Polioencephalomalacia in cattle consuming water with elevated sodium sulfate levels: A herd investigation

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Abstract
Polioencephalomalacia (PEM), hereafter used to refer to the specific lesion of cerebrocortical necrosis, developed in 11 of 110 mature cattle on pasture in central Saskatchewan. The primary water source contained a markedly elevated level of sodium sulfate (7200 ppm). The significant clinical findings of the herd investigation included depression, ataxia, cortical blindness, dysphagia, and death. Diagnosis of PEM was confirmed by histopathological evidence of cerebrocortical and subcortical necrosis with microvascular fibrinoid necrosis predominantly in the thalamic region of three affected cattle. The histopathology of sulfate-associated PEM observed in this herd appears to be unique and its features are presented and discussed. Mean levels for serum transketolase, copper, red blood cell transketolase activity, and thiamine (vitamin B₁) in all exposed young (n = 100) and mature (n = 99) animals did not reveal evidence of deficiencies. Although the blood thiamine status of the seven surviving, affected animals was not evaluated before treatment with exogenous thiamine, 199 members of the herd had blood thiamine levels within the reference range at the time of the outbreak. The outbreak resolved after cattle were moved to a water source containing acceptable levels of sodium sulfate.

Résumé
Polioencéphalomalacie chez les bovins s’abreuvant à une source d’eau à teneur élevée en sulfate de sodium: étude de troupeau
La polioencéphalomalacie, terme se référant ci-après aux lésions spécifiques de nécrose cérébrocorticale, s’est développée chez 11 des 110 bovins adultes d’un troupeau du centre de la Saskatchewan, gardé au pâturage. La principale source d’approvisionnement en eau contenait un taux élevé de sulfate de sodium (7200 ppm). Les signes cliniques comprenaient de la dépression, de l’ataxie, de la dysphagie, une cécité d’origine corticale et la mort. Le diagnostic a été confirmé par l’étude histopathologique de trois bovins atteints de la maladie. Les lésions comportaient une nécrose cérébrocorticale et souscorticale avec une nécrose fibrinoïde microvasculaire de la région thalamique. L’image histopathologique de la polioencéphalomalacie associée aux sulfates, observée dans cet élevage, semble unique en son genre et les caractéristiques y sont présentées et discutées. Les taux sériques moyens de la transkétolase, du cuivre, de l’activité transkétolase des globules rouges et de la thiamine (vitamine B₁) chez les jeunes animaux exposés (n = 100) ou les adultes (n = 99) n’ont pas révélé de déficiences. Bien que le taux de thiamine sérique n’ait pas été évalué avant un traitement à base de thiamine exogène, chez sept animaux atteints, mais ayant survécu, les taux de thiamine de 199 animaux du troupeau ont été déterminés au moment de l’apparition de la maladie et les valeurs étaient dans les limites de la normale. La condition s’est résolue lorsque les animaux ont été transférés dans un endroit où la source d’approvisionnement en eau contenait des taux acceptables de sulfate de sodium.

(Traduit par Dr Thérèse Lanthier)

Introduction
Polioencephalomalacia (PEM) is a noninfectious disease of wild and domestic ruminants characterized by blindness, depression, and incoordination (1-5). Severe cases may progress rapidly to recumbency, convulsions, and death. Clinical signs are referable to pathological changes involving the central nervous system. Necropsy findings of edema and laminar necrosis of cerebrocortical grey matter are pathognomonic for PEM (2,3). The lesions of PEM are not that of a single etiopathological entity, as similar cerebrocortical lesions have been reported in cattle with acute lead encephalopathy and salt toxicosis/water deprivation (6,7). Polioencephalomalacia has also been attributed to selenium toxicosis (2) and deficiency (1), poisonous plants (1,2,8,9), mycotoxins (1), cobalt deficiency (1,3), and sulfate toxicity (3,7,10-14).
Regardless of the cause, it has long been believed that thiamine (vitamin B₁) deficiency plays a major role in the pathogenesis (3) and that early stages of PEM are responsive to parenteral administration of thiamine. Historically, it was hypothesized that PEM may result from alterations in carbohydrate metabolism in the brain of thiamine-deficient cattle (3). Thiamine is necessary for the production of thiamine diphosphate, a coenzyme, which plays a role in the activation of transketolase. Transketolase, found in giall cells and erythrocytes, is an important enzyme involved in glucose metabolism. Since the brain is glucose-dependent, giall cell transketolase plays an important role in brain metabolism. Thiamine deficiency is evidenced by decreased erythrocyte transketolase activity and by excess production of intermediate compounds of glucose metabolism (e.g., elevated blood pyruvate concentrations). Direct measurements of blood thiamine may be unreliable because most of it is stored in erythrocytes (3); therefore, erythrocyte or tissue thiamine levels or erythrocyte transketolase activity may be more indicative of the animal's thiamine status (3).

Recently, Gould et al (14) has questioned the role of thiamine in the pathogenesis of PEM in cattle and has experimentally produced PEM through excess dietary sulfate intake without significant changes in tissue, blood, cerebrospinal fluid, brain, or liver thiamine levels.

Although PEM occurs conventionally in young cattle on high carbohydrate diets (17), we report herein the investigation of clinical, hematological, necropsy, and histopathological findings in 11 older cattle affected by PEM and an assessment of blood thiamine, erythrocyte transketolase, transketolase thiamine pyrophosphate (TPP) effect, and plasma copper on 199 sulfate-exposed, nonaffected herdmates.

History
A cow-calf herd with 110 Angus and Angus/Hereford crossbred cows had grazed a quarter section of pasture for five weeks. The cattle had free access to mineral mix containing selenium, copper, phosphorus, iron, iodine, manganese, sodium, cobalt, zinc, fluoride, and vitamins A, D₃, and E (Feed-Rite-Hi Boot-C-n-Z, Feed-Rite Limited, Winnipeg, Manitoba). The cattle had access to a stock tank supplied with water by a newly dug 7.6 meter well. In late July 1989, the cattle were moved to an adjacent pasture with access to the same stock tank. At this time the feeder pipe was lowered in the well. The reliability of the water supply was in question and the rancher felt that some of the cattle may have been deprived of water for several days. The ambient temperature was unusually high (37°C) on the day of movement and it was felt that this may have resulted in water deprivation.

On July 26, three days following the movement of the cattle, heifer #1 was found staggering. She was brought into the corral and given fresh water by stomach tube. The rancher observed the heifer to have difficulty swallowing and attempted to force feed hay by hand. The following morning two animals were found dead and two were recumbent and comatose. The rancher sought veterinary assistance from the Ambulatory Clinic of the Western College of Veterinary Medicine (WCVM).

Affected cattle were brought to a corral and offered water from well #1. Dysphagic animals and those with moderate to severe dehydration were given water and oral electrolytes by stomach tube. Daily parenteral thiamine (Thiamine, 500 mg, Rafter 8 Products, Calgary, Alberta) was given at a minimum dose of 1 gram/animal until clinical signs resolved. Cattle #1, #4 and #6 were given exogenous thiamine by attending veterinarians. The rancher treated all clinical cases with exogenous thiamine, but did not keep detailed treatment notes.

Materials and methods
Clinical examinations were done on affected cattle on days 1, 2, 3 and 9 of the outbreak. Blindness was assessed by menace response and, on day 9, by ophthalmologic examination of the fundus. Animals believed to be blind were examined while they were walking around in a corral, and assessed as people and obstacles were placed in their path. Animals able to walk were examined under either minimal physical restraint or in a chute. Unaffected members of the herd were given cursory visual examination on days 1 and 2, and on day 3, were examined and sampled in a chute.

Complete blood counts and serum biochemical analyses were performed on affected cattle over the course of days 1, 2 and 3 of the outbreak on an automated cell counter (Coulter Counter S + 4, Coulter Electronics Inc., Hialeah, Florida, USA) and a Discreet Analyzer with Continuous Optical Scanning (DACSOS, Coulter Electronics Inc.), respectively.

Trace mineral and vitamin analyses were performed on serum from affected cattle on day 9 of the outbreak. Plasma copper, blood thiamine, and red blood cell (RBC) transketolase (TK) analyses were performed on all cattle on day 3 of the outbreak by methods described previously (10,15).

Histological examinations were done on brain tissue previously fixed in 10% buffered formalin. Paraffin-embedded slices were sectioned at 0.5 µm and stained with hematoxylin and eosin.

Well water analysis was performed on day 2 of the outbreak.

For statistical analysis the variables blood thiamine, RBC transketolase, transketolase thiamine pyrophosphate (TK-TPP) % increase, and plasma copper were normally distributed and means were analyzed by Student's t-test. A p value of less than 0.05 was used as indicative of statistical significance.

Results
Clinical examination-Day 1
Two animals (#2 and #3) were found dead. Heifer #2 was necropsied and tissues were submitted for histopathological examination. The head was severed and submitted for gross and histological examination. The carcass of cow #3 was severely autolysis and a necropsy was not performed. Clinical examinations of six affected animals revealed ataxia in three, blindness in five, dysphagia in four, depression in six, and recumbency in two. The rest of the herd of cows and calves was observed in a cursory manner. The pasture
growth was ample but mature. Within the pastured area were several fields without crop that had been cultivated and were devoid of vegetation. At this time some of the adult cattle were noticed to be segregated from the herd, gaunt, and some had loose manure.

**Clinical examinations—Days 2 and 3**

Over the course of two days, both recumbent cows (4 and 5), aged ten and seven years, respectively, deteriorated and were euthanized. Heads were severed from the dead animals and submitted for gross and histopathological examination.

**Clinical examinations—Day 9**

Four surviving cattle, including two three-year-old heifers, a cow aged seven years, and a two-year-old bull, initially identified as cases on day 1, were reexamined. Clinical signs included blindness in four, depression in two, and dry, alopecic, depigmented hair coats in four. All four animals had hyperkeratotic lesions surrounding the eyes, with females having lesions on the vulva, teats, udder, and perianal region. Three additional affected animals, two cows aged four and eight years, and a heifer were identified and examined. Clinical signs observed in the additional cases included depression in one and blindness and dry, depigmented hair coats with hyperkeratotic alopecia in three.

**Hematology—Days 1, 2 and 3**

Complete blood counts and serum biochemical analyses were done on blood from four of the affected animals, two of which were recumbent. Complete blood counts were unremarkable. Serum biochemical analyses revealed elevated creatine phosphokinase levels in both recumbent cows (1185 and 15696 U/L for cows 4 and 5, respectively; reference range <350 U/L). Cow 5 also showed mild to moderate elevations in magnesium (1.48 mmol/L; reference range 0.80–1.32 mmol/L) and urea (12.0 mmol/L; reference range 1.8–3.9 mmol/L). Two ataxic, blind, and dysphagic animals had mild elevations in serum urea levels (8.3 and 9.9 mmol/L). Serum sodium levels were within the reference range.

**Necropsy findings**

The carcasses of cases 2, 4 and 5 were in good body condition and gross lesions were confined to the brain. The brains in all three cases revealed the dorsal surfaces of the gyri of the occipital, parietal, and to a lesser extent the frontal lobes to be bilaterally and multifocally flattened and yellowish. The brains were soft but did not show tentorial herniation or cerebellar coning typical of severe cerebral edema. On the cut surfaces, the sides and depths of the cerebral cortical sulci were multifocally yellow and occasionally fluoresced under the ultraviolet light of a Wood’s lamp. Bilaterally, the central thalamic and mesencephalic (midbrain) regions contained large areas of hemorrhage and malacia; in two cases the areas were up to 2 cm in diameter. These hemorrhagic and malacic foci were also consistently present in the caudal colliculi of the midbrain and, in one case, extended into the medulla oblongata. In two cases, petechial hemorrhagic foci also extended cranially from the thalamus into the basal ganglia and internal capsules (Figure 1).

**Figure 1.** Multiple transverse sections of brain from one cow showing areas of hemorrhage and necrosis in the thalamus (1), midbrain (2), caudal colliculi (3), and vermis of the cerebellum (4). Cortical lesions of malacia not clearly visible in this photograph.

Multiple routine sections of all brains showed bilateral and multifocal, acute necrosis of cortical grey matter, most consistently involving outer cortical laminae. These foci were most abundant in the parietal and occipital regions and typically showed diffuse hyperesinophilia of the neuropil, the acutely necrotic neurons being shrunken and eosinophilic. These areas were well delineated from the surrounding, more pale-staining and vacuolated parenchyma and most consistently involved the bottoms and sides of the sulci; occasionally, however, the full thickness of the cortex was involved, including the tops of the gyri. In most areas, blood vessel endothelial cells showed mild to moderate hypertrophy. In two brains, fibrinous thrombosis, circumferential fibrinoid degeneration of small vessel walls, and perivascular hemorrhage were common, variably involving capillaries, venules, arterioles, and small muscular arteries of the cortical grey matter regions. Sections of thalamus, midbrain, and medulla showed similar severe acute parenchymal necrosis but demarcation with adjacent viable tissue was less obvious. These regions tended to show more edema, with prominent perineuronal and perivascular vacuolations. Intraneuronal and perivascular hemorrhage was also much more severe and widespread in tissue areas associated with thrombosis, fibrinoid necrosis of vessel walls, and karyorrhexis of nuclei of necrotic vessel walls. Commonly, very large veins were tremendously dilated and thrombosed. Large areas of the central thalamus and midbrain, with smaller areas of the basal ganglia and medulla, were involved. The hippocampus did not show any lesions and the cerebellum showed only small hemorrhages in the granular layer, with hypoxic damage to the Purkinje cells.

The brain sodium level of cow 5 was 1300 ppm. Levels greater than 1800 ppm were considered indicative of sodium salt toxicosis (16). Renal and hepatic tissues of heifer 2 were negative for lead and a biopsy was done on several areas of depigmented, hyper-
**Table 1.** Mean (± SEM) effects on blood thiamine, erythrocyte transketolase, and thiamine pyrophosphate in cattle consuming water containing elevated sodium sulfate

<table>
<thead>
<tr>
<th></th>
<th>Clinically affected and thiamine-treated adults (n = 7)</th>
<th>Nonaffected adults (n = 99)</th>
<th>Nonaffected calves (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood thiamine(^a) (µmol/L)</td>
<td>337.1 ± 128.9</td>
<td>148.0 ± 3.66(^d)</td>
<td>200.8 ± 3.99(^d)</td>
</tr>
<tr>
<td>Erythrocyte transketolase (µmol/min/µl)</td>
<td>0.52 ± 0.038(^c)</td>
<td>0.44 ± 0.015(^c)</td>
<td>0.43 ± 0.011(^c)</td>
</tr>
<tr>
<td>Transketolase TPP effect(^b)</td>
<td>18.3 ± 5.5</td>
<td>15.1 ± 1.6(^f)</td>
<td>38.7 ± 2.3(^f)</td>
</tr>
<tr>
<td>Plasma copper (µmol/L)(^f)</td>
<td>10.9 ± 1.68</td>
<td>10.1 ± 0.21(^g)</td>
<td>11.2 ± 0.21(^g)</td>
</tr>
</tbody>
</table>

\(^a\)Reference range 75–185, marginal 50–75, deficient < 50
\(^b\)The percentage of increase in transketolase activity after addition of thiamine pyrophosphate (TPP) to the assay
\(^c\)Reference range > 11, marginal 7.9–11, deficient < 7.9

Statistically significant differences between values are indicated at

\(^d\)where p < 0.05 and \(^g\)where p < 0.005

keratotic, alopecic skin from case #9 and revealed an eosinophilic dermal infiltration consistent with insect bites.

**Serum vitamin and trace mineral analysis of affected animals**

Serum zinc levels of seven affected animals were within the reference range. The mean value was 18.2 ± 0.78 (SEM) µmol/L with a range of 15.3 to 20.8 µmol/L. Serum vitamin A levels of seven affected animals were within the reference range (>0.87 µmol/L) for three animals and low in four. Animals demonstrating low vitamin A levels had levels between 0.45 and 0.70 µmol/L. Serum levels for vitamin E for the seven affected animals tested were within the reference range. Serum copper levels for the seven affected animals revealed that two had levels within the reference range (>11 µmol/L) and that five had marginal levels between 7.9 and 10.2 µmol/L with a mean level for marginal animals of 8.9 ± 0.37 (SEM) µmol/L. The seven affected animals tested had thiamine levels greater than the reference range (75–185 nmol/L) with a mean of 337.1 ± 128.9 nmol/L. However, it was unknown how many of these animals received exogenous thiamine injections by the rancher prior to sampling.

**Herd analyses (Table 1)**

Affected cattle had significantly greater RBC TK when compared to nonaffected, exposed herdmates (p<0.05). TK-TPP effect % increase was not significantly different between affected and nonaffected adult animals. Clinically affected animals had greater blood thiamine levels when compared to nonaffected, exposed herdmates. The difference was not statistically significant, however, due to the large standard deviation of the thiamine values of affected animals. The role of exogenously administered thiamine on the levels of affected animals was unknown. Nonaffected calves had significantly greater blood thiamine levels (p<0.005) compared to nonaffected mature animals. Immature animals had significantly greater copper values compared to nonaffected, exposed, mature animals (p<0.005). Approximately 27% of nonaffected, mature animals and 11% of calves had copper concentrations of <9.44 µmol/L.

**Well water analyses (Table 2)**

The ranch is served by four wells: #1, #2, #3 and #4. Wells are 7.6 meters deep with the exception of well #4 which is 18.3 meters deep. All wells serve livestock, and water from wells #1 and #4 was consumed by humans. Cattle had been supplied with water from well #1 from May until mid-June and well #2 from mid-June until the onset of the PEM outbreak. Water analysis revealed a markedly elevated sodium sulfate level of 7200 ppm from well #2.

**Discussion**

Polioencephalomalacia (PEM) is a term that has been used to describe softening and necrosis of the brain's grey matter. Most of the time this has been specifically associated with a thiamine deficiency that may be overt or may develop because of the presence of thiaminases in the digestive tract of the ruminant (17). In our report the term PEM has been used to provide a morphological-anatomical description of the lesions seen in cases of sulfate-exposed neurological disease.

The cattle had clinical signs of variable severity which included ataxia, blindness, depression, dysphagia, recumbency, and sudden death. The herd history of recent pasture change, hot weather, and possible water deprivation, in conjunction with the initial clinical and postmortem examinations, led us to consider water deprivation/salt toxicosis, lead-induced encephalopathy, and polioencephalomalacia as possibilities for the primary disease.
Table 2. Well water analysis of four wells on the property. All cattle were watered from well #2 during the five weeks prior to the polioencephalomalacia outbreak

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Well #1</th>
<th>Well #2</th>
<th>Well #3</th>
<th>Well #4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/L)</td>
<td>1000*</td>
<td>248</td>
<td>421</td>
<td>79</td>
</tr>
<tr>
<td>Cl (mg/L)</td>
<td>250*</td>
<td>34</td>
<td>280</td>
<td>5</td>
</tr>
<tr>
<td>K (mg/L)</td>
<td>not available</td>
<td>7.9</td>
<td>22</td>
<td>1.9</td>
</tr>
<tr>
<td>Na (mg/L)</td>
<td>300*</td>
<td>130</td>
<td>1250</td>
<td>12</td>
</tr>
<tr>
<td>Mg (mg/L)</td>
<td>200*</td>
<td>71</td>
<td>1050</td>
<td>14</td>
</tr>
<tr>
<td>Sulfates (mg/L)</td>
<td>1000*</td>
<td>745</td>
<td>7200</td>
<td>39</td>
</tr>
<tr>
<td>Total hardness</td>
<td>not available</td>
<td>912</td>
<td>5350</td>
<td>258</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>3000*</td>
<td>2000</td>
<td>9610</td>
<td>577</td>
</tr>
<tr>
<td>pH</td>
<td>6.5–9.0*</td>
<td>7.22</td>
<td>7.59</td>
<td>7.58</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>not available</td>
<td>368</td>
<td>529</td>
<td>242</td>
</tr>
</tbody>
</table>

*From Canadian Water Quality Guidelines, Environmental Quality Guidelines Division, Water Quality Branch, Inland Water Directorate, Environment Canada, Ottawa, Ontario K1A 0H3

Sodium salt toxicosis occurs commonly in poultry and swine, and occasionally in cattle and sheep (17). It may occur indirectly when excessive volumes of water are consumed following prolonged deprivation, or directly by consumption of saline water or feed. Indirect salt toxicosis generally manifests as neurological signs resulting from cerebral edema, whereas direct toxicosis manifests as gastrointestinalitis. Clinical signs reported in affected cattle include severe muscular weakness, dehydration, diarrhea, belligerence, and convulsions (16). Salt toxicosis/water deprivation was ruled out on the basis of the brain sodium level from cow #5 and the serum sodium levels of five affected animals.

Lead-induced encephalopathy may be manifested as neurological disease with sudden death (6), and was ruled out by the absence of lead in the renal and hepatic tissues of heifer #2.

The diagnosis of polioencephalomalacia was confirmed by postmortem findings of cerebrocortical and subcortical necrosis (malacia) with microvascular fibrinoid necrosis and thrombosis predominantly in the thalamic-midbrain region of three affected animals. Herd history and well water analysis suggested a causal role for increased levels of drinking water sulfates in the development of PEM in this herd. Unlike many PEM outbreaks, where young animals are most affected (2,3,12,17), calves in this herd were unaffected. This may be attributed to the limited intake of the high sulfate water by nursing calves. The outbreak of PEM ceased and surviving affected animals recovered when the herd was removed from access to well #2.

The role of increased sulfate intake, the biochemical interactions among copper, zinc, iron, and molybdenum, and the role of sulfate in rumin thiamine metabolism have been investigated (10). Precise mechanisms for the induction of sulfate-related B1 deficiency have not been elucidated, although evidence suggests that high sulfates reduce the microbial synthesis of thiamine (18). However, if thiamine played a critical role in the development of PEM in this herd, one might expect the exposed, clinically unaffected cattle to show signs of marginal or deficient blood thiamine status. The herd mean blood thiamine level of 148.0 ± 3.66 nmol/L for the 99 adults was well within the reference range (19). Conclusions cannot be drawn about the blood thiamine levels of the seven affected cattle because they may have been influenced by the administration of unknown quantities of exogenous thiamine by the rancher, and thus may be falsely elevated. The blood thiamine levels of the four cattle that died at the onset of the outbreak were also unknown.

Alternatively, elevated rumen sulfide concentrations, from the consumption of high sulfate water and subsequent rumin microbial degradation to sulfide, may produce PEM by inhalation of eructated rumen gas (14). Hydrogen sulfide (H2S) is highly neurotoxic (20). Interestingly, when dietary sulfate toxicosis PEM was experimentally induced in five calves, thiamine concentrations in blood, CSF, brain, and liver were not significantly decreased from the reference range (14). Therefore, sulfate toxicosis may account for the PEM outbreak in this herd and explain the adequate mean blood thiamine levels of the herd during the outbreak.

The consistent, extensive thrombosis and vascular necrosis seen histologically in the midbrain and thalamus of the three animals necropsied has not been described in other PEM cases, to our knowledge, and may be a unique feature of sulfate toxicosis PEM. Gould et al (14) describe histological lesions ranging from cerebrocortical neuronal necrosis to cerebrocortical poliolomalacia and cavitation in calves with experimentally induced sulfate toxicosis PEM. Importantly, calves were necropsied within 48 hours of the first clinical signs of disease. Therefore, lesions seen by Gould et al (14) would be expected to differ from those seen in the outbreak reported herein where chronic sulfate exposure was allowed to run a full clinical course. Additionally, the level of sulfate exposure under experimental conditions may differ greatly from exposure under natural conditions.

The interesting finding of dry, depigmented hair coats and hyperkeratotic alopecia may suggest a role for copper deficiency or hypovitaminosis A in this herd.
outbreak of PEM. Increased levels of dietary sulfate decrease the bioavailability of copper to the animal (10). Clinically, this sulfate-copper interaction may result in copper deficiency and to present dermatologically as decreases in hair coat pigmentation and quality. Five of the seven affected animals in this outbreak showed marginal serum copper levels. Although dermatological changes seen in members of this herd may be due to marginal copper status, an alternative explanation may be hypovitaminosis A (17). Four of the seven affected animals sampled for vitamin A revealed marginal levels. Polioencephalomalacia-affected cattle in this report were observed to have decreased ability to avoid flystrike, when compared to unaffected members of the herd, and this was supported by biopsy of depigmented lesions. Therefore, the diagnosis of PEM due to sulfate toxicosis may account for the entire range of clinical signs seen in affected animals of this herd.

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References