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CASE REPORT

Early-onset muscle weakness syndrome (MW) in an Australian Holstein calf

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Early-onset muscle weakness syndrome (MW) is a recessive genetic disorder known to affect Holstein cattle. This report describes the clinical findings in an Australian Holstein calf diagnosed with MW. The calf initially presented for examination at a dairy farm at 3 days of age, being recumbent since birth but able to stand with assistance. A presumptive diagnosis of traumatic injury was made at first. However, the calf was re-examined multiple times due to ongoing intermittent episodes of recumbency, prompting further diagnostic investigation. Given the non-specific nature of the clinical and laboratory findings, a presumptive diagnosis of MW was made after reviewing the calf's breeding pedigree. A definitive diagnosis, however, required genotype testing. To the authors knowledge, this case report represents the first peer-reviewed manuscript to describe the clinical presentation of MW in an Australian Holstein calf.

Keywords calf recumbency; early-onset muscle weakness syndrome; Holstein; recessive genetic disorder

Abbreviations BVDV, bovine viral diarrhoea virus; CDH, cholesterol deficiency haplotype; MW, early-onset muscle weakness syndrome

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Early-onset muscle weakness syndrome (MW) is a recessive genetic disorder known to affect Holstein cattle. The development of artificial insemination, in vitro fertilisation, and embryo transfer reproduction programmes has allowed for genomic selection to increase milk and component yield and select desirable physical attributes in dairy cattle. The reported 115% increase in milk yield for Holstein cows has been attributed to the implementation of genomic selection; however, despite these positive trends, genomic selection has accelerated inbreeding, producing issues such as lethal mutations and the expression of new recessive conditions.[1]

In 2019, Dechow et al.[2] reported a condition categorised by recumbency despite normal mentation and appetite that was reported in 34 Holstein calves from multiple farms in New York, Florida, and Pennsylvania, United States. All affected calves had no evidence of metabolic, infectious or neurologic abnormalities. No cholesterol, vitamin, selenium deficiencies nor anaemia were noted based on serum testing. Gross necropsies and histopathology samples of the central nervous system, peripheral nervous system and muscle tissue were unremarkable. The severity of recumbency varied from complete recumbency from parturition to progressive loss of mobility, with resultant recumbency typically within the neonatal period.

Initial genomic testing of the affected calves identified a shared homozygous recessive haplotype at the end of chromosome 16.[2] Further exploration identified 30 potential mutations, with a moderate impact missense mutation at 79,613,592 (single-nucleotide polymorphism [SNP] rs3423414874) on the calcium channel, voltage dependent, L-type, alpha 1 subunit (CACNA1s) protein coding gene as the most plausible.[3] The mutation is encoded on the negative strand and appears to change the CCG codon to ACG (glycine to serine), with SIFT prediction indicating a deleterious impact on amino acid function.[3]

This manuscript describes the clinical findings and subsequent diagnostics of a Holstein calf diagnosed with MW and an overview of MW in Holstein cattle.

Clinical features

On 17 July 2024, veterinary attention was sought for a 3-day-old Holstein heifer calf from a 650-cow, split-calving dairy herd in northern Victoria. The calf had been unable to stand unassisted since birth but was able to rise and ambulate with assistance.

The calf was conceived via artificial insemination and born without intervention on 12 July 2024 to a multiparous Holstein cow, that had previously delivered three clinically normal calves. The calf's dam's first calf, a heifer, born in July 2021, died from neonatal diarrhoea at 19 days of age; the dam's second calf was a heifer and was currently in the milking herd; and the dam's fourth calf was a bull calf that was sold at a week of age.

On clinical examination, the calf was recumbent. Its heart rate was 76 beats per minute, respiratory rate 22 breaths per minute and body temperature 38.7°C. No abnormalities were detected upon abdominal or naval examination, and faeces were semi-formed. Musculoskeletal assessment revealed no signs of pain on manipulation of the joints, pelvis, spine or head, and the limbs appeared grossly normal. A preliminary diagnosis of a soft tissue traumatic injury occurring at or around the time of parturition was made. The calf was treated with meloxicam (MELOXXI™ Injection, Abbey Animal Health Pty Ltd, 20 mg/mL) at a dosage of 0.5 mg/kg SC. The owner reported

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that the calf was able to rise and become ambulatory without assistance on 18th July 2024.

On 7th November 2024, the calf was re-examined, as it was once again unable to stand. The owner provided the following history: On 25th July 2024, the calf had been treated with sulfadiazine and trimethoprim (Ilium Trimethoprim-480 Anti-bacterial Injection, Troy Animal Healthcare) at doses of 20 and 5 mg/kg, respectively, via SC injection once daily for three days for undifferentiated neonatal calf diarrhoea. The calf was also veterinary disbudded on 2nd of October 2024. During this time, intermittent hindlimb paresis was noted, manifesting as mild ataxia. The calf was easily knocked to the ground by its herd mates during feeding.

On clinical examination, the calf was recumbent (Figure 1), and its vital signs were within normal limits. The calf was able to rise and become ambulatory when assisted. Musculoskeletal examination revealed kyphosis, gross atrophy of the quadriceps, semitendinosus and gluteal muscles in the hindlimbs (Figure 2) and an exaggerated appearance of the extensor tendons in the forelimbs. When ambulatory, the calf displayed a short-stepping gait.

Differential diagnoses included musculoskeletal trauma, subacute nutritional muscular dystrophy, congenital neosporosis, in utero infection with bovine viral diarrhoea virus (BVDV), cholesterol deficiency haplotype (CDH) and hypokalaemia. After reviewing the calf's genetic pedigree, a presumptive diagnosis of early-onset MW was made.

The calf was re-examined multiple times due to ongoing intermittent episodes of recumbency, prompting further diagnostic investigation to determine a definitive diagnosis. On 12th November 2024, the calf was re-examined for further diagnostic work-up, including tissue sampling and blood collection. A tissue sample was obtained from the calf's ear using a tissue sampling unit (TSU) and sent to a genetic testing laboratory (STgenetics Head Office, 31 Hovell Street, Wodonga, VIC, 3690), before being forwarded to the USA (Genetics Visions-ST, 8137 Forsythia St., Suite 100 Middleton, WI 53562), for early-onset MW genetic marker testing. Blood samples were collected via the jugular vein and submitted for haematology and biochemistry testing (Regional Laboratory Services, 136 Samaria Road, Benalla, VIC, 3672) (Table 1) and *Neospora caninum* ELISA serology testing (Gribbles Veterinary Pathology, 1868 Dandenong Road, Clayton, VIC, 3168). The Neospora ELISA was negative. From these results, the differential diagnosis of subacute nutritional muscular dystrophy, cholesterol deficiency, hypokalaemia and congenital neosporosis was unlikely to be the cause of the calf's clinical signs.

On the 20th of November, the calf (Figure 3) had an ear tissue sample collected to perform a BVDV antigen test (IDEXX Bovine Viral Diarrhoea Virus Antigen Test Point-of-Care, IDEXX Laboratories, Inc., Westbrook, Maine, USA). The test result was negative. The differential diagnoses of congenital BVDV could not be definitely ruled out; however, given the herd's BVDV vaccination history and the negative test result, BVDV was considered unlikely to be the cause of the calf's clinical signs.

The early-onset MW genetic marker test results were reported on 12th December 2024. The calf was found to be affected (homozygous for MW). The calf was euthanased as the calf's clinical signs were progressing (having more difficulty to rise to its feet), failing to thrive and concerns for the calf's welfare.

Discussion

Early-onset MW is a recessive genetic disorder observed in Holstein cattle. The authors propose that MW, as an emerging recessive genetic disease in Australia, may be underreported as affected calves could be misdiagnosed as having sustained traumatic injuries at parturition or in the neonatal period, leading to their premature culling from herds. Conditions associated with the CACNA1s have been described in people with similar phenotypic expression as muscle weakness in calves. Hypokalaemic periodic paralysis and dihydropyridine receptor congenital myopathy have reported myotonia from birth; however, they are notably associated with respiratory distress.[4,5]

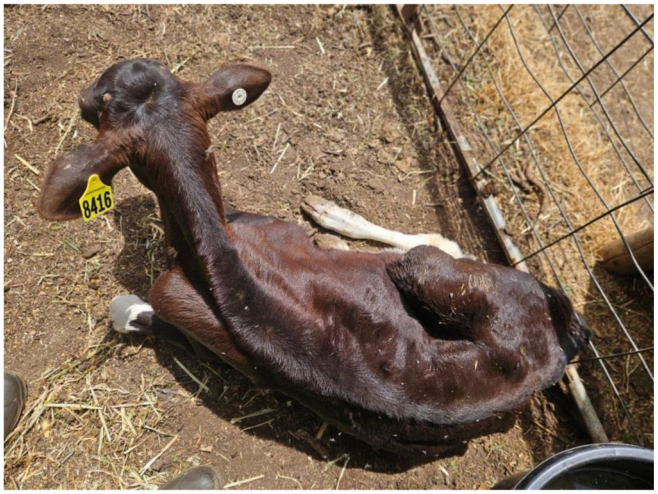

Figure 1. The calf in recumbency, unable to stand unassisted, at 118 days of age.

Figure 2. The calf standing after being assisted at 118 days of age.

Table 1. Summary of the biochemistry, electrolytes, haematology and serology finding of the calf

Parameter	Reference range[a]	Result
Red blood cell count	5.00–8.00 × 10^{12}/L	10.59
Haematocrit	23%–44%	36
Haemoglobin	8.0–15.0 g/dL	10.2
Mean erythrocyte volume	44–62 fL	34
Mean haemoglobin volume per red blood cell count	14–20 pg	10
Mean corpuscular haemoglobin concentration	30–35 g/dL	28
White blood cell count	4.0–12.0 × 10^{9}/L	11.3
Neutrophils	0.60–4.00 × 10^{9}/L	5.09
Band neutrophils	0.00–0.12 × 10^{9}/L	0.00
Lymphocytes	2.50–7.50 × 10^{9}/L	5.65
Monocytes	0.03–0.84 × 10^{9}/L	0.57
Eosinophils	0.00–2.40 × 10^{9}/L	0.00
Basophils	0.00–0.20 × 10^{9}/L	0.00
Platelet count	100–800 × 10^{9}/L	333
Protein (refractometer)	65–85 g/L	66
Fibrinogen	3–7 g/L	6
Protein/fibrinogen ratio	15–100	10
Gamma-glutamyl transferase	0-35 U/L	23
Glutamate dehydrogenase	0-30 U/L	39
Aspartate aminotransferase	0-120 U/L	66
Bilirubin	0.0–24.0 umol/L	3.5
Creatine kinase	0-300 U/L	229
Urea	2.1–10.7 mmol/L	4.2
Creatine	0–186 umol/L	77
Phosphorus	0.80–2.80 mmol/L	2.49
Urea/creatine ratio	0.00–0.07	0.05
Protein	60.0–85.0 g/L	59.9
Albumin	25.0–38.0 g/L	34.4
Globulins	30.0–45.0 g/L	26.5
Albumin/globulin ratio	0.7–1.1	1.3
Beta-hydroxybutyrate	0.00–0.80 mmol/L	0.43
Calcium	2.00–2.75 mmol/L	2.40
Magnesium	0.74–1.44 mmol/L	0.93
Sodium	132–152 mmol/L	148
Potassium	3.9–5.8 mmol/l	5.6
Chloride	97–111 mmol/L	101
Cholesterol	1.60–5.00 mmol/L	1.40
Haptoglobin	0.00–0.30 g/L	0.20
Serum haemoglobin	0.00–0.20 g/dL	0.06
Glutathione peroxidase	40–300 u/gHb	292

[a] NATA Accredited Laboratory.

The differential diagnoses for this bovine included musculoskeletal trauma, subacute nutritional muscular dystrophy, congenital neosporosis, in utero infection with BVDV, cholesterol deficiency and hypokalaemia. Based on the clinical findings, the following conditions were deemed unlikely:

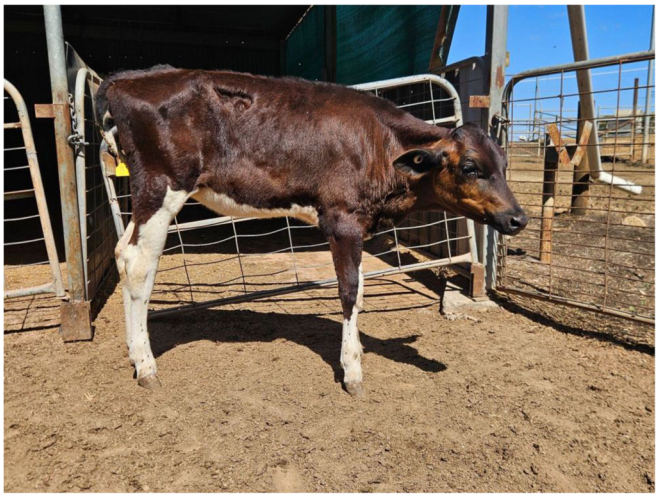

Figure 3. The calf standing at 131 days of age.

Musculoskeletal trauma: Initially considered the most likely diagnosis, musculoskeletal trauma was ruled out after repeated clinical examinations failed to detect any evidence of pain or injury.

Subacute nutritional muscular dystrophy: This condition has many similar clinical features, such as stiff-legged gait, paresis, and recumbency. The whole blood selenoenzyme glutathione peroxidase can be used as a proxy to measure selenium status (at the time erythropoiesis) and was found to be within the normal range. It should be noted that calves clinically affected with subacute nutritional muscular dystrophy can have the same whole blood levels of glutathione peroxidase as unaffected calves. The differential of subacute nutritional muscular dystrophy was considered unlikely as the clinical history was not typical of subacute nutritional muscular dystrophy, as the farm is not in a known selenium deficiency area, a single animal was affected, the calf was not grazing lush, high-quality pastures that contain a high level of polyunsaturated fatty acids, and the calf had no history of chronic diarrhoea.[6]

Congenital neosporosis: This condition was considered unlikely, as the herd had no recent history of mid-to-late-term abortions, stillbirths and premature calves, and only one calf exhibited mild neurological signs.[6] Congenital neosporosis was ruled out by a negative serum *Neospora caninum* ELISA test result.

In utero BVDV infection: Unlikely, as the herd currently uses a commercial BVDV vaccine, and the affected calf tested negative on the IDEXX Bovine Viral Diarrhoea Virus Antigen Point-of-Care Test.

Cholesterol deficiency haplotype (CDH): CDH is another genetic condition observed in Holstein calves. Kipp et al.[7] reported median cholesterol levels of 0.15 mmol/L (range: 0.07–0.25 mmol/L) in affected calves. The calf in this case did not have severely low cholesterol or the typical clinical signs of emaciation and chronic diarrhea associated with CDH.

Hypokalaemia: Hypokalaemia can present clinically as muscle weakness and muscle fasciculations, and as the condition progresses, recumbency may result.[6] Hypokalaemia was ruled out, as the calf's potassium levels were within the normal range according to electrolyte analysis.

The haematology and biochemistry results revealed several parameters outside the normal range. Although these results did not indicate an underlying disease process to explain the clinical presentation, they are discussed below for completeness.

Low mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH), as well as an increased red blood cell count (RBC) have been reported in calves across multiple studies.[8–11] Mohri et al.[8] reported that over the first 84 days of life, there is an upwards trend in RBCs, and at the concluding day of study (Day 84) both MCV and MCH were still markedly lower than the adult reference interval.

Serum total protein and globulins in calves are reported lower than the given clinical reference ranges. The decreased total protein and globulins in calves are credited to the breakdown of immunoglobulins absorbed from colostrum.[8]

The definitive diagnosis of MW was made on genetic marker test results when the calf was found to be homozygous for the MW haplotype. The case presentation aligns with the reported findings by Dechow et al.[2]

The periodic episodes of varying degrees of ataxia and recumbency exhibited by the calf support the hypothesis of partial penetrance and variable expressivity of the mutation by Al-Khudhair et al.[3] The degree of penetrance and expression for the mutation is still unclear. Dechow et al.[2] reported a calf that was homozygous for the mutation yet did not have phenotypic expression, and Al-Khudhair et al.[3] reported that one of two homozygous bulls (Rocketfire) had mobility problems as a calf; however, through nursing care, it became functional.[2,3] The second homozygous bull did not present with weakness or recumbency. Causes of variability in phenotypic expression and penetrance are difficult to articulate. Variations in phenotypic expression have been observed in mice with identical genetic and environmental conditions, notably variation in lethality of genes despite identical variants.[12]

Further investigation at the herd level, incorporating genomic analysis of dams and their offspring, could help identify additional homozygous individuals, thereby providing a more comprehensive understanding of the penetrance and expressivity of MW. Moreover, further case reports could help identify factors, such as environmental, genetic or management-related variables, that may predispose individuals to increased penetrance and expression.

The pedigree analysis of the affected calf (Figure 4) traced the sire and dam lines back to two known MW carrier sires *Roylane Socra Robust-ET* (*Robust*) and *Seagull-Bay Supersire-ET* (*Supersire*). Dechow et al.[2] initially traced the haplotype to the 2008-born sire, *Robust*. One prolific son of *Robust*, *Supersire*, was present in 30 of 33 paternal and 23 of 24 maternal lineages.[2] Al-Khudhair et al.[3] continued tracing the suspect haplotype to a 1984 bull, *Southwind Bell*

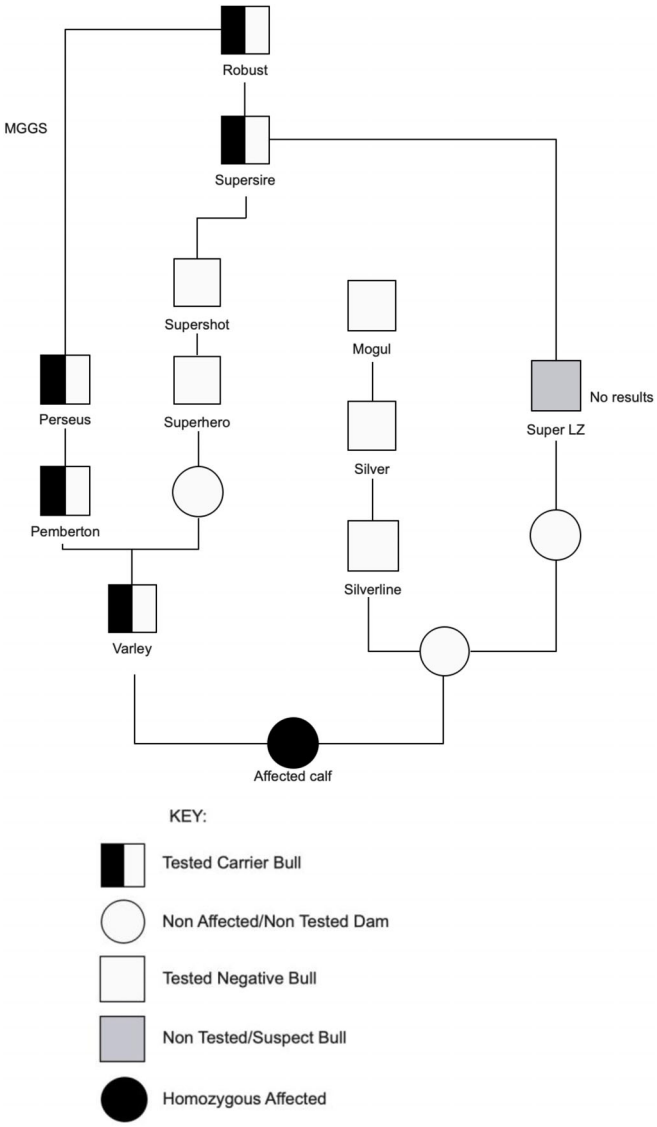

Figure 4. Pedigree of affected calf and key.

of Bar-Lee (*Southwind*) who was identified as homozygous for the haplotype.

One limitation identified in the analysis was that not every bull had been tested for the mutation. *Calister Super LZ* is the maternal great grandsire of the affected calf and is a direct progeny of *Supersire*; however, no MW genomic testing results are available. The calf was confirmed to be homozygous for MW, leading to the presumption that *Calister Super LZ* is a carrier. It is suggested that *Calister Super LZ* transmitted the MW haplotype to the affected calf's granddam, who subsequently passed it to the calf's dam (Figure 5). The mating of two carrier animals, *Carenda Varley* (sire) and the calf's dam, resulted in the production of an MW homozygous-affected calf. Assessment of the calf's dam's three other offspring pedigrees suggests that all three calves would have been either MW non-carriers or MW carrier animals, as each of the inseminations was from semen from bulls that have been tested MW non-carriers.

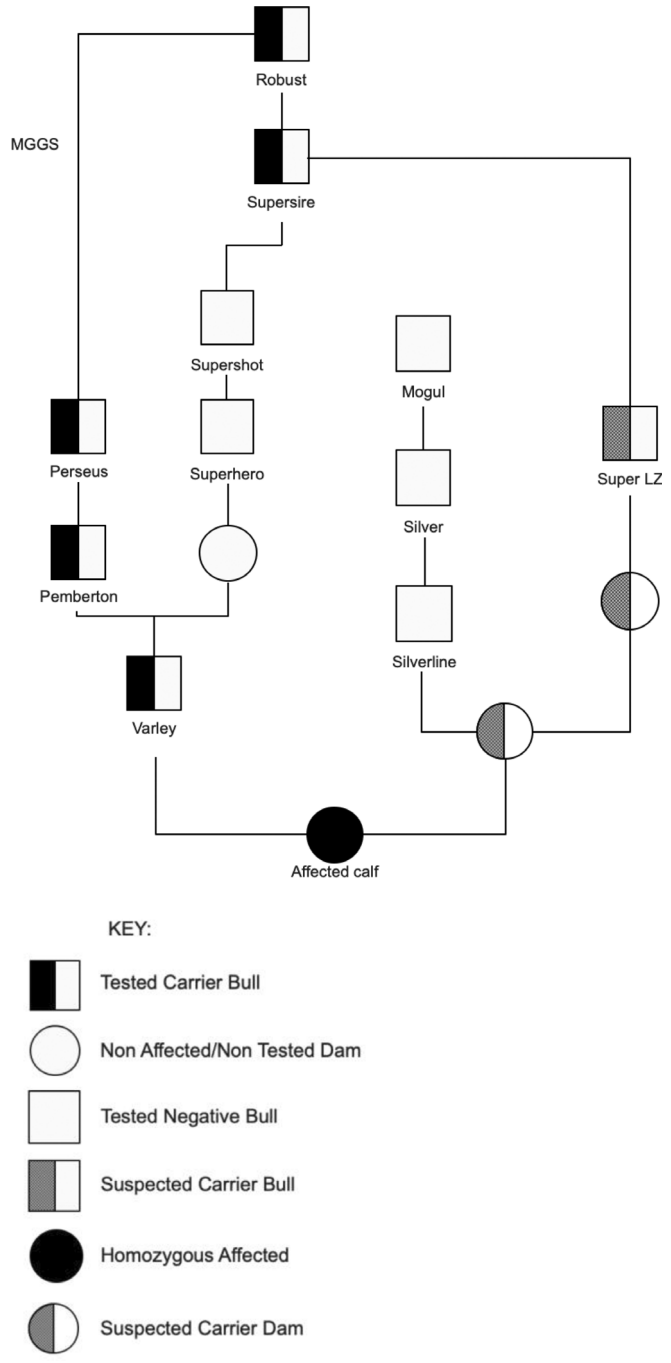

Figure 5. **Suggested pedigree of calf with suspected carriers and key.**

In the affected calf's herd, the females are not routinely tested for MW carrier status with the current genomic testing service provider, making it difficult to ascertain the extent of MW carriers and possibly homozygous animals in the wider Holstein population. A crude pedigree analysis of the current herd estimates that approximately 12% of the females may be MW carriers. Al-Khudhair et al.[3] estimate the frequency of MW carrier animals in the Holstein population (since the year 2000) to be 2.09% known carriers and 8.15% suspect carriers and estimate the frequency of MW homozygous-affected animals to be 0.01%.

With the knowledge of a MW affected calf and an estimate of 12% of the females that may be MW carriers in the current herd, it is recommended for the herd owner to perform MW haplotype testing on the entire herd to identify carrier cows and heifers, which would provide the most accurate information on herd incidence and assist with breeding decisions. However, this approach may be neither practical nor cost-effective; purchasing semen exclusively from MW-tested non-carrier bulls is recommended. Alternatively, implementing strategic individual mating decisions is suggested. For example, MW carrier semen could be used with females unlikely to be carriers, whereas MW-tested non-carrier semen should be prioritised for females likely to be MW carriers. However, with the former approach, there remains a risk of producing homozygous MW animals, as the precise MW carrier status of each female would remain unknown.

The calf was euthanased at 132 days of age (4.4 months) as the calf's clinical signs were progressing (having more difficulty to rise to its feet), failing to thrive and concerns for the calf's welfare. Al-Khudhair et al.[3] reported that 52% of MW homozygous-affected calves died before 18 months of age, with an average age of 1.7 ± 1.6 months. A small number of MW homozygous-affected calves can go on to have productive lives and survive in herds for three to four lactations.[3]

As of 7 August 2024, ABS Australia (American Breeding Services—ABS Global) ceased the marketing and sale of semen from bulls identified as carriers of the haplotype.[13] Testing conducted by ABS Australia identified 12 carrier bulls, prompting the decision to discontinue sales in an effort to enhance profitability and improve the welfare of future herds.[13] In contrast, several other Australian semen companies continue to market semen from known MW carrier bulls, providing haplotype testing results as Supporting Information.[14] It would be beneficial if semen sellers provided transparent information in regard to haplotype testing, including MW, on every bull marketed, so dairy producers and service providers can make informed decisions when it comes to making breeding decisions for dairy herds.

A separate but important issue of inbreeding was noted during the pedigree analysis. The inbreeding of Holstein cattle is likely a significant contributor to the current and future prevalence of the deleterious recessive genes such as MW. In the case of MW, the extensive use of semen from Robust and Supersire prior to the mutation discovery has facilitated the rapid spread of MW carrier animals and the affected animals.

Conclusion

To the authors' knowledge, this case report is the first peer-reviewed manuscript to describe a clinical presentation of early-onset muscle weakness disease in an Australian Holstein calf. With growing awareness of MW in Holstein calves, it is expected to lead to an increase in reported or suspected cases. Effective herd-level management is crucial to mitigate the negative health and productivity impacts of the disease. Ideally, testing the entire herd to identify carrier cows and heifers would provide the most accurate information on herd incidence. However, for many producers, this approach may

be neither practical nor cost-effective. Instead, it is recommended to evaluate herd lineage and strategically select bulls based on confirmed negative MW status. Additionally, reducing the overall degree of inbreeding within the herd is vital to minimise the expression of new and deleterious recessive genes.

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Conflicts of interest and sources of funding

The authors declare no conflicts of interest or sources of funding for the work presented here.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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