A Fatal Case of MSTN Mutation Calf Pancreatitis

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Case Report

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Abstract

Background:

Myostatin (MSTN) is related to growth and development. MSTN gene-mutated animals are prone to dystocia and fetus enlargement, but there is no report of pancreatitis. Pancreatitis is a disease of the pancreas caused by the self-digesting effect of trypsin. In animals, pancreatitis often occurs in dogs or cats and manifests as edema, congestion, bleeding, or necrosis of the pancreas. Clinically, symptoms such as abdominal pain, bloating, nausea, vomiting, and fever appear. Elevated levels of amylase in blood and urine. Pancreatitis is very dangerous to the survival of animals. We must pay attention to it.

Case presentation:

This is a fatal case report of a myostatin (MSTN) gene-mutated calf diagnosed with pancreatitis. Analyses of the blood biochemistry, blood routine, postmortem anatomy and tissue section of the 2-month old calf showed that the pancreatitis indexes α -amylase and serum lipase were higher than those of normal MSTN gene-mutated and wild-type cattle. The pancreas was found to be swollen, dark red and bloody. The structure of the acinar cells of the pancreas was unidentifiable, and massive coagulation necrosis and lysis necrosis filled in the lobules of the pancreas. The interlobular spaces became enlarged, the vessels among the lobules were dilated and congested with obvious edema and blood stasis.

Conclusions:

Evidence from anatomy, histology, blood physiology, and biochemistry parameters indicate that the myostatin mutated calf died of pancreatitis. There are few reports on the pathology of gene-mutated animals. This is the first case of MSTN gene-mutated bovine pancreatitis.

Background

Myostatin (MSTN), also known as GDF-8, belongs to the TGF- β superfamily and is a negative regulator of skeletal muscle growth and development [1, 2]. The loss or reduction of its activity will cause the excessive development of animal muscles, which is manifested as a double muscle (DM) trait [3]. McPherron et al. identified the MSTN gene in 1997 with a double-muscle hip phenotype [1]. Researchers used artificial mutation techniques to obtain the MSTN gene mutant mice [1] and cattle [4–7] that exhibited double muscle phenotype. DM animals may provide some advantages for farmers, the meat industry and consumers, such as the efficient conversion of feed into high valuable carcasses, tender meat with low-fat content. However, the MSTN gene mutant animals are also characterized to some extent by lower robustness. They are more susceptible to dystocia, respiratory disease, lameness, muscle degeneration, and heat stress [8]. Pancreatitis refers to non-infectious inflammation in the pancreatic site, including acute and chronic. Acute pancreatitis is an acute inflammation caused by trypsin digestion of the pancreas and its surrounding tissues, which is characterized by edema, bleeding, necrosis of the pancreas, and increased amylase levels in blood and urine [9]. Acute pancreatitis can be divided into a

common type and hemorrhagic necrosis type. Bleeding necrosis is rare, but the disease is severe, and the mortality rate is high. Chronic pancreatitis is a disease of chronic pancreatic damage caused by repeated episodes of acute pancreatitis. The main manifestations of pancreatitis pathology are the thinness or hardening of the pancreas, the pancreas has bleeding points, bleeding spots or calcium fibrosis.

In this study, an MSTN gene mutant calf 190223 died at two month-old with abnormal symptoms such as vomiting, diarrhea, frequent small urination, and anorexia. Continuous treatment with antibiotics for several days, the symptoms were alleviated but recurred two or three days later. Finally, the symptoms worsened and showed stooping and flexion, arching, restlessness, abdominal pain; weakness in hind legs, tiptoe, and hobbled walking.

Case Presentation

Phenotype

The calf (190223) was born on February 23, 2019, and with a birth weight of 25 kg. After birth for one month, the calf looked healthy and without obvious abnormal performance, and reached 62 kg (Fig. 1A). However, from then, the abnormal calf symptoms appeared, such as vomiting, diarrhea, frequent small urination, and anorexia. Continuous treatment with antibiotics for several days, the symptoms were alleviated but recurred two or three days later. Finally, the symptoms worsened and showed stooping and flexion, arching, restlessness, and abdominal pain; weakness in hind legs, tiptoe, hobbled walking (Fig. 1B). Weakness in the hind legs, stoic, and abnormal walking are typical symptoms of pancreatitis[10–14].

The calf was died on April 28, 2019, at 2-month-old and weighed 70 kg. Suffering from the disease, the calf gained only 8 kg in the second month of life. At death, the calf had no obvious traumatic defects and no edema (Fig. 1C).

Anatomical observation revealed that the calf had strong gluteal muscles, semitendinosus muscles, and gastrocnemius muscles, which showed obvious double muscle phenomena resulted from MSTN mutation (Fig. 1D) as occurred in MSTN knockdown cattle [6]. When the chest cavity was opened, no obvious lesions of the heart, liver, spleen, lungs and kidneys were observed. The pancreas, however, became enlarged, swollen, dark red, and blood spots spread the pancreas area. A large amount of contents accumulated in the rumen and some blood was mixed in the intestinal contents. Enlarged mesenteric lymph nodes were distributed.

Histological observation

In the tissues of the spleen and kidney, red infiltration was filled, while the other tissues of the heart, liver, and lung were with no obvious differences compared to the controls (Fig. 2).

Histology of the normal pancreas tissue was pale pink with clear and uniform contours; the lobules were neatly arranged; the pancreatic cells had complete morphology; no congestion and edema and

hemorrhage and necrosis were observed (Fig. 3B). However, the pancreas of the dead calf was in dark brown (Fig. 3A, in the lower tube), while the normal calf's is in slightly yellow (Fig. 3A, in the upper tube).

In the dead calf, the pancreatic acinar cells were blurred; large coagulative necrosis and lytic necrosis in the lobules; the acinar cells in the necrotic area shrank with dissolved nuclei and disappeared acinar. The interlobular space became enlarged, and the blood vessels between the lobules were dilated and congested. A large number of inflammatory cells infiltrated into the interstitium, edema, blood stasis and bleeding were observed. The leaflet gap increased and pink slurry existed in the gap (Fig. 3C).

Blood routine parameters

The blood parameters of the dead calf showed that the white blood cell (WBC) was ($4.5 \times 10^9/L$), significantly lower than the normal average index ($8.31 \pm 2.01 \times 10^9/L$). The average platelet volume was 10.6 fL, higher than the normal calves (7.3 ~ 7.6 fL) (Table 1). These results implied that the immunity of the dead calf was weaker and less resistance, which could be indeed induced by pancreatitis.

Table 1
Routine blood parameters of the dead MSTN gene-edited calf

	190223	Normal MSTN gene-edited cattle	
		Mean	Value range
White blood cell count WBC ($10^9/L$)	4.5	8.31 ± 2.01	11.3 ~ 6.2
Lymphocyte ratio LYM (%)	3.3	2.97 ± 0.55	3.7 ~ 2.0
Median cell ratio MON (%)	6.1	7.04 ± 0.99	9.2 ~ 6.1
Lymphocyte count LYM ($10^9/L$)	0.2	0.24 ± 0.05	0.3 ~ 0.2
Median cell count MON ($10^9/L$)	0.3	0.57 ± 0.18	0.9 ~ 0.4
Red blood cell count RBC ($10^9/L$)	8.8	8.64 ± 0.77	9.5 ~ 7.5
Hematocrit HCT (%)	3235.0	3506 ± 315.2	4079.0 ~ 3119.0
Platelet count PLT ($10^9/L$)	912	1276.29 ± 750.58	2293 ~ 434
Platelet hematocrit PCT (%)	0.97	0.38 ± 0.1	0.49 ~ 0.32
Mean platelet volume MPV (fL)	10.6	7.3 ± 0	7.3 ~ 7.6
Giant immature cells LIC ($10^9/L$)	0.46	1.66 ± 0.93	3.14 ~ 0.94
The percentage of huge immature cells LIC ($10^9/L$)	11.41	24.5 ± 11.01	44.07 ~ 14.34
Absolute value of basophils BAS ($10^9/L$)	0.04	0.11 ± 0.05	0.17 ~ 0.05
Basophil percentage BAS ($10^9/L$)	0.9	1.29 ± 0.34	1.7 ~ 0.8
Abnormal lymphocyte percentage ALY ($10^9/L$)	0.6	0.55 ± 0.13	0.73 ~ 0.36
Abnormal lymphocyte absolute value ALY ($10^9/L$)	0.03	0.05 ± 0.01	0.07 ~ 0.03
Hemoglobin HGB (g/dL)	111.0	110.29 ± 10.03	129.0 ~ 97.0

Blood physiological and biochemical analysis

As shown in Table 2, in the dead calf, the concentration of α -amylase, the major indicator of pancreatitis, was 364.0 U/L, at least 11 times higher than the normal calves (ranged 32.50 ~ 24.5 U/L). The lipase content (243 U/L) was extremely higher in the dead calf than the average normal calves (21.40 ± 4.99 U/L). Alpha-amylase, mainly secreted from the pancreas, usually occurs outburst in acute

pancreatitis [15] and keep the high level in chronic pancreatitis [16]. Also, the increase of serum lipase tends to happen in acute pancreatitis and pancreatic cancer for a long time [17].

The other essential serum components, such as lipase, alanine aminotransferase, creatinine, lactate dehydrogenase, creatine kinase, vitamin B12, and adrenocorticotrophic cortex hormone were significantly higher in the dead calf than those normal calves. Alpha-amylase and lipase are essential clinical diagnostic indicators of pancreatitis [18, 19]. Clinically, many diseases or necrosis are most likely to cause blood glutamate transaminase increase [20]. The high level of glutamate transaminase should be produced by pancreatic inflammation in the present case. Under the action of various etiology, the pancreas defence mechanism is destroyed, cause pancreatic excretion disorder, which in turn induces pancreatitis [21–23].

Table 2
The blood physiology and biochemistry of the dead calf

	Dead calf	Normal calves	
		Mean	Value range
Alpha-amylase (AMY) (U/L)	364	29.35 ± 3.58	32.50 ~ 24.5
Lipase (LIPC) (U/L)	243	21.40 ± 4.99	24.70 ~ 12.8
Glucose (GLU) (mmol/L)	8.74	5.32 ± 0.61	6.36 ~ 4.55
Aspartate aminotransferase (AST) (U/L)	51.92	51.28 ± 17.82	84.9 ~ 29.6
Aspartate aminotransferase (ALT) (U/L)	97.8	15.98 ± 9.12	27.6 ~ 5.8
Total protein (TP) (g/L)	48.7	59.73 ± 5.42	67.7 ~ 51
Creatine Kinase (CK) (U/L)	518	280.75 ± 252.92	850.0 ~ 109.0
High-density lipoprotein (HDL-C) (mmol/L)	1.97	2.82 ± 0.53	3.57 ~ 2.19
Low-density lipoprotein (LDL-C) (mmol/L)	0.61	1.08 ± 0.27	1.47 ~ 0.73
Creatinine (CRE) (μmol/L)	314.0	117.63 ± 32.67	157.0 ~ 71.0
Lactate dehydrogenase (LDH-L) (U/L)	5249.0	857.8 ± 147.25	990.0 ~ 620.0
Lactate dehydrogenase (CO2-L) (mmol/L)	18.0	24.24 ± 2.82	27.4 ~ 19.9
Cholinesterase (CHE) (U/L)	97.0	150.5 ± 32.77	188.0 ~ 86.0
Cholesterol (CHOL2) (mmol/L)	2.2	3.12 ± 0.57	3.91 ~ 2.49
Lactic acid (LACT) (mmol/L)	4.66	3.47 ± 1.31	5.13 ~ 1.61
Triglyceride (TRIGL) (mmol/L)	0.69	0.4 ± 0.22	0.87 ~ 0.2
Urea (UREAL) (mmol/L)	5.9	3.86 ± 1.31	6.3 ~ 1.8
Vitamin B12 (pg/ml)	126.8	111.51 ± 27.77	156.3 ~ 74.7
Folic acid (FOL) (FOL) (ng/ml)	5.08	4.82 ± 1.83	7.74 ~ 2.29
Free Triiodothyronine (FT3) (pmol/L)	18.09	18.97 ± 7.11	27.17 ~ 6.21
Free thyroxine (FT4) (ng/dl)	2.13	1.85 ± 0.78	2.83 ~ 0.53

	Dead calf	Normal calves	
Thyroxine binding force (TBI) n	0.83	1.03 ± 0.06	1.12 ~ 0.96
ACTH (pg/ml) (ACTH) (pg/ml)	36.45	22.7 ± 15.03	52.66 ~ 7.57
Vitamin D (Vitamin D) (ng/ml)	4.86	9.02 ± 4.16	16.27 ~ 5.3
Thyroxine (T4) (mmol/L)	100.8	110.81 ± 48.41	191.0 ~ 37.1

Discussion

Existing studies have reported that MSTN-edited cattle will have difficulty in production, fatigue, heat stress, and respiratory diseases. The MSTN gene-mutated animal's reduction of the skeleton, and especially the reduced development of the hip bone [24], may have repercussions on reproduction performance. When the MSTN allele was included the pelvic area was decreased [25]. The calf's pelvic muscles are well-developed, which hinders the expansion of the pelvis and increases the chance of dystocia[26]. A drawback of the application of the cesarean section is the significant reduction in subsequent pregnancy rate [24] resulting in an extended calving interval. Furthermore, the endurance of MSTN gene-mutated cattle is lower, leading more quickly to exhaustion after severe exercise [27], which may even terminate in sudden death. Indicating that the degree of metabolic acidosis is aggravated, indicating that the blood circulation rate is reduced, the amount of oxygen transport is reduced by 1/3, and the aerobic metabolism of muscles is reduced [28].

MSTN gene-mutated cattle are more susceptible to heat stress than non- MSTN gene-mutated cattle [29]. Besides over the body surface, heat loss can also occur via the lungs [30]. The reduced lung capacity is another disadvantage for MSTN gene-mutated cattle. Furthermore, cattle that had severe respiratory disease early in life will have a decreased ability to regulate their heat load [31].

Heat stress can negatively affect aspects of reproduction [32]. MSTN gene-mutated cattle are more susceptible to heat stress than non- MSTN gene-mutated cattle. Furthermore, MSTN gene-mutated cattle appear to be predisposed to develop alveolar hypoxia and hypoxemia in comparison with conventional cattle

Pancreatitis is infrequent in herbivores as cattle. Evidence from anatomy, histology, blood physiology, and biochemistry parameters indicate that the myostatin mutated calf died of pancreatitis. Whether or not pancreatitis occurred in the calf is related to the mutation of gene myostatin is open to question.

Declarations

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Contributions

Guangpeng Li and Miaomiao Yang conceived and designed the experiments; Miaomiao Yang and Zhuying Wei performed the experiments; Miaomiao Yang, Xinyu Zhou and Ying Zhang analyzed the data. Guangpeng Li and Miaomiao Yang drafted and revised the manuscript. All authors read and approved the final version.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written informed consent was obtained from the owner for publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The dead MSTN mutated calf. A. Calf with MSTN mutation before disease ; B. The hobbled walking phenotype; C. The postmortem observation; D. The strong gluteal muscle masses.

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The dead MSTN mutated calf. A. Calf with MSTN mutation before disease ; B. The hobbled walking phenotype; C. The postmortem observation; D. The strong gluteal muscle masses.

Figure 2

The morphology of the heart, liver, spleen, lung and kidney.

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Figure 3

The morphology of the pancreas of the dead calf. A. The colours of the normal pancreas (the upper tube) and the dead calf pancreas (the lower tube); B. The normal pancreatic histology; C. The dead calf pancreatic histology (The black arrow points to blood stasis and necrotic area)

Figure 3

The morphology of the pancreas of the dead calf. A. The colours of the normal pancreas (the upper tube) and the dead calf pancreas (the lower tube); B. The normal pancreatic histology; C. The dead calf pancreatic histology (The black arrow points to blood stasis and necrotic area)

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