Case report: Diagnostic findings of bovine neurological cases at the Iowa State University Veterinary Diagnostic Laboratory between January 2017 and May 2021

David Villar,[1] DVM, PhD, DABVT; David Gomez-Beltrán,[1] DVM, MS; Scott Radke,[2] DVM, MS, DABVT; Kent Schwartz,[2] DVM, MS; Drew Magstadt,[2] DVM, MS, DACVP

[1]CIBAV Group, Faculty of Agrarial Sciences, University of Antioquia, Medellin 050010, Colombia
[2]Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa 50011-1134
***Corresponding author:** Dr. David Villar, davidvillar2003@yahoo.com

Abstract

This study reviews the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) computer records of bovine neurological cases submitted from January 2017 to May 2021. There were 236 cases diagnosed as follows: listeriosis (83), polioencephalomalacia (44), thrombomeningoencephalitis (23), miscellaneous bacteria (22), lead toxicosis (21), nitrate toxicosis (18), urea toxicosis (9), nervous coccidiosis (5), hypomagnesemia (5), sodium toxicosis (3), rabies (1), herpesvirus type 6 (1), and epsilon toxin from *Clostridium perfringens* type D (1). Most histories did not provide specific descriptions of the type of neurological signs, which could better target diagnostic investigation. However, each condition had specific peculiarities relevant from a post-mortem diagnostic perspective. For listeriosis, confirmation of *Listeria monocytogenes* using IHC was considered superior to bacterial culture, with 16/19 (84%) positive by IHC compared to 23/59 (38.9%) of cases positive by culture. In 44/83 (53.0%) of cases, histopathological examination mentioned that cerebellum and cerebrum were unremarkable, implying that without submission of the entire brain, particularly brain stem and obex, listeriosis cannot be ruled out. In 44 cases histologically diagnosed as polioencephalomalacia, when ultraviolet light (Wood's lamp) was performed, it revealed multifocal areas of apple-green autofluorescence in 13/21 (61.9%) of brains, indicating that false negatives can occur with this technique. A substantial number of submissions were toxicoses that required specific sample types to confirm a diagnosis. For lead, whole blood, liver or kidney was necessary. For nitrate and urea toxicosis, appropriate samples were ocular fluid or the incriminating feed/water source. Confirmation of sodium toxicosis relied on sodium analysis of brain tissue.

Key words: bovine, neurological diseases, toxicosis, infectious agents, recommendations

Introduction

Clinical presentations of neurologic disease are relatively frequent in bovines, with etiologies that may be infectious, nutritional, metabolic or toxicological. Observation and accurate reporting of clinical signs with clinical context is important for differential diagnosis and targeting diagnostic testing. However, many cerebral or hindbrain diseases cause overlapping and diffuse (symmetric) clinical signs that cannot be easily differentiated.[1] For example, seizures in cattle can be triggered by intracranial structural lesions or extracranial causes such as metabolic disturbances or toxicants.[2] This may explain why many veterinarians report clinical signs simply as central nervous system disease (CNS disease) or "downers" in their submission forms to diagnostic laboratories. Definitive diagnosis usually requires a complete postmortem examination, the proper collection of appropriate samples, and the application of relevant laboratory tests, including histopathology. Selection of specific tests also relies on the clinical context, including experience and knowledge of the clinical setting, diseases most common in various locales and production settings, age of the animals, season and feedstuffs or feeding practices. A review of bovine neurological disease cases presented at the two Georgia Veterinary Diagnostic Laboratories between 2001 and 2017 were 6 and 4% of the bovine submissions.[3] A similar study in Scotland (UK) reported that 5.4% of all bovine submissions were for neurological disease.[4] In the Georgia study, suppurative meningoencephalitis was the most common diagnosis in 28% of cases and was frequently associated with bacterial sepsis.[3] A potential bias of their study was omitting neurologic dysfunction cases in which toxicosis might have been present. Toxicants such as lead, urea, sodium (with or without water deprivation), nitrate, organophosphates, carbamates and metabolic causes (e.g., hypomagnesemia and hypocalcemia) were not included. This study reviews the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) computer records of 236 bovine neurological cases submitted from January 2017 to May 2021, providing insights to the most common neurological conditions and their predisposing factors that affect cattle in Iowa, as well as the limitations to establish a diagnosis.

Case description

A search was performed of electronic records from the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) for all bovine submissions with a clinical diagnostic code(s) of neurologic disease. Cases were included only if a final diagnosis was achieved by gross and/or histopathological examinations or other laboratory tests related to central nervous system (CNS) disease. The year and month of submission, age of the animals affected, and history (when provided) were recorded. Etiologies associated with "sudden death" or animals found dead, including plants such as Japanese yew (*Taxus cuspidata*), microcystins, cholinesterase inhibiting insecticides, and metabolic disturbances such as ketosis, hypocalcemia, or hypovitaminosis A, were excluded.

Diagnostic findings

ISU-VDL gets approximately 2,300 cases per year for diagnostic investigation that include tissues for histopathology, with "CNS cases being a minority (roughly 4%) of cases" (Table 1). Between 2017 and 2021, 386 accessions were submitted with a history of CNS disease, of which a definitive diagnosis was made in 236 (61%). Reasons for failure to confirm an etiologic diagnosis included: a) proper samples to confirm a diagnosis were not submitted, where autolytic or contaminated, b) lesions were present but an agent was not confirmed, c) monetary constraints prevented confirmatory tests. Therefore, about 1/3 of accessions with reported CNS signs could not identify a primary etiology or achieve level of confidence for the diagnostician to assign a specific/singular etiologic agent.

In 2017, cattle inventory for Iowa was reported at 3,950,920 head of cattle, of which 5.7% (223,579) were dairy cows/heifers, 23.8% (938,818) beef cows/heifers, and 70.5% (2,788,523) replacement heifers (dairy and beef) and calves weighing less than 500 pounds (USDA, 2019).[5] A summary of the major causes identified in neurological disease submissions to the ISU-VDL is provided in Table 2 including listeriosis, thromboemeningoencephalitis (TEME), polioencephalomalacia (PEM), bacterial encephalitis, sodium toxicosis, nitrate toxicosis, urea toxicosis, lead toxicosis, hypomagnesemia, nervous coccidiosis, rabies and herpesvirus. Information includes the offending agent, the number of accessions and whether they originated in Iowa or other states, results of the main ancillary laboratory tests used to rule out the suspected cause, and the median and range of ages of the animals affected. There were 128/236 (54.2 %) submissions that had a bacterial origin that are presented in the following sections.

Listeriosis

Listeria monocytogenes (Lm) was the most common cause of neurological disease in this survey. The diagnosis was by observation of typical microscopic lesions as described below, regardless of the results of ancillary tests. The total number of listeriosis cases represented 83/236 (35.2%) of all neurologic cases and 83/128 (64.8%) of bacterial neurological cases. Eighteen of 83 submissions originated from states other than Iowa. The majority of cases 56/83 (67.46%) were diagnosed in March, April and May (Figure 1). Submission histories often did not provide specific description of clinical signs; 48/83 (57.8%) of submissions either did not describe signs or simply stated "CNS or lateral recumbency." The most common clinical signs and the number of times reported were: circling (13), blindness (10), unilateral facial paralysis (9), ataxia/staggering (9), tongue hanging out (5), ear drooping (4), salivation (4). Animals that were either ≤ 1 year of age or between 1 and 2 years represented the majority of the age groups, with 27.7% (23/83) and 36.1% (30/83), respectively (Table 2). Bacterial isolation was attempted in 59 cases, with 23 samples positive by direct culture and 2 samples positive only after cold enrichment. Antimicrobial therapy before sampling is likely to have occurred, but usually was not stated on submission forms. Nineteen cases were examined by immunohistochemistry (IHC), with 16 found positive. However, only 5/16 (31.3%) of cases positive by IHC were also positive by culture. Thus, the confirmation of the presence of Lm using IHC is considered superior to bacterial culture in field submissions. The diagnosis was made by the presence of typical histopathological lesions in the brainstem, which include: a) areas of the neuropil with malacia, rarefaction and gitter cells forming microabscesses, b) perivascular infiltrates (cuffing) of mixed populations of inflammatory cells, c) dilated axon sheaths (spheroids). Interestingly, 44/83 (53.0%) of cases mentioned that cerebellum and cerebrum were unremarkable, implying that listeriosis cannot be ruled out without submission of the entire brain, particularly brain stem and obex.

Polioencephlomalacia

Polioencephalomalacia (PEM) was the second most common cause of neurological disease. Forty-four cases of PEM were confirmed by typical histopathological lesions as described below. Twenty-seven cases were in animals less than 1 year (27/44 [61.4%]), and 11 cases were between 1 and 2 years (11/44 [25%]). Unlike listeriosis, there was no clear seasonal pattern for submissions. Unfortunately, clinical signs were poorly described in the submission history for most cases, often only a simple mention of "neurologic" or lateral recumbency. When described, the most common signs were paddling (7), opisthotonus/stargazing (7), circling (4), blindness (4), convulsions (3), or teeth grinding (2). Ultraviolet light beaming of the brain (Wood's lamp) demonstrated multifocal areas of apple-green autofluorescence in 13 of 21 brains examined, indicating that false negatives can occur with UV light technique. Typically, fluorescence occurred in swollen flat gyri and gray matter of the cerebral cortex (Figure 2). Classical histopathological lesions of laminar cortical necrosis were: a) vacuolation or rarefaction of the perivascular neuropil in the cortical gray matter (moth-eaten appearance – malacia), b) remaining neurons were shrunken, angular, hypereosinophilic, with pyknotic nuclei, c) reactive endothelium characterized by swollen or plumped endothelial cells.

Table 1: Number of bovine tissue accessions received at the ISU-VDL from January 2017 to May 2021.

2017	2018	2019	2020	2021 to Jun 1st	TOTAL	
2,439	2,448	2,148	2,064	1,186	10,285	# Accessions with gross and/or histopathology (BVD IHC/earnotch surveillance cases are excluded)
85(est)*	85(est)*	77	100	39	386	# of cases with a history of neurological disease

* Data capture of CNS clinical signs was not reliable until 2019, hence 2017-2018 are estimates.

Table 2: Neurological bovine cases diagnosed at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) between January 2017 and May 2021.

Diagnosis	# of accessions Iowa (other States)	Diagnostic test	Median age (range)
Listeria monocytogenes	65 (18)	Brain culture 23 + 36 -	1 year (2 months – 8 years)
Polioencephalomalacia	34 (10)	Wood´s lamp 13 + 8 -	<1 year (4 months – 10 years)
TEME	18 (5)	Brain culture/PCR 14 + 6 -	< 1 year (9 weeks – 16 months)
Lead intoxication	10 (11)	Pb in liver (Median, range) 15,9 (5 – 93) ppm	2.5 months (1 month – 10 years)
Miscellanous bacteria	15 (7)	Culture and histopathology	Variable: mostly newborn calves
Nitrate toxicosis	7 (11)	Nitrate in ocular fluid (median, range in ppm) 71.3 (27.8 – 927.9)	2 years (8 months – 8 years)
Urea toxicosis	2 (7)	Ammonia in ocular fluid (median, range in ppm) 50 (21.4-207.8)	2 years (6 months – 3 years)
Nervous coccidiosis	4 (1)	Fecal flotation and histopathology of the colon	7 months (1.5 – 12 months)
Hypomagnesemia	2 (3)	Serum magnesium <2.1 mg/L	5 years (3-10 years)
Sodium toxicosis	1 (2)	Brain sodium > 1,800 ppm	Newborn calves
Rabies	1	Fluorescent antibody	4 months
Herpesvirus type 6	1	PCR	1 year
Epsilon toxin Cl. *Perfringens type D*	1	Epsilon toxin of *Cl. perfringens* type D.	20 months

Thromboembolic meningoencephalitis/*Histophilus somni*

Thromboembolic meningoencephalitis (TEME) caused by *Histophilus somni* (Hs), was histologically diagnosed in 23 cases, with 18 originating in Iowa. When age was included in the submission, 12 animals were ≤ 1 year old (12/23 [52.2%]), and 3 were 1 to 2 years old (3/23 [13.0%]). There were too few cases to identify a seasonal pattern for submissions. Of the 23 cases diagnosed by histopathology, 20 had the brain tested by bacterial culture or PCR, showing evidence of *Histophilus somni* in 14 cases (14/20 [70.0%]). Again, clinical signs were poorly described in most submissions, being recumbency and thrashing/paddling the most common one in 19/23 (82%), and opisthotonus/stargazing reported in 8/23 (35%) submissions. Classical histopathological brain lesions included: a) multifocal vascular fibrinous thromboemboli with fibrinoid degeneration of the vessel walls (necrotizing vasculitis) and perivascular cuffing, b) mixed pyogranulomatous inflammation of the neuropil with random necrotic foci associated with hemorrhage (encephalitis), c) suppurative meningitis. In addition, most cases were associated with myocardial or pulmonary lesions. Occasionally, multifocal, randomly distributed hemorrhage and necrosis were reported, which is nearly pathognomonic for TEME due to Hs (Figure 3).

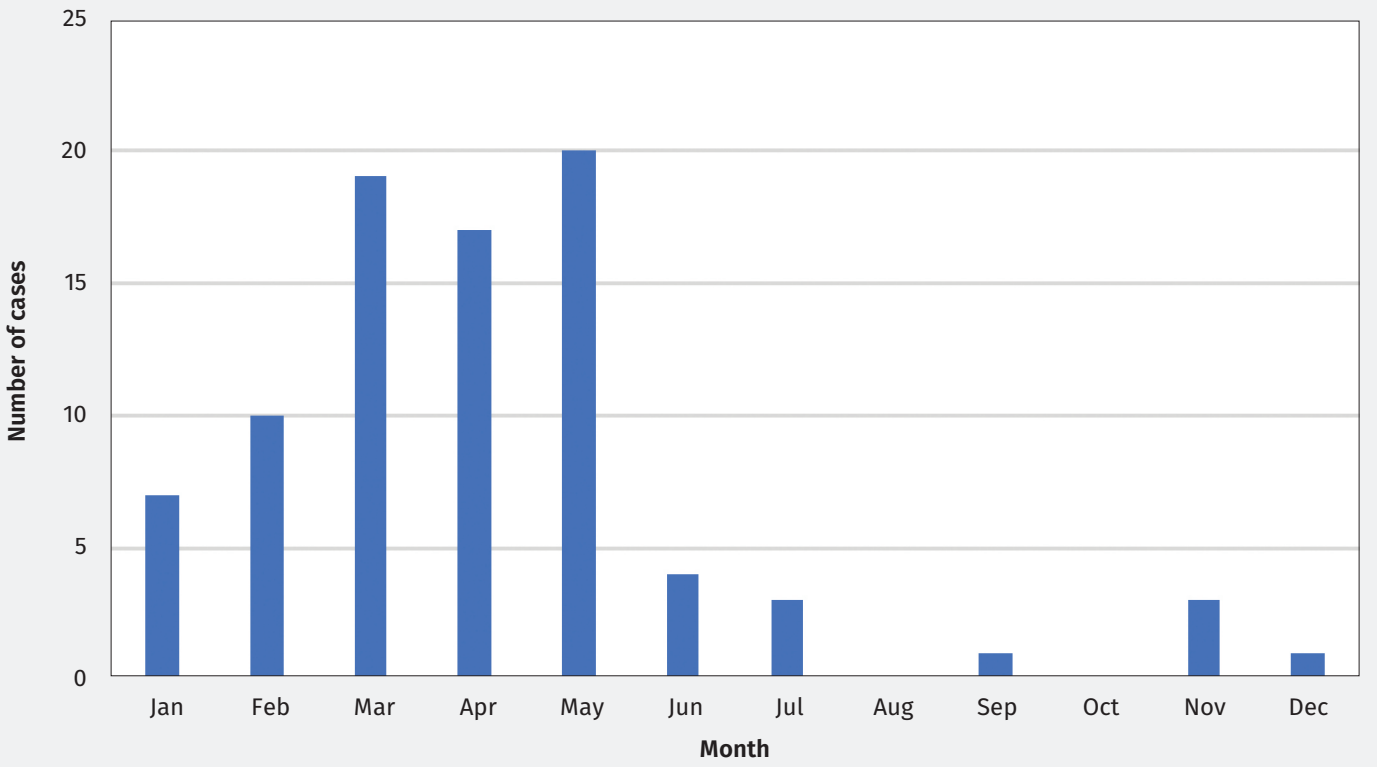
Lead toxicosis

Lead intoxication was diagnosed in 21 bovines, with 10 originating in Iowa and 11 from other states. The median age of affected animals was 2.5 months. Although clinical signs were not reported in 10 cases, the most commonly reported ones were blindness (5), circling (3), grinding of teeth (3), salivating (3), tremors (2), seizures (2), ear twitching (1), and head tilt (1). Liver concentrations were measured in 14 cases at ≥ 5 ppm wet weight. Whole blood samples were analyzed 7 times, and concentrations were ≥ 0.5 ppm in all cases.

Nitrate toxicosis

Of 18 submissions attributed to nitrate toxicosis, 7 originated in Iowa. A diagnosis was made when nitrate in the ocular fluid was ≥20 ppm.[6] A total of 25 analyses for nitrate were performed in ocular fluids, with a median concentration of 71.3 ppm, average (± SD) of 123.8 (± 174.4) ppm, and a range between 27.8 and 927.9 ppm. Nitrite concentrations were not correlated with nitrate concentrations in ocular fluid. In 9/18 (50.0%) of cases, nitrite concentrations were < 1 ppm. Therefore, measurement of nitrate is necessary, as measurement of only nitrite in the ocular fluid can result in many false negatives. For 4 cases, dry weight nitrate concentrations in the new

Figure 1: Number of bovine listeriosis cases diagnosed at the ISU-VDL between January 2017 and May 2121 (n = 83 cases).



hay bales used as feed the previous day were available and reported as 3.2%, 1.95%, 1.2%, and 2.6%. When cows are not accustomed to diets high in nitrates, levels ≥ 1.0% in hay can be lethal.[7] No history of a suspected or known source of nitrate was provided in 14/18 cases. However, in 2 cases, water supplementation was provided with tanks previously used to transport fertilizers. In those 2 cases, water nitrate concentrations were reported at 4,800 and 11,000 ppm, far surpassing lethal concentrations of ≥ 1,500 ppm. In one of those cases, the water remnant of the fertilizer left in the tank hose was associated with the death of 15 rodeo bulls.

Urea toxicosis

Nine cases were diagnosed, of which 7 were from states other than Iowa. Histories of transporting water in tanks previously used to transport fertilizers were the most typical case scenario. Confirmation of diagnosis was obtained by testing ocular fluid for ammonia, and in all cases, concentrations exceeded the toxicity level of 20 ppm.[8] Median ammonia concentration in 16 ocular fluid samples was 50 ppm (range 21.4-207.[8] ppm). In 2 cases where the water source was also submitted, urea concentrations were 0.3% and 0.74% (3,000-7,400 ppm). In one case, ingestion of molasses containing high levels of urea was the source of the intoxication, resulting in ocular fluid ammonia concentration of 29.6 ppm. Some cases had high morbidity and mortality; for example, losses in 2 cases were 21/72 (29.1%) and 150/1,000 (15%) animals.

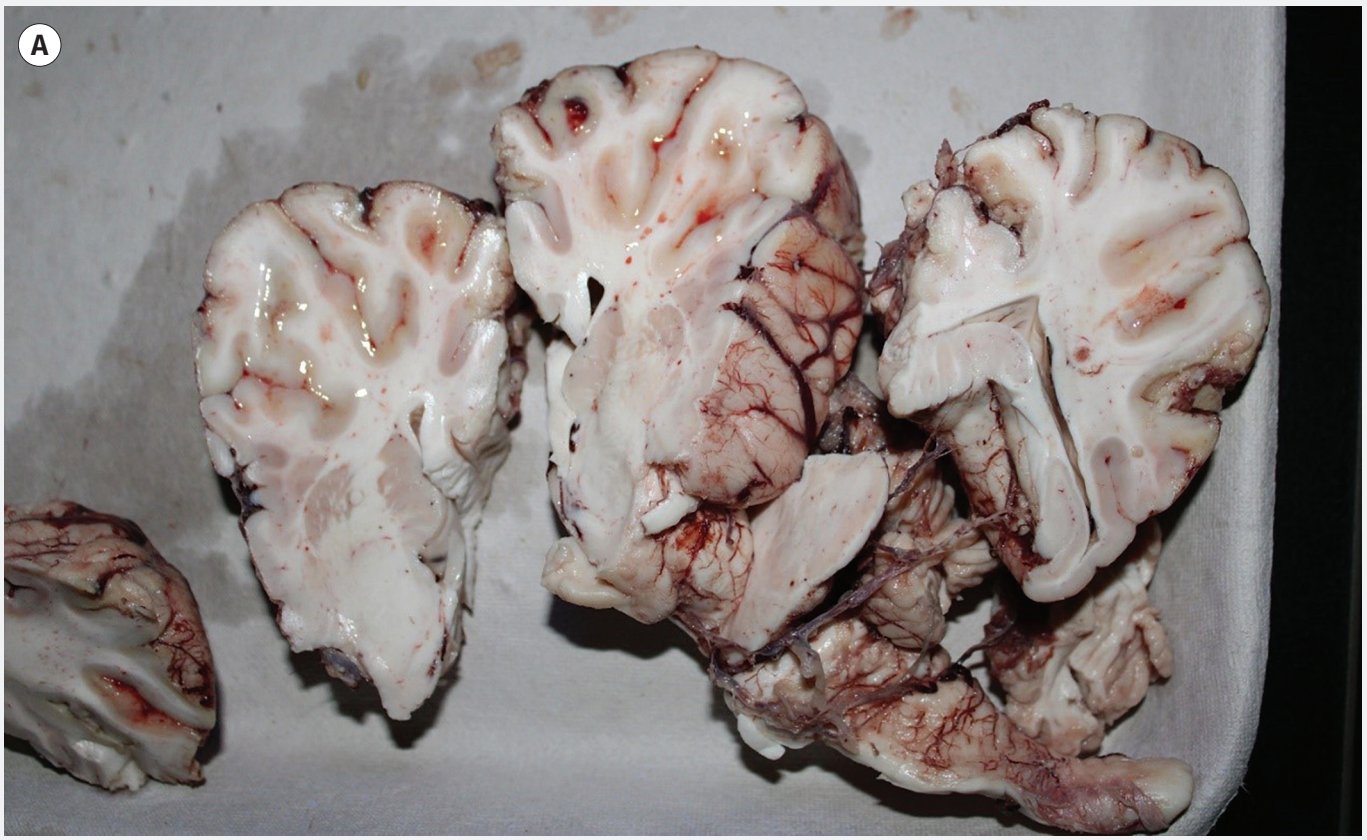
Hypomagnesemia

Five cases of hypomagnesemia were diagnosed in adult cows. In 4 cases, serum Mg (1.3, 1.05, 1.02, and 0.56 mg/dL) was below half the expected normal range (2.1-2.9 mg/dL).[9] In the remaining case, where a full postmortem examination was conducted, both ocular fluid and urine had low Mg (1.52 mg/dL and 1.33 mg/dL, respectively), substantiating hypomagnesemia. Urine is the preferred fluid to test for magnesium because concentrations in urine will decrease rapidly to zero as the animal becomes increasingly hypomagnesemic (at plasma Mg concentrations below 1.7 mg/dL), and urine concentrations <2 mg/dL are consistently observed in hypomagnesemia compared to expected normal Mg concentrations of 12-25 mg/dL.[9] Ideally, the preferred method to report Mg in urine is corrected against creatinine, with a ratio of less than 1 mmol/L for Mg/creatinine considered deficient.[10] Unfortunately, in the only urine sample submitted Mg/creatinine was not analyzed.

Nervous coccidiosis

Fatal nervous disease was associated with the presence of acute colitis and severe coccidiosis in 5 cases, with clinical signs only vaguely described. The main ones reported were lateral recumbency with paddling in 4 cases with 1 case describing tremors, twitching nictitans and stiffness that wane and reappeared. However, previous reports indicate that typical, early clinical signs often include intermittent and progressive seizures.[11,12] Histopathologic lesions of severe colitis with eosinophils infiltrating the lamina propia and the presence of high numbers of immature coccidia and oocysts were reported in all 5 cases. There were no brain lesions observed, as would be expected for cases of nervous coccidiosis.[11]

Figures 2a and 2b: Polioencephalomalacia. Segmental laminar pattern of yellow discoloration in the superficial cerebral cortex and same brain sections showing apple-green autofluorescence when examined with ultraviolet light (Wood's lamp).



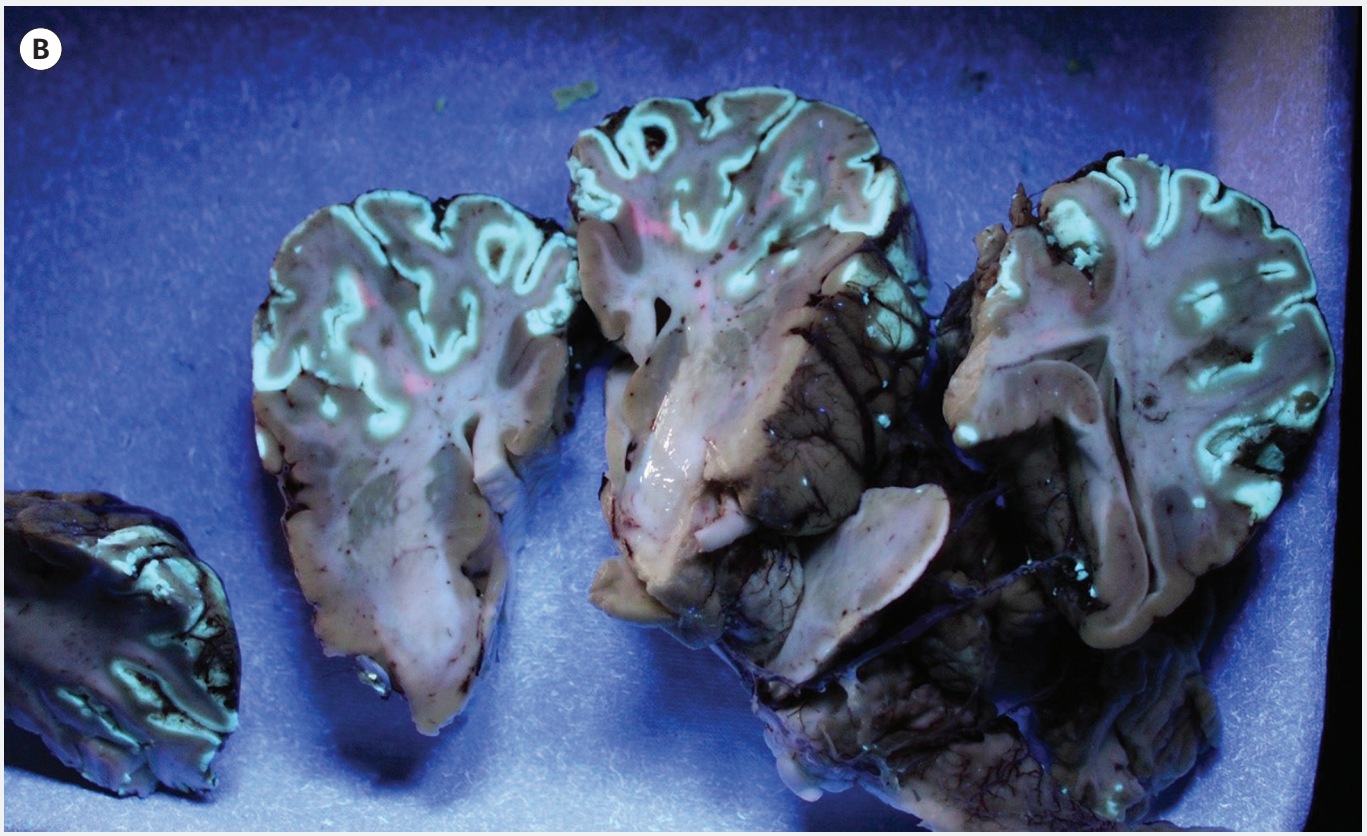
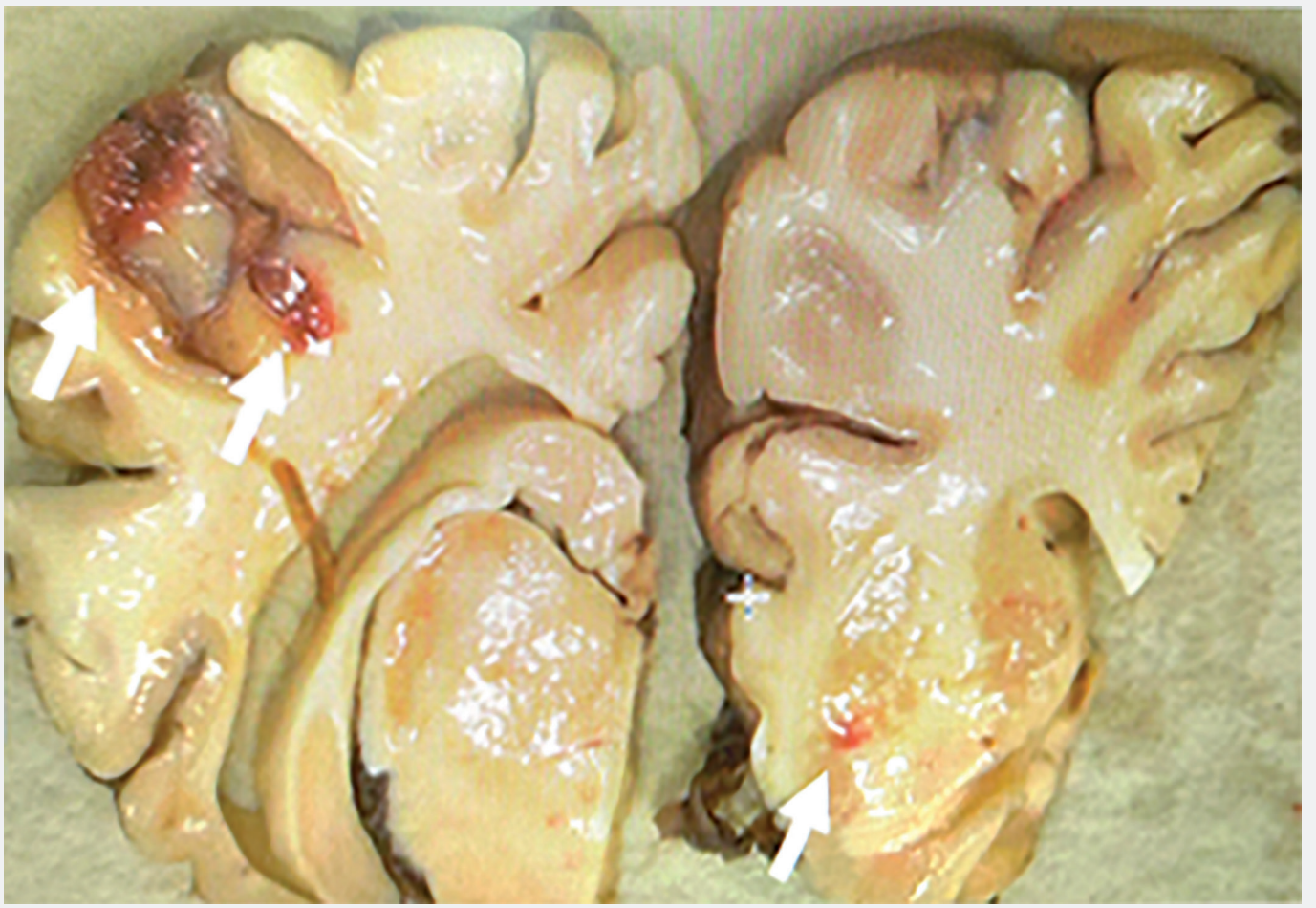


Figure 3: Thromboembolic meningoencephalitis (TEME). Two necrohemorrhagic foci in the right cerebral cortex of a calf at the level of the hippocampus. Another hemorrhagic focus can be seen in the right basal ganglia (courtesy Dr. Alyona Michael).



Sodium toxicosis

Three cases were diagnosed as sodium toxicosis. Two cases were nursing calves given milk replacers. One case involved 25 calves, of which 6 were sick and 14 died. Clinical signs reported in both cases were lateral recumbency, paddling, opisthotonus and blindness. A diagnosis was made by analyzing concentration of sodium in the brain. Concentrations were 1,954, 1,973 and 1,766 ppm. Concentrations > 1,800 ppm are sufficient to make a tentative diagnosis of sodium toxicosis.[13] The third bovine case did not provide any history, but sodium concentrations were reported as 2,329 and 2,762 ppm. This case probably occurred by providing free choice salt to deprived cattle that were not in immediate proximity to water.

Miscellaneous bacteria

Twenty-one cases were classified as miscellaneous bacterial systemic infections that also had suppurative meningoencephalitis (Table 2). These included 13 cases of bacterial septicemia with meningitis in newborn and suckling calves from 2 days to 3 weeks of age. In 9/13 (69.2%) of cases, the offending bacteria could not be cultured. In the remaining 4 cases, smooth/mucoid *E. coli* (2 cases), *Salmonella dublin* (1 case), and *Streptococcus* sp. (1 case) were isolated. Two cases of *Trueperella pyogenes* in 9- and 18-month-old calves were diagnosed and were considered the cause of necrosuppurative encephalitis (brain abscess).

Discussion

This study describes the most common causes of neurological diseases diagnosed by post-mortem examinations and laboratory testing at the ISU-VDL. The results revealed peculiarities for each condition that are important to make a definitive diagnosis. It also highlights the importance of a complete history provided by referring veterinarians to facilitate an initial tentative diagnosis. For example, a study that described the clinical signs in 98 cattle diagnosed with listeriosis described that the most frequent cranial neurological signs were facial nerve paralysis, salivation, strabismus, reduced or absent pupillary light reflexes, and reduced or absent tongue movement and head tilt.[14] Based on our findings, we would add that age (≤ 2 years) and season (winter months) are two presumptive factors to consider in listeriosis. We found that listeriosis accounted for the largest number (83/236, 35.1%) of all neurological cases submitted. Bacterial culture was not as reliable as IHC to make a definitive diagnosis of listeriosis, as only 23/59 (39%) of cultures for isolation attempts were positives compared to 16/19 (84%) IHC positives. The gold standard for diagnosing listeriosis in all 83 cases were the presence of typical

histopathological lesions in the brain stem and obex. This is consistent with a study that compared 3 different methods to detect listeria and showed success as follows: culture (28.5%), Gram stains (47.6%), and immunohistochemically positive (80.9%).[15] A similar study that also evaluated the sensitivity of laboratory tests for confirming a diagnosis of listeriosis showed that cultures were only 42% successful compared to 47% by Gram stains and 82% by IHC.[16] More importantly, in 44/83 (53.0%) of cases in our study, lesions were not observed in the cerebrum nor the cerebellum, implying that without submission of the brain stem and obex, listeriosis cannot be ruled out. Other reports also describe histologic lesions limited to the brain stem and anterior spinal cord as the case definition criteria for listeriosis.[16]

The presence of gross lesions in the brain and perhaps other systemic locations can often lead to a presumptive, if not definitive, diagnosis of neurological disease. For example, the presence of hemorrhagic-necrotic foci grossly visible is virtually pathognomonic for TEME and is characteristic of the multiple septic emboli lodging in parts of the brain that result in infarctions.[1,2] Unfortunately, it is unknown how many submissions that resulted in a TEME diagnosis had grossly visible lesions, as they were not mentioned in submission forms or examinations by pathologists. Like most commensal pathogens, *Histophilus somni* (Hs) is probably not a pathogen by choice but by chance. Here, it was mostly diagnosed in feedlot cattle < 1 year old that were probably being exposed to the stress of transportation, concurrent viral infections, overcrowding, and weaning. In the experience of one author (KS), TEME and polyserositis manifestations of Hs are common in feeder cattle placed > 30 days, after cases of Bovine Respiratory Disease Complex (BRDC). In a study that evaluated the prevalence of diseases that cause mortality in feedlot cattle 60 days after arrival, *H. somni* myocarditis was diagnosed in 8% of the cases, and commonly in association with *Mannhemia haemolytica* or *Pasteurella multocida*, causing fibrinosuppurative bronchopneumonia.[17] Therefore, *H. somn*i can not only cause TEME, but also be involved in cases of BRDC, polyserositis and myocarditis.[17]

Gross lesions of PEM can sometimes be observed as softening restricted to the cerebrocortical gray matter and laminar necrosis at the gray and white matter interface.[18,19] Also useful is examining the cortex under Wood's lamp (ultraviolet light), which can reveal multifocal areas of apple-green autofluorescence.[18,20] Typically, fluorescence occurs in swollen flat gyri and gray matter of the cerebral cortex.[18,21] However, 13/21 (69.2%) of brains examined did not fluoresce, indicating the UV light method had false negatives. Autofluorescence may likely be absent in acute cases that die rapidly. Noteworthy is that PEM is a lesion that has several causes, including thiamine deficiency, sulfur toxicity, lead toxicity or water deprivation/salt toxicosis.[19] Therefore, PEM is descriptive terminology for a specific pattern of histological lesions and not a specific disease. Veterinarians submitted the ration being fed and drinking water to test for sulfur in conjunction with the animal samples in only 2/44 (4.5%) of cases in this study, while the rest of the submissions did not provide results of potential analyses performed at other laboratories. A lack of analysis results for feed and water limited the ability to diagnose the exact etiology of PEM. Alternatively, the ISU-VDL can rule out exposure to high sulfur intake by analyzing for thiosulfate in the urine. A study aimed to determine when feedlot cattle most frequently expressed signs of PEM revealed that of 246 cattle that were diagnosed with PEM, 192 (78.0%) showed clinical signs between days 15 and 30 following arrival at the feedlot.[22] Two hundred forty (97.6%) of these cases were detected within 59 days after entering the feedlot. The authors estimated that the overall dietary sulfur intake (diet and water) was 0.67% of DM during the summer. Although the diets contained on average 0.2% sulfur, they consumed water with 2,500 ppm of sulfate (SO4). An experimental study that exposed yearling calves to various concentrations of sulfate in water showed that peak ruminal hydrogen sulfide concentrations coincided with the period of greatest risk to develop PEM.[23] The surge of hydrogen sulfide concentrations on day 13 was attributed to the introduction to the finishing ration by day 0 and the consequent drop in rumen pH. However, blood thiamine concentrations were not different between control animals and animals exposed to high sulfate in the water. It is known that inorganic sources of sulfur, such as SO4, pose a greater challenge for PEM than undegradable sources of sulfur as they can be easily reduced to S2- and then to the toxic H2S gas.[24] The National Research Council suggests that diets fed to feedlot cattle greater than 85% concentrates should not exceed 0.3% sulfur, assuming negligible exposure through the water.[25]

Twenty-one cases were classified as miscellaneous bacterial systemic infections and were associated with suppurative meningoencephalitis. These infections occurred mostly in calves under 30 days of age. Newborn calves are particularly at risk for developing septicemia because they depend on colostral antibodies for immunity.[18] Although the type of bacteria was not determined in 9/13 cases, *E. coli* has been the most common pathogen associated with meningitis in calves; however, other bacteria such as *Salmonella, Campylobacter, Klebsiella* and different *Staphylococcus* species, have also been isolated.[18] As it happens with enteric infections, prevention is most likely achieved by feeding adequate amounts of high quality colostrum in time for optimum absorption.[26] If multiple calves are affected, environmental and farm management practices should be examined, including poor umbilical hygiene, overcrowding and dirty environments.

Nervous coccidiosis was diagnosed in 5 cases. Although history was not provided, some studies report that the clinical picture is highly presumptive and distinctive to allow a diagnosis on clinical grounds alone.[12] The unique feature is that animals appear near normal in interictal periods (between seizures), and a proctitis palpable as a thick, roughened rectal mucosa, is commonly present; in other words, affected calves may get up and experience periods of apparent normalcy between seizures. Menace and pupillary light reflexes are intact during the interictal periods. Another feature commonly reported in affected herds is clinical signs of coccidiosis, i.e., diarrhea (±bloody), tenesmus and coccidia in feces.[11] In fact, the neurological syndrome can occur in up to 30% of the calves affected with the enteric form.[12] For example, in one outbreak of 58 calves affected with clinical coccidiosis, 7 (12.1%) exhibited CNS signs.[7] The diagnosis remains one of exclusion of other possible explanations for the signs accompanied by the evidence of acute enteric coccidiosis. In this study a diagnosis was made by finding histological lesions of colitis with numerous developmental stages of coccidian within the epithelial cells. The ages of calves observed in published reports ranged from 7 months to one year; in our study, the median age was 7 months.[11,12] Gross, and histopathological examination of the brains did not identify any lesions, which is similar to what the other studies reported. [11,12]

Fifty-one cases requested specific toxicological tests, of which 20 originated in Iowa. Typically, most referring veterinarians called the ISU-VDL to request assistance on the type of samples to submit for ruling out toxicosis. Lead was the most common toxicosis (21 cases), followed by nitrate toxicosis (18 cases), urea toxicosis (9 cases), and sodium toxicosis (3 cases). A diagnosis of lead toxicosis was reached by analyzing whole blood or liver and/or kidney. The median age of animals affected was 2.5 months, partly attributed to a greater ability to absorb up to 50% of the lead ingested, whereas older animals only absorb 1-3% of ingested lead.[27] Clinical signs were infrequently reported, although those that described them were similar to experimental observations. In a study that evaluated edetate (ethylene-diamine-tetraacetic acid) and thiamine for the treatment of experimentally induced lead poisoning, clinical signs were scored to test the efficacy of the treatments.[28] The criteria used was based on scores from 0 to 3 for the following 5 signs: ptyalism (hypersalivation), bruxism (grinding of teeth), muscle tremors and twitching, tongue wallowing, and hyperesthesia (high sensitivity of the skin). In the present study, the most commonly reported ones were blindness (5), circling (3), grinding of teeth (3), salivating (3), tremors (2), seizures (2), ear twitching (1), and head tilt (1). Comparing the signs from the above experimental study and field case observations, it appeared that severity is worse in field reports.

Nitrate toxicosis was confirmed by measuring nitrate concentrations in the ocular fluid or incriminating feed/water. Interestingly, nitrite concentrations were not correlated with nitrate concentrations in ocular fluid, as there were 9/18 cases where nitrite was > 1 ppm while nitrate was ≥ 20 ppm. Therefore, measurement of only nitrite in the ocular fluid can result in high incidence of false negatives.

Similarly, urea toxicosis resulting from drinking water contaminated with fertilizers was diagnosed by measuring ammonia in ocular fluid or urea in the incriminating water source. Three cases of accidental acute fertilizer intoxications were previously reported in Iowa.[30] Water transported in tanks previously contaminated with a nitrogen-based fertilizer was the source in all cases. In 2/3 cases, urea was 1,640 and 2,300 ppm, and ammonia-nitrogen was 640 and 750 ppm. In the present study, urea was determined on 2 occasions in the alleged water sources and measured 3,000 and 7,000 ppm.

Sodium toxicosis was diagnosed in newborn calves fed milk replacers. Unfortunately, the incriminating source was not submitted to test for sodium or total salt content. Formulations of milk replacers containing excess salt or that were incorrectly mixed have been associated with death in veal calves housed without access to water.[29] Presenting signs were mostly referrable to as dysfunction of the CNS and included hyperesthesia, opisthotonus, nystagmus, muscle twitching, and intermittent convulsions. The salt content of the milk replacer originated in the whey included in the formula at 2.6%, that is, 10 times the levels recommended by the National Research Council.[26] The case was diagnosed by measuring serum sodium concentrations in some of the calves at levels of ~200 mEq/L (normal range 131-144 mEq/L). A histological examination of the brains of several calves did not reveal any lesions. In our submissions, a diagnosis was reached by measuring total brain sodium at concentrations > 1,800 ppm (normal 1,600 – 1,800 ppm).[13,30] It is likely that sodium toxicity in those cases occurred by free choice salt offered to cattle that were not in immediate proximity to water. Sodium passively enters the CNS but requires energy to be transported out of the CNS.[30] This mechanism explains the elevation of Na within the brain.

Conclusions

Given the diversity of neurological problems in cattle, a comprehensive approach is necessary to reach a definitive diagnosis, including the submission of appropriate samples, reporting of pertinent clinical sings, and the application of relevant laboratory tests, especially, histopathology. About half of the submissions had bacterial origin (listeriosis, thromboembolic meningoencephalitis, miscellaneous bacteria) for which the diagnosis was based on observation of typical microscopic lesions, regardless of the results of ancillary tests such as culture and IHC. A seasonal pattern was observed for listeriosis, but not other bacterial infections, and submission of the entire brain (including brain stem and obex) was necessary as many cases mentioned lack of lesions in the cerebellum and cerebrum. Toxicological causes were diagnosed in 55 cases and required submission of specific biological specimens (described next) to reach a diagnosis as brain lesions are typically absent. Depending on the type of toxicant, the appropriate samples included: 1) liver/kidney or whole blood for lead toxicosis, 2) ocular fluid, feed and water for nitrate and urea toxicosis, and 3) brain for sodium toxicosis. Nutritional causes of neurological disease included hypomagnesemia and polioencephalomalacia. Although few hypomagnesemia cases were identified, diagnosis requires testing of Mg concentrations in ocular, serum, and, ideally, in urine. Polioencephalomalacia was diagnosed based on classical histopathology lesions, but submission of water and feed for sulfur analysis is strongly recommended. If these analyses are performed at a different laboratory, the results should be reported in the submission or as soon as possible to aid the pathologists in the differential diagnosis.

A search of electronic records from the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) on submissions of bovines with clinical presentations of neurologic disease showed that etiologies were preferentially infectious, nutritional, metabolic and toxicological. Many cerebral and hindbrain diseases result in overlapping clinical signs that cannot be easily differentiated. A major limitation for achieving a good diagnosis was the lack of proper reporting of clinical signs in the submission form. Therefore, diagnostic laboratories could provide a form that includes checkboxes for the major neurological signs that can help in making a list of differential diagnoses of neurological disease. Obtaining a final diagnosis is a team effort that requires synergy between the submitting veterinarians and the pathologists.

In conclusion, common causes of neurologic disease in cattle encompass a wide range of etiologies, and the sample types needed to diagnose these causes often extend beyond neurologic tissue. Practitioners are encouraged to consider all differentials that may correlate with the information they have obtained, and to collect and submit samples such as ocular fluid, liver, feed/water and colon in diagnostic investigations of neurologic disease. The correct sample is the key to confirm a diagnosis or to confidently rule out a differential.

Declaration of conflicting interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Middleton JR. Cerebral disorders of the adult ruminant. *Vet Clin North Am Food Anim Pract* 2017; 33:43-57.

2. D´Angelo A, et al. Seizure disorders in 43 cattle. *J Vet Intern Med* 2015; 29:967-971.

3. Clarke LL, et al. Central nervous system diseases of cattle in Georgia, 2001-2017. *J Vet Diag Invest* 2019; 31:588-593.

4. Giles L, et al. Ruminant neurological disease: a retrospective cohort study. *Vet Rec* 2017; 181:372-373.

5. United States Department of Agriculture (USDA) 2019 Census of Agriculture. Summary and State Data. Volume 1. Geographic Area Series – Part 51.

6. Boermans HJ. Diagnosis of nitrate toxicosis in cattle, using biological fluids and a rapid ion chromatographic method. *Am J Vet Res* 1990; 51(3):491-495.

7. Clay BR, et al. Toxic nitrate accumulation in the sorghums. *Bov Pract* 1976; 11:28–32.

8. Villar D, et al. Acute poisoning of cattle by fertilizer-contaminated water. *Vet Hum Toxicol* 2003; 45:88-90.

9. Martens H, Schweigel M. Pathophysiology of grass tetany and other hypomagnesemias. *Vet Clin North Am Food Anim Practice* 2000; 16(2):339-368.

10. Tebbe AW, Weiss WP. Evaluation of creatinine as a urine marker and factors affecting urinary excretion of magnesium by dairy cows. *J Dairy Sci* 2018; 101(6): 5020-5032.

11. Fanelli HH. Observations on "nervous" coccidiosis in calves. *Bov Pract* 1983; 18:50-53.

12. Jubb TF. Nervous disease associated with coccidiosis in young cattle. *Aust Vet J* 1988; 65:353-354.

13. Martin T, et al. Evaluation of normal brain sodium levels in cattle. *Vet hum Toxicol* 1986; 28(4):308-309.

14. Schweizer G, et al. Clinical findings and treatment of 94 cattle presumptively diagnosed with listeriosis. *Vet Rec* 2006; 158:588-592.

15. Loeb E. Encephalitic listeriosis in ruminants: immunohistochemistry as a diagnostic tool. *J Vet Med A Physiol Pathol Clin Med* 2004; 51:453-455.

16. Johnson GC, et al. Evaluation of laboratory tests from confirming the diagnosis of encephalitic listeriosis in ruminants. *J Vet Diag Invest* 1995; 7:223-228.

17. Gagea MI, et al. Diseases and pathogens associated with mortality in Ontario beef feedlots. *J Vet Diag Invest* 2006; 18:18-28.

18. Dore V, et al. Cerebral disorders of calves. *Vet Clin Food Anim* 2017; 33:27-41

19. McGuirk SM. Polioencephalomalacia. *Vet Clin Noth Am Food Anim* 1987; 3:107-117.

20. Vandevelde M, Higgins R, Oevermann A. editors. *Veterinary Neuropathology: Essentials of Theory and Practice*. Ames (IA): Wiley Blackwell; 2012 pp. 108-111.

21. Nietfeld JC. Neuropathology and diagnostics in food animals. *Vet Clin North Am Food Anim Pract* 2012; 28:515-534.

22. McAllister MM, et al. Evaluation of ruminal sulfide concentrations and seasonal outbreaks of polioencephalomalacia in beef cattle in a feedlot. *J Am Vet Med Assoc* 1997; 211:1275-1279

23. Loneragan G, et al. The magnitude and patterns of ruminal hydrogen sulfide production, blood thiamine concentration, and mean pulmonary arterial pressure in feedlot steers consuming water of different sulfate concentrations. *Bov Pract* 2005; 39:16-22.

24. Nichols CA, et al. The effect of sulfur and use of ruminal available sulfur as a model to predict incidence of polioencephalomalacia in feedlot cattle. *Bov Pract* 2013; 47:47-53.

25. *National Academy Press. Nutrient requirement of beef cattle*, update of 7th ed. Washington DC. 2000

26. Saif LJ, Smith KL. Enteric viral infections of calves and passive immunity. *J Dairy Sci* 1985; 68(1):206-228.

27. Niles GA. Toxicosis of the ruminant nervous system. *Vet Clin North Am Food Anim Pract* 2017; 33:111-138.

28. Coppock RW, et al. Evaluation of edetate and thiamine for treatment of experimentally induced environmental lead poisoning in cattle. *Am J Vet Res* 1991; 52:1860-1865.

29. Pearson EG, et al. A case of presumptive salt poisoning (water deprivation) in veal calves. *Cornell Vet* 1982; 72:142-149.

30. Romano et al. Sodium distribution in the bovine brain. *J Vet Diagn Invest* 2021; 33(2):384-387.

