Maple syrup urine disease (MSUD) and further cases were identified in herd mates of a small Hereford herd in Indiana based on history, clinical signs, microscopic lesions, and biochemical and genetic testing. This aminoacidopathy has been diagnosed in polled Shorthorn, polled Hereford, and Hereford cattle in Australia, Uruguay, Argentina, and Canada and is the result of a mutation of the branched-chain alpha-ketoacid dehydrogenase complex. The Indiana index calf case was confirmed by showing the classic accumulation of ketoacids in liver that results from a defect in the E1-alpha subunit (248 C/T haplotype) in the mitochondrial branched-chain \( \alpha \)-ketoad dehydrogenase complex. The presence of the mutation was confirmed in the index case, the dam, and four related herd mates that represent the first confirmed cases of bovine MSUD mutation in United States cattle.

1. Introduction

Maple syrup urine disease (MSUD) is an inherited, autosomal recessive, aminoacidopathy resulting from branched-chain \( \alpha \)-ketoad dehydrogenase complex (BCKDH) dysfunction (Skvorak [1]). The BCKDH complex is a mitochondrial multisorsubunit enzyme composed of three catalytic components including E1, E2, and E3 [1–3]. MSUD naturally occurs in humans and polled Shorthorn, polled Hereford, and Hereford calves resulting in central nervous system dysfunction approximately 2–4 days after birth in calves [1, 4]. In polled Herefords, disease is caused by premature termination of translation, of the E1-alpha subunit, that is induced by a cytidine to thymidine transition at nucleotide 248 (248C-->T) that converts the glutamine codon –6 to a stop codon (Zhang et al. [5]). Since MSUD is an autosomal recessive condition, consanguineous breeding in cattle, as has been shown in humans, would presumably lead to a greater incidence of disease (Skvorak [1]). A deficiency in BCKDH results in an inability to oxidize the branched-chain ketoacids of the branched-chain amino acids leucine, isoleucine, and valine (Maxie and Youssef [4]). Loss of this activity results in the accumulation of the branched-chain amino acids along with their respective ketoacids: ketoisocaproic, keto-\( \beta \)-methylvaleric, and ketoisovaleric acids in cerebrospinal fluid, blood, and tissues. The mechanism by which these metabolites cause central nervous system dysfunction is not fully understood (Maxie and Youssef [4]).

MSUD could represent a subset of the hereditary neurraxial edema disease complex described in Hereford calves in the United States but these cases were never confirmed to have the currently known genetic mutations or biochemical changes consistent with MSUD and spongy vacuolation was described in the spinal cord which is not consistent with MSUD (Cordy et al. [6]). A syndrome similar to MSUD was also reported in Gelbvieh-cross calves in Nebraska but genetic analysis failed to show a mutation in the El-\( \alpha \) subunit (O’Toole et al. [7]). To the authors’ knowledge, no case report of naturally occurring MSUD in cattle has been published in the United States. In the current report, MSUD in an Indiana
Hereford herd is described to inform veterinarians and owners of the presence of this disease mutation in the Hereford cattle population and the possibility of this mutation resulting in clinical disease.

2. Case Presentation

The affected male Hereford calf came from a small herd (7 cow/calf pairs) in Indiana. This was the owners’ first year raising cattle and they obtained the herd from the previous owner without any known pedigree information. The cattle were kept on a small pasture with a small calf barn for shelter. No other calves from this farm were affected and all calves had the same sire. At three days of age, the owner noted that the calf was “not acting normally.” The following day the calf was recumbent and depressed. The calf was given oxytetracycline and milk replacer with no clinical improvement. On the subsequent morning, the animal was presented to the Purdue University Veterinary Teaching Hospital for evaluation.

Upon presentation to the Purdue University Veterinary Teaching Hospital, the animal was depressed, was laterally recumbent, and had bilateral nystagmus. Temperature, pulse, and respiration were within normal limits. Based on the age of the calf and limited diagnostic work-up, the suspected diagnosis at that time was bacterial meningitis. Due to financial restrictions associated with diagnostics and treatment, euthanasia was elected and the calf was submitted to the Animal Disease Diagnostic Laboratory for necropsy. Neither a chemistry panel nor a complete blood count was performed prior to euthanasia.

Gross necropsy examination was unremarkable. No gross abnormalities were observed in the brain. No obvious urine odor was noted. Specimens of major organs and tissues were collected, fixed in neutral buffered 10% formalin, routinely processed, embedded in paraffin, sectioned, stained with H&E, luxol fast blue, oligodendrocyte transcription factor (Olig-2), and glial fibrillary acidic protein (GFAP), and examined by light microscopy. Histologic abnormalities on H and E staining were confined to the white matter of the cerebrum, cerebellum, and brain stem and consisted of severe spongy vacuolation of myelin with the long axis of vacuoles parallel to axons (status spongiosus) (see Figure 1). Lesions were not observed in sections of peripheral nerve. Virchow Robin's space in the white matter was also variably expanded by increased clear space (edema). Astrocytes in the white matter had an increased amount of eosinophilic cytoplasm (reactive). No abnormalities in myelination were observed with luxol fast blue staining compared to routinely used control brain sections. The relative number of oligodendrocytes and astrocytes were compared using Olig-2 and GFAP, respectively, between the calf with MSUD and an age-matched control calf. No appreciable difference was observed between the calf with MSUD and the age-matched control calf by two observers (William L. Wigle and Mark E. Robarge). The author’s acknowledge that a difference may have been present since edema in the calf with MSUD made observations on numbers of oligodendrocytes and astrocytes in these areas difficult to compare to the age-matched control.

Each of the nine amplicons corresponding to the exons of bovine BCKDHA were successfully amplified using the genomic DNA isolated from the original calf, and herd mates, submitted for analysis. Direct sequencing of the PCR products revealed that the affected calf was homozygous for the previously identified mutation in polled Herefords caused by premature termination of translation, of the E1-alpha subunit, that is induced by a cytidine to thymidine transition in exon 2 (248C-->T) that converts the glutamine codon –6 to a stop codon (Zhang et al. [5]). In polled Shorthorns, the mutation results in a substitution of leucine in place of a highly conserved proline at codon 372 due to a cytidine to thymidine transition at nucleotide 1380 (1380C-->T) resulting in dysfunction of the E1-alpha subunit (Dennis and Healy [8]). The dam of the affected calf was shown to be heterozygous for the 248 C/T haplotype, which is consistent with the reported recessive inheritance of the disease. Of the eight additional individual samples collected from the subject herd, four were found to be heterozygous for the mutation (corresponding to one carrier cow and three carrier calves) with the remainder being homozygous for the normal allele. The sire of the affected calf had been sold and was unavailable for testing.

Sections of frozen liver from the calf affected with MSUD and three “healthy” age-matched but not breed-matched control calf frozen livers were evaluated for branched-chain ketoacid levels (Olson et al. [9]) of ketoisovaleric (KIV), ketoisocaproic (KIC), and keto-β-methylvaleric (KMV) acids which are the ketoacids of valine, leucine, and isoleucine, respectively, and showed marked elevations consistent with MSUD. “Healthy” control calves of a comparable age but different breed were diagnosed with pneumonia, ventricular septal defect, and coccidiosis, respectively, which presumably should minimally affect the branched-chain ketoacid levels in the liver. Results are summarized in Table I.

3. Discussion

The history, histologic findings, homozygous mutation in the E1-α subunit, and increased branched-chain ketoacid detection in liver of submitted Hereford calf are consistent with MSUD. Multiple animals (one cow and three calves)
Table 1: Branched-chain ketoacid concentrations.

<table>
<thead>
<tr>
<th>Animals</th>
<th>KIV (nmol/g)</th>
<th>KIC (nmol/g)</th>
<th>KMV (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSUD Suspect calf</td>
<td>24.92</td>
<td>&gt;362</td>
<td>24.25</td>
</tr>
<tr>
<td>3 “Healthy Calves”</td>
<td>1.97</td>
<td>10.92</td>
<td>0.63</td>
</tr>
<tr>
<td>Increase in MSUD calf from healthy calves</td>
<td>12.6X</td>
<td>&gt;33X</td>
<td>38.4X</td>
</tr>
</tbody>
</table>

Ketoad concentrations in the liver of the Hereford calf with maple syrup urine disease versus the average of three “healthy” age-matched but not breed-matched control calves are summarized. KIV: ketoisovaleric; KIC: ketoisocaproic, and KMV: keto-β-methyl-valeric acids, respectively.

in the herd were heterozygous for the mutation in the E1-α subunit as well.

Status spongiosus, the histologic hallmark of MSUD, is a term that describes neural tissue that has a microvacuolar, sieve-like change by light microscopy [2, 10, 11]. This appearance may be from swelling of astrocyte/oligodendrocyte cytoplasm, processes in neuropil, or myelin sheaths [4, 11, 12]. Although the presence of this lesion in myelin is distinctive on light microscopic evaluation, electron microscopy is needed to definitively define the change (Maxie and Youssef [4]). In MSUD, myelin vacuolation is due to splitting of myelin lamellae at the intraperiod line which produces vacuoles within the myelin sheath mostly involving the outer myelin lamellae [13]. Status spongiosus has many causes in animals including idiopathic, toxic, metabolic, and infectious conditions [4, 5, 7, 11, 14, 15].

Many different categories of disease, as mentioned above, in calves can cause status spongiosis of white matter and were considered in this index case before ancillary testing confirmed this calf to have MSUD. One such cause is idiopathic spongiform myelopathy that has been documented in horned Hereford calves in New Zealand and in polled Hereford calves in Britain (Maxie and Youssef [4]). Idiopathic myelopathies were ruled out after positive ancillary testing confirmed MSUD. Hepatic and to a lesser extent renal encephalopathy are possible etiologies; however, the kidney and liver were normal in this case, and Alzheimer type II cells, reactive astrocytes in the gray matter with a clear nucleus, and increased amount of cytoplasm found singly or in groups were not observed [4, 11]. Hexachlorophene, a polychlorinated phenolic compound used as a topical antiseptic, and halogenated salicylanilide, an anthelmintic, cause status spongiosus of white matter in both the central and peripheral nervous system (Maxie and Youssef [4]). Idiopathic compounds like these can be ruled out based on lack of exposure to the compound and no lesions seen in peripheral nervous tissue. Ingestion of corn towards the end of growing season infected with Stenocarpella maydis can cause status spongiosus as a result of mycotoxicosis but was ruled out since this fungus is found in southern Africa and Argentina (Maxie and Youssef [4]). Many toxic plants should be considered including Stypandra sp., Hemerocallis sp., Tylecodon wallichii, Ornithogalum toxicares, and Helichrysum sp.; however, these plants grow in various countries around the world and are not reported in Indiana (Maxie and Youssef [4]).

To the authors’ knowledge, this represents the first documented naturally occurring case of MSUD in cattle in the United States. This finding indicates the presence of the genetic mutation for MSUD within the US cattle population and suggests the possible need for genetic screening to eliminate the trait.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References


