mutation. The clinical phenotype of the affected calf included muscular atrophy, retarded growth, and chronic diarrhea. Hypocholesterolemia and low TG concentrations was present in the affected calf. Additionally, the cholesterol concentration of the mother of the affected calf was also lower. The pathological phenotype of homozygous calf was steatorrhea with a segmental enteritis. Although the animal, whilst alive, did not present neurological signs, the brain presented hyperemia of meningeal vessels and a slight cerebral edema. CD must be considered as a possible differential diagnosis for chronic diarrhea and failure to thrive in Holstein calves with no evidences of primary infections. Confirmation of the associated APOB mutation is needed.

Key words: Apolipoprotein B; Diarrhea; Cholesterol Deficiency; Cattle; Holstein
List of Abbreviations, Acronyms and Symbols

**APOB** – Apolipoprotein B gene
**APOB** – Apolipoprotein B protein
**bp** – Base pairs
**BTA** – Bovine Chromosome
**BVDV** – Bovine Viral Diarrhea Virus
**CD** – Cholesterol Deficiency
**CDH** – Cholesterol Deficiency Haplotype
**cells/IL** – cells per interleukins
**cm** – centimeter
**EDTA** – Ethylenediaminetetraacetic Acid
**FHBL** – Familial Hypobetalipoproteinemia
**GGT** – Gamma-Glutamyl Transferase
**GWAS** – Genome-Wide Association Study
**Hb** – Hemoglobin
**HDL** – High Density Lipoproteins
**HE** – Hematoxylin and Eosin Stain
**Ht** – Hematocrit
**IU** – International Units
**kb** – kilo base pairs
**kg** – kilogram
**LDH** – Lactate Dehydrogenase
**LDL** – Low Density Lipoproteins
**LDL-C** – Low Density Lipoprotein Cholesterol
**LTR** - Long Terminal Repeat
**Mb** – Mega base pairs
**mg/kg** – milligram per kilogram
**mg/mL** – milligram per milliliter
**mL** – milliliter
**mmol/L** – millimol per liter
**NaCl** – Sodium chloride
**PCR** – Polymerase chain reaction
**pH** – Potential of Hydrogen
RBC – Red Blood Cell Count
RNA – Ribonucleic Acid
RT-PCR – Reverse Transcription Polymerase Chain Reaction
SE – Standard Error
TBil – Total Bilirubin
TEM – Transmission Electron Microscopy
TG – Triglycerides
VLDL – Very Low Density Lipoprotein
µg/L – microgram per liter
µm – micrometer
% - Percentage
°C – Celsius degrees
> – greater than
~ – approximately equal
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Casuistic of the curricular traineeship

a) Organization of the curricular traineeship

The curricular traineeship, included in the scope of the study plan of the Integrated Master in Veterinary Medicine, was performed at the Clinic for Ruminants of Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy. The traineeship had a duration of 3 months, starting on the 1st of September until the 1st of December 2017.

b) Program of the traineeship

The traineeship program consisted in internal medicine, surgery, herd health management and milk quality. Pathologic anatomy was also an important subject of the traineeship. The main objectives included the practical application of knowledge acquired during the course of Veterinary Medicine and the acquisition of new experiences. It was possible to accompany different specialties. Considering internal medicine, the program offered by the University of Bologna included the completion of a detailed clinical examination, diagnosis, complementary methods of diagnosis, treatment, detailed post-mortem examination and implementation of different necropsy techniques in cattle and calves. In surgery, it was possible to attend all the procedures including the pre-surgery preparation, surgery performance and the post-surgery period of all the animals. Therefore, pre-surgery and/or a pre-anesthetic examination was executed prior, as well as the anesthetic protocol and the anesthetic monitoring. Being the surgeon’s assistant was also possible very often. After surgery, all the animals were accompanied.

The clinic routine consisted of a morning briefing at 8h regarding the discussion of clinical cases. From 8h30 am until 9h, a complete clinical exam was performed to all the hospitalized animals. During this period the medications were also administered. From 9h until 12h, the complementary methods of diagnosis were performed, necropsies, surgeries, and if there was free time study of the clinical cases. From 12h until 13h, clinical exam to the hospitalized animals was performed. The period between 13h – 14h was destined to lunch and to study time, unless there were emergencies or critical care patients. Furthermore, from 14h – 17h, complementary methods of diagnosis, necropsies
and surgeries were also performed. When there were practical classes taught by the department of ruminants, assist the Professor was often possible. From 17h – 18h, we discussed articles regarding the clinical cases. Night shifts were performed when there were critical care patients or if there were emergencies. Once a week there were visits to dairy farms in Emilia Romagna region to do reproductive management and herd health management.

Given the high casuistic of the Clinic, a various procedures such as: performance of a minacious general objective exam and a particular objective exam to all the animals, myelography, endoscopy of respiratory tract, endoscopy of digestive tract, reproductive echography, abdominal echography, thoracic echography, orthopedic echography, application of ear catheters, performance and interpretation of laboratorial diagnosis methods (blood hematological, biochemical analysis, ionogram, protein electrophoresis, urinalysis, measure of ketosis in cows), necropsy and orthopedic radiography were observed. Furthermore, there was the opportunity to perform coprology, milk quality, buccal swabs and histology.

c) Statistical data of the observed casuistic

During the traineeship a total of 692 animals were observed, 67 percent (%) were dairy cows, 32% calves and 1,4% bulls (Graphic 1).

The casuistic distribution per specialty (Graphic 2) reveled a higher incidence at the Herd Health Management, with 32% of the observed cases. The veterinary should play a central role in preventing disease and maintaining the health of dairy cows within a framework of economic, environmentally sustainable farming. Maintaining excellent cow health and welfare are the primary and overarching aims of a herd health program. When it comes to medical procedures, we include vaccinations, deworming, blood sample to test for Brucellosis, Bovine Viral Diarrhea and Paratuberculosis, intradermal tuberculin test, welfare management and nutritional management as some of the relevant dimensions to look at.
Milk quality was the second major area, with 15% of the cases. Every dairy operation must ensure that its milk is of high quality and safe for consumption. High quality milk is crucial for processing high quality dairy products. This area is fundamental to ensure food safety.

**Graphic 1** – Distribution of the cases considering cows, calves and bulls.

**Graphic 2** – Distribution of observed casuistic per specialty.
Chapter 1

1. Introduction

It is common knowledge that it is crucial to dairy farmers that they successfully raise healthy calves (Kipp et al., 2016). Diseases during the first weeks of a calf's life not only influence the vitality and well-being of the calf in the short term, but also compromise health and productivity in later life (Correa et al., 1988). Illnesses in calves and heifers can hinder them from achieving their genetic potential as mature cows (Heinrichs & Heinrichs, 2011). Lost production, combined with the costs associated with disease treatment, can limit dairy farm profitability (Gelsinger & Heinrichs, 2017).

Calf morbidity and mortality is associated with higher costs for the farmer, such as compensation for calf losses, the costs for medical treatment, and the costs of raising calves (Mohd Nor et al., 2012). Moreover, calf morbidity and mortality are important animal welfare issues (Mee, 2013). Calf mortality plays a major role in the rearing success in cattle breeding (Sanderson & Dargatz, 2000; Hanzlicek et al., 2013).

Calf survival is mainly influenced by management factors, but there is also a genetic background for the mortality of young cattle, facilitating its inclusion in future breeding strategies (Olesen et al., 2000). Over the last decades, several genetic disorders have been discovered in cattle. However, the genetic background of disorders in calves is not widely reported (Kipp et al., 2016).

Cholesterol deficiency (CD), a new autosomal recessive inherited genetic defect in Holstein cattle, has been recently reported to have an influence on the rearing success of calves (Menzi et al., 2016). The affected animals show unresponsive diarrhea, buccal lesions and retarded growth of unknown etiology (Mock et al., 2016). They suffer highly visible hypocholesterolemia and hypolipidemia, indicating an inherited fat metabolism disorder (Mock et al., 2016). Affected calves do not respond to symptomatic medical treatment (Kipp et al., 2016). These animals usually die within the first 6 months of their life, and it has been assumed that about 80% of homozygous affected calves do not survive more than one year (Kipp et al., 2015). Even though heterozygous carrier animals do not show any clinical signs, they have reduced levels of blood cholesterol and triglycerides (Kipp et al., 2015). Breeding organizations in Switzerland, Germany and other countries have reported an increasing occurrence of cases in Holstein cattle (Mock et al., 2016; Kipp et al., 2016).
The recent discovery of the causal mutation for the Holstein haplotype for CD has been identified in the apolipoprotein B gene (*APOB*) (Charlier, 2016; Menzi et al., 2016; Schütz et al., 2016). The disease-associated haplotype on cattle chromosome 11 traces back to the Canadian Holstein sire Maughlin Storm born in 1991 (Kipp et al., 2015). The causal mutation representing an insertion of a transposable element in the coding sequence of the exon 5 of *APOB*, which results in truncated transcripts and abnormal splicing, was recently discovered by Menzi et al. (2016). The lack of apolipoprotein B protein (*APOB*) in homozygous mutant animals extends to 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 the liver (Gross et al., 2016). A significant recent advancement has been the development of a polymerase chain reaction (PCR) based direct gene test, allowing the detection of animals with CD without pedigree information (Menzi et al., 2016).

In human patients, truncating mutations in *APOB* give rise to familial hypobetalipoproteinemia (FHBL) (Mock et al., 2016). FHBL is caused by mutations in the *APOB* gene on chromosome 2p23–24 which interfere with the translation of full-length APOB and/or impair secretion of very low density lipoprotein (VLDL) (Tarugi & Averna, 2011). There are more than 60 mutations described in the literature for FHBL in the *APOB* (Lee & Hegele, 2014). Homozygous FHBL have the following as clinical phenotype: steatorrhea, neurological dysfunction, vision problems, and non-alcoholic fatty liver (Chan, 2014). Implementing a low-fat diet routine along with vitamin supplementations will improve most symptoms except for hepatic steatosis (Chan, 2014).

2. **Etiology**

The rapid growth in the number of genotyped dairy cattle, which recently surpassed 1 million in the United States (Council on Dairy Cattle Breeding, 2017), has resulted in the identification of several recessive disorders (Adams et al., 2016; Cooper et al., 2014; Daetwyler et al., 2014; Mc-Clure et al., 2014) and permitted the determination of carrier status of genotyped animals using haplotypes in place of laboratory tests (Cole et al., 2013).

Cholesterol Deficiency is a new autosomal monogenic recessive defect in Holstein cattle (Online Mendelian Inheritance in Animals [OMIA], 2017). Calves being
recessive homozygotes die within a period of days to months after birth as a consequence of the onset of idiopathic diarrhea (Kipp et al., 2015).

A combined approach of a genome-wide association study (GWAS) and homozygosity mapping revealed an approximately equal (~) 2.7 mega base pairs (Mb) disease-associated haplotype on bovine chromosome (BTA) 11 (Kipp et al., 2015). The disease-associated haplotype traces to the Holstein sire Maughlin Storm born in 1991 (VanRaden & Null 2015). Maughlin Storm’s sire and maternal grandsire were genotyped and do not carry this disease-associated haplotype (Menzi et al., 2016). Maughlin Storm’s great grandsire along the maternal lineage, Fairlea Royal Mark, born in 1966, has not been genotyped but is the sire of Willowholme Mark Anthony, born in 1975, the origin of the ancestral version of the haplotype (Figure 1) (Menzi et al., 2016). It was assumed that Willowholme Mark Anthony must carry the ancestral normal haplotype, from which the mutant haplotype arose, because several artificial insemination sires received a copy of this haplotype through their maternal lines, and homozygous calves descending from these bulls live normal lives (Kipp et al., 2015; VanRaden & Null, 2015). In conclusion, it was speculated that the mutation causing CD must have occurred in the three generations between Fairlea Royal Mark and Maughlin Storm, or even earlier, if this sire was homozygous for the BTA 11 haplotype.

Menzi et al. (2016) re-sequenced the entire genome of an affected calf and a healthy partially inbred male carrying one copy of the critical 2.24-Mb segment and detected a causal mutation – 1.3 kilo base pair (kb) insertion of a transposable long terminal repeat (LTR) element (endogenous retrovirus 2-1) located in the coding sequence of the APOB. This insertion generated premature stop codon resulting in a truncation of the APOB to less than 140 aminoacids (Kamiński & Ruść, 2016). The 1.3kb insertion was confirmed by Schütz et al. (2016), who reported that the LTR element was inserted into exon 5 of the APOB (at BTA11:77,959 kb) and is flanked by 6 base pairs (bp) target site duplications typical of insertions mediated by retroviral integrases. APOB is an essential compound of chylomicrons and low-density lipoproteins (Kamiński & Ruść, 2016). It seems then that the mutation represents a loss-of-function mutation similar to autosomal recessive inherited FHBL in humans (Young et al., 1988). Gross et al., (2016), reported that the causal mutation for CD affects lipid metabolism, steroid biosynthesis and cell membrane function in homozygous as well as heterozygous carriers, and may result in unspecific signs like reduced fertility, growth, and health. The rapid exchange of genetic material by means of artificial insemination, import of semen, and
transfer of embryos enables rapid transmission of mutation across populations (Kamiński & Ruść, 2016).

3. Geographic distribution and Prevalence

CD in Holstein calves was reported for the first time in the summer of 2015 in Germany (Kipp et al., 2015; Vanraden & Null, 2015).

Breeding organizations in Switzerland and other countries have reported an increasing occurrence of cases in Holstein cattle (Mock et al., 2016). According to the Swiss cattle breeding organizations, approximately 25 cases in calves with diarrhea and inbreeding to this sire have been recorded in Switzerland in the last few months (Mock et al., 2016). Animal Teaching Hospital, Obihiro University of Agriculture and Veterinary Medicine, Japan also reported cases of CD in Holstein calves (Inokuma et al., 2017).
Furthermore Kamiński & Ruść (2016) described cases of CD in Poland. Additionally, Cole et al. (2016) reported cases in the United States of America.

The cholesterol deficiency haplotype (CDH) has a higher frequency (2.5 percentage [%, based on all known and suspected heterozygotes) than many other recessives that also result in calf deaths, such as Complex Vertebral Malformation (1.37%) and Bovine Leucocyte Adhesion Deficiency (0.25%) (Cole et al., 2016). Cole et al. (2016) identified 56,641 Holsteins as carriers of CDH in their August 2015 genomic evaluation. Of those animals, 30,928 (54.6%) were heterozygous for the harmful haplotype and parental origin could be determined, 275 (0.48%) were homozygous for the harmful haplotype, 25,077 (44.2%) were heterozygous for the recessive haplotype but parental origin could not be determined, and 358 (0.63%) were homozygous for the recessive haplotype but parental origin could not be determined (Cole et al., 2016). Approximately half of the putative carriers and affected animals may carry the normal form of the recessive haplotype, which means that the economic impact of CDH is further increased because a number of unaffected animals may be culled (Cole et al., 2016).

4. Economic Impact

Cattle breeding populations are susceptible to the propagation of recessive diseases. Individual sires generate tens of thousands of progenies through artificial insemination (Pausch et al., 2015). The frequency of deleterious alleles carried by such sires may increase considerably within a few generations (Pausch et al., 2015).

Assuming random mating of all tested bulls, 3,175 animals homozygous for the haplotype are born per year in Germany, given a haplotype frequency of 4.2% and about 1.8 million Holstein calving’s per year (Kipp et al., 2016). Given an average value of 400€ per calf (average lifetime 85 days, raising costs and medical treatment), the economic loss per year in Germany alone amounts up to approximately 1.3 million Euros (Kipp et al., 2016).

Cole et al. (2016) also reported that, in Holsteins, CDH (1,696,555 Dollars) had lower values than other recessive diseases because of a lower carrier frequency. However, these results underestimate the total effect of recessives because there are large populations of those breeds in other countries that use semen from United States bulls (Cole et al., 2016).
5. Clinical Phenotype

Clinical findings of the disease in homozygous calves include failure to thrive and intermittent or chronic diarrhea which is unresponsive to treatment (Mock et al., 2016). Kipp et al. (2016) reported that the general attitude of affected calves was bright, alert, and responsive in three calves and mildly to moderately lethargic in two. Eyeballs were deeply sunken into the orbit in all calves while the skin tent duration suggested adequate hydration in three animals, mild dehydration in one, and moderate dehydration in another calf (Kipp et al., 2016). Affected calves present considerably retarded growth, with a body condition classified as considerably emaciated or cachectic and a dull and rough hair coat (Kipp et al., 2016).

Mock et al. (2016), reported that the affected calves’ feces had a yellow to olive-green color, normal smell, and, at variable intervals over an observation period of up to 14 days, a fecal consistency that changed between soft and liquid.

A recent article has described that muscular atrophy was observed in all homozygous calves; this was significantly more frequent than what was observed in non-homozygous calves (Inokuma et al., 2017). Additionally, most homozygous calves showed ataxia at the final stage of the disease, likely due to the severe muscular atrophy (Inokuma et al., 2017). Anorexia with bloat was also reported in one case (Kipp et al., 2016). However, most of the affected calves do not lose their appetite until just before death or euthanasia (Inokuma et al., 2017). A higher rate of severe muscular atrophy and lower rate of anorexia and ataxia are other characteristic clinical signs of this disease (Inokuma et al., 2017). Moreover, one calf showed subtle clinical signs of a radial nerve paresis in one forelimb (Kipp et al., 2016). Mock et al., (2016), also reported that affected calves are often severely emaciated despite normal appetites. Inokuma et al., (2017), described that eight of the twelve homozygotes had history or presented findings of fever higher than 39.5 Celsius degrees (°C).

A recent report has additionally described a heifer that had shown poor development and intermittent diarrhea in the first months of its life but had no longer shown diarrhea later on; it was brought to the Clinic for Ruminants of the Vetsuisse Faculty, University of Bern, Switzerland with a primary complaint of lesions in the buccal cavity (Mock et al., 2016). Despite resolution of the digestive problem, restored appetite and activity of the gastrointestinal tract; hypermetria and pacing, suggestive of a diffuse
cortical lesion, remained apparent upon neurologic examination (Mock et al., 2016). The cerebrospinal fluid showed a slight pleocytosis (9.7 cells per interleukins [cells/IL], norm: 3 cells/IL) with 39% monocytes and 61% lymphocytes (Mock et al., 2016). The pandy test for proteins was slightly positive (Mock et al., 2016). The blood concentrations of cholesterol (0.11 millimol per liter [mmol/L], norm: 1.20–3.84 mmol/L) and triglycerides (0.03 mmol/L, norm: 0.19–0.51 mmol/L) were similar to those of the affected calves (Mock et al., 2016).

As laboratory findings go, the affected calves reveal marked hypocholesterolemia and hypolipidemia, indicating an inherited fat metabolism disorder (Mock et al., 2016). Blood analyses reveal lowered cholesterol levels in the range of 15% of the reference range for cattle (greater than [>]1.8 mmol/L; reference laboratory values in Western College of Veterinary Medicine at the University of Saskatchewan et al., 2007) as hallmark findings (Kipp et al., 2016). The most remarkable and consistent abnormality of the laboratory standard blood biochemical panel is a pronounced hypocholesterolemia with low density lipoprotein cholesterol (LDL-C) concentrations below the detection limit (Kipp et al., 2016).

Red blood cell count (RBC), hemoglobin (Hb), and hematocrit (Ht) of homozygous calves are significantly lower than those of non-homozygous calves (Inokuma et al., 2017). In a previous report, a moderate amount of acanthocytes was observed in the blood smear in one calf (Mock et al., 2016). Another calf showed a slight anemia with a Ht of 17% (norm: 19–34%) and a low selenium concentration (47 microgram per liter [µg/L], norm: >50 µg/L) (Mock et al., 2016). Because cholesterol is an essential component of the reticulocyte membrane, RBC of the affected animals may be fragile, which may lead to acanthocytosis and lower RBC, Hb, and Ht; and higher mean corpuscular volume in some affected animals (Inokuma et al., 2017).

In another study, all calves showed a pronounced leukocytosis and tended to have slightly elevated serum urea nitrogen concentrations (Kipp et al., 2016). Mild to moderate hypokalemia, with values between 2.5 and 3.1 mmol/L, was diagnosed in three calves (Kipp et al., 2016).

Liver enzyme activities in serum are moderately elevated in some calves, with glutamate dehydrogenase, aspartate aminotransferase, and gamma-glutamyl transferase above the reference range (Kipp et al., 2016). Total bilirubin (TBil) is above the reference range in several cases (Kipp et al., 2016). Concentrations of vitamin A and E are usually markedly lowered (Kipp et al., 2016).
Heterozygous carrier animals show no apparent clinical signs but have lower blood cholesterol levels (Kipp et al., 2015).

6. Diagnosis

6.1. Laboratory diagnosis

Cholesterol and lipid metabolism have been widely investigated in humans and in rodent animal models as important factors contributing to lipid-associated diseases (Maxfield & Tabas, 2005).

Lipoproteins are composed of a core of hydrophobic lipids (triglycerides [TG] and cholesterol esters) and an envelope comprised of apolipoproteins and amphiphilic lipids (phospholipids and free cholesterol) (Kessler et al., 2014). Hepatocytes secrete cholesterol and triglycerides in VLDL, which are processed in the circulation into intermediate-density lipoproteins by hydrolysis of the TG (Kessler et al., 2014). Intermediate-density lipoproteins are rich in cholesteryl esters and are also metabolized to low density lipoproteins (LDL) or taken up by the liver (Cornell University College of Veterinary Medicine [CUCVM], 2017). In humans, LDL are the main carriers of cholesterol in blood and deliver cholesterol from the liver to the peripheral tissues (Kessler et al., 2014). High density lipoproteins (HDL) are synthesized in the liver and gastrointestinal tract and transport cholesterol from tissues to the liver (so-called “reverse” cholesterol transport) (CUCVM, 2017).

Kipp et al. (2016), explained that the serum cholesterol concentration was found to be significantly associated with the number of haplotype copies an animal carried. Animals not carrying the haplotype had an estimated mean cholesterol level of 1.82 mmol/L (standard error [SE] 0.11 mmol/L) (Kipp et al., 2016). Animals with one copy of the haplotype had a significantly lower mean cholesterol level of 1.25 mmol/L (SE 0.13 mmol/L). Animals with two copies had a significantly lower mean cholesterol level of 0.4 mmol/L (SE 0.10 mmol/L) (Kipp et al., 2016). These large differences between noncarrier, carrier, and homozygous defective carrier suggest a codominant inheritance (Kipp et al., 2016). Due to the association of the disease-associated haplotype and blood cholesterol values, the haplotype was denominated as CDH (Kipp et al., 2016).
Furthermore, CD in homozygotes carriers causes a fatal inability to maintain a level of blood cholesterol sufficient for life (Duff et al., 2016). Therefore, hypocholesterolemia and low triglyceride concentrations in the cattle are the main characteristic laboratory findings of this disease (Inokuma et al., 2017).

6.2. Genetic diagnosis

Pedigree analysis indicated familial relationships among affected calves, and the distribution of affected animals in both genders was suggestive of an autosomal monogenic recessive genetic defect inherited fat metabolism disorder, which was named cholesterol deficiency (OMIA, 2017; Menzi et al., 2016; Mock et al., 2016). GWAS and homozygosity mapping revealed a ~2.7-Mb disease-associated haplotype on BTA 11 (Kipp et al., 2015).

The disease-associated haplotype traces to the Canadian Holstein sire Maughlin Storm born in 1991 (VanRaden & Null 2015). Maughlin Storm was the first known carrier bull of the disease, for which the primary abnormality is a 1,299-bp insertion of a transposable element located in exon 5 of the APOB (Kipp et al., 2015; Kipp et al., 2016).

Mock et al., reported that the analysis of the genealogy of six affected animals revealed a relation to the sire Maughlin Storm on the maternal and on the paternal side. This Holstein sire has been reported to be the possible founder of the inherited CD disorder in previous investigations (Kipp et al., 2015; Menzi et al., 2016). The homozygous presence of the APOB insertion was confirmed in all six affected animals. Most of these calves died within a couple of days or weeks (Mock et al., 2016).

The APOB insertion is validated by a diagnostic PCR using three primers, and subsequent automated capillary electrophoresis (Fragment Analyzer; Advanced Analytical Technologies) (Kipp et al., 2016). Thereby, Kipp et al. (2016), confirmed that non-affected carriers were indeed heterozygous for this APOB variant, whereas the affected calves were homozygous mutant compared to the reference sequence.
6.3. Pathological Phenotype

At macroscopical examination, affected animals show very poor body condition (Mock et al., 2016; Kipp et al., 2016). Some animals exhibit serous atrophy of the bone marrow (“starvation atrophy”; Figures 2 and 3. A) and structural fat tissue (Figure 3. B) and are consequently classified as cachectic (Kipp et al., 2016). Some of the calves with cachexia displayed severe subcutaneous edema, accentuated over the ventral thorax and abdomen (Kipp et al., 2016).

The anus and perianal region of the calves are usually soiled with feces, which can be interpreted as a sign of diarrhea (Mock et al., 2016).

The abomasum is generally filled with moderate amounts of partly digested ingesta (Kipp et al., 2016). The affected animals have also large amounts of greasy, sticky, bright yellow stool (Kipp et al., 2016).

Mock et al. (2016), reported that the small intestine of affected animals was diffusely filled with a moderate amount of bright yellow, beige to lime-green, liquid, and partially foamy to fatty content (Figure 4. A). The mucosa of the distal two thirds of the small intestine was severely edematous and appeared whitish (Figure 4. A) (Mock et al., 2016). The large intestine was filled with a yellow to green liquid with a partially foamy to fatty content, consistent with steatorrhea (Mock et al., 2016).

An additional pathological finding is a moderate to severe, multifocal to confluent, suppurative bronchopneumonia affecting about 5 to 20% of the lung in some animals (Kipp et al., 2016). A few calves additionally show mild to moderate, multifocal degeneration of the musculature with a moderate lymphohistiocytic inflammatory infiltration (Kipp et al., 2016).
A severe, ulcerative abomasitis with a perforating lesion resulting in severe, diffuse, fibrinopurulent peritonitis was diagnosed in one calf (Kipp et al., 2016). This same animal also displayed a severe, focal, suppurative-to-necrotizing colitis and a moderate to severe dilatation of the renal tubuli with intraluminal hyaline and granular casts, multifocal adenomatous proliferation of the tubular epithelium, and a moderate interstitial fibrosis in the kidney (Kipp et al., 2016).

Histological changes are most prominent in the small intestine and accentuated in the jejunum (Mock et al., 2016). The enterocytes covering the tips of the villi contain large amounts of optically empty, round to oval vacuoles, ranging from 2 to 20 micrometers in diameter (Fig 4. B) (Mock et al., 2016). Multifocally, the nuclei of the affected enterocytes are displaced to the basal cell borders (Mock et al., 2016). In frozen sections, these intracytoplasmic vacuoles and large parts of the intestinal content are positively stained by Sudan stain indicating lipid origin (Fig 4. B) (Mock et al., 2016). Furthermore, multiple lacteals are commonly moderately to severely dilated, correlating with the macroscopically visible edema (Mock et al., 2016).

Presently, no pathomorphological or pathohistological lesions were observed in the liver (Mock et al., 2016; Kipp et al., 2016). Transmission electron microscopy (TEM) corroborated the presence of large intracytoplasmic lipid droplets in the small intestinal enterocytes (Mock et al., 2016). The droplets filled most of the supranuclear cytoplasm but also occurred in an infranuclear position (Figure 4. C) (Mock et al., 2016). Additionally, isolate lipid droplets are seen in hepatocytes but to a minor extent as compared with the enterocytes, excluding a hepatic steatosis on TEM level (Mock et al., 2016).

Other pathological findings include a moderate, focal, ulcerative, and fibrinopurulent, subacute ruminitis and a moderate-to-severe, multifocal, lymphohistiocytic endocarditis and myocarditis (Kipp et al., 2016).

Mock et al. (2016), reported a case where single dilated myelin sheaths containing fragmented axons and macrophages without any particular distribution pattern were observed in the central nervous system. The variation of nerve fiber size and the proportion of small nerve fibers appeared to be increased in the sciatic nerve, compared to a control animal of the same age (Figure 4. D) (Mock et al., 2016). Myelin sheaths of numerous axons were decreased in thickness and, multifocally, hypertrophied Schwann cells enclosed the myelin sheaths (Mock et al., 2016). In the eyes, no retinal degeneration was present (Mock et al., 2016).
Figure 3. Histology of femoral bone marrow and of the structural fat tissue. (A) Histology of the femoral bone marrow. Micrograph of the femoral bone marrow. The bone marrow is severely hypocellular with only single, small accumulations of hematopoietic cells (arrows). Adipocytes are atrophied and replaced by an eosinophilic amorphous material. Hematoxylin and eosin stain, 200×. (B) Histology of structural fat tissue. Micrograph of the structural fat tissue of the Hoffa’s fat pad. Adipocytes are atrophied and replaced by fibrillary to amorphous, eosinophilic material intermixed with single macrophages (arrows). Hematoxylin and eosin stain (HE), 400×. (Adapted: Kipp et al., 2016)

Figure 4. Lesions of congenital cholesterol deficiency in Holstein cattle. (A) Macroscopic appearance of the small intestine and its content. The intestinal content is foamy and greasy, varying from light-yellow to green in color, and the mucosa is edematous (scale bar = 1 centimeter [cm]). (B) In routinely processed histological sections of jejunum, many enterocytes contain optically empty cytoplasmic vacuoles, which are shown to represent lipid inclusions in Sudan-stained frozen sections (left) (1009). (C) Electron micrograph of enterocytes containing fat vacuoles within the apical cytoplasm (scale bar = 5 micrometer [µm]). (D) Sciatic nerve (right), showing thin and irregular myelin sheaths (arrow) with Schwann cell activation (arrowheads) in comparison to a control animal of the same age (left) (HE, 409). (Mock et al., 2016)
6.4. Differential Diagnosis

6.4.1. Diarrhea

Calf diarrhea remains the leading cause of mortality in dairy calves (United States Department of Agriculture, 1996). The diarrhea can be classified as primary enteritis (unspecific or specific), diarrhea secondary to a gastroenteric pathology or a symptomatic enteritis (generalized infection, intoxication, metabolic pathology or organic pathology) (Dirksen et al., 2004). Differentials diagnosis presented in Table 1 include infectious and non-infectious causes.

Rotavirus, cryptosporidium, coronavirus, Escherichia coli, and Salmonella spp. are recognized as the major infectious pathogens associated with diarrhea in calves (Doll, 2004; Klee, 2004 b). Campylobacter spp., Bovine Viral Diarrhea Virus (BVDV), Toxocara vitulorum, Eimeria spp., Giardia spp. are other possible infectious agents (Doll & Moennig, 2004; Gründer, 2004 b; Klee, 2004 a).

Intussusception, abomasal displacement and nutritional diarrhea are described as non-infectious causes of diarrhea in calves (Francoz & Guard, 2015; Fecteau & Guard, 2015). Intussusception occurs most commonly in the jejunum, but the frequency of ileocecal and colon intussusceptions appears higher in calves than adults (Dirksen & Doll, 1986). Commonly, there is a history of diarrhea (Francoz & Guard, 2015). Abomasal displacement is rare in young ruminants (Fecteau & Guard, 2015). An association of left-sided abomasal displacement with pneumonia in calves suggests that an altered vagal function may be involved in the pathogenesis of the condition (Dennis, 1984; Medina-Cruz et al., 1990). Concerning nutritional diarrhea, deliberate underfeeding of healthy calves may also predispose to diarrhea.

6.4.2. Buccal Mucosal Lesions

Oral lesions are found in relation to a number of conditions. In general, they result in some degree of dysphagia or reluctance to eat because of pain (MacLachlan & Mayo, 2015). The lesions include vesicles, erosions, ulcers, crusts, or growths; in or on the lips, tongue, gums, palate, or pharynx (B.P. Smith, 2015). Oral lesions are often associated with champing and increased amounts of saliva (ptyalism) being observed on the lips or
running from the mouth (B.P. Smith, 2015). Pseudoptyalism refers to a normal volume of saliva that, because it is not swallowed, is visible to the observer and may be confused with dysphagia (B.P. Smith, 2015). Buccal lesions may be caused by physical, chemical or infectious agents, the last causing the greater number of events (B.P. Smith, 2015). Some of the possible agents are listed at Table 2.

6.4.3. Failure to thrive

Cachexia and Weak Calf Syndrome are possible differentials diagnosis of failure to thrive (Maas, 2015). It is highly possible that several cases of autosomal recessive cholesterol deficiency may have been misdiagnosed as weak calf syndrome or diseases of unknown etiology with poor growth in the past (Inokuma et al., 2016).

Calves with Weak Calf Syndrome exhibit anaemia, depression, weakness, variable body temperature, difficulty nursing, growth retardation, and increased susceptibility to infection (Takasu et al., 2008). The pathological features of perinatal weak calf syndrome are anaemia with bone marrow dysfunction and foeto-placental dysfunction. The incidence of perinatal weak calf syndrome is dependent on the paternal and maternal family, and genetic factors have been implicated (Ogata et al., 1999).

6.4.4. Hypcholesterolemia

Decreased absorption, decreased production, altered metabolism and increased uptake of lipoproteins are possible causes of hypocholesterolemia (CUCVM, 2017). Moreover, the administration of egg yolk concentrate over 3 days can be initiated as part of a differential diagnostic therapy to facilitate distinction between intestinal cholesterol malabsorption and disturbed cholesterol synthesis as the underlying cause of disease (Kipp et al., 2016).
Table 1 – Clinical signs and diagnosis of infectious and non-infectious differential diagnosis of diarrhea.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Clinical signs</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>Weakness and collapse (septicemia). Diarrhea. Dehydration. Complications such as meningitis.</td>
<td>Culture of organism and serotyping.</td>
</tr>
<tr>
<td><strong>Salmonella spp.</strong></td>
<td>Septicemia in neonatal ruminants with high case fatality rate. Acute diarrhea and dysentery, fibrinous fecal casts, fever, marked dehydration, toxemia; chronic enteritis; abortion; dry gangrene of extremities; arthritis and foci of osteomyelitis. Severe diarrhea and dehydration.</td>
<td>Culture of organism and serotyping.</td>
</tr>
<tr>
<td><strong>Campylobacter spp.</strong></td>
<td>The disease may be so mild as to be unapparent, without fever, and may be manifested only by mild depression and soft feces with occasional strands of mucus.</td>
<td>Real-time quantitative PCR.</td>
</tr>
<tr>
<td><strong>Rotavirus, Coronavirus, Bovine torovirus (Breda virus)</strong></td>
<td>Calves: outbreak of diarrhea at 5 – 14 days of age and older up to 3 – 4 weeks. Profuse liquid diarrhea; pale, yellow, mucoid feces and may contain flecks of blood.</td>
<td>ELISA in feces.</td>
</tr>
<tr>
<td><strong>BVDV</strong></td>
<td>Inapparent subclinical bovine virus diarrhea acute mucosal disease. In persistently infected cattle 6-24 months of age with fever, diarrhea, oral erosions and high case – fatality rare. Peracute BVDV in cattle of all ages including adults with severe diarrhea, fever, agalactia and rapid death in few days, thrombocytopenia and hemorrhagic disease in veal calves. Reproductive failure.</td>
<td>Virus isolation from blood and tissues. Antigen detection (antigen capture ELISAs and immunohistochemical tests). PCR amplification of RNA. Viral neutralization serum antibody and ELISA test.</td>
</tr>
<tr>
<td><strong>Malignant catarrhal fever</strong></td>
<td>Diarrhea and dysentery. Severe stomatitis. Persistent high fever, conjunctivitis, hematuria, enlarged lymph nodes, skin lesions, encephalitis.</td>
<td>Serologic test.</td>
</tr>
<tr>
<td><strong>Cryptosporidium spp.</strong></td>
<td>Occurs in calves from 5 – 15 days of age and is characterized by persistent diarrhea which may last for several days.</td>
<td>The cryptosporidia may be detected by Giemsa stain of fecal smears or by fecal flotation.</td>
</tr>
<tr>
<td><strong>Eimeria spp.</strong></td>
<td>May include diarrhea, ill thrift, increased susceptibility to pneumonia, tenesmus, increased mucus in feces, and hematochezia. Pyrexia, dehydration, and anemia may also be observed. Calves appear weak and listless with pasty feces, drooping eyes, and a staring coat. Calves start shedding at about 1 month of age and shed for 3 to 4 months.</td>
<td>Salt or sugar filtration of the feces.</td>
</tr>
<tr>
<td><strong>Intussusception</strong></td>
<td>May have history of diarrhea, now scant bloodstained feces. Depressed, will not suck or drink, dehydrated, contour of abdomen may appear normal or slightly distended, fluid-splashing sounds and small “ping” may be audible, bloodstained peritoneal fluid, presurgical diagnosis often difficult, surgery necessary.</td>
<td>Abdominal ultrasound.</td>
</tr>
<tr>
<td><strong>Abomasal displacement</strong></td>
<td>Occurs in calves between 6 and 14 weeks of age, but younger calves may be affected. Reduced appetite, poor weight gain, recurrent tympany, and diarrhea. Affected animals may have a hypochloremic metabolic alkalosis.</td>
<td>Auscultation and percussion.</td>
</tr>
<tr>
<td><strong>Nutritional Diarrhea</strong></td>
<td>Diarrhea.</td>
<td>History of deliberate underfeeding of healthy calves and clinical signs.</td>
</tr>
</tbody>
</table>
Table 2 – Oral lesions and other lesions description of possible differentials diagnosis of buccal mucosal lesions.

<table>
<thead>
<tr>
<th>Lesion Description</th>
<th>Oral Lesions</th>
<th>Other Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluetongue</td>
<td>Large oral ulcers; dental pad and tongue most affected; generalized vasculitis.</td>
<td>Coronitis, muscle degeneration, lameness, pulmonary edema, edema of face and ears.</td>
</tr>
<tr>
<td>BVDV/ mucosal disease</td>
<td>Ulcers in mouth, particularly on hard palate; erosive stomatitis.</td>
<td>May have skin lesions; a few have corneal edema or enlarged lymph nodes; pneumonia and lesions in esophagus and gastrointestinal.</td>
</tr>
<tr>
<td>Bovine Papular Stomatitis</td>
<td>Round, dark red, raised papules on muzzle and on hard palate.</td>
<td>Occasionally in esophagus.</td>
</tr>
<tr>
<td>Vesicular stomatitis</td>
<td>Vesicles for short time, then large ulcers; tongue usually severely involved.</td>
<td>Teats and feet may be involved.</td>
</tr>
<tr>
<td>Malignant catarhal fever</td>
<td>Erosive stomatitis with ulcers; generalized vasculitis.</td>
<td>Purulent nasal discharge, corneal edema, enlarged lymph nodes, cracking skin, central nervous system signs; severe diarrhea; high fever.</td>
</tr>
<tr>
<td>Alimentary form of infectious bovine rhinotracheitis in calves</td>
<td>Gray pinpoint pustules on soft palate.</td>
<td>Rhinotracheitis, conjunctivitis, pneumonia.</td>
</tr>
<tr>
<td>Actinobacillosis (wood tongue)</td>
<td>Nodular lesions are often slightly ulcerated. The base of the tongue is most frequently affected, but the shaft may also be involved.</td>
<td>Lymph nodes may present granulomas or abscesses. Most granulomatous abscesses in a herd outbreak of actinobacillosis involved the tongue, muzzle, and lips and the submandibular, parotid, and cranial cervical areas. Cutaneous lesions of the facial may be observed.</td>
</tr>
<tr>
<td>Fusobacterium necrophorum (Necrotic laryngitis)</td>
<td>Inflammation and necrosis in oral mucosa.</td>
<td>Inflammation, necrosis, and edema in the laryngeal mucosa. If infection extends into the laryngeal cartilage, laryngeal chondritis develops, which may lead to a chronically deformed larynx.</td>
</tr>
<tr>
<td>Actinomycosis</td>
<td>Cavities (caries) in the dentin or dental pulp.</td>
<td>Hard, immovable, paintless, bony mass on the mandible and maxilla. Preponderance of mandibular lesions, with the development of periosteal new bone and brosis (&quot;lumpy jaw&quot;). Lesions occasionally occur in so tissues of the head, esophagus, forestomachs, and trachea. Occasionally may cause granulomatous abscesses in other so tissues.</td>
</tr>
<tr>
<td>Bristle grass ulcers</td>
<td>Ulcers in mouth.</td>
<td>Ulcers in digestive tract.</td>
</tr>
<tr>
<td>Traumatic stomatitis and Oral foreign body</td>
<td>Inflammation, ulceration, hemorrhage.</td>
<td></td>
</tr>
<tr>
<td>Irritant or caustic chemicals</td>
<td>Full-thickness burn of oral cavity.</td>
<td>Full-thickness burn of esophagus and stomach.</td>
</tr>
</tbody>
</table>
Chronic iodine toxicity is a differential diagnosis of hypocholesterolemia that also needs to be taken into account (Hillman & Curtis, 1980). Preliminary data from farm herds fed excessive dietary iodide and displaying signs of iodism indicated hyperglycemia, hypocholesterolemia, and a neutrophilic-lymphopenic shift in blood leukocytes (Hillman & Curtis, 1980).

Furthermore, Elissalde et al. (1983) reported that Babesia bovis infected cows showed cholesterol and cortisol values markedly reduced (less than 50% of normal values) during the acute phase of the disease.

Fat Cow Syndrome is another possible differential diagnosis. It is a multifactorial condition occurring in dairy cows after parturition (G.W. Smith, 2015). The syndrome is characterized by progressive depression and failure to respond to treatment of other predisposing diseases (G.W. Smith, 2015). This condition may increase the non-esterified fatty acid levels and decrease TG and cholesterol (G.W. Smith, 2015).

7. Treatment

Firstly, the implemented treatment should be the symptomatic treatment (Kipp et al., 2016). As a specific treatment, recommendations described for humans are: dietary fat restriction to prevent steatorrhea, and long-term high-dose vitamin E and A supplementation to prevent or at least slow the progression of neuromuscular and retinal degenerative disease (Chowers et al., 2001; Kane & Havel, 2001; Lee & Hegele, 2014). However, this treatment is not applicable to young cattle (Mock et al., 2016). Exceptionally older CD cattle, are particularly interesting animals for further investigation concerning the pathogenesis of the disease (Mock et al., 2016).

A sugar supplementation can also be used in retarded growth calves because it accommodates the rumen protozoa profile and stimulates papillae development (Sato et al., 2010).
7.1. Diarrhea

Diarrhea in calves can result in potentially serious metabolic derangements including profound acidemia due to strong ion (metabolic) acidosis, hyper-D-lactatemia, hyper-L-lactatemia, azotemia, hypoglycemia, hyperkalemia and hyponatremia (Trefz et al., 2017). Critically ill calves with diarrhea typically exhibit variable degrees of dehydration, depression, decreased or loss of the suckling reflex, and impaired ability to stand (Table 3 – 4) (Berchtold, 2009).

The treatment of diarrheic calves should consist in oral electrolyte solutions and if indicated with constant drip infusions consisting of sodium bicarbonate, saline, and glucose solutions (Table 4) (Trefz et al., 2017). Oral fluid and electrolyte therapy is initiating in calves that appear mildly dehydrated or showed electrolyte imbalances (Kipp et al., 2016).

Table 3 – Guidelines for Assessing Dehydration in Calves. (Izzo et al., 2015 b)

<table>
<thead>
<tr>
<th>Dehydration (%)</th>
<th>Eyeball Sunkenness</th>
<th>Neck Skin Tent (Seconds)</th>
<th>Mucous Membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>&lt;1</td>
<td>Moist</td>
</tr>
<tr>
<td>1-5</td>
<td>None to slight</td>
<td>1-4</td>
<td>Moist</td>
</tr>
<tr>
<td>6-8</td>
<td>Slight separation of eyeball and globe</td>
<td>5-10</td>
<td>Tacky</td>
</tr>
<tr>
<td>9-10</td>
<td>Gap, &lt;0.5 cm, between eyeball and orbit</td>
<td>11-15</td>
<td>Tacky to dry</td>
</tr>
<tr>
<td>11-12</td>
<td>Gap, 0.5-1 cm, between eyeball and orbit</td>
<td>16-45</td>
<td>Dry</td>
</tr>
</tbody>
</table>

Intravenous administration of sodium bicarbonate solutions is the treatment of choice in diarrheic calves with clinical signs of acidosis, and studies in diarrheic calves (Table 4) (Lorenz & Vogt, 2006; Trefz et al., 2012), and neonatal kids have indicated that markedly elevated plasma D-lactate concentrations normalize after correction of metabolic acidosis (Bleul et al., 2006). Intravenous fluid therapy with 0.9% sodium chloride (NaCl), 0.5% glucose solution combined with sodium bicarbonate is administered when calves develop profuse diarrhea resulting in dehydration and acidemia (Kipp et al., 2016). This therapy must be continued until clinical signs of metabolic acidosis or dehydration normalize and calves are able to counterbalance the enteral fluid and electrolyte losses by the oral intake of milk and an electrolyte solution (Trefz et al.,
Table 4 – Prediction of severity of metabolic acidosis from body position, strength of suck reflex, and age. (Izzo et al., 2015 b)

### A – Bicarbonate requirements for diarrheic calves (≤8 days of age)

<table>
<thead>
<tr>
<th>Clinical assessment</th>
<th>Base deficit (mmol/L)</th>
<th>Therapy</th>
<th>Therapy description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>Descriptive</td>
<td>30kg</td>
<td>35kg</td>
</tr>
<tr>
<td>Standing, strong suck reflex</td>
<td>5</td>
<td>Oral</td>
<td>(should contain at least 60 mmol/L of acetate or bicarbonate)</td>
</tr>
<tr>
<td>Standing, weak suck reflex</td>
<td>10</td>
<td>Intravenous</td>
<td>(total bicarbonate requirement for intravenous fluid therapy, mmol)</td>
</tr>
<tr>
<td>Sternal recumbency</td>
<td>15</td>
<td>175</td>
<td>200</td>
</tr>
<tr>
<td>Lateral recumbency</td>
<td>20</td>
<td>150</td>
<td>175</td>
</tr>
</tbody>
</table>

### B – Bicarbonate requirements for diarrheic calves (>8 days of age)

<table>
<thead>
<tr>
<th>Clinical assessment</th>
<th>Base deficit (mmol/L)</th>
<th>Therapy</th>
<th>Therapy description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>Descriptive</td>
<td>30kg</td>
<td>35kg</td>
</tr>
<tr>
<td>Standing, strong suck reflex</td>
<td>5</td>
<td>Oral</td>
<td>(should contain at least 60 mmol/L of acetate or bicarbonate)</td>
</tr>
<tr>
<td>Standing, weak suck reflex</td>
<td>10</td>
<td>Intravenous</td>
<td>(total bicarbonate requirement for intravenous fluid therapy, mmol)</td>
</tr>
<tr>
<td>Sernal recumbency</td>
<td>15</td>
<td>225</td>
<td>262.5</td>
</tr>
<tr>
<td>Lateral recumbency</td>
<td>20</td>
<td>300</td>
<td>350</td>
</tr>
</tbody>
</table>

(A) Calves 8 days of age or less. (B) Calves more than 8 days of age.
Bicarbonate requirements can be calculated from base deficit values (based on blood gas measurements or estimated from physical findings) as follows:

\[
\text{mmol bicarbonate} = \text{Body weight (kilogram [kg])} \times \text{Base deficit (mmol/L)} \times \text{Volume of distribution (0.6 in calves)} \quad (\text{Izzo et al., 2015})
\]

Additional supportive therapy consists of the parenteral administration of nonsteroidal drugs, probiotics and the supplementation of vitamin E and selenium (Trefz et al., 2017). Antimicrobial therapy should be initiated in cases of a concurrent bacterial infection, suspected septicemia, fever, or the presence of hypothermia (Lofstedt et al., 1999).

Figure 5 illustrate the correct way of decision-making concerning to calves’ diarrhea treatment.

---

**Figure 5.** Decision-making flowchart to determine treatment for calves with diarrhea. (Adapted: Pereira et al., 2017)
7.2. **Buccal Mucosal Lesions**

Treatment of stomatitis in affected calves is nonspecific and aimed at supportive and nursing care. Animals with severe oral lesions are reluctant to eat or drink (B.P. Smith, 2015). Young individuals may need to be tube-fed if the lesions are severe enough to preclude suckling (Radostits, 2007). Older animals can be fed gruels of alfalfa pellets by stomach tube and can be encouraged to eat feeds or green grass (MacLachlan & Mayo, 2015). Antimicrobial treatment can be used to help prevent secondary bacterial infections (Radostits, 2007). Debilitated cattle should be given broad-spectrum antibiotics in an effort to control secondary bacterial pneumonia (MacLachlan & Mayo, 2015).

7.3. **Hypocholesterolemia**

Kipp et al. (2016), reported oral supplementation of cholesterol through administration of egg yolk concentrate (200 milliliters [mL] per animal in 12-hour intervals, equivalent to 12 yolks every 12 hours). Overall, there are insufficient data to recommend the routine administration of egg yolk concentrate to calves with hypocholesterolemia.

In human medicine the therapeutic approach consists in early treatment with high oral doses of vitamins E and A (Chowers et al., 2001). As vitamin E transport strongly relies on APOB-containing lipoproteins, high doses of vitamin E (100–300 milligram per kilogram [mg/kg] per day orally) are required to circumvent the chylomicron pathway, perhaps by incorporation into HDL (Berriot-Varoqueaux et al., 2000; Lee & Hegele, 2014). Erythrocyte and platelet vitamin E levels provide the best assessment of tissue vitamin E status (Clarke et al., 2006). Supplementation with vitamins A, D, and K are also recommended (Hooper et al., 2015). Medium chain TG, which are not packed into chylomicrons, have been suggested as a form of delivering calories, but their use and safety has not been established (Lee & Hegele, 2014; Welty, 2014). Evidences of using this treatment in cattle are not recorded.
7.4. Management

The CDH is clearly associated with calf mortality and high impact on the worldwide Holstein population (Kipp et al., 2015).

Each human genome contains approximately 100 loss-of-function mutations, including about 20 genes that are completely inactive (MacArthur et al., 2012), and the total is probably similar for cattle (Cole et al., 2016). Most mutations are spread throughout the population because the founder sired many daughters directly, and then the founder’s sons provided many granddaughters (Cole et al., 2016). Popular sire effects can be amplified when a bull is heavily used in a small population (Cole et al., 2016).

It is easy to reduce the frequency of a deleterious allele in a population under selection but is extremely difficult to eliminate it entirely from the population (Cole et al., 2016). Known carriers may be removed from the population, but in practice it is more common to avoid carrier-to-carrier matings because carrier bulls may have high genetic merit for economically important traits. Segelke et al. (2016) recently suggested that selection of cows on an index, including haplotypes of interest and bulls on breeding values, can be used to balance selection for or against specific alleles with genetic gain, and Cole (2015) has demonstrated a strategy for mate allocation that can accommodate many recessives simultaneously.

Furthermore, the information about the haplotype status including the availability of pedigree data allows for the prevention of future risk-matings (Kipp et al., 2015). Identifying carriers for this genetic disorder and considering this information in breeding programs can prevent calf mortality and improve animal welfare and health (Kipp et al., 2015).

Dairy farmers are unlikely to completely avoid the use of carriers, thus the inclusion of recessives in selection programs is needed to ensure that harmful allele frequencies remain low (Cole et al., 2016).

8. Familial hypobetalipoproteinemia (FHBL)

Cholesterol and TG are almost insoluble in plasma; therefore, they are transported in spherical lipoprotein particles which contain a central core of varying amounts of nonpolar lipids, TG and cholesterol ester, covered on the surface by polar lipids
comprised of phospholipids, one or more apolipoproteins and unesterified cholesterol (Havel & Kane, 1995). APOB exists in two isoforms in plasma, APOB-100 and APOB-48, both of which are products of the same structural gene on chromosome 2p24-p23 (Young, 1990). APOB-100 is synthesized by the liver and secreted in the form of VLDL, a TG-rich-lipoprotein (Chen et al., 1987).

Familial hypobetalipoproteinemia belongs to a heterogeneous group of monogenic disorders characterized by reduced plasma levels of low-density lipoprotein cholesterol (LDL-C) and APOB below the fifth percentile for age and sex in the population (Rimbert et al., 2016). This disease is the most frequent monogenic form of hypobetalipoproteinemia (Di Costanzo et al., 2017). The frequency in the heterozygous form is estimated to be 1:1000e1:3000 (Tarugi et al., 2007; Hooper & Burnett, 2014). Heterozygous FHBL is often asymptomatic and not diagnosed unless a lipid profile is obtained (Welty, 2014).

FHBL is caused by mutations in the APOB on chromosome 2p23–24 which interfere with the translation of full-length APOB and/or impair secretion of VLDL (Tarugi & Averna, 2011). It has been linked to heterozygous mutations in the APOB (Tarugi & Averna, 2011), which in most cases interferes with the complete translation of APOB messenger ribonucleic acid (RNA) (Di Costanzo et al., 2017). This, in turn, causes the production of truncated proteins of various lengths, ranging from APOB-2 to APOB-89 (from 2% to 89% of APOB-100 size, according to a centile nomenclature) (Schonfeld et al., 2005). Only the truncated APOB with a size above that of APOB-29/30 located in the APOB region spanning from exon 26 to exon 29 are detectable in plasma (Schonfeld et al., 2005; Schonfeld, 2003).

Although there is one normal allele in heterozygous FHBL, plasma APOB-100 levels are approximately 24% of normal rather than the predicted 50% (Welty et al., 1997). Stable isotope studies have shown that these lower than expected levels result from a 74% lower secretion rate of VLDL APOB-100 from the liver, decreased production of LDL APOB-100, increased catabolism of VLDL and extremely low secretion of the truncated APOB (Elias et al., 1999). This decreased secretion of APOB from the liver results in decreased TG export from the liver, which in turn leads to the development of fatty liver (Tanoli et al., 2004). Therefore, hepatic steatosis and mild elevation of liver enzymes are the main clinical manifestations of heterozygous FHBL although oral fat
intolerance and intestinal fat malabsorption have been infrequently reported (Welty, 2014).

In homozygous FHBL, the clinical and biochemical features are indistinguishable from those of abetalipoproteinemia (Burnett & Hooper, 2015). Clinical signs consist in fat malabsorption with steatorrhea, vomiting, abdominal distension, and failure to thrive in the neonatal period, and later in life, progression to atypical retinitis pigmentosa and spinocerebellar ataxia (Berriot-Varoqueaux et al., 2000). Deficiency of vitamin E leads to the most debilitating clinical manifestations which are neurological disorders, that in turn lead to progressive degeneration of the central nervous system and death (Welty, 2014). Acanthocytosis, decreased erythrocyte survival, anemia, hyperbilirubinemia and hemolysis, and coagulopathy due to vitamin K deficiency can occur (Kane & Havel, 2001). Liver abnormalities include hepatomegaly, increased amino-transferases, and hepatic steatosis (Berriot-Varoqueaux et al., 2000), which can progress to steatohepatitis, fibrosis, and cirrhosis (Burnett & Hooper, 2015).

Table 5 outlines the clinical evaluation and the laboratory investigation on homozygous FHBL, and Table 6 outlines a treatment strategy.

Currently, genetic diagnosis in FHBL relies largely on Sanger sequencing of APOB (Rimbert et al., 2016). Because APOB is a large gene composed of 29 exons covering 14,121 bp, Western-blotting may be used to detect truncated protein species that are >30% of full-length protein size (Tarugi et al., 2007; Hooper & Burnett, 2014). When Western blotting is negative, sequencing the 25 first exons of APOB (exons 1 to 25) - a time consuming and costly approach - is mandatory (Burnett et al., 2015). Next generation sequencing technologies enable rapid and cost-effective sequencing of targeted genomic regions (Rimbert et al., 2016). Next generation sequencing is thus a powerful approach for genetic diagnostics in inherited Mendelian disorders (Johansen et al., 2014; Sikkema-Raddatz et al., 2013).

The standard treatment consists of a low-fat diet with replacement of fat-soluble vitamins such as vitamins A and E in order to prevent, or at least slow the progression of, neuromuscular and retinal degenerative disease (Kane & Havel, 2001; Lee & Hegele, 2014; Chowers et al., 2001).
Table 5 – Homozygous Hypobetalipoproteinemia Follow-up Outline. (Adapted: Lee & Hegele, 2014)

<table>
<thead>
<tr>
<th>Clinical Evaluation (every 6 – 12 months)</th>
<th>Laboratory investigations (every year)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td><strong>Lipids</strong></td>
</tr>
<tr>
<td>• Height/weight for growth Curve</td>
<td>• Total cholesterol</td>
</tr>
<tr>
<td></td>
<td>• Triglyceride</td>
</tr>
<tr>
<td></td>
<td>• LDL-C</td>
</tr>
<tr>
<td></td>
<td>• HDL-C</td>
</tr>
<tr>
<td></td>
<td>• APOB</td>
</tr>
<tr>
<td></td>
<td>• apoA1</td>
</tr>
<tr>
<td><strong>Diet</strong></td>
<td><strong>Hepatic</strong></td>
</tr>
<tr>
<td>• Adequate caloric intake</td>
<td>• AST</td>
</tr>
<tr>
<td>• Low-fat (&lt;30 % total calories) diet with EFA supplements</td>
<td>• ALT</td>
</tr>
<tr>
<td>• MCTG intake generally not required</td>
<td>• GGT</td>
</tr>
<tr>
<td>• Vitamin supplementation</td>
<td>• Total and direct bilirubin</td>
</tr>
<tr>
<td></td>
<td>• ALP</td>
</tr>
<tr>
<td></td>
<td>• Albumin</td>
</tr>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td><strong>Vitamins</strong></td>
</tr>
<tr>
<td>• Appetite</td>
<td>• Beta-carotene</td>
</tr>
<tr>
<td>• Diarrhea</td>
<td>• 25-OH Vitamin D</td>
</tr>
<tr>
<td>• Vomiting</td>
<td>• Plasma or RBC Vitamin E</td>
</tr>
<tr>
<td>• Esophagitis</td>
<td>• INR</td>
</tr>
<tr>
<td>• Abdominal distention</td>
<td></td>
</tr>
<tr>
<td>• Hepatomegaly</td>
<td></td>
</tr>
<tr>
<td><strong>Neurological</strong></td>
<td><strong>Other</strong></td>
</tr>
<tr>
<td>• Expected development for age</td>
<td>• CBC</td>
</tr>
<tr>
<td>• Ataxia</td>
<td>• Vitamin B12</td>
</tr>
<tr>
<td>• Dysarthria</td>
<td>• Folate</td>
</tr>
<tr>
<td>• Hyporeflexia</td>
<td>• Calcium</td>
</tr>
<tr>
<td>• Proprioception loss</td>
<td>• Phosphate</td>
</tr>
<tr>
<td>• Muscle pain or weakness</td>
<td>• Uric acid</td>
</tr>
<tr>
<td></td>
<td>• Thyroid Stimulating Hormone</td>
</tr>
</tbody>
</table>

EFA, essential fatty acid; MCTG, medium chain triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; APOB, apolipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; RBC, red blood cell; INR, international normalized ratio; CBC, complete blood count.
### Table 6 – Special Dietary Counseling and Treatment. (Adapted: Lee & Hegele, 2014)

<table>
<thead>
<tr>
<th>Special Dietary Counseling and Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General Lipids</strong></td>
</tr>
<tr>
<td>- Ensure adequate caloric intake</td>
</tr>
<tr>
<td>- Low fat (less than 30% of total calories), with reduced long-chain fatty acids</td>
</tr>
<tr>
<td>- Oral essential fatty acids</td>
</tr>
<tr>
<td>- Medium chain triglycerides generally unnecessary</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
</tr>
<tr>
<td>Oral fat-soluble vitamins:</td>
</tr>
<tr>
<td>- Vitamin E 100–300 (IU/kg/day)</td>
</tr>
<tr>
<td>- Vitamin A 100–400 IU/kg/day</td>
</tr>
<tr>
<td>- Vitamin D 800–1200 IU/day</td>
</tr>
<tr>
<td>- Vitamin K 5–35 mg/week</td>
</tr>
</tbody>
</table>

IU/kg/day, International units per kilogram per day; IU/day, International units per day; mg/week, milligram per week.

### 9. Objectives

This dissertation intends to describe a clinical and pathological phenotype showed by the calf confirmed to be affected by CD, understand the steps needed to perform a correct diagnosis and execute a treatment. Furthermore, it intends to show to the farmers and veterinarians that CD is a possible differential diagnosis for chronic diarrhea and failure to thrive in Holstein calves from three weeks to six months of age.
Chapter 2

1. Materials and Methods

1.1. Animal Ethics Statement

Endoscopy on the affected calf was performed by accredited veterinarians. Blood samples of affected and unaffected animals were also collected by accredited veterinarians. No ethical approval was required for this study. The animals’ owner had consented to the diagnostic work-up for research purposes and the inclusion of his animals in this study.

1.2. Description of Phenotype

A female Holstein calf with 5 months was referred to the Clinic for Ruminants of Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy on 19th of August 2017. The calf had a history of failure to thrive, intermittent diarrhea and had shown poor development. It was presented to the Clinic with a primary complaint of lesions in the buccal cavity.

Upon admission, the affected animal underwent a complete clinical examination. Blood samples were obtained by venipuncture of a jugular vein for a complete blood count, a blood chemistry profile, an electrophoresis of proteins, and an ionogram. A urine exam was also performed. In addition, total cholesterol and TG were measured.

Fecal samples were sent for routine viral and parasitological analyses to exclude the most usual pathogens causing calf diarrhea (cryptosporidia, coccidia, BVDV). Diarrhea caused by dietetic management was excluded by clinical history. The animal was tested for BVDV using a reverse transcription polymerase chain reaction (RT-PCR) on feces and blood sample. Furthermore, a buccal swab and an endoscopy of the nasal cavity and larynx was performed. Swab samples were collected from ulcers in oral mucosa. All the coprologic exams and the oral swabs were processed by Servizio di Malattia Infettive, Parassitarie e Aviarie from the Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy. A genetic test was also performed.
The animal suffered a natural death 33 days after admission to the Clinic. A complete necropsy was performed.

After the necropsy, blood was collected to ethylenediaminetetraacetic acid (EDTA) from 3 familiar related healthy cows (mother, sister 1, sister 2). A complete blood count, a blood chemistry profile, total cholesterol and TG, and genetic test were performed. The father’s semen was also submitted to the genetic test.

1.3. Blood Hematological and Biochemical Analysis

Blood samples were obtained on day 30 and day 33 (day of the calf’s death); however, the samples from the relatives were obtained afterwards; blood was collected in tubes not containing any anticoagulant and in vials containing EDTA and syringes containing Li-heparin. All the samples were collected and analyzed by Servizio di Patologia Clinica from the Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy; the only exception was the analysis of APOB that was performed by Doctor Cord Drögemüller from Institut fur Genetik der Universität Bern, Switzerland. Samples were analyzed the day of sample collection for the parameters of the standard hematology and blood biochemical panel. Serum was used to perform protein electrophoresis and then was stored at −21°C. The standard hematological analysis was conducted on an automated analyzer and included a platelet cell count, red blood cell count, packed cell volume, hemoglobin concentration, erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) as well as a white blood cell count, lymphocytes, monocytes, neutrophils, eosinophils, basophils and reticulocytes. The standard blood biochemical analysis included total protein, albumin, albumin/globulin, total bilirubin, indirect bilirubin, direct bilirubin, total cholesterol, serum urea nitrogen, creatinine, the enzyme activities of aspartate aminotransferase, gamma-glutamyl transferase, glucose, alkaline phosphatase, creatine kinase, lactate dehydrogenase and the electrolytes sodium, potassium, chloride, calcium, phosphorus, and magnesium. Moreover, the potential of hydrogen (pH), partial pressure of carbon dioxide, anion gap, osmolality and plasma bicarbonate concentration were measured. A protein electrophoresis was also performed (albumin, gamma, total protein, albumin/globulin). These analyses were conducted on an automated analyzer.
1.4. Urinalysis

The urine exam was processed by Servizio di Patologia Clinica from the Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy. The urine analysis included macroscopical aspects (color and transparency), pH, specific gravity, protein, hemoglobin, leucocytes, glucose, ketone bodies, bilirubin. Furthermore, the urine sediment exam was also performed.

Urine sampling was obtained by spontaneous urination; urine was collected in an aseptic plastic container. It was centrifuged for 10 minutes (1000 rotation per minute) and then the density was measured with a refractometer. By then, a urine stick was performed (Roche Combur10 test® UX) and it was analyzed by Roche Urisys 1100. The sediment analysis was done with the microscope.

1.5. Coprology

The coprology was processed by Servizio di Malattia Infettive, Parassitarie e Aviarie from the Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy. This procedure was performed using the sedimentation technique and then microscopy observation as described by Garcia & Bruckner (1988).

1.6. BVDV Test

The BVDV test was processed by Laboratorio di Virologia from Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy, using a RT-PCR on the feces and blood samples. The analysis was performed as described by Vilcek et al. (1994).

1.7. Buccal Swab

Swab samples were collected from ulcers in oral mucosa and were used to research fungi. The samples have been processed by Servizio di Malattia Infettive,
Parassitarie e Aviarie from the Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy. An isolation streaking technique has been performed. The sample was inoculate at the top of an half of petri dish. The loop was flamed and then one streak was made in a zigzag pattern from half of Petri dish. Then touching the last line of the first streaking another line in zigzag pattern started in a quarter of the other half of Petri dish. Finally, the last quarter of Petri dish was streaked without touching the previous lines.

1.8. Endoscopy

In the endoscopy, the animal was sedated with 0,020 mg/kg of xylazine chloridrate (20 milligram per milliliter [mg/mL]; Rompun®, Bayer, Milan). It was performed an orthograde rhinoscopy followed by a laryngoscopy and a tracheoscopy using the mouth as the access way. This procedure was performed with the endoscopic Pentax EG-1840 (6 millimeters of diameter and 1050 millimeters of length).

1.9. Genetic Analysis

The genetic test was processed by Doctor Cord Drögemüller from the Institut für Genetik, Universität Bern, Switzerland, and it was performed as described by Menzi et al. (2016) using blood and semen as sample.

1.10. Treatment

A solution of iodine glycerin was given to the affected calf, administered locally on the oral mucosal lesions three times a day for the first 7 days. At day 1, 3, 5, 7, 32, was administrated 10 ml intramuscular of selenium, cyanocobalamin, adenosine-5’-monophosphoric acid and sorbitol (Selevit®, Fatro, Ozzano Emilia). From day 8 until day 14, iodopovidone (10%; Betadine®, Meda Pharma, Milan) and honey were administered locally on the oral lesions twice a day. From day 14 until day 18, 7,5 mL subcutaneous of benzylpenicillin+diidrostreptomicine (200.000 International Units per
milliliter + 250 mg/mL; Repen®, Fatro, Ozzano Emilia) was administrated on the animal. From day 18 until 21, it was administered 3 mL subcutaneous of enrofloxacin (100 mg/mL; Baytril®, Bayer, Milan). At day 32 it was administered 5 mL subcutaneous of vitamin A, vitamin D3, vitamin E (vitamin A 10.000.000 international units [IU], vitamin D3 2.500.000 IU, vitamin E 10.000 milligram; Adisole A-D-E®, Vetem S.p.A., Lungomare Pirandello). A total blood transfusion (university donor) and an intravenous administration of 5 liters of NaCl 0,9% with 80 milliequivalents of potassium chloride were also performed. At day 33 it was given 5 liters of intravenous NaCl 0,9%.

1.11. Necropsy and Histology

The necropsy and the histology were performed by Dipartimento di Scienze Mediche Veterinarie form Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy. The affected calf was submitted for postmortem examination and a complete necropsy. The body condition was evaluated based on the subcutaneous and abdominal fat reserves. Fresh samples of colon, encephalon, esophagus, liver, ileus, lymph node, spleen, muscle, lung, body of stomach, adrenal, thyroid, trachea, bladder, thymus, aorta, subclavian artery, tongue, eye and rumen were also collect and were fixated in formalin (4% aqueous solution of formaldehyde). Since water and paraffin do not mix, the first step in embedding with paraffin was to replace the water in the tissues with a solvent that is miscible with paraffin. Dehydration was the first part of the process. It was accomplished by transferring the block of tissue through a series of alcohol-water solutions beginning with 50 % and running up to absolute alcohol. Clearing was the second part of the process. The alcohol was replaced by cedar oil, which is readily soluble in alcohol, and in turn, was replaced by melted paraffin. The third part was the embedding. The actual embedding took place when the paraffin-infiltrated tissue was placed in fresh paraffin and the latter was allowed to cool. Celloidin embedding was the fourth part. Celloidin consisted in dissolving in equal parts of absolute alcohol and ether. The tissue was dehydrated in alcohol in the same way as for paraffin except that it was transferred from absolute alcohol to a dilute solution of celloidin. As the alcohol and ether evaporated, they were replaced by more concentrate celloidin. It was finally hardened in chloroform and stored in 80 percent alcohol. Therefore, the epoxy embedding was performed. Finally, the samples were stained with hematoxylin and eosin.
2. Results

2.1. Clinical Presentation of the Case

The calf admitted to the Clinic for Ruminants of Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Italy, had a history of poor growth and progressing emaciation. The animal presented a reduced skeletal development and constitution for its age. The nutritional score was cachectic with an increased transverse ventral abdominal diameter. The muscular tonicity was hypotonic. At mastication, the calf revealed a dry rumor with prehension of small quantities of food. The animal elevated the head frequently and opened the mouth moving the tongue from one side to another, showing signs of pain. During the mastication, the calf revealed increased production of saliva (Figure 6. A). It presented a dull and rough hair coat. Some gray alopecic zones in level of the distal cervical and left ear were also noticed. The ocular and vaginal mucosae were rose. At buccal level, the calf revealed gingival (Figure 6. C, D, E, I, J), sub-lingual (Figure 6. F, G, H) and palatine (Figure 6. B) lesions compatible with fibrinous-ulcerative. The animal exhibited an increased volume of parotid lymph nodes (especially the right) and the right pre-scapular lymph node. It presented a rectal temperature of 38.7°C, an arterial pulse of 104 pulsations per minute, and a respiratory frequency of 48 respirations per minute.

All the main organic functionalities were present. The pulmonary auscultation revealed a murmur slightly increased in the right cranial quadrant. At forced respiration, the calf showed presence of stridor in the level of the right medium-dorsal quadrant.

To the particular objective exam of the cardio-circulatory system no alterations were observed. However, to the particular objective exam of digestive system the presence of diarrhea was noticed.
2.2. Blood Hematological and Biochemical Analysis

The results of the hematological and blood biochemical analyses of the affected calf are summarized in Table 10, and the protein electrophoresis in Table 8.

All the results of the hematological and blood biochemistry of the three animals related of the calf were normal except for the total cholesterol of the mother, which was lower than the reference range. The results of total cholesterol are described in Table 9.
2.3. Urinalysis

At the macroscopical examination, the urine had a yellow color and was transparent. In the sediment exam, there was presence of amorphous material 2+. The other parameters that were measured are summarized in Table 7.

Table 7 – Biochemical analysis of the urine of the affected calf.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.00</td>
<td>7.50 – 8.00</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.014</td>
<td>1.020 – 1.040</td>
</tr>
<tr>
<td>Protein</td>
<td>Negative</td>
<td>Absent</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>250 erythrocytes/µL</td>
<td>Absent</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>Negative</td>
<td>Absent</td>
</tr>
<tr>
<td>Glucose</td>
<td>Negative</td>
<td>Absent</td>
</tr>
<tr>
<td>Ketonic bodies</td>
<td>Negative</td>
<td>Absent</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Negative</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Table 8 – Results of protein electrophoresis of the affected calf at day 33.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>1.94</td>
<td>3.0 – 4.3</td>
</tr>
<tr>
<td>Gamma (g/dl)</td>
<td>1.61</td>
<td>1.69 – 2.25</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>5.61</td>
<td>6.8 – 8.6</td>
</tr>
<tr>
<td>Albumin / Globulin</td>
<td>0.53</td>
<td>0.8 – 0.9</td>
</tr>
</tbody>
</table>

Table 9 – Results of the cholesterol of the affected calf at day 30, the mother, sister 1 and sister 2.

<table>
<thead>
<tr>
<th>Total Cholesterol (mg/dl)</th>
<th>Reference Range:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected calf</td>
<td>2.0</td>
</tr>
<tr>
<td>Mother</td>
<td>62</td>
</tr>
<tr>
<td>Sister 1</td>
<td>137</td>
</tr>
<tr>
<td>Sister 2</td>
<td>129</td>
</tr>
</tbody>
</table>

Reference Range: 80 – 120 mg/dl

, Decreased
Table 10 – Results of hematological and blood biochemical analysis of the affected calf.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results Day 30</th>
<th>Results Day 33</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cell count (cells/mm³)</td>
<td>5420</td>
<td>9160</td>
<td>4000 – 12000</td>
</tr>
<tr>
<td>Basophils (cells/mm³)</td>
<td>9160</td>
<td>4000 – 12000</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (cells/mm³)</td>
<td>2500 – 7500</td>
<td>25 – 840</td>
<td></td>
</tr>
<tr>
<td>Monocytes (cells/mm³)</td>
<td>1620</td>
<td>2500 – 7500</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (cells/mm³)</td>
<td>70</td>
<td>60</td>
<td>0 – 2400</td>
</tr>
<tr>
<td>Eosinophils (cells/mm³)</td>
<td>4000 – 12000</td>
<td>2500 – 7500</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes (cells/mm³)</td>
<td>70</td>
<td>60</td>
<td>0 – 2400</td>
</tr>
<tr>
<td>Mean platelet volume (MPV; fL)</td>
<td>8.2</td>
<td>6.6</td>
<td>6.6 – 10.9</td>
</tr>
<tr>
<td>Red blood cell count (cells/mm³)</td>
<td>544 0000</td>
<td>50 0000 – 100 00000</td>
<td>100 000 – 800 000</td>
</tr>
<tr>
<td>Hemoglobin (gr %)</td>
<td>7.2</td>
<td>8.0</td>
<td>8.0 – 15.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>19.8</td>
<td>24.0</td>
<td>24.0 – 46.0</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV; fl)</td>
<td>36.5</td>
<td>40 – 60</td>
<td></td>
</tr>
<tr>
<td>Red Cell Distribution Width (RDW; %)</td>
<td>21.2</td>
<td>15.0 – 23.0</td>
<td></td>
</tr>
<tr>
<td><strong>Blood biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>5.61</td>
<td>6.56</td>
<td>6.8 – 8.6</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.96</td>
<td>3.0 – 4.3</td>
<td></td>
</tr>
<tr>
<td>Albumin/Globulin</td>
<td>0.54</td>
<td>0.8 – 0.9</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST; IU/L)</td>
<td>72</td>
<td>78 – 132</td>
<td></td>
</tr>
<tr>
<td>Lactate Dehydrogenase (LDH; IU/L)</td>
<td>1567</td>
<td>690 – 1445</td>
<td></td>
</tr>
<tr>
<td>Creatine Kinase (IU/L)</td>
<td>92</td>
<td>105 – 409</td>
<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase (IU/L)</td>
<td>159</td>
<td>27 – 107</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.47</td>
<td>0.9 – 1.3</td>
<td></td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>43.88</td>
<td>8 – 23</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>78</td>
<td>45 – 75</td>
<td></td>
</tr>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.43</td>
<td>0 – 0.1</td>
<td></td>
</tr>
<tr>
<td>Indirect Bilirubin (mg/dl)</td>
<td>0.20</td>
<td>0 – 0.1</td>
<td></td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dl)</td>
<td>0.23</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-</td>
<td>12 – 31</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>2</td>
<td>80 – 120</td>
<td></td>
</tr>
<tr>
<td>Gamma-glutamyl transferase (GGT; IU/L)</td>
<td>10.9</td>
<td>15 – 39</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>7.5</td>
<td>9.7 – 12.4</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mEq/L)</td>
<td>5.58</td>
<td>5.6 – 6.5</td>
<td></td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>128</td>
<td>132 – 152</td>
<td></td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>2.9</td>
<td>3.9 – 5.8</td>
<td></td>
</tr>
<tr>
<td>Chlorine (mEq/L)</td>
<td>86.8</td>
<td>97 – 111</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>1.71</td>
<td>1.8 – 2.3</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.394</td>
<td>7.36 – 7.44</td>
<td></td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>50.7</td>
<td>35 – 44</td>
<td></td>
</tr>
<tr>
<td>Anion GAP (mmol/L)</td>
<td>12.9</td>
<td>13 – 20</td>
<td></td>
</tr>
<tr>
<td>Osmolality (mOsm/Kg)</td>
<td>260.7</td>
<td>260 – 300</td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>29.7</td>
<td>22 – 28</td>
<td></td>
</tr>
</tbody>
</table>

↓ Decreased; ↑ Increased
2.4. Coprology

Fecal samples of the calf were submitted for parasitological examination. The analyzed samples were negative to endoparasites. In particular, neither *Eimeria* spp. nor *Cryptosporidium* spp. could be identified.

2.5. BVDV Test

Virus isolation of BVDV was negative.

2.6. Buccal Swab

The culture of buccal swab was positive to *Candida albicans*.

2.7. Endoscopy

When the endoscopic examination of the right nasal cavity was performed, moderate degree of hyperemia of the mucosa associated with the presence of purulent material in moderated quantity free in the lumen was noticed. Considering the larynx, the function is in the limit of normality. The entrance of the larynx was normal. However, a light level edema of the mucosal of arytenoid and epiglottis was observed. Distally to the larynx, at the level of dorsal portion of the tracheal rings, the presence of purulent mucous strongly attached to the tracheal wall was noticed.

In conclusion, there was a rhinolaryngitis of light level associated to a tracheitis of high level.

2.8. Genetic Analysis

The results of the genetic analysis of the affected calf, the mother, the father and sister 1 and sister 2 are summarized on Table 11.
Table 11 – Genetic analysis results of the affected calf, the mother, the father and the sister 1 and sister 2.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected Calf</td>
<td>Homozygous for the APOB Mutation</td>
</tr>
<tr>
<td>Mother</td>
<td>Heterozygous, carrier of the APOB Mutation</td>
</tr>
<tr>
<td>Father</td>
<td>Heterozygous, carrier of the APOB Mutation</td>
</tr>
<tr>
<td>Sister 1</td>
<td>Free of the APOB Mutation, non-carrier</td>
</tr>
<tr>
<td>Sister 2</td>
<td>Free of the APOB Mutation, non-carrier</td>
</tr>
</tbody>
</table>

APOB – Apolipoprotein B gene

2.9. Necropsy and Histology

At macroscopical 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.

At the buccal cavity opening, the calf presented buccal lesions compatible with a fibrinous-ulcerative stomatitis. The animal presented multiple ulcerative lesions on the oral mucosa (gingival, sub-lingual, labial and palatine) with regular sides, well limited. A hemorrhagic background filled partially with an adherent and detachable membrane with a yellowish to pink compatible with fibrin was also noticeable.

Figure 7. Macroscopic lesions of larynx, epiglottis, brain and liver (A) Laryngitis; moderate congestion of the larynx mucosa and above the epiglottis. (B) Hyperemia of meningeal vessels; slight cerebral edema. (C) Perihepatitis and probably hepatitis. (Author’s photos)
The larynx presented a moderate congestion of the mucosa and above the epiglottis (Figure 7. A). These features are consistent with laryngitis. The presence of small deposits of greenish above the first rings of the trachea was also observed (Figure 7. A).

The esophagus showed a profound segmental ulceration of the cranial third of the esophageal mucosa with exposed submucosa and a marked tissue retraction (Figure 8). The ulcerative areas were covered by a thin membrane with a yellowish/orange coloration partially adherent compatible with fibrin (Figure 8).

At the abdominal cavity opening, the liver presented a variegated coloration. It was possible to identify multiples focus of congestion of irregular profile and ill-defined limits (Figure 7. C). The Glisson capsule revealed increased thickness, showing various grades of opacity, more exacerbated at the diaphragmatic face and at the visceral face of the hepatic lobes. Above the capsule, adherent to it, it was possible to identify a deposit of yellowish color with flocculate texture a friable consistency, compatible with fibrin. Keeping in mind the lesions of the liver, this is compatible with perihepatitis and probably hepatitis. Furthermore, the small intestine revealed generalized congestion, and its mucosa presented a marked hyperemia (Figure 9. A). The intestine was diffusely filled with a moderate amount of bright yellow, liquid, and partially foamy to fatty content. Additionally, it was possible to observe the congestion of the serous that covers the colon (Figure 9. B; Figure 9. C). The intestinal lesions described were compatible with segmental enteritis.

Figure 8. Esophagitis; profound segmental ulceration of the cranial third of the esophageal mucosa with exposing of the submucosa and a marked tissue retraction; presence of thin membrane with a yellowish/orange coloration partially adherent compatible with fibrin. (Author’s photo)
The brain presented hyperemia of meningeal vessels (arachnoid and piamater). A slight cerebral edema was also noticed (Figure 7. B).

At the histology, no alterations were found.

![Image of histology](image)

**Figure 9.** Segmental enteritis. (A) Small intestine: generalized congestion; presence of marked hyperemia. (B) and (C) Congestion of serous that covers the colon. *(Author's photos)*
3. Discussion

Autosomal recessive cholesterol deficiency in Holstein cattle is a new inherited fat metabolism disorder secondary to a genetic defect (Gross et al., 2016). Maughlin Storm, a Canadian Holstein sire, was the first known carrier bull of the disease (VanRadan & Null, 2015), for which the primary abnormality is a 1,299 bp insertion of a transposable element located in exon 5 of the APOP (Kipp et al., 2015; Kipp et al., 2016).

CD was identified recently because farmers in Germany reported problems with calves that suffered from decreased birth weight and diarrhea that were not responsive to veterinary intervention (Kipp et al., 2015). Moreover, breeding organizations in Switzerland have reported an increasing frequency of cases in Holstein cattle (Mock et al., 2016). In Japan (Inokuma et al., 2017), Poland ( Kamiński & Ruś, 2016) and United States of America (Cole et al., 2016) were also reported cases of CD.

The CDH has a haplotype frequency of 4.2% in Germany (Kipp et al., 2016). With this frequency, given an average value of 400€ per calf (average lifetime 85 days, raising costs and medical treatment) and considering 1.8 million Holstein calving’s per year, the economic loss per year in Germany alone amounts up to approximately 1.3 million Euros. In United States the haplotype frequency is 2.5% (based on all known and suspected heterozygotes) (Cole et al., 2016). In another study, Schütz et al. (2016) found 12.5% carriers among Holstein bull born between 2012 and 2015 in Germany. Furthermore, CDH has a higher frequency when compared with other recessive haplotypes that also result in calf deaths (Cole et al., 2016).

Modern breeding of dairy cattle increasingly involves programs based on the international trade of semen from elite bulls with high genetic merit (Meydan et al., 2010). With the widespread use of advanced reproductive technologies, including artificial insemination and multiple ovulation embryo transfer, individual bulls are able to quickly sire thousands of calves in many countries (Windsor & Agerholm, 2009).

Since Holstein cattle is one of the most inbred dairy cattle breed, closer international integration of national associations of Holstein cattle breeders and coordination of genetic defects discovery programs is necessary to decrease the risk of new recessive disorders and reduce their prevalence (Ruś & Kamiński, 2016).

Therefore, it is important that carriers and affected animals are identified as quickly as possible (Cole et al., 2016). Uncontrolled spreading of CD will decrease the fertility of cows since the higher number of carriers increase the chance of producing
recessive homozygotes (Kamiński & Ruś, 2016). Presently, fertility is one of the most important traits and consequently any factors leading to its deterioration should be limited (Kamiński & Ruś, 2016).

The most reliable clinical features found in this report, were intermittent diarrhea and failure to thrive associated with hypocholesterolemia and low TG concentrations. In this study, the calf presented a very marked hypocholesterolemia (2.0 mg/dl; reference range: 80 – 120 mg/dl) and low triglyceride concentrations. Although the clinical signs were unspecific and of moderate intensity, the CD affected calf revealed poor growth and progressing emaciation, intermittent diarrhea and buccal lesions. Similar results have been reported by Mock et al. (2016). The culture of buccal swab was positive for Candida albicans. At the pulmonary auscultation the sounds were slightly increased in the right cranial quadrant and when the forced respiration was performed, the calf showed the presence of stridor in the level of the right medium-dorsal quadrant. Kipp et al. (2016) had also reported abnormal pulmonary sounds in two CD affected calves. The heart and respiratory rate of the calf were above the normal limits. Additionally, in the endoscopy a rhinolaryngitis of light level associated to a tracheitis of high level was noticed. It remains undefined if the various, presumably secondary infectious processes that were diagnosed together in the calf are a manifestation of an increased susceptibility to secondary infections resulting from vitamins deficiency. Hypovitaminosis in any case was shown to decrease the resistance to infections in cattle (Spears, 2000; Xiuyuan et al., 2012).

Indeed, the pathological phenotype of the homozygous APOB mutant calf, such as cachexia, muscular atrophy, signs of diarrhea, intestine diffusely filled with a moderate amount of bright yellow, liquid, and partially foamy to fatty content and enteritis were also described by Kipp et al. (2016).

Taking the pathological finding of enteritis and the clinical phenotype of failure to thrive, hypocholesterolemia, low triglyceride concentration into account, CD is highly similar to human FHBL (Welty, 2014). Although there were no neurological signs in life, post mortem examination revealed hyperemia of meningeal vessels (arachnoid and piamater) and a slight cerebral edema. FHBL is additionally characterized by malabsorption of lipid-soluble vitamins (A, D, E, K), leading to retinal degeneration, neuropathy, and coagulopathy (Lee & Hegele, 2014). These neurologic disorders are associated with cerebellar dysfunction and compromising of the posterior column.
function with demyelination in the central nervous system and peripheral nervous system (Lee & Hegele, 2014; Welty, 2014).

Furthermore, the CD calf of this report presented esophagitis. In human patients with FHBL, esophagitis is described as one of the possible complications of the disease (Lee & Hegele, 2014). However, there are no previous reports of esophagitis in calves.

Perihepatitis and hepatitis with presence of fibrin, described as pathological findings of FHBL in humans, were present in the CD calf (Welty, 2014). In the present study, an increased alkaline phosphatase, TBil, indirect bilirubin and direct bilirubin was found in the calf. However, the investigated calf, according to the results of postmortem examination, had no signs of fatty liver (hepatic steatosis). Thus, this might be attributed to the young age of the animal at the time of death (Burnett & Hooper, 2015).

Over 60 truncating APOB mutations have been identified as causes for FHBL (Welty, 2014). The mutant proteins in the liver do not fulfill any physiological function in cattle homozygous for the bovine APOB mutation (Menzi et al., 2016). Therefore, it is highly probable that both physiological APOB isoforms are missing (Mock et al., 2016). Presently, it is not acknowledged if mutant APOB are expressed in CD-affected cattle or not, and this should be subjected to further investigation (Menzi et al., 2016). Reduced function of human APOB-100 results in declined TG export (by LDL receptor-mediated endocytosis) from the liver, which in turn leads to the development of fatty liver (Tanoli et al., 2004). Until now, none of the investigated cattle had signs of fatty liver (hepatic steatosis), leading to the assumption that hepatic steatosis due to APOB-100 dysfunction is not a feature of CD in cattle (Mock et al., 2016). Otherwise, the nonappearance of hepatic steatosis in the CD affected calf might be explained by the differences in fat metabolism between ruminants and humans. Mostly, the de novo fatty acid synthesis in humans takes place in the liver with glucose as a basic substrate (Vernon, 1980). Acetate absorbed from the rumen in ruminating animals is the substrate for direct de novo synthesis of fatty acids (Vernon, 1980). Acetate is produced by microbial fermentation of cellulose in the rumen and stored in adipose tissue as well as used for milk fat synthesis in the mammary gland of lactating animals (Vernon, 1980). Therefore, the primary site of de novo fatty acid synthesis is the adipose tissue and the secondary site is mammary tissue (lactating cows), not the liver (Bergen & Mersmann, 2005). This only applies for ruminating animals, as in this case. Lipids are transported in the blood plasma as lipoproteins, complexes of various lipid materials with specific proteins (Bergen & Mersmann, 2005). The large chylomicron and VLDL contain considerable amounts of
triacylglycerol with small amounts of cholesterol (Bergen & Mersmann, 2005). Chylomicrons are synthesized in the intestine and are found in lymph and in plasma after a meal containing fat (Drackley, 2005). Ruminants have few or no chylomicron particles because consumption of fats 5% diet dry matter interferes with the rumen microbial feed digestion; under such circumstances, few long-chain fatty acids are absorbed (Drackley, 2005). The predominant carriers of cholesterol are the LDL and HDL (Bergen & Mersmann, 2005).

Apolipoproteins form the structural proteins of lipoproteins allowing to transport lipophilic cholesterol and triacylglycerol in hydrophilic blood (Kipp et al., 2016). APOB is a structural protein that binds cholesterol to form LDL-C and very low density lipoprotein cholesterol to transport cholesterol (Kipp et al., 2016). In general, APOB-containing lipoproteins carry lipids from liver (site of synthesis) and gut (site of absorption) to various sites of utilization for energy production, storage, membrane assembly, or steroid hormone production (Marcovina & Packard, 2006). Kipp et al. (2016) reported that all of the homozygous CDH carrier calves had markedly decreased concentrations of LDL-C (below the detection limit) consisting of cholesterol bound to APOB. Although, in this report, there are no data available for the values of LDL-C.

In this study, the total cholesterol of the mother (heterozygote, carrier of the APOB mutation) of the CD calf was decreased and did not present any clinical signs of malabsorption. However, it was not possible to measure the TG and phospholipids. Fascinatingly, none of the heterozygous carriers of the APOB mutation (calves and adult animals) show any clinical signs of maldigestion compared with homozygous affected cattle (Gross et al., 2016). Despite lower plasma concentrations of TG, phospholipids, and total cholesterol, heterozygous animals apparently are able to maintain cholesterol and lipoprotein homeostasis for, for example, steroid hormone biosynthesis and cell membrane function (Gross et al., 2016). Moreover, heterozygous humans with FHBL are generally asymptomatic (Schonfeld, 2003). However, fatty liver often developed, and oral fat intolerance and intestinal malabsorption were reported in some cases (Schonfeld, 2003; Tarugi et al., 2007). Beyond malabsorption of dietary lipids, deleterious effects of APOB deficiency on hepatic lipid metabolism, steroid biosynthesis, and cell membrane function can be expected (Gross et al., 2016). However, it could be speculated that these effects might not be fully overt in heterozygous carriers of the APOB mutation, resulting in possible unspecific signs of reduced fertility, growth, and health (Gross et al., 2016).
RBC, Hb, and Ht of the homozygous calf were below the reference range. Although there are no data available for the blood smear observation, acanthocytosis is thought to be an early laboratory feature of FHBL in humans (Lee & Hegele, 2014; Welty, 2014). Since cholesterol is an essential component of the reticulocyte membrane, red blood cells of the affected animals may be fragile, which may lead to acanthocytosis and lower RBC, Hb, and Ht (Inokuma et al., 2017). Inokuma et al. (2017) reported that 5 affected homozygous calves had similar results. Additionally, Mock et al. (2016) also reported these findings in one CD affected calf.

Total protein, albumin, globulin and albumin/globulin ratio of the CD calf were also under the reference range. In a previous report, analogous results were obtained (Kipp et al., 2016). Panhypoproteinemia occurs when there is a reduction in the amount of plasma proteins in the vascular space in the presence of normal or almost normal plasma volume (Elsevier, 2018). The reduced protein concentration can be the result of impaired production or accelerated loss (Constable et al., 2017). Reduced production of all plasma proteins occurs only as part of malnutrition and starvation (Constable et al., 2017). Liver disease can cause a reduction in the concentration in plasma of those proteins produced by the liver but in large animals is an unusual cause of hypoproteinemia (Elsevier, 2018). Loss of protein is a more common cause of hypoproteinemia (Elsevier, 2018). The loss of proteins can be either from the vascular space into the extravascular compartment (endotoxemia, vasculitis) or from the body (compensated hemorrhage, glomerulonephritis, protein-losing enteropathy) (Constable et al., 2017). This situation is evident as a reduction in concentrations of both albumin and globulins (Elsevier, 2018).

In the urinanalysis of the calf, the pH and the specific gravity were decreased. A urine with the specific gravity below 1.020 might be caused by polydipsia, metabolic disorders or renal insufficiency (Gründer, 2004 a). The calf also presented hemoglobinuria. However, no macroscopical or microscopical lesions were found on the kidneys.

Calves with CD are described to usually die within the first 6 months of life (Kipp et al., 2015). The treatment described for humans includes, for example total fat restriction and lipid-soluble vitamin supplementation (Lee & Hegele, 2014; Welty, 2014), but is not applicable to young cattle. However, older CD cattle, like the calf in this study, are particularly interesting animals for further investigation concerning the pathogenesis of the disease. The possibility of a natural large animal model for human FHBL could be explored with further investigations (Kipp et al., 2016).
Unspecific clinical signs of diarrhea and failure to thrive in young calves are the main limitations for the diagnosis of CD in cattle (Kipp et al., 2016). It is important, as demonstrated by the affected animal described here, which belongs to an age group most susceptible for various diarrhea-causing pathogens, to first exclude common agents causing diarrhea. In case of intermittent or chronic diarrhea resistant to treatment in young Holstein cattle, analysis of total cholesterol and triglycerides can lead to a suspicion of CD (Kipp et al., 2016). Pathological investigations are only diagnostic if samples of the small intestine are immediately fixed in formalin (within minutes after euthanasia) (Kipp et al., 2016). Under other circumstances, the lipid vacuoles in the enterocytes of the villi tips are lost due to immediate autolytic changes in the small intestine (Kipp et al., 2016).

Regardless if there are clinical or pathological suspicion of CD, a confirmation using the genetic test detecting the APOB mutation after consideration of pedigree information indicating inbreeding linked to the founder sire Maughlin Storm should be performed (Menzi et al., 2016). This recently developed diagnostic tool will allow a reduction in unnecessary treatment costs, use of antibiotics, and time-consuming care of affected animals (Kipp et al., 2016). The early diagnosis of CD will allow targeted eradication of the APOB mutation from the Holstein population and consequently prevent economical losses in the future (Kipp et al., 2016).

4. Conclusion

This work reports the occurrence of a recent Holstein haplotype of autosomal codominant inheritance that is associated with a markedly disturbed cholesterol metabolism with a tremendous effect on health and survival of homozygous haplotype carriers. This congenital defect, termed CDH, is associated with a phenotype characterized by growth retardation, emaciation, muscular atrophy and chronic or intermittent diarrhea in calves and shows striking similarities with the human homozygous with FHBL.

CD must be considered as a possible differential diagnosis for chronic diarrhea and failure to thrive in Holstein calves from three weeks to six months of age. At present, definitive diagnosis rests on testing for the genetic defect.
Chapter 3

1. Bibliographic References


### 2. Figures Bibliographic References


### 3. Tables Bibliographic References


Chapter 4

Appendix: Resumo das Secções em Português
JOANA GONÇALVES PONTES JACINTO

DEFICIÊNCIA EM COLESTEROL AUTOSSÓMICA RECESSIVA NUMA VITELA HOLSTEIN

RESUMO DAS SECÇÕES EM PORTUGUÊS

Orientador: Professor Doutor João Cannas da Silva
Coorientador: Professor Doutor Arcangelo Gentile

Universidade Lusófona de Humanidades e Tecnologias
Faculdade de Medicina Veterinária

Lisboa
2018
Resumo

A deficiência em colesterol (CD) é um defeito genético hereditário autossómico recessivo recentemente identificado no efetivo Holstein. Foram reportados como fenótipos clínicos diarreia sem resposta à medicação, atraso no crescimento, hipocolesterolemia e os animais geralmente morrem nas primeiras semanas ou meses de vida. A CD é causada por uma mutação no gene da apolipoproteína B bovina (APOB). O objetivo do presente trabalho é descrever o fenótipo clínico e patológico e compreender os passos necessários para realizar um diagnóstico e, consequente, tratamento de uma vitela Holstein homozigótica para a mutação no APOB. Uma vitela Holstein com história clínica de diarreia intermitente e erosões na cavidade bucal foi admitida na Clínica de Ruminantes da Facoltà di Medicina Veterinaria dell’Università degli Studi di Bolonha, Itália. Nesse sentido, recolheu-se sangue a 3 animais saudáveis aparentados (mãe, irmã 1, irmã 2) e sêmen ao pai. Este estudo incluiu uma descrição clínica completa do fenótipo clínico e fenótipo patológico, análise hematológica e bioquímica do sangue e os valores de colesterol e triglicéridos (TG). O animal teve uma morte natural 33 dias após a admissão na clínica. Um teste genético foi realizado conforme descrito por Menzi et al. (2016) usando sangue como amostra (vitela afetada, mãe, irmã 1, irmã 2) e sêmen (pai) para determinar o genótipo do APOB. A vitela foi confirmada como homozigótica para a mutação no APOB. O pai e a mãe, como esperado, eram portadores heterozigóticos da mutação no APOB. As irmãs foram consideradas livres da mutação no APOB. O fenótipo clínico da vitela afetada incluía atrofia muscular, falha no crescimento e diarreia crónica. Verificou-se a presença de hipocolesterolemia e baixas concentrações de TG na vitela afetada. Ademais, verificou-se que a concentração de colesterol da mãe da vitela afetada também se encontrava abaixo do limite normal. O fenótipo patológico observado da vitela homozigótica incluía esteatorreia com enterite segmentar. Embora o animal não tenha manifestado sinais neurológicos em vida, o cérebro apresentava hiperemia dos vasos meníngeos e discreto edema cerebral. A CD deve ser considerada como um possível diagnóstico diferencial para diarreia crónica e atraso no desenvolvimento de vitelos Holstein sem evidências de infeções primárias. A confirmação da mutação no APOB associada é necessária.

Palavras-chave: Apolipoproteína B; Diarreia; Deficiência em Colesterol; Bovinos; Holstein.
Introdução

A CD – um novo defeito genético hereditário autossômico recessivo no efetivo Holstein – foi recentemente reportada como influente no sucesso da produção de vitelos (Menzi et al., 2016). Os animais afetados apresentam diarreia não responsiva ao tratamento, lesões bucais e crescimento retardado de etiologia desconhecida (Mock et al., 2016). Estes animais sofrem de hipocolesterolemia e hipolipidemia altamente visíveis, indicando um distúrbio hereditário do metabolismo lipídico (Mock et al., 2016). Esses animais por via de regra morrem nos primeiros 6 meses de vida (Kipp et al., 2015). Embora os animais heterozigotos portadores da mutação não apresentem sinais clínicos, estes têm valores de colesterol e triglicerídeos no sangue inferiores ao limite normal (Kipp et al., 2015). Organizações de reprodução na Suíça, Alemanha e outros países relataram uma ocorrência crescente de casos no efetivo Holstein (Mock et al., 2016; Kipp et al., 2016).

A mutação responsável pelo haplótipo Holstein para a CD foi recentemente identificada no gene da APOB (Charlier, 2016; Menzi et al., 2016; Schütz et al., 2016). O haplótipo associado à doença no cromossoma bovino 11 remonta ao touro canadiano Maughlin Storm, nascido em 1991 (Kipp et al., 2015).

A ausência de proteína apolipoproteína B (APOB) em animais homozigóticos mutantes leva a uma má absorção de gordura dietética e vitaminas lipossolúveis no intestino. Com efeito, presume-se que esta circunstância prejudica o metabolismo do colesterol e o seu transporte na circulação sanguínea e no fígado (Gross et al., 2016). Atualmente é possível utilizar o teste genético direto baseado na reação em cadeia da polimerase (PCR), permitindo a detecção de animais com CD sem informação de pedigree (Menzi et al., 2016).

Em pacientes humanos, mutações truncadas no APOB dão origem à hipobetalipoproteinemia familiar (FHBL) (Mock et al., 2016). Os homozigóticos FHBL apresentam os seguintes sintomas clínicos: esteatorreia, disfunção neurológica, problemas de visão e esteatose hepática não alcoólica (Chan, 2014). A implementação de uma dieta com baixo teor de gordura, juntamente com suplementos vitamínicos, permite melhorar a maioria dos sintomas, à exceção da esteatose hepática (Chan, 2014).
Etiologia

A Deficiência em Colesterol é um novo defeito autossómico recessivo monogênico no efetivo Holstein (OMIA, 2017).

Uma abordagem tendo simultaneamente em conta o estudo de associação genómica ampla (GWAS) e mapeamento de homozigóticos revelou um haplótipo associado à doença aproximadamente igual a (~) 2,7 mega pares de bases (Mb) no cromossoma bovino (BTA) 11 (Kipp et al., 2015). O touro Maughlin Storm é o possível animal fundador da CD. Assim, a mutação pode ter ocorrido nas gerações de Fairlea Royal Mark, Wykholme Dewdrop Gail ou Wykholme Dewdrop Tacy ou durante o desenvolvimento embrionário inicial de Maughlin Storm. Devido ao ciclo de endogamia fruto do ancestral Fairlea Royal Mark, o macho Dudoc Mr Burns herdou duas versões do haplótipo ancestral. A sua cópia materna do haplótipo ancestral carrega a mutação CD, enquanto que a sua cópia paterna ainda está no seu estado ancestral do tipo selvagem.

Menízi et al. (2016) re-sequenciaram o genoma inteiro de um vitelo afetado e um macho parcialmente consanguíneo saudável, carregando uma cópia do segmento crítico de 2,24-Mb e detetaram uma mutação causal - 1,3 kilo de par de bases (kb) inserção de um elemento de transposição LTR (retrovírus endógeno 2-1) localizado na sequência codificadora do APOB. A inserção de 1,3kb foi confirmada por Schütz et al. (2016), que relataram que o elemento LTR foi inserido no exão 5 do APOB (em BTA11: 77,959 kb). A APOB é essencial na produção de quilomicrons e lipoproteínas de baixa densidade (Kamiński & Ruś, 2016). Deste modo, parece legítimo afirmar que a mutação no APOB representa uma mutação de perda de função similar à FHBL hereditária autossómica recessiva em humanos (Young et al., 1988). Gross et al., (2016), reportaram que a mutação causal da CD afeta o metabolismo lipídico, a biossíntese de esteroides e a função da membrana celular em portadores homozigotos e heterozigotos, podendo resultar em sintomas inespecíficos como redução da fertilidade, crescimento e saúde. A troca rápida de material genético por meio de inseminação artificial, importação de sêmen e transferência de embriões permite a rápida transmissão da mutação entre as populações (Kamiński & Ruś, 2016).
Distribuição Geográfica e Prevalência


O haplótipo da deficiência em colesterol (CDH) tem uma frequência maior (2,5%, baseada em todos os heterozigotos conhecidos e suspeitos) do que muitos outros haplótipos recessivos que resultam igualmente na morte de vitelos, como a Malformação Vertebral Complexa (1,37%) e Deficiência na Adesão de Leucócitos Bovina (0,25%) (Cole et al., 2016).

Cole et al. (2016) identificaram 56.641 Holsteins, portadores do CDH, na sua avaliação genómica em agosto de 2015. Desses animais, 54,6% eram heterozigóticos para o haplótipo prejudicial e 0,48% eram homozigóticos para o haplótipo prejudicial. Neste caso, a origem parental pode ser determinada (Cole et al., 2016). Adicionalmente, 44,2% eram heterozigóticos para o haplótipo recessivo e 0,63% eram homozigóticos para o haplótipo recessivo. Não obstante, a origem parental não pode ser determinada (Cole et al., 2016). Assim sendo, aproximadamente metade dos supostos portadores e animais afetados podem ser portadores do haplótipo recessivo, o que significa que o impacto econômico da HCD pode ser superior ao expectado (Cole et al., 2016).

Impacto econômico

Os efetivos de bovinos são suscetíveis à propagação de doenças recessivas. Um touro pode gerar dezenas de milhares de progénitos através da inseminação artificial (Pausch et al., 2015). A frequência de alelos deletérios transportados por estes touros pode aumentar consideravelmente dentro de algumas gerações (Pausch et al., 2015).

Assumindo o acasalamento aleatório de todos os touros testados, nascem por ano na Alemanha 3.175 animais homozigóticos para o haplótipo, considerando uma frequência haplotípica de 4,2% e cerca de 1,8 milhões de nascimentos de Holsteins por
ano (Kipp et al., 2016). Dado um valor médio de 400 Euros por animal (tempo médio de vida de 85 dias, custos de produção e tratamento médico), a perda econômica por ano na Alemanha representa cerca de 1,3 milhões de Euros (Kipp et al., 2016).

**Fenótipo Clínico**

Os achados clínicos da doença em vitelos homozigóticos incluem atraso no crescimento e diarreia crônica ou intermitente não responsiva ao tratamento (Mock et al., 2016). Kipp et al. (2016) descreveram a atitude geral dos vitelos afetados como alerta e responsiva em três bezerros e levemente a moderadamente letárgica em dois.

Os globos oculares encontram-se normalmente profundamente afundados na órbita em todos os vitelos, indicando um grau de desidratação de moderado a grave (Kipp et al., 2016). Os vitelos afetados apresentam ainda crescimento consideravelmente retardado, com uma condição corporal classificada como emaciada ou caquética e com um pêlo enfraquecido e áspero (Kipp et al., 2016). Além disso, os animais afetados exibem atrofia muscular (Inokuma et al., 2017), mostrando ataxia no estádio final da doença.

Mock et al. (2016), reportaram que as fezes dos animais afetados apresentavam coloração amarela a verde-oliva, cheiro normal e uma consistência fecal de mole a líquida durante um período de observação de 14 dias.

A maioria dos vitelos afetados não apresenta perda de apetite até pouco antes da morte natural ou da eutanásia (Inokuma et al., 2017).

Adicionalmente, Kipp et al. (2016) reportaram um caso de um animal que apresentava sinais clínicos de paresia do nervo radial num dos membros anteriores.

Foi descrito também um caso de uma novilha homozigótica recessiva que manifestou atraso no crescimento e diarreia intermitente nos primeiros meses de vida (Mock et al., 2016). Foi levada para a Clínica de Ruminantes da Faculdade Vetsuisse, Universidade de Berna, Suíça, apresentando como queixa primária a presença de lesões na cavidade bucal (Mock et al., 2016). Porém, após a realização do exame neurológico, verificou-se que este animal apresentava também hipermetria e pacing, sugerindo assim a presença de uma lesão cortical difusa (Mock et al., 2016). A análise ao líquido cerebroespinhal mostrou uma leve pleocitose com 39% de monócitos e 61% de linfócitos
(Mock et al., 2016). A novilha apresentava ainda valores de colesterol e de triglicerídeos semelhantes aos vitelos afetados.

Os achados laboratoriais mais relevantes dos animais afetados revelam hipocolesterolemia e hipolipidemia com a LDL-C abaixo do limite de detecção, indicando um distúrbio hereditário do metabolismo lipídico (Mock et al., 2016; Kipp et al., 2016).

O RBC, a Hb e o Ht dos vitelos homozigóticos apresentam-se significativamente menores em comparação a vitelos não homozigóticos (Inokuma et al., 2017). A maioria dos animais afetados apresentam leucocitose e valores acima dos valores de referência de glutamato desidrogenase, aspartato aminotransferase, gama-glutamil transferase e bilirrubina total (Kipp et al., 2016). As concentrações de vitamina A e E e selênio são geralmente acentuadamente reduzidas (Kipp et al., 2016).

Animais portadores heterozigotos não apresentam sinais clínicos aparentes, no entanto têm níveis mais baixos de colesterol no sangue (Kipp et al., 2015).

**Diagnóstico**

As lipoproteínas são compostas por lipídios hidrofóbicos (triglicerídeos e ésteres de colesterol) e um envelope composto de apoproteínas e lipídios anfipáticos (fosfolipídios e colesterol livre) (Kessler et al., 2014). As lipoproteínas de densidade intermediária são ricas em ésteres de colesterol e também são metabolizadas em lipoproteínas de baixa densidade (LDL) ou absorvidas pelo fígado (CUCVM, 2017). Em humanos, as LDL são as principais transportadoras de colesterol no sangue e distribuem o colesterol do fígado para os tecidos periféricos (Kessler et al., 2014). As lipoproteínas de alta densidade (HDL) são sintetizadas no fígado e no trato gastrointestinal e transportam o colesterol dos tecidos para o fígado (o chamado transporte “reverso” do colesterol) (CUCVM, 2017).

Kipp et al. (2016), mostraram que a concentração sérica de colesterol está significativamente associada ao número de cópias de haplótipos que um animal carrega. Os animais que não transportam o haplótipo têm valores de colesterol dentro do normal. Os animais com uma cópia do haplótipo apresentaram valores de colesterol inferiores ao valor de referência. Por fim, os animais com duas cópias apresentam valores de colesterol
significativamente inferiores aos dois grupos enumerados anteriormente (Kipp et al., 2016). Estas diferenças entre portadores, não portadores e homozigóticos portadores da doença sugerem um patrimônio genético codominante (Kipp et al., 2016). Este haplótipo é denominado de CDH (Kipp et al., 2016).

Ademais, a CD em portadores homozigóticos causa uma incapacidade fatal de manter um nível de colesterol no sangue suficiente para a vida (Duff et al., 2016). Neste sentido, a hipocolesterolemia e baixas concentrações de triglicerídeos no efetivo Holstein são os principais achados laboratoriais desta doença (Inokuma et al., 2017).

A deficiência de colesterol é uma doença provocada por um defeito genético autossômico recessivo monogênico (OMIA, 2017; Menzi et al., 2016; Mock et al., 2016). O GWAS e o mapeamento de homoigosidade revelaram um haplótipo associado à doença de ~ 2,7 Mb no BTA 11 (Kipp et al., 2015).

O haplótipo associado à doença tem origem no touro Holstein canadiano Maughlin Storm, nascido em 1991 (VanRaden & Null 2015). Maughlin Storm foi o primeiro touro conhecido portador da doença, para o qual a anormalidade primária é uma inserção de 1.299-pb de um elemento transponível localizado no exão 5 do APOB (Kipp et al., 2015; Kipp et al., 2016).

A inserção do APOB é identificada através de um PCR de diagnóstico usando três primers e eletroforese capilar automatizada (Analisador de fragmentos; Advanced Analytical Technologies) (Kipp et al., 2016).

Ao exame macroscópico, os animais afetados apresentam uma fraca condição corporal (Mock et al., 2016; Kipp et al., 2016). Alguns animais exibem atrofia grave da medula óssea e do tecido adiposo estrutural, sendo, consequentemente, classificados como caquéticos (Kipp et al., 2016). Por sua vez, alguns vitelos caquéticos apresentaram edema subcutâneo acentuado, principalmente sobre o tórax e abdómen ventral (Kipp et al., 2016).

O ânus e a região perianal dos vitelos apresentam-se sujos de fezes, o que pode ser interpretado como um sinal de diarreia (Mock et al., 2016).

O abomaso está geralmente preenchido com quantidades moderadas de ingesta parcialmente digerida (Kipp et al., 2016). Os animais afetados também têm grandes quantidades de fezes oleosas, pegajosas e brilhantes (Kipp et al., 2016).

A mucosa dos dois terços distais do intestino delgado da maior parte dos animais apresenta-se edemaciada e esbranquiçada (Mock et al., 2016). O intestino grosso
encontra-se preenchido com um líquido de cor amarelo-esverdeado com um conteúdo parcialmente espumoso a gorduroso, consistente com esteatorreia (Mock et al., 2016).

Um achado patológico adicional que urge ser tido em consideração é a broncopneumonia supurativa multifocal a confluentes moderados a graves, afetando cerca de 5 a 20% do pulmão em alguns animais (Kipp et al., 2016). Alguns vitelos também apresentam degeneração multifocal leve a moderada da musculatura com infiltrado inflamatório linfo-histiocitário moderado (Kipp et al., 2016).

As alterações histológicas são mais proeminentes no intestino delgado, destacando-se no jejuno (Mock et al., 2016). Os enterócitos contêm grandes quantidades de vacúolos redondos a ovais, tipicamente vazios, variando de 2 a 20 micrômetros de diâmetro (Mock et al., 2016). Em seções congeladas, estes vacúolos intracitoplasmáticos e grande parte do conteúdo intestinal foram positivamente corados pela coloração de Sudão, indicando uma origem lipídica (Mock et al., 2016).

Atualmente, não foram observadas lesões patomorfológicas ou patohistológicas no figado (Mock et al., 2016; Kipp et al., 2016).

Outros achados patológicos incluem: ruminite subaguda moderada, focal, ulcerativa e fibrinos purulenta, endocardite linfo-histiocitária multifocal de moderada a grave e miocardite (Kipp et al., 2016).

**Diagnósticos Diferenciais**

**Diarreia**

A diarreia pode ser classificada como enterite primária, diarreia secundária a uma patologia gastrointestinal ou enterite sintomática (infeção generalizada, intoxicação, patologia metabólica ou patologia orgânica) (Dirksen et al., 2004).

A intussuscepção, o deslocamento do abomaso e a diarreia nutricional estão descritos como causas não infecciosas de diarreia em vitelos (Francoz & Guard, 2015; Fecteau & Guard, 2015).

**Lesões na Mucosa Oral**

Geralmente as lesões na mucosa oral resultam num certo grau de disfagia ou relutância em comer por causa da dor (MacLachlan & Mayo, 2015). As lesões incluem vesículas, erosões, úlceras, crostas ou crescimentos nos lábios, língua, gengivas, palato ou faringe (Smith, 2015). As lesões vestibulares podem ser causadas por agentes físicos, químicos ou infecciosos, sendo que este último causa o maior número de eventos (Smith, 2015). No tocante aos agentes infecciosos a título ilustrativo podem-se enumerar os seguintes: BVDV, estomatite vesicular, actinobacilose, *Fusobacterium necrophorum*, *Candida* spp., entre outros.

**Atraso no crescimento**

A caquexia e a síndrome do vitelo fraco são possíveis diagnósticos diferenciais de atraso no crescimento (Maas, 2015). É altamente possível que vários casos de animais autossómicos recessivos com CD possam ter sido diagnosticados de forma incorreta como síndrome do vitelo fraco (Inokuma et al., 2016).

**Hipocolesterolemia**

A diminuição da absorção, a diminuição da produção, a alteração do metabolismo e o aumento da captação de lipoproteínas são possíveis causas de hipocolesterolemia (CUCVM, 2017).

A intoxicação crônica por iodo é um possível diagnóstico diferencial de hipocolesterolemia que também deve ser considerado (Hillman & Curtis, 1980). Os achados clínicos incluem hiperglicemia, hipocolesterolemia e uma mudança neutrofilica-linfopénica nos leucócitos na circulação sanguínea (Hillman & Curtis, 1980).

Além disso, Elissalde et al. (1983) descreveram que vacas infetadas com *Babesia bovis* apresentavam valores de colesterol e cortisol marcadamente reduzidos (menos de 50% dos valores normais) durante a fase aguda da doença.
A síndrome da vaca gorda é outro diagnóstico diferencial possível. É uma condição multifatorial que ocorre em vacas leiteiras após o parto (Smith, 2015). Esta condição pode aumentar os níveis de ácidos gordos não esterificados e diminuir os TG e o colesterol (Smith, 2015).

**Tratamento**

Em primeiro lugar, o tratamento implementado deve ser um tratamento sintomático (Kipp et al., 2016). Como tratamento específico, as recomendações descritas para humanos são: restrição em gordura dietética para prevenir a esteatorreia e suplementação de longa duração de doses altas de vitamina E e A para prevenir ou, pelo menos, retardar a progressão da doença degenerativa neuromuscular e da retina (Chowers et al., 2001; Kane & Havel, 2001; Lee & Hegele, 2014). Repare-se que este tratamento não é aplicável em vitelos jovens (Mock et al., 2016). Animais mais velhos com CD são particularmente interessantes para investigação adicional sobre a patogénese da doença (Mock et al., 2016).

A suplementação de açúcar pode também ser usada em vitelos com crescimento retardado, na medida em que instala o perfil dos protozoários do rúmen e estimula o desenvolvimento das papilas (Sato et al., 2010).

**Maneio**

O CDH está claramente associado à mortalidade de vitelos e tem um elevado impacto na população mundial de Holsteins (Kipp et al., 2015).

A generalidade das mutações está disseminada por toda a população, visto que o touro fundador gerou milhares descendentes (Cole et al., 2016). Por essa razão, os efeitos negativos do touro podem ser amplificados quando este é muito utilizado numa população pequena (Cole et al., 2016).

É fácil reduzir a frequência de um alelo deletério numa população sob seleção, no entanto é extremamente difícil eliminá-lo completamente da população (Cole et al., 2016). Os portadores conhecidos podem ser removidos da população, não obstante, na prática, é mais comum evitar acasalamentos entre portadores, pois os touros portadores
podem ter alto mérito genético para características economicamente importantes. Segelke et al. (2016) sugeriram recentemente que a seleção de vacas num índice, incluindo haplótipos de interesse e touros com valor genético, pode ser utilizada para equilibrar a seleção para (ou contra) alelos específicos com ganho genético, e Cole (2015) demonstrou uma estratégia para alocação de correspondência que pode acomodar inúmeros recessivos simultaneamente.

Em adição, as informações sobre o status do haplótipo, incluindo a disponibilidade de dados de pedigree, permitem a prevenção de acasamentos futuros de risco (Kipp et al., 2015). A identificação de portadores de CD e a consideração dessas informações em programas de reprodução podem prevenir a mortalidade em vitelos e melhorar o seu bem-estar e saúde (Kipp et al., 2015).

**Hipobetalipoproteinemia Familiar Humana (FHBL)**

A FHBL pertence a um grupo heterogêneo de doenças monogénicas caracterizado por níveis reduzidos de LDL-C e APOB no plasma (Rimbert et al., 2016). Esta doença é causada por mutações no APOB no cromossoma 2p23-24 que interferem com a tradução da APOB e adicionalmente ou em contraste prejudicam a secreção de VLDL (Tarugi & Averna, 2011).

Os heterozigóticos portadores FHBL são normalmente assintomáticos e não diagnosticados a não ser que se realize um perfil lipídico (Welty, 2014).

Em homozigóticos da FHBL, os achados clínicos e laboratoriais são indistinguíveis da abetalipoproteinemia (Burnett & Hooper, 2015). Os sinais clínicos consistem numa má absorção de gordura com esteatorreia, vômitos, distensão abdominal e atraso no crescimento no período neonatal. Mais tarde, pode haver uma progressão para uma retinite pigmentosa atípica e ataxia espinocerebelar (Berriot-Varoqueaux et al., 2000). A deficiência em vitamina E conduz à manifestação clínica mais debilitante que se traduz em alterações neurológicas que, por sua vez, levam a uma progressiva degeneração do sistema nervoso central e à morte (Welty, 2014). A acantocitose, a diminuição da vida dos eritrócitos, a anemia, a hiperbilirrubinemia e hemólise, e a coagulopatia podem ocorrer devido à deficiência em vitamina K (Kane & Havel, 2001). As alterações hepáticas incluem: hepatomegália, aumento das amino-transferases e
esteatose hepática (Berriot-Varoqueaux et al., 2000), que podem progredir e originar uma esteatohepatite, fibrose e cirrose (Burnett & Hooper, 2015).

Atualmente, o diagnóstico genético da FHBL utiliza a sequência de Sanger no APOB (Rimbert et al., 2016).

O tratamento consiste numa dieta pobre em gordura com o suplemento de vitaminas solúveis em lípidos como as vitaminas A e E. Este tratamento é utilizado com intuito de a prevenir ou, pelo menos, retardar a progressão das doenças degenerativas neuromusculares e da doença retinal (Kane & Havel, 2001; Lee & Hegele, 2014; Chowers et al., 2001).

**Objetivos**

Esta dissertação de mestrado pretende apresentar um caso clínico de uma vitela com Deficiência em Colesterol, perceber os passos necessários para a realização de um diagnóstico correto e implementação de um tratamento. Além disso, pretende mostrar aos produtores e aos médicos veterinários que a CD é um possível diagnóstico diferencial para a diarreia crônica e o atraso no crescimento em vitelos Holstein.

**Materiais e Métodos**

Este estudo teve por base uma vitela Holstein com 5 meses de idade que foi referenciada para a Clínica de Ruminantes da Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Itália. O animal tinha como história clínica atraso no crescimento, diarreia intermitente e progressiva emaciação. O principal motivo que levou a vitela à Clínica foi a presença de lesões na cavidade bocal.

Após a admissão, o animal afetado foi submetido a um exame clínico completo. Foram recolhidas amostras de sangue através de venipuntura da veia jugular para a realização de um perfil hematológico, perfil bioquímico, uma electroforese de proteínas, um ionograma e a medição dos valores de colesterol total e TG. Foi também realizada uma urianálise.

Adicionalmente, foram recolhidas amostras fecais submetidas a análises de modo a excluir os agentes patogêneos causadores de diarreia mais comuns (Cryptosporidium
spp., coccidiose, BVDV). A diarreia causada por mau maneio nutricional foi, desde logo, excluída através da história clínica.

Foi realizada uma zaragatoa oral e uma endoscopia à cavidade nasal e à laringe. Os exames coprológicos e a zaragatoa nasal foram processados pelo Servizio di Malattia Infettive, Parassitarie e Aviarie from the Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Itália. Foi também realizado um teste genético processado pelo Doutor Cord Drögemüller do Institut für Genetik, Universität Bern, Suíça.

Foi implementado como tratamento a iodina glicerinada administrada localmente nas lesões de mucosa oral três vezes por dia durante os primeiros sete dias. No dia 1,3,5,7 e 32 foi administrado selênio, cianocobalamina, ácido adenosina-5’-monofosfórico e sorbitol (Selevit®, Fatro, Ozzano Emilia). Do dia 8 ao dia 14, foi administrado localmente iodopovidona (10%; Betadine®, Meda Pharma, Milan) e mel nas lesões orais. Do dia 14 até ao dia 18 foi administrado subcutaneamente benzylpenicilina+diidrostreptomicina (200.000 Unidades Internacionais por mililitro + 250 mg/mL; Repen®, Fatro, Ozzano Emilia). Do dia 18 até ao dia 21, foi administrado enrofloxacina subcutâneo (100 mg/mL; Baytril®, Bayer, Milão). No dia 32 foi administrado vitamina A, vitamina D3, vitamina E (vitamina A 10.000.000 unidades internacionais [IU], vitamina D3 2.500.000 IU, vitamina E 10.000 miligramas; Adisole A-D-E®, Vetem S.p.A., Lungomare Pirandello). Foi administrada uma transfusão de sangue total e 5 litros de NaCl 0,9% com 80 miliequivalentes de cloreto de potássio. No dia 33 foram administrados 5 litros intravenosos de NaCl 0,9%.

O animal teve uma morte natural 33 dias após a admissão na Clínica. Foi realizada uma necropsia completa e exame histológico (cólon, encéfalo, esófago, fígado, ilio, linfonodos, baço, músculo, pulmão, corpo do estômago, adrenal, tiroide, traqueia, bexiga, timo, aorta, artéria subclávia, língua, olho e rúmen).

Após a necropsia, foi colhido sangue de 3 familiares saudáveis da vitela em estudo (mãe, irmã 1, irmã 2). Foi realizado um perfil hematológico completo, um perfil bioquímico, procedeu-se à medição dos valores de colesterol total e TG e, por fim, foi realizado um teste genético. O sêmen do pai da vitela foi também submetido a um teste genético.
Resultados

A vitela admitida na Clínica para Ruminantes da Facoltà di Medicina Veterinaria dell’Università degli Studi di Bologna, Itália, com história de atraso no crescimento e emaciação progressiva, apresentava hipotonia muscular e diarreia intermitente. A nível oral, a vitela apresentava lesões gengivais, sublinguais e palatinas compatíveis com estomatite fibrino-ulcerativa. Durante a auscultação pulmonar revelou um aumento do murmúrio vesicular ao nível do quadrante cranial direito. Quando se realizou a prova da respiração forçada o animal mostrou a presença de estridores ao nível do quadrante medio-dorsal direito.

Tendo em conta as análises sanguíneas, os achados mais significantes da vitela foram: hipocolesterolemia e baixas concentrações de TG; RBC, Hb e Ht inferiores aos valores de referência; panhipoproteinemia; fosfatase alcalina, bilirrubina total, bilirrubina indireta e bilirrubina direta acima do valor de referência. A mãe da vitela apresentou valores de colesterol inferiores aos valores de referência.

Na urianálise verificou-se que o pH e a densidade específica da urina da vitela se encontrava abaixo dos valores referência e que continha eritrócitos.

O exame coprológico foi negativo à análise parasitológica, bacteriológica e viral. Já a zaragatoa oral foi positiva à Candida albicans.

À endoscopia observou-se uma rinolaringite de baixo grau associada a uma traqueíte de alto grau.

Ao exame genético verificou-se que a vitela é homozigótica para a mutação no APOB, a mãe e o pai da vitela são heterozigóticos portadores da mutação no APOB, e as irmãs 1 e 2 são não portadoras da mutação no ABOB.

No exame macroscópico efetuado durante a necropsia, o animal apresentou lesões na mucosa oral (gengival, sublingual, labial e palatina) compatíveis com estomatite fibrino-ulcerativa. Apresentava também lesões compatíveis com laringite, esofagite, perihepatite e provavelmente hepatite, e hiperemia dos vasos meníngeos e ligeiro edema cerebral. Além disso, o intestino delgado revelou uma congestão generalizada e estava difusamente preenchido com conteúdo amarelado, bege a verde lima, líquido e particularmente com conteúdo gorduroso. As lesões do intestino eram compatíveis com enterite segmentar.
Discussão

A CD é uma doença autossômica recessiva recentemente descrita no efetivo Holstein (Gross et al., 2016). Consiste numa alteração secundária a um defeito genético (Gross et al., 2016). O touro Holstein Canadiano Maughlin Storm foi o primeiro touro conhecido como sendo portador da doença (VanRaden & Null, 2015). A CD é provocada por uma mutação localizada no exão 5 do APOB (Kipp et al., 2015; Kipp et al., 2016).

Os achados clínicos mais relevantes encontrados neste trabalho foram diarreia intermitente e atraso no crescimento associados a hipocolesterolemia e baixas concentrações de triglicéridos. Apesar dos sinais clínicos terem sido inespecíficos e de intensidade moderada, a vitela com CD revelou um crescimento deficiente, progressiva emaciação, diarreia intermitente e lesões orais. Resultados semelhantes foram descritos por Mock et al. (2016). A cultura da zaragatoa oral foi positiva para Candida albicans. Na auscultação pulmonar os sons encontravam-se ligeiramente aumentados no quadrante cranial direito e quando se realizou a prova da respiração forçada o animal mostrou a presença de estridores ao nível do quadrante medio-dorsal direito. Kipp et al. (2016) reportaram também a presença de sons pulmonares anormais em dois vitelos doentes. Adicionalmente, à endoscopia verificou-se a presença de uma rinolaringite de baixo grau associado a uma traqueíte de alto grau. Permanece indefinido se os processos infecciosos secundários diagnosticados na vitela são uma manifestação do aumento da suscetibilidade a infeções secundárias resultantes da deficiência em vitaminas. De qualquer modo, já foi estudado que a hipovitaminose tem como consequência uma diminuição da resistência a infeções no efetivo bovino (Spears, 2000; Xiuyuan et al., 2012).

Além disso, o fenótipo patológico da vitela homozigótica mutante para o APOB, como caquexia, atrofia muscular, sinais de diarreia, o intestino preenchido difusamente com conteúdo moderado amarelado, líquido e parcialmente espumoso a gordurento e enterite também foram descritos por Kipp et al. (2016).

Considerando o achado patológico de enterite e o fenótipo clínico de atraso no crescimento, hipocolesterolemia e baixas concentrações de TG, a CD é altamente similar à FHBL humana (Welty, 2014). Apesar de não se terem verificado sinais neurológicos em vida, o exame post mortem revelou a presença de hiperemia dos vasos meníngeos e um leve edema cerebral. A FHBL é ainda caracterizada pela má absorção das vitaminas lipossolúveis (A, D, E, K), levando à degeneração reticular, neuropatias e coagulopatias (Lee & Hegele, 2014). Estas desordens neurológicas estão associadas à disfunção
cerebelar e comprometem a função da parte posterior da coluna com desmielinização do sistema nervoso central e periférico (Lee & Hegele, 2014; Welty, 2014).

A vitela deste estudo apresentava esofagite. Em doentes humanos com FHBL, a esofagite está descrita como uma das possíveis complicações da doença (Lee & Hegele, 2014). Contudo, até agora nenhum estudo pregresso reportou esofagite em vitelos com CD.


Presentemente, não se sabe se as APOB são ou não expressas nos bovinos com CD, devendo esta questão ser objeto de investigação mais aprofundada (Menzi et al., 2016). A redução da função da APOB-100 resulta numa redução da exportação de TG do fígado, o que, por sua vez, leva ao desenvolvimento da esteatose hepática (Tanoli et al., 2004). Até agora, nenhum dos bovinos estudados apresentou fígado gordo, o que leva a concluir que a esteatose hepática decorrente da disfunção da APOB-100 não ocorre nos bovinos afetados com CD (Mock et al., 2016). Por outro lado, a ausência de esteatose hepática na vitela afetada com CD pode ser explicada pelas diferenças de metabolismo entre bovinos e humanos. Fundamentalmente, a síntese de ácidos gordos de novo em humanos ocorre no fígado usando a glicose como substrato (Vernon, 1980). Nos bovinos, o acetato absorvido no rúmen é o substrato direto para a síntese de ácidos gordos de novo (Vernon, 1980). O acetato é produzido através da fermentação microbiana da celulose no rúmen e é armazenado no tecido adiposo e é também usado para a produção da gordura do leite na glândula mamária de animais em lactação (Vernon, 1980). Assim sendo, o local primário da síntese de ácidos gordos de novo é no tecido adiposo e o local secundário é na glândula mamária (animais em lactação), e não no fígado (Bergen & Mersmann, 2005). Isto apenas se aplica a animais ruminantes, como no caso em apreço. Os lípidos são transportados no plasma sanguíneo como lipoproteínas (Bergen & Mersmann, 2005). Os quilomicrons e as VLDL contêm grandes quantidades de triglicerol e pequenas quantidades de colesterol (Bergen & Mersmann, 2005). Os quilomicrons são sintetizados no intestino e podem ser encontrados na linfa e no plasma após uma refeição contendo...
gordura (Drackley, 2005). Os ruminantes têm apenas algumas ou mesmo nenhuma partículas de quilomicrons uma vez que o consumo de gordura de apenas 5% em matéria seca interfere com a digestão microbiana no rúmen (Drackley, 2005). Nestas condições, algumas cadeias longas de ácidos gordos são absorvidas (Drackley, 2005). Os principais transportadores de colesterol são as LDL e as HDL (Bergen & Mersmann, 2005).

As apolipoproteínas formam as proteínas estruturais das lipoproteínas permitindo o transporte lipofílico do colesterol e do triglicerol no sangue (Kipp et al., 2016). A APOB é a proteína estrutural que liga o colesterol formando a LDL-C e a VLDL-colesterol de forma a transportar o colesterol (Kipp et al., 2016). Geralmente, as lipoproteínas que contêm a APOB transportam lipídios do figado (local de síntese) e do intestino (local de absorção) para vários locais de utilização de forma a obter produção de energia, produção da membrana celular, ou ainda para produção de hormonas esteroides (Marcovina & Packard, 2006). Kipp et al. (2016) reportaram que todos os vitelos homozigóticos portadores do CDH apresentavam concentrações de LDL-C muito reduzidas. No entanto, neste caso, não foi possível fazer a medição dos valores de LDL-C.

No presente estudo, o colesterol total da mãe (heterozigótica, portadora da mutação no \( APOB \)) da vitela com CD estava abaixo dos limites referência e não apresentava sinais clínicos de má absorção. Porém, não foi possível medir os valores de TG e de fosfolípidos. Nenhum dos heterozigóticos portadores da mutação no \( APOB \) (vitelos e bovinos adultos) mostrava sinais de má digestão quando comparados com os animais homozigóticos afetados (Gross et al., 2016). Além das concentrações diminuídas de TG, fosfolípidos e colesterol total, os bovinos heterozigóticos são capazes de manter a homeostasia do colesterol e das lipoproteínas de biossíntese de hormonas esteroides, por exemplo, e a função da membrana celular (Gross et al., 2016). Ademais, humanos heterozigóticos portadores da FHBL são geralmente assintomáticos (Schonfeld, 2003).

Devido à má absorção de lipídios, são esperados efeitos deletérios da deficiência em APOB no metabolismo lipídico hepático, na biossíntese esteroide e na função da membrana celular (Gross et al., 2016). Contudo, poderia ser especulado que efeitos deste podem não ser evidentes nos animais heterozigóticos, resultando em sinais inespecíficos que afetam a fertilidade, o crescimento e a saúde (Gross et al., 2016).

O RBC, a Hb e o Ht da vitela homozigótica eram inferiores aos valores de referência. Apesar de não existirem dados sobre a observação do esfregaço sanguíneo da vitela, a acantocitose é um achado laboratorial em humanos com FHBL (Lee & Hegele, 2014; Welty, 2014). Uma vez que o colesterol é um componente essencial da
reticulocitose da membrana, os eritrócitos dos animais afetados podem ser frágeis, o que pode conduzir a valores diminuídos de RBC, Hb e Ht (Inokuma et al., 2017).


Ao exame urinário, a vitela com CD apresentou um pH e uma densidade específica diminuídos. Uma urina com uma densidade inferior a 1.020 pode ser causada por polidipsia, doenças metabólicas ou insuficiência renal (Gründer, 2004 a). Contudo, tanto a nível microscópico como a nível macroscópico, não foram encontradas lesões nos rins.

Os vitelos com CD normalmente morrem nos primeiros seis meses de vida (Kipp et al., 2015). O tratamento descrito para doentes humanos inclui, nomeadamente, restrição total em gordura e suplementação em vitaminas lipossolúveis (Lee & Hegele, 2014; Welty, 2014). Todavia, esta terapêutica não é exequível em animais muito jovens. Por outro lado, bovinos com mais idade, como a vitela apresentada neste estudo, são animais particularmente interessantes para investigar a patogénese da doença.

Os sinais clínicos inespecíficos de diarreia e atraso no crescimento em vitelos jovens limitam a realização do diagnóstico de CD (Kipp et al., 2016). Assim sendo, é importante descartar todos os possíveis agentes que causem diarreia. Caso se verifique a presença de uma diarreia intermitente ou crónica não responsiva ao tratamento em vitelos Holstein, a análise do colesterol total e dos triglicéridos pode levar a uma suspeita de CD (Kipp et al., 2016).

O diagnóstico final de CD deve ser sempre confirmado realizando um teste genético para deteção da mutação no APOB (Menzi et al., 2016). A realização de um diagnóstico precoce permitirá a redução de custos em tratamentos, a utilização de antibióticos e o tempo de tratamento dos animais (Kipp et al., 2016). Por conseguinte,
permitirá também a erradicação da mutação no *APOB* da população Holstein e, consequentemente, prevenir perdas económicas no futuro (Kipp *et al.*, 2016).

**Conclusão**

Este trabalho reporta a ocorrência de um haplótipo autossômico de herança codominante, que está associado a uma alteração significativa no metabolismo do colesterol com tremendos efeitos na saúde e na sobrevivência dos portadores homozigóticos do haplótipo. Este defeito congênito, designado CDH, está relacionado com um fenótipo caracterizado por atraso no crescimento, emaciação, atrofia muscular e diarreia crónica ou intermitente em vitelos e mostra notáveis similaridades com os homozigóticos humanos com FHBL.

Assim sendo, a CD deve ser considerada como possível diagnóstico diferencial de diarreia crónica e atraso no crescimento em vitelos Holstein entre os três e os seis meses de idade. Atualmente, o diagnóstico definitivo só é possível realizando um teste genético.