Japanese Journal of Veterinary Research 65(2): 107–112, 2017

JJVR

SHORT COMMUNICATION Clinical Case Report

Retrospective study of clinical and laboratory findings of autosomal recessive cholesterol deficiency in Holstein calves in Japan

Hisashi Inokuma[1,*], Noriyuki Horiuchi[2], Ken-ichi Watanabe[2] and Yoshiyasu Kobayashi[2]

[1] Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080–8555, Japan

[2] Department of Basic Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080–8555, Japan

Received for publication, March 22, 2017; accepted, April 5, 2017

Abstract

This retrospective study was conducted to clarify the clinical findings of an autosomal recessive cholesterol deficiency disorder in Holstein calves. Thirty-three calves with poor growth by uncertain causes from 2007 to 2016 comprised the study population of calves that were homozygous (n = 12) and non-homozygous (n = 21) for the disease. No significant group-dependent differences were found in the proportion with diarrhea or fever. Fewer calves with ataxia and anorexia were found in homozygotes. All affected calves showed muscular atrophy. Median RBC, Hb, and Ht values in affected calves were significantly lower. Median total cholesterol (TC) in homozygotes was extremely low, at 6.5 mg/dl (range, 5.0–15.0). TC readings under 10 mg/dl were thought to be suggestive of the disease.

Key Words: autosomal recessive cholesterol deficiency, Holstein, retrospective study

Autosomal recessive cholesterol deficiency in Holstein Frisian cattle was first reported by Kipp *et al.* in 2015[2]. A Canadian Holstein sire named Maughlin Storm was the first known carrier bull of the disease[8], for which the primary abnormality is a 1,299-bp insertion of a transposable element located in exon 5 of the apo lipoprotein B gene (*APOB*)[2,3]. Clinical findings of the disease described in a recent report include poor growth and chronic diarrhea unresponsive to treatment[6]. Although hypocholesterolemia and low triglyceride concentrations in the cattle are characteristic laboratory findings and suggestive for disease diagnosis[6], clinical signs of the disease are non-specific and similar to other diseases which cause chronic diarrhea. It is highly possible that several cases of autosomal recessive cholesterol deficiency might have been misdiagnosed as weak calf syndrome or diseases of unknown etiology with poor growth in the past. In the present report, a retrospective study was conducted to clarify the clinical and laboratory findings of autosomal recessive cholesterol deficiency by comparing the findings with other diseases that

*Corresponding author: Hisashi Inokuma, DVM, PhD, Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080–8555, Japan
Phone: +81–155–49–5370. E-mail: inokuma@obihiro.ac.jp
doi: 10.14943/jjvr.65.2.107

caused poor growth or chronic diarrhea in the past.

In total, 33 Holstein Frisian calves with poor growth by uncertain causes were selected among patients that presented at the Animal Teaching Hospital, Obihiro University of Agriculture and Veterinary Medicine from April 2007 to October 2016. The chief complaints for all 33 calves were poor growth, emaciation, chronic diarrhea, or weakness at the first consultation by local veterinarians. Routine clinical examinations were performed for the calves at the time of presentation to the university hospital. Complete blood counts (CBC) and blood chemical analysis, including total cholesterol (TC), non-esterified fatty acid (NEFA), total protein (TP), albumin, aspartate aminotransferase activity (AST), lactate dehydrogenase activity (LDH), and creatine phosphokinase activity (CPK) were also examined as part of our routine examination. Final diagnosis by the complete necropsy of these 33 calves did not reveal any obvious primary causes such as congenital malformation, severe pneumonia, or enteritis. Thus, these calves were diagnosed with weak calf syndrome with unknown causes in the past. All animals tested negative for bovine viral diarrhea virus (BVDV) by reverse transcription polymerase chain reaction (PCR).

Preserved EDTA blood samples of the 33 calves that had been kept at −30°C were used to extract DNA for the diagnosis of autosomal recessive cholesterol deficiency. PCR to distinguish between homozygotes and non-homozygotes (hetero and wild) was performed by the method described by Menzi *et al.*[5]. Of the 33 calves examined by the present study, 12 (36%) were found to be homozygous by PCR. The PCR also identified 2 hetero and 20 wild types, which were collectively defined as non-homozygous calves. All 12 homozygotes were born after April 2011. Pedigree information from a retrieval system for the Holstein Cattle Association in Japan (http://www.rg.liaj.jp/hol/index) confirmed that the 12 affected calves were linked to the founder sire Maughlin Storm. Of the calves with poor growth of unknown etiology and thought to have weak calf syndrome in the past, one third were confirmed to have autosomal recessive cholesterol deficiency. Median age of the patients at death or at the time of euthanasia were 110 and 85 days old in the homozygote and non-homozygote groups, respectively. This finding is consistent with a non-specific finding in a previous report that noted that affected animals usually die within the first 6 months of life[2]. Ulcers in the esophageal membrane were noted in 4 affected calves and an abomasal ulcer was found in another.

History and present clinical signs at the time of presentation, as well as laboratory findings of homozygous and non-homozygous calves were compared. Numbers of patients with history and/or the presence of clinical signs of diarrhea, fever more than 39.5°C, ataxia, anorexia, and muscular atrophy in the two groups were compared by Fisher's exact test. Laboratory findings, including red blood cell counts (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell counts (WBC), TC, NEFA, TP, albumin, AST, LDH, and CPK of both groups were compared by Mann–Whitney U test. A p-value less than 0.05 was considered statistically significant.

Median ages at the first consultation with local veterinarians were 44 and 50 days old in homozygotes and non-homozygotes, respectively. The numbers of calves in each group for which a history was obtained and/or who presented with clinical signs of diarrhea, fever more than 39.5°C, anorexia, ataxia, and muscular atrophy in both groups were summarized in Table 1. Eight of the 12 homozygotes and 14 of the 21 non-homozygotes had a history or present signs of diarrhea. We found no significant difference between the ratio of homozygous and non-homozygous calves with diarrhea. Notably, chronic diarrhea is not considered a specific clinical sign of autosomal recessive cholesterol deficiency disease, even though the most consistent clinical

Table 1. History and present clinical signs in calves with homozygous and non-homozygous autosomal recessive cholesterol deficiency

	Homozygous (N = 12)	Non-homozygous (N = 21)	*p*-value
Age of the first consultation (days)*			
	44	50	Not evaluated
History or clinical signs at the first presentation (heads (%))**			
Diarrhea	8 (66.7)	14 (66.7)	1.000
Fever (>39.5°C)	8 (66.7)	5 (23.8)	0.108
Anorexia***	2 (16.7)	14 (66.7)	0.016
Ataxia***	0 (0.0)	10 (47.6)	0.014
Muscular atrophy	12 (100.0)	13 (61.9)	0.042

*: Median of both groups
**: Numbers of animals showing signs of disease in the history or at the first presentation
***: Most homozygous calves also showed astasia and anorexia at the end stage of the disease

features of affected calves are intermittent diarrhea and a failure to thrive associated with hypocholesterolemia and low triglyceride concentrations[6]. Mock *et al.*[6] also reported that the feces of 5 affected calves 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. Indeed, two-thirds of the affected calves showed intermittent diarrhea in the present study, while the rest had no history of diarrhea and showed normal or hard feces during our observation period at the university hospital.

Eight of the 12 homozygotes showed history or present findings of fever more than 39.5°C. No significant group-dependent differences were found in the proportion of calves with fever, and significantly fewer calves with ataxia and anorexia were found among the homozygous calves than the non-homozygous calves. Muscular atrophy was observed in all homozygous calves; this was significantly more frequent than what was observed in non-homozygous calves. Additionally, most homozygous calves showed ataxia at the final stage of the disease, likely due to the severe muscular atrophy. However, most of the affected calves did not lose their appetite until just before death or euthanasia. A higher rate of severe muscular atrophy and lower rate of anorexia and ataxia are other characteristic clinical signs of this disease. Mock *et al.*[6] also reported that affected calves are often severely emaciated despite normal appetites.

A previous examination of the cardiovascular, respiratory, urinary, musculoskeletal, and neurologic systems showed no abnormalities in affected calves[6]. Meanwhile, in our study, bradyarrhythmia was recorded in 2 of the 12 affected calves, who had heart rates between 40–60 beats per minute. The reason for the decreased heart rates in the 2 affected calves is unknown.

Median CBC values obtained from both the homozygous and non-homozygous groups are shown in Table 2. Median RBC, Hb, and Ht of homozygous calves were significantly lower than those of non-homozygous calves. Five of the 12 affected calves had lower RBC counts ($< 5.0 \times 10^6/\mu l$), Hb <6.0 g/dl, and Ht <20%, while median MCV values of homozygous calves were significantly higher than those of non-homozygous calves. There were no significant differences in MCHC and WBC between the two groups. Although there are no data available for the blood smear observation, acanthocytosis was thought to be an early laboratory feature of familial hypobetalipoproteinemia in humans[1,4,9]. Because cholesterol is an essential component of the reticulocyte membrane, red blood cells of the affected animals may be fragile, which may lead

Table 2. Median values for bloodwork in homozygous and non-homozygous calves with autosomal recessive cholesterol deficiency

	Homozygous (N = 12)	Non-homozygous (N = 21)	*p*-value
RBC ($\times 10^6$/μl)	6.18	9.09	0.001
Hb (g/dl)	7.5	9.7	0.022
Ht (%)	23.0	30.0	<0.001
MCV (fl)	39.9	34.0	<0.001
MCHC (g/dl)	32.3	32.8	0.837
WBC (/μl)	9,470	9,700	0.708

Table 3. Median values for blood chemistry findings in homozygous and non-homozygous calves with autosomal recessive cholesterol deficiency

	Homozygous (N = 12)	Non-homozygous (N = 21)	*p*-value
T. Chol (mg/dl)	6.5	47.0	<0.001
NEFA (μEq/L)	155	290	0.023
TP (mg/dl)	5.9	6.0	0.793
Albumin (mg/dl)	2.8	2.8	0.311
AST (U/L)	128	84	0.359
LDH (U/L)	1041	1040	0.656
CPK (U/L)	310	338	0.758

to acanthocytosis and lower RBC, Hb, and Ht, and higher MCV in some affected animals. In the previous report, as only 1 of the affected calves showed slight anemia with a hematocrit of 17%, and no abnormalities were recorded in the CBC in 3 affected calves among 5 cases[6], more direct evidence is required to clarify whether or not the affected calves had hemolytic anemia.

Median values of blood chemistry findings in homozygous and non-homozygous calves are shown in Table 3. The median TC of 6.5 mg/dl in homozygous calves was extraordinarily low, relative to the 47.0 mg/dl observed in non-homozygous calves, with a statistically significant difference observed between the two groups ($p < 0.001$). The median NEFA of homozygous calves was also significantly lower than that in non-homozygous calves. No significant group-dependent differences were found in median values of TP, albumin, AST, LDH, and CPK. A previous study also found distinctly lower blood concentrations of TC (3.5–9.3 mg/dl) in 5 affected calves with autosomal recessive cholesterol deficiency[6]. Although TG was also quite a bit lower in the affected calves of the previous study[6], TG values for the present study patients were not available, as these are not routinely examined in our laboratory.

TC levels for each of the calves in both groups are shown in Fig. 1. TC levels of homozygous calves were between 5.0 and 15.0 mg/dl, while those of non-homozygous calves showed more variation, with values ranging between 14.0 and 113.0 mg/dl. Ten of the 12 homozygotes (83%) had TC levels <10 mg/dl, while none of the 21 non-homozygote calves had TC levels <10 mg/dl. Thus, TC levels <10 mg/dl may be suggestive of autosomal recessive cholesterol deficiency. Because the clinical signs of the disease are non-specific, extremely lower TC levels could serve as a good marker to suspect this disease, as mentioned by Mock *et al.*[6]. As some of the non-affected calves also showed very low TC concentrations (< 20 mg/dl), veterinarians should note that the

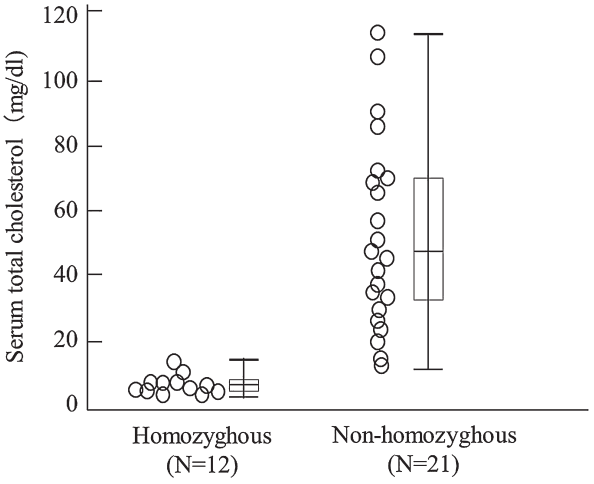

Fig. 1. Serum total cholesterol concentration of homozygous and non-homozygous calves with autosomal recessive cholesterol deficiency. Dot shows total cholesterol value of individual calves. Bar represents the interquartile range of the data.

specificity of lower TC levels is not 100%. Autosomal recessive cholesterol deficiency should be confirmed through genetic testing after confirmation of pedigree information that indicates inbreeding linked to the founder sire Maughlin Storm, as suggested by Menzi *et al.*[5]. Heterozygous carrier animals reportedly show no clinical signs but have lower TC levels[2]. In the present study, 2 heterozygous calves showed lower TC levels (35 and 69 mg/dl). As these heterozygous calves showed clinical signs of poor growth, it is impossible to determine how lower TC levels are affected by heterozygosity. Further study is needed to clarify the pathogenesis in heterozygotes.

In conclusion, clinical signs of autosomal recessive cholesterol deficiency are non-specific, with intermittent diarrhea observed in two-thirds of affected calves, ataxia and anorexia observed in fewer homozygous calves than heterozygous calves, and all affected calves exhibited severe muscular atrophy. TC levels $<$10 mg/dl would be suggestive of the disease, but genetic testing and pedigree information should be obtained to confirm the diagnosis.

Acknowledgements

We thank all staff members of the Diagnostic Center for Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine for the RT-PCR testing for BVDV. We also thank the local veterinarians in Hokkaido who brought the clinical cases of calves to our attention.

References

1) Kane JP, Hardman DA, Paulus HE. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. Proc Natl Acad Sci U S A 77, 2465–2469, 1980.
2) Kipp S, Segelke D, Schierenbeck S. A new Holstein haploytpe affecting calf survival. Interbull Bull 49, 49–53, 2015.
3) Kipp S, Segelke D, Schierenbeck S, Reinhardt F, Reents R, Wurmser C, Pausch H, Fries R, Thaller G, Tetens J, Pott J, Haas D, Raddatz BB, Hewicker-Trautwein M, Proios I, Schmicke M, Grünberg W. Identification of a haplotype associated with cholesterol deficiency and increased juvenile mortality in Holstein cattle. J Dairy Sci 99, 8915–8931, 2016.
4) Lee J, Hegele RA. Abetalipoproteinemia and homozygous hypobetalipoproteinemia: a framework for diagnosis and management. J Inherit Metab Dis 37, 333–339, 2014.
5) Menzi F, Besuchet-Schmutz N, Fragnier M. A transposable element insertion in APOB causes cholesterol deficiency in Holstein cattle. Anim Genet 47, 253–257, 2016.
6) Mock T, Mehinagic K, Menzi F, Studer E, Oevermann A, Stoffel MH, Drogemuller F, Meylan M, Regenscheit N. Clinicopathological phenotype of autosomal recessive cholesterol deficiency in Holstein cattle. J Vet Intern Med 30, 1369–1375, 2016.
7) Schütz E, Wehrhahn C, Wanjek M, Bortfeld R, Wemheuer WE, Beck J, Brenig B. The Holstein Friesian lethal haplotype 5 (HH5) results from a complete deletion of TBF1M and cholesterol deficiency (CDH) from an ERV-(LTR) insertion into the coding region of APOB. PLoS One 11, e0154602, doi: 10.1371/journal.pone.0154602, 2016.
8) Vanraden P, Null D. Holstein haplotype

for cholesterol deficiency (HCD) [Internet]. Council on Dairy Cattle Breeding; 2015 [cited 2015 Dec 20]. Available at: https://www.cdcb.us//refer ence/changes/HCD_inheritance. pdf, 2015.
9) Welty FK. Hypobetalipoproteinemia and abetalipoproteinemia. Curr Opin Lipidol 25, 161–168, 2014.