Case report: cerebellar hypoplasia associated with bovine viral diarrhoea (BVD) virus infection in a calf, in Turkey

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SUMMARY

This is the first published report of cerebellar hypoplasia associated with bovine viral diarrhoea (BVD) virus infection in a calf from the Elazig province, Turkey. A 3-weeks old female Holstein calf was unable to stand with its head pulled backwards and opisthotonos. As cerebellar hypoplasia was evoked, euthanasia and necropsy were performed. Numerous severe cavities were found in cerebellum; folial structures were hypoplastic (cell depletion in the external granular layer, disappearance of white matter in some foliae, and loss of Purkinje cells) and the diagnosis of cerebellar hypoplasia was confirmed. Additionally, nodular costochondral lesions were observed and tissue homogenates gave positive results with specific ELISA test and RT-PCR for the BVD virus. These results show that the BVD virus infection can cause cerebellar hypoplasia and this association may be included in the differential diagnosis with cerebrocortical necrosis, hypovitaminosis A and encephalitic colibacillosis in calves.

Keywords: calf, cerebellar hypoplasia, bovine viral diarrhoea, virus antigen, RT-PCR, Turkey

RESUME

Cas Clinique: hypoplasie cérébelleuse associée à une infection par le virus de la diarrhée virale bovine (BVD) chez un veau, en Turquie

Un veau femelle Holstein de 3 semaines était incapable de se tenir debout, présentait sa tête rejetée en arrière et un opisthotonos. Comme la possibilité d’hypoplasie cérébelleuse a été évoquée, l’animal a été euthanasié et autopsié. De nombreuses et parfois importantes cavités ont été observées dans le cervelet ; les structures foliaires étaient hypoplasiques (déléition cellulaire dans la couche granuleuse externe, disparition de la substance blanche et perte importante des cellules de Purkinje) et le diagnostic d’hypoplasie cérébelleuse a donc été confirmé. De surcroît, des lésions nodulaires costochondrales ont été notées et le virus de la BVD a été mis en évidence par un test ELISA spécifique et par RT-PCR dans les homogénats tissulaires. Il s’agit du premier cas décrit dans la province d’Elazig en Turquie de l’association entre le virus de la BVD et une hypoplasie cérébelleuse. Ces résultats montrent qu’une infection par le virus de la BVD peut conduire à une hypoplasie cérébelleuse et qu’une telle association doit être incluse dans le diagnostic différentiel avec la nécrose du cortex cérébral, l’hypovitaminose A et les formes encéphalitiques de la colibacillosis chez les veaux.

Mots-clés : veau, hypoplasie cérébelleuse, diarrhée virale bovine, antigène viral, RT-PCR, Turquie

Introduction

Cerebellar hypoplasia is abnormal development of the cerebellum during the foetal period. Even though observed in many animal species (calf, lamb and foal), it is mostly reported in the Hereford, Holstein-Friesian, Guernsey, Shorthorn, Ayrshire and Angus breeds of cattle [8, 11]. The congenital form of cerebellar hypoplasia is reported to be non-progressive [1] and sporadic [8].

The emergence of the malformation depends on not only genetic factors, but also some intra-uterine viral infections including bovine viral diarrhoea (BVD), blue tongue and border disease, feline panleukopenia, hog cholera [1, 6, 8, 9]. The BVD virus infection of sensitive cattle during their 90-170th day of gestation terminates with abortion, stillbirth, hydranencephaly or cerebellar hypoplasia in foetuses [6, 8]. RADOSTITIS et al. [8] report that BVD virus is the first viral agent determined to have teratogenic effect on cerebellum leading to cerebellar hypoplasia but in Turkey, the influence of this virus on cerebellum malformation is still unknown.

However, in the present clinical case, it is demonstrated association between cerebellar hypoplasia and BVD virus infection in a calf from the Elazig province, in Turkey.

Case report

The material of the study consisted of a female Holstein calf, 3-weeks old, brought to the Internal Diseases Clinic of the Veterinary Faculty, Firat University. The animal was unable to stand and its head was pulled backwards, despite having sucking reflex.

The animal was treated with Baytril 10% injection (involving 100 mg enrofloxacin in 1 mL, Bayer, Turkey), Nervit injection (involving 100 mg vitamin B12, 10 mg vitamin B1 in 1 mg., Vetaş, Türkiye) and Ademin injection (involving 500.000 IU vitamin A, 75.000 IU vitamin D3, 50 mg vitamin E in 1 mL., Ceva-DİF, Türkiye) because cerebrocortical necrosis (CCN), pneumonia and omphalitis were diagnosed one week before; however, the animal showed no recovery.
Further physical examination showed that the animal was unable to stand, to lay in a lateral recumbency, was weak despite having sucking reflex, it has soft hoofs, un-erupted teeth, umbilical cord inflammation, and exhibited hyperaemic mucosa and harsh vesicular sound in lungs in auscultation, and it was in opisthotonos (figure 1), but it lacked papilla and palpebral reflexes. Body temperature, heart and respiration frequencies were 39.1°C, 128 beats / min and 88 beats / min, respectively. A possible diagnosis was cerebellar hypoplasia, and the euthanasia and necropsy were recommended.

On post-mortem examination, after the removal of calvarium, there was an apparent reduction in the size of cerebellum measuring 1.40 x 2.90 x 1.00 cm$^3$ and had shrunken or foamy external surface (figure 2). The lateral lobes and lateral vermis were much severely affected than the vermis. The cut surfaces of cerebellum showed focal irregular tiny cavities measured from 1 to 3 mm. There was 1.5-2.7 cm diameter, round, nodular lesions at the costochondral junctions (figure 3). No other gross lesions were noted in teeth, skeletal bones and visceral organs.

During necropsy, tissue samples from cerebellum, cerebrum, eyes, optic nerves, liver, lungs, kidneys and thymus were fixed in 10% formalin. After embedding in paraffin, 5 µm thickness tissue sections were stained with haematoxylin and eosin. Microscopically, most of the folial structures in cerebellum were apparently hypoplastic. The external granular layer was thinner or irregular due to focal or diffuse cell depletion. The white matter of some foliae was decreased or even totally absent causing cavitations (figure 4) which was covered by neutrophil infiltration and meninges. A considerable loss in the Purkinje cells as well as focally malpositioned or degenerated Purkinje cells were observed in the foliar architecture. The leptomeninges were fibrotic in appearance and contained focal mild lymphohistiocytic cell infiltrations (figure 5). The costochondral lesions were characterized by aggregation of connective tissue cells surrounded by the normal osseous matrix (figure 6). In the eyes, retinal dysplasia was seen characterized by retinal folding in fibrosed retina. Optic nerves were atrophic and showed gliosis. No cataract was seen. Additionally, no remarkable changes were found in brain, spinal cord or in other visceral organs.

Figure 1: Opisthotonos in a female Holstein calf, 3-weeks old, from the Elazig Province (Turkey) suspected for cerebellar hypoplasia.

Figure 2: Gross aspect after formalin fixation of the brain of the presented female Holstein calf, 3-weeks old, from the Elazig Province (Turkey), showing moderate to severe hypoplastic cerebellar lateral lobes (arrows) and partly spared vermis (arrow head). Bar: 1 cm.

Figure 3: Nodular swellings (arrows) in costochondral junctions in the presented female Holstein calf, 3-weeks old, from the Elazig Province (Turkey).

Figure 4: Totally cavitary appearance (arrow) of white matter of the folia in cerebellum of the presented female Holstein calf, 3-weeks old, from the Elazig Province (Turkey). Haematoxylin-eosin, Bar: 500 mm.
For BVDV antigen survey, brain, lymph nodes, lungs and liver were sampled, and tissues were pooled and mixed with 2 mL PBS diluted to 1:10. The samples were homogenized in beaded tubes at 3000 g for 3 minutes using a tissue homogenizer (MagNa Lyser, Roche). Homogenate was centrifuged in microtubes at 12000 g for 3 minutes. Solid materials and beads at the bottom were removed. The supernatant was kept at –80°C until analysis. In one hand, a commercial BVD virus Antigen ELISA (IDEXX, USA) kit was used to determine the presence of viral antigen in tissue homogenate [4] according to the manufacturer’s instructions. The results were read using a 450-nm absorbance filter. The calf was considered as positive for BVD viral antigens in nervous and visceral tissues.

On the other hand, resin-based extraction kit (RNAeasy Mini Kit Qiagen) was used for viral RNA extraction from tissue homogenate according to the manufacturer’s instructions. The obtained RNA was analyzed using one-step RT-PCR [5]. Since cDNA synthesis was placed in one-step RT-PCR kit, before adding RNA extraction into kit, 2 μL extracted RNA was mixed with 1 μL deionized formamide, and was denatured by heating at 100°C for 40 seconds. The mixture, consisting in 9.0 μL water, 12.5 μL Qiagen one step RT-PCR buffer, 1.0 μL enzyme mix, 0.25 μL forward primer (18 μM), 0.25 μL of the reverse primer (18 μM) and 0.25 μL 100 nM MGB probe, was added to the one-step PCR master mix (One Step RT-PCR Kit Qiagen) [5]. The mixture was put in LC capillaries. Prepared capillaries were centrifuged in an LC Carousel (Roche) centrifuge at 3000 g for 15 seconds. Tubes containing reaction mixture were put in a Real-time PCR device, which includes softwares about primarily cDNA synthesis and then multiplication of the relative areas. Samples were kept in the real-time PCR device at 50°C for 15 minutes, which achieved cDNA synthesis. Primary denaturation was realized at 95°C in 5 minutes, and for the following 50 cycle PCR, denaturation was realized at 95°C for 15 minutes and bonding and stretching application at 60°C for 30 seconds. The results were evaluated using the Qualitative Detection program Channel 530 of the real-time PCR device. Since the cycle threshold (CT) was 28, the result was evaluated as positive. The primaries and probes used in the real-time RT-PCR [12] are indicated in the Table I. PCR amplified DNA fragments corresponding to the BVD virus were obtained from tissue homogenates from the present female calf.

Discussion

Clinical findings including inability to stand, the laying in lateral position, weakness despite having sucking reflex, opisthotonos, the lack of papilla and palpebral reflexes are consistent with cerebellar hypoplasia as described in earlier reports in the literature [8]. Additional findings of soft hoofs and un-erupted teeth support the claims [8, 11] that preterm delivery can be observed in BVD virus cases. Cerebellar hypoplasia was diagnosed via specific clinical and necropsy findings (post-mortem examination) [11] in the present report.

The BVD virus infections, presumed to be among the viral factors causing cerebellar hypoplasia, can be diagnosed directly via virus isolation and viral nucleic acid identification, as well as by virus neutralisation test in order to detect blood serum BVD virus-specific antibodies, and by commercial ELISA kits evidencing specific BVD virus antigens in tissues [6, 8, 11]. In this way, BVD virus antigens can be detected in spleen, kidney and lymph nodes [6, 8, 11]. Rapid detection of BVD virus antigens was performed in brain, lungs, liver and lymph nodes using a commercial specific ELISA kit in the case reported here. In addition, the ELISA test is commonly used as a screening test in the literature [5, 13], while the RT-PCR is usually used for verification because of its specificity. In the present case, the presence of the BVD virus was confirmed using RT-PCR.

It is well known that BVD virus cause cerebellar hypoplasia by infecting and destroying primarily external granular layer.
of cerebellum containing mitotoc cells during gestational and early neonatal periods [2, 3]. Microscopic lesions in the cerebellum characterized by loss of cerebellar cortex, thinner or irregular external granular layer, decreased number of Purkinje cells and cavities in white matter, retinal dysplasia and atrophy in optic nerve are in complete agreement with the earlier reports in the literature [2, 3, 7].

To the authors’ knowledge, macroscopic nodular bone lesions in costochondral junctions have not been reported earlier. These lesions were very similar to rickets in macroscopic examinations. However, microscopically, the lesions had no cartilaginous element, being reminiscent to primary callus, including fibrous tissue elements and secondary spongy elements. Hence, secondary bone fractures due to rickets were finally diagnosed. It would be speculative to associate the present lesions to BVD infection, however osteopetrotic-like bone lesions and osteopetrosis have been previously described from BVD virus-induced abortions in cattle and in 2 month old calves, respectively [7, 10].

As a conclusion, the present findings suggest that care should be taken to avoid confusing neural symptoms observed in cerebellar hypoplasia cases with the cerebrocortical necrosis (CCN), hypovitaminosis A and encephalitis in colibacillosis of bovine fetus during gestational period [2, 3, 7]. Secondary bone fractures caused by rickets have also been associated with cerebellar hypoplasia in calves. Moreover, this is the first published report of cerebellar hypoplasia caused by BVD virus infection determined in a calf in the Elazig region, Turkey.

References
