Infectious diseases of the central nervous system (CNS) in cattle can have important economic and public health implications. Some of them, such as rabies, listeriosis, or bovine spongiform encephalopathy (BSE), are zoonoses and thus can threaten human health.1

Examination of brain tissue collected in Switzerland in the time frame of BSE and rabies surveillance indicated that a causative diagnosis could only be made for some, but not all cattle presented with neurologic disease. Several retrospective studies in different countries have aimed at the detection of bacteria, viruses, or parasites as causative agents of neurologic disease, often without success, especially in cases of nonsuppurative encephalitis.2–7 This type of CNS lesion also has been referred to as European sporadic bovine meningoencephalitis (SBE)8 and has been diagnosed in 9.8% of cattle subjected to histological brain examination as part of the Swiss statutory reporting of rabies and BSE suspects between 1985 and 1994. No etiologic agent could be found in these cases at that time, but brain tissue showed a histological pattern characteristic of SBE. Another 16.9% of the 532 cases of cattle displaying neurologic clinical signs included in that study remained without diagnosis, and histological examination of the brain of those animals showed no common pattern of lesions.5

Bovine astrovirus is a member of the genus Mamastrovirus within the family Astroviridae. Astroviruses are small nonenveloped RNA viruses.9 They are recognized as an important cause of diarrhea in humans, have been found in diarrheic fecal samples of mammals, and also can cause disease in birds.9,10 In addition to their known gastrointestinal pathogenicity, astroviruses also have been detected in brain tissue of immunocompromised human patients with encephalitis,11,12 and in an outbreak of shaking mink syndrome.13 By means of unbiased next-generation sequencing, a neurotropic bovine astrovirus (BoAstV CH13/NeuroS1) has been found in brain tissue of cattle suffering from encephalitis of undetermined origin nearly simultaneously in Switzerland and the United States.14,15 In the meantime, these viruses have been found in additional bovine cases,16–18 and diagnostic tools such as in situ hybridization (ISH) or reverse transcriptase polymerase chain reaction (RT-PCR) have been developed.19,20

Neurologic Clinical Signs in Cattle With Astrovirus-Associated Encephalitis

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Background: Evidence of neurotropic astroviruses has been established using novel genetic methods in cattle suffering from viral encephalitis of previously unknown origin.

Objective: To describe the clinical signs observed in cattle with astrovirus-associated encephalitis.

Animals: Eight cattle (4 cows, 3 heifers, and 1 bull of 4 different breeds) admitted to the Clinic for Ruminants for neurologic disease and 1 cow investigated in the field.

Methods: Cases were selected based on neuropathologic diagnosis of nonsuppurative encephalitis, positive in situ hybridization result for astrovirus, and availability of the results of physical and neurologic evaluations. Laboratory results were evaluated if available.

Results: The most frequently observed clinical signs were decreased awareness of surroundings (7), cranial nerve dysfunction (5), and recumbency (5). The cow seen in the field was the only animal that had severe behavioral changes. Cell counts in cerebrospinal fluid (CSF) were increased in 4 animals, and protein concentration was increased in 3 of 5 specimens. In 1 case, the presence of astrovirus could be identified in a CSF sample by reverse transcriptase polymerase chain reaction. Other laboratory abnormalities were nonspecific.

Conclusions and Clinical Importance: Astrovirus infection may be an important differential diagnosis in cattle with clinical signs of brain disease and should be considered after exclusion of other causes. The clinical and epidemiological relevance of encephalitis associated with astrovirus infection should be further investigated.

Key words: Atypical; Bovine; Central nervous system; Viral.

Abbreviations:

BSE bovine spongiform encephalopathy
CNS central nervous system
CSF cerebrospinal fluid
FFPE formalin-fixed paraffin-embedded tissue
ISH in situ hybridization
RT-PCR reverse transcriptase polymerase chain reaction
SBE sporadic bovine meningoencephalitis
Approximately 25% of the tested brain samples from cattle with nonsuppurative encephalitis of undetermined etiology were positive for BoAstV CH13/NeuroS1, and none of the control animals was positive for this virus in case-control studies, which supports the conclusion that the infection is likely associated with the disease.

Our aim was to describe the clinical presentation of cattle with neurologic disease associated with the presence of astrovirus in brain samples, so as to allow clinicians to recognize this new entity as a possible diagnosis in cattle with corresponding signs of neurologic disease.

Materials and Methods

Formalin-fixed paraffin-embedded (FFPE) and frozen tissue from cattle with histologically confirmed nonsuppurative encephalitis was collected from the archives of the Vetsuisse Faculty Bern, Switzerland. Only those cases for which neither histological examination of brain tissue by an expert neuropathologist nor follow-up diagnostic tests had allowed for an etiological diagnosis were selected. All available CNS regions of brain tissue of affected animals were tested for the presence of BoAstV-CH13/NeuroS1 by ISH, RT-PCR, or both. An example of a positive ISH result is shown in Figure 1.

Cattle that had been referred to the Clinic for Ruminants were selected from the ISH-positive cases. These animals had received a standardized clinical examination including complete neurologic examination. Further laboratory diagnostic investigations had been performed depending on the individual case.

Results

Study Group and Available Data

Including the last cow seen in the field, the study involved 9 cattle, 8 females, and 1 male. Ages ranged from 1.5 to 8 years (mean, 3.14 years; median, 2.5 years). The study group included 4 Simmental, 2 Simmental × Red Holstein crosses, and 2 Brown Swiss female cattle, as well as 1 young Eringer bull. Three animals were heifers, and the cows were 2–3 months (3 animals), 8 months, or >12 months (1 each, of which the first was 2 months and the second 7 months pregnant) after parturition, and in their first (3 animals), second, or fourth (1 each) lactation. The first case was seen in 1989 and the last in 2015. No seasonal pattern was evident, with 2 animals presented in the first trimester of the year, 3 in the second, 3 in the third, and 1 in the last trimester (Table S1).

Animals were humanely euthanized and the brain submitted for neuropathologic examination. In 2 animals (#s 4 and 6), the spinal cord also was evaluated.

Fig 1. Detection of the BoAstV-CH13/Neuro-S1 on RNA level by in situ hybridization (ISH). Arrows indicate positive neurons. Like most BoAstV-CH13/Neuro-S1-positive cattle, case # 4 shows a diffuse positive ISH signal in different regions of the central nervous system, for example, cerebellum (A) and the cerebral cortex (B). Animals # 2 (C) and # 8 (D) are BoAstV-CH13/Neuro-S1 positive mainly in the brainstem. The hematoxylin and eosin staining shows histopathologic lesions typical for nonsuppurative encephalitis including perivascular cuffs (asterisk), neuronal degeneration (black arrow), and gliosis (white arrow) in the brainstem of case # 8 (E). The staining of the cases tested for this study is comparable to what we observed in positive controls that were previously tested positively for the presence of the BoAstV-CH13/Neuro-S1 (F), while negative controls from healthy animals clearly remain unstained (F, inset).
Six animals (หมายเลข 1 และ 4-8) ผ่านการผ่าตัดคนเรียกเก็บ。

**History**

The onset of clinical signs as observed by the owners ranged from the previous day to 3 weeks (mean, 1 week; median, 2.5 days). Some reported problems were nonspecific (e.g., anorexia, apathy, decreased general condition) and were first attributed to other conditions such as traumatic reticuloperitonitis or enzootic pneumonia (4 cases,หมายเลข 2, และ 5-7). The clinical signs observed in the remaining 5 animals, such as tremor, coordination deficits, dysphagia, compulsive walking, or aggressive behavior, were suggestive of neurologic disorders (ตาราง S1).

**Results of Clinical Examination**

All animals were presented in poor general condition. Specific signs of disease reported included apathy (หมายเลข 4 และ 7) or decreased peripheral temperature (หมายเลข 3), but in most cases, only an overall statement of poor general condition was noted. Decreased digestive activity also was noted all animals; feces were more liquid than normal in cows (หมายเลข 2 และหมายเลข 9 และ 1 และหมายเลข 6) and appeared well digested and of sticky consistency in cow (หมายเลข 1). Respiratory signs were noted in 2 animals: Cow (หมายเลข 1) had inspiratory sounds on lung auscultation (a history of enzootic pneumonia had been recorded on this animal’s farm before referral), and cow (หมายเลข 3) had a productive cough.

The most commonly observed neurologic signs, in decreasing order of frequency, were decreased awareness of the surroundings (7), signs indicative of cranial nerve dysfunction (5), and recumbency (5) (ตาราง S1).

All but 1 animal (หมายเลข 4) exhibited decreased awareness of their surroundings (ie, obtundation).

Four animals were recumbent upon arrival to the clinic, and 1 (หมายเลข 8) became recumbent shortly thereafter. All 3 nonrecumbent animals examined at the Clinic for Ruminants showed gait abnormalities (ie, short steps especially in the pelvic limbs [หมายเลข 3], ataxia and falling to 1 side [หมายเลข 6], and unsteady gait [หมายเลข 7]). Proprioception could not be used to assess clinical signs associated with astrovirus infection in our study, because 5 of 8 affected animals were recumbent and 2 showed loss of balance.

Five of the 8 animals (62.5%) showed at least 1 sign indicative of cranial nerve dysfunction, including dysphagia in 1 animal (หมายเลข 5), decreased lingual tone in 2 animals (หมายเลข 1 และ 5), and jaw tone also was decreased in cow (หมายเลข 5). The menace response was decreased in 3 animals (หมายเลข 1, และ 5, และ 8), the palpebral reflex in 2 (หมายเลข 1 และ 5), and ear sensitivity in animals (หมายเลข 5, และ 6, และ 8). The corneal reflex was absent in cow (หมายเลข 1, และ a ventral rotation of the eye also was noted in cow (หมายเลข 6). Facial sensitivity was completely absent in cow (หมายเลข 8). Cranial nerve deficits without further specification were noted in cow (หมายเลข 7).

Lateralization of signs was observed in 50% of the animals, meaning that they either were present on 1 side only or more severe on 1 side, or that the animal leaned to 1 side in its gait or posture. Heifer (หมายเลข 1) always held its head to the left; in cow (หมายเลข 5), the menace and palpebral reflexes as well as ear sensitivity were only decreased on the left side; cow (หมายเลข 6) had a head tilt to the right and fell on its left side after manipulation of the neck; and the unspecified cranial nerve deficits in cow (หมายเลข 7) were observed to be more severe on the right side.

Seizure Activity was Observed in 2 Animals (หมายเลข 2 และ 8).

Finally, the last animal observed in the field (หมายเลข 9) is reported separately because complete clinical examination was not possible because of its aggressive behavior. It was the only animal with behavioral changes as the main clinical sign. The cow showed mainly hyperexcitability and aggressive behavior. A neurologic examination could not be performed.

**Laboratory Findings**

A CBC with differential cell count was available for 6 animals (หมายเลข 2 และ 8), whereas only the total leukocyte count was available for cow (หมายเลข 3). The total leukocyte count was markedly increased at 23.54 x 10^3/uL, due to neutrophilia with a regenerative left shift in 1 animal (หมายเลข 8). No or slight and nonspecific changes were present in the other available CBCs (ตาราง S2).

A serum biochemistry panel was available for 7 animals (หมายเลข 2-8), whereas only the total leukocyte count was available for cow (หมายเลข 3). The total leukocyte count was markedly increased at 23.54 x 10^3/uL, due to neutrophilia with a regenerative left shift in 1 animal (หมายเลข 8). No or slight and nonspecific changes were present in the other available CBCs (ตาราง S2).

Samples of cerebrospinal fluid (CSF) were collected in 5 animals antemortem (หมายเลข 1, 3, และ 6-8). An increased CSF protein concentration was observed in 2 of 5 samples (หมายเลข 1, และ 6), and nonsuppurative pleocytosis was observed in 4 of 5 (หมายเลข 1, และ 6-8; range, 26-141 cells/μL; normal, <3 cells/μL). Another set of 4 CSF samples (from animalหมายเลข 5-8) was tested for astroviruses by RT-PCR. The CSF sample of cow (หมายเลข 5) was collected postmortem and submitted for RT-PCR only. One of these 4 samples (หมายเลข 7) gave a positive result.
Necropsy and Neuropathologic Examination

Complete necropsy was performed in 6 animals (#s 1 and 4–8). Additional lesions in the form of abscesses were found in cow #s 3 and 4 (the latter also had myocarditis with Sarcocystis). Animal #s 6 and 7 had ulcerative abomasal inflammation (accompanied by cholangitis due to Fasciola hepatica in cow # 7) (Table S2).

Features of nonsuppurative encephalitis and meningitis were observed to a variable extent in all animals. The brainstem was moderately to severely affected in all animals. Nuclei of the cranial nerves were in particular affected in animal #s 2, 5, 7, and 8, for all of which, except cow # 2, cranial nerve deficits had been recorded. Cranial nerve deficits had been recorded for another animal (# 1), but no histopathologic lesions were identified in the brainstem.

The cerebellum generally was less affected than the brainstem, mainly by gliosis and meningitis. In animal #s 3, 7, and 8, the hippocampus had especially severe lesions. Nonsuppurative myelitis was diagnosed in those animals for which spinal cord tissue was available (#s 4 and 6), and ganglioneuritis was evident in 2 animals for which the trigeminal ganglia were available (#s 6 and 7). Besides nonsuppurative inflammation, case # 9 showed severe neuronal vacuolation in the red nucleus of the rostral midbrain. All animals had tested negative for BSE.

Discussion

Nine cattle with neurologic disease and positive for BoAstV CH13/NeuroS1 in brain tissue were identified. The duration of disease before presentation was variable, but this information must be interpreted with caution because it depends on the ability of owners to recognize subtle changes (eg, changes in gait and behavior). All animals referred to the Clinic for Ruminants had poor general condition, possibly because they were presented late in the course of the disease.

The brainstem was moderately to severely affected in all animals, which relates to the most frequent neurologic sign of obtundation and also fits with the frequently observed gait abnormalities. Nuclei of the cranial nerves were affected in cow #s 2, 5, 7, and 8, for all of which, except cow # 2, signs indicative of cranial nerve deficits were recorded. However, cow # 2 was presented in a late state of disease, already showing tremor and seizures; thus, it is unclear whether it really was possible to test for cranial nerve deficits in this animal. In cow #s 3, 7, and 8, the hippocampus had particularly severe lesions. Based on its function in the limbic system, pronounced behavioral abnormalities were expected, but were not noted in these cases. Cow # 9 had severe neuronal vacuolation in the red nucleus of the rostral midbrain. Because it is part of the extrapyramidal motor system, marked gait abnormalities would be expected, but hyper excitability primarily was noted in this animal, which was seen by a veterinarian in the field. It was thus reported separately because it had a different clinical presentation with mainly behavioral changes, and a thorough clinical examination was not possible because of hyperexcitability.

Clinical laboratory results were nonspecific, with some animals appearing to have chronic inflammation (eg, increased serum protein concentrations, normal or low serum albumin concentration, shortened glutaraldehyde test), 1 animal an acute inflammatory response (cow # 8 with leukocytosis, neutrophilia, and a regenerative left shift), and others only with dehydration (high total protein and albumin concentration, low PCV, and corresponding clinical signs). The increased activity of creatine kinase and at least 1 other serum enzyme activity were the most consistent finding in the serum biochemistry panel and likely were related to the recumbency observed in all but 1 animal. Cerebrospinal fluid was altered in all cases (high protein concentration in 3 and pleocytosis in 4 of 5 cases), but not all samples had the same changes, and the number of CSF samples was limited. These results therefore should be interpreted with caution. 1 of 4 samples, a positive RT-PCR for BoAstV CH13/NeuroS1 result was obtained.

Two single-case reports of neurotropic astroviruses described a sudden onset of clinical signs. 16,17 One study that evaluated 21 cases of SBE also described animals with a sudden onset and others with a slower progression of disease. 8 So far, no report mentions several deceased animals in 1 herd, and based on available information, such was not the case in the cattle of the present study either.

Clinically, several similarities to SBE could be noted, such as the presence of gait abnormalities, decreased awareness of the surroundings, or signs indicative of cranial nerve dysfunction. 8 Brain tissue samples originating from the SBE study recently have been investigated for the presence of neurotropic astroviruses, and 12 of 14 cases were positive by ISH. 18

To our knowledge, there are 3 other single-case reports of neurotropic bovine astrovirus infection. One animal was found in lateral recumbency with opisthotonus and limbs in extensor rigidity, 15 possibly showing a final stage of the disease, as with the 2 case reports with tonic-clonic convulsions seen at our clinic. Another animal showed marked behavioral abnormalities (“suddenly running off in the field”) similar to our field case (# 9), but also presented with “oblique bearing of the head, circling movements, inappetence, and somnolence,” which were not reported in our case. In 1 of 3 of our cases (#s 3, 7, and 8), lesions were especially severe in the hippocampus, as in the recent study about astroviruses in SBE cases, 16 infection tended to be most severe in this area, which is related to behavior. However, altered behavior was not reported in these animals, and subtle changes might have been missed because all 3 animals were presented in a poor general condition.

Astroviruses are an important cause of diarrhea in humans 9,10 and also have been isolated from fecal samples of cattle in Europe and Asia. 22,23 A link between
diarrhea and the presence of astroviruses was not established in these studies. One study noted that the presence of astroviruses was strongly associated with the presence of group A rotaviruses and postulated that age may protect against clinical disease after infection with astroviruses as is known for rotaviruses. The genetic sequences identified in astroviruses from fecal samples did not correspond to those of neurotropic genetic sequences identified in astroviruses from fecal case reports of astrovirus-positive animals. Neither blood nor feces of herd euthanasia of the diseased animal, and blood and fecal samples were collected. Neither blood nor feces of herd mates were positive for neurotropic astrovirus by RT-PCR. Over a prolonged period of time allowed for the report of the largest case series of bovine nonsuppurative encephalitis associated with astrovirus infection to date.

The fact that the presence of neurotropic astroviruses has been shown independently by 2 groups on 2 continents and later found in more animals strongly supports the hypothesis of the clinical relevance of CNS infections with astroviruses. In addition, none of the control animals was positive for neurotropic candidate astroviruses in a recent case-control study. However, astrovirus infection may only explain some but not all nonsuppurative encephalitis cases. Because the clinical signs of astrovirus-associated encephalitis can be similar to those of neurologic diseases of high public health relevance such as rabies or BSE, it is important that additional investigations be conducted in the future to better define the clinical presentation and diagnosis of this disease. Veterinary clinicians should be aware of astrovirus infection as a potential differential diagnosis in cattle with corresponding signs of neurologic disease.

Footnote

* Graeub AG, Bern, Switzerland

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References


Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Summary of study group, history and neuroclinical signs.
Table S2. Laboratory findings in astrovirus positive animals.