Cerebellar cortical atrophy in a Belgian Blue cow with lesions described in human Norman-Jaeken disease

Cerebellaire corticale atrofie bij een Belgisch Witblauwe koe met letsels beschreven bij de humane Norman-Jaekenziekte

[1]S. Roels, [2]P. Lausberg, [3]C. Lettelier , [1]E. Vanopdenbosch

[1]Veterinary and Agrochemical Research Centre (CODA/CERVA), Pathology Unit, Department of Biocontrol, Groeselenberg 99, 1180 Brussels (Ukkel), Belgium
[2]Veterinary Inspection of the Belgian Food Agency (FAVV-AFSCA) and practicing veterinarian, Rue Bruyère 19, 4720 Kelmis, Belgium.
[3]Veterinary and Agrochemical Research Centre (CODA/CERVA), General Virology Unit, Department of Virology, Groeselenberg 99, 1180 Brussels (Ukkel), Belgium

stroe@var.fgov.be

ABSTRACT

Cerebellar cortical atrophy has been described both in animals and in humans. In cattle, it has already been identified in several breeds, with the main histopathological lesions being located in the cerebellar Purkinje layer and ranging from chromatolysis to loss of neurons. This case, however, is the first described in a Belgian Blue cow. Additionally, the histopathological lesions were different from those described in other cattle breeds, with prominent focal granular layer loss and abnormal Purkinje cells comparable to those described in Norman-Jaeken disease or primary degeneration of the granular layer of the cerebellum in humans.

SAMENVATTING

Cerebellaire corticale atrofie is beschreven bij dier en mens. Er zijn gevallen bekend bij verschillende runderrassen. De voornaamste histopathologische letsels situeren zich ter hoogte van de cerebellaire Purkinjelaag. Deze letsels kunnen variëren van chromatolyse tot een totaal verlies van de neuronen. In het voorliggend artikel wordt deze aandoening voor het eerst gerapporteerd bij een Belgisch Witblauwe koe. Het histopathologisch beeld is anders dan van de tot nu bekende gevallen bij andere runderrassen. Bij de Belgisch Witblauwe koe werden een uitgesproken haardvormig verlies van de korrellaag en abnormale Purkinjecellen ter hoogte van het cerebellum vastgesteld. Analoge histopathologische bevindingen zijn ook beschreven bij de Norman-Jaekenziekte bij de mens, beter bekend als de primaire degeneratie van de korrellaag van het cerebellum.

CASE REPORT

Cerebellar cortical atrophy (CCA) is characterized by selective degeneration of Purkinje cells of the cerebellar cortex (Cho and Leipold, 1978). In humans, CCA is mainly described as primary degeneration of the granular layer, and this form of CCA is also known as the "Norman type" (Norman, 1940). CCA can be divided into two major groups. One group is called hypoplasia, which indicates that the cerebellum has failed to develop to its full potential. This can result from in utero viral infections such as Bovine Viral Diarrhea virus (BVD). The other group is called abiotrophy. This has been described in most domestic animal species and in a few rodents and primates. The abiotrophic process is defined as degeneration due to an intrinsic developmental abnormality of the cell which causes its premature death. Thus, the clinical hallmark of the abiotrophic group of CCAs (with few exceptions) is neurological normality at birth, followed by the development of cerebellar deficits that progressively worsen in the postnatal period. In contrast, with the hypoplasic group of CCAs, the viral agents, which can damage the developing cerebellum at a very precise stage of fetal life, provoke cerebellar ataxia from the time of birth. Because the injury is not ongoing, the clinical deficiency tends to remain static or even very slowly improve as the animal compensates for its deficit (Summers *et al*., 1995).

In April 2007, a twenty-one month old Belgian Blue cow originating from a 105-unit herd (mixed and meat types) was presented for slaughter with neurological symptoms including ataxia, a tendency to fall, myoclonia, hypersalivation and hyperexcitation. The owner declared that the animal was one of a non-identical twin and had presented nervous problems

since birth, more specifically a tendency to fall down. Initially, the animal could be prevented from falling down by attaching it, but later on the clinical signs grew worse. Another interesting feature was that the clinical signs were more pronounced when strangers came into the pen (panic reaction). The sire was of the Belgian blue breed (BBB) and the dam was of the Holstein x BBB mixed type. The twins were both very well muscled, which resulted in a difficult delivery at birth. In fact, the farmer mentioned that the present case was suspected of having lacked oxygen during birth. The other calf of the twin, as well as both parents, had not shown any neurological problems up to the time of the study. Subsequently, the veterinary inspector included the animal in the routine protocol for ruminants that are suspected of transmissible spongiform encephalopathy (TSE) (Vanopdenbosch *et al*., 1998).

According to the protocol, no full necropsy could be performed. On removal of the brain, no prominent hypoplasia could be noticed. After excluding rabies as a routine precaution, using a direct immunofluorescence technique and isolation on cultures of neuroblastoma cells (Vanopdenbosch *et al*., 1998), samples of the brain stem, the cerebrum and the cerebellum were taken for further TSE control and histological examination (Vanopdenbosch *et al*., 1998). For this purpose, large samples of the three major brain parts mentioned were fixed in a 4% phosphate-buffered formaldehyde solution, processed routinely, paraffin-embedded, and sectioned at 5-µm thickness. The sections were primarily stained with hematoxylin-eosin staining. The rest of the brain was stored in the freezer at –20°C. The TSE control (using rapid TeSeE Elisa, Western blotting and immunohistochemistry) was negative. For BVDV analysis, homogenate extracts of the brain were prepared and tested in virus isolation. Briefly, after 2 passages on MDBK cells, the plate was stained by immunoperoxidase assay using a polyclonal bovine serum against BVDV. BVDV-specific real-time RT-PCR was also performed on homogenate extracts, as described previously (Letellier and Kerkhofs, 2003). Both virus isolation and real-time Rt-PCR were negative for BVDV. Furthermore, after viral isolation, no cythopathogenic effect indicative of a herpesvirus infection was recorded.

The histopathological lesions were mainly concentrated in the cerebellum, with focal loss of granular cells in different stages (varying from limited loss over a remnant part of the layer to total absence of the granular cells) and Purkinje cells that were frequently arranged in clumps (Figure 1) or dislocated into the molecular layer (Figure 2). The brainstem and cerebrum appeared normal on multiple sections. These findings are very comparable to those described in several cases of the Norman type of cerebellar atrophy in man.

The cases described in cattle are all of young age, varying from one week to several months of age, and the histopathological lesions described are mainly limited to the Purkinje cells (Cho and Leipold, 1978; Innes *et al.,* 1940; Swan and Taylor, 1982; Whittington *et al.,* 1989). In human cases, the variability in onset of the symptoms is larger than in cattle, with patients affected from a few hours of age till 42 years of age (Lapresle and Annabi, 1980; Pascual-Castroviejo *et al*., 2006).

The clinical signs in affected calves can be progressive, starting with repetitive seizures to gradual development of cerebellar ataxia. This ataxia can be present for more than 2 years (Barlow, 1979). Several clinical signs in the present case also indicate a cerebellar problem, namely the ataxia and the myoclonus. The hypersalivation could be due to the reduced muscular power of the muscles responsible for swallowing (hypotonia, hyporeflexia and asthenia) that has also been described in cases with damage to the lateral cerebellar hemisphere (Dichgans, 1984).

Although the histopathological lesions could be more indicative for a non-progressive disease, the present case seemed to exhibit progressive neuro-

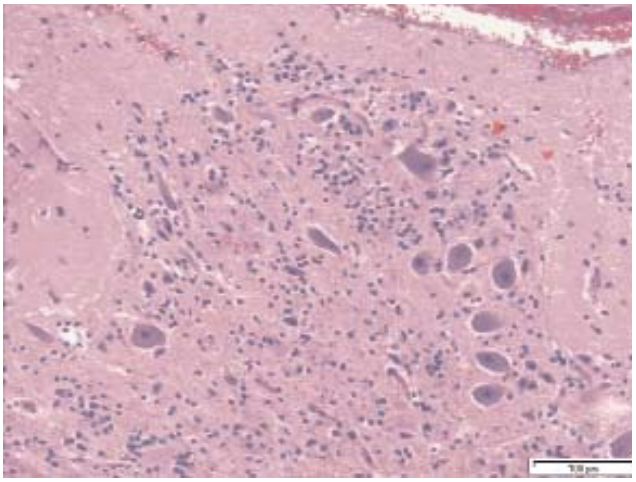

Figure 1. Section of the cerebellar cortex stained with H&E showing remnants of the granular layer and the Purkinje cells, which are not aligned in a single row but are grouped in clumps.

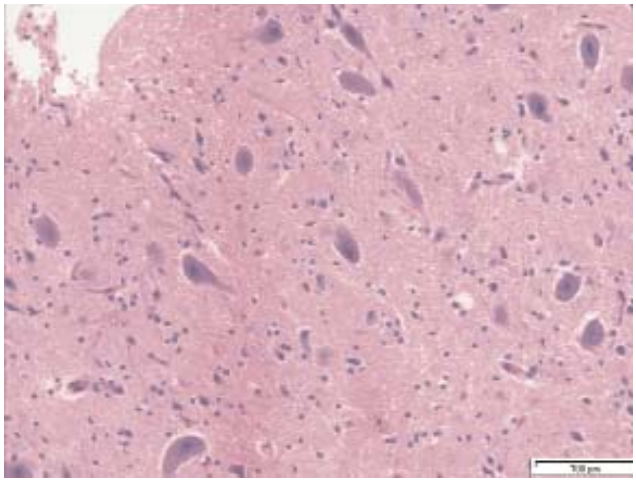

Figure 2. Section of the cerebellar cortex stained with H&E showing an almost complete disappearance of granule cells, and Purkinje cells that are dispersed and dislocated in the molecular layer.

logical signs. Similar ideas have been mentioned in the Norman-Jaeken cases in man, several of which led researchers to believe that this disease could be progressive with variable time of onset and histological evolution in each family (Pascual-Castroviejo *et al*., 1994). Norman-Jaeken's cerebellar atrophy is a congenital, autosomal recessive disorder associated with characteristic clinical features such as hypotonia, strabismus, delayed motor development, nonprogressive ataxia, delayed language development with dysarthria and mental retardation (Lapresle and Annabi, 1980; Pascual-Castroviejo *et al*., 1994; 2006).

The present study reports for the first time on cerebellar cortical atrophy in the Belgian Blue breed. The clinical signs were present at birth, having been described only in pigs (Kidd *et al.,* 1986) and horses (Palmer *et al.,* 1973), but until now it has never been seen in cattle. The histopathological lesions were also different from those currently described in cattle, but are comparable to those seen in pigs (Kidd *et al.,* 1986), horses (Palmer *et al.,* 1973), mice (Summers *et al*., 1995), dogs (Summers *et al*., 1995) and humans (Lapresle and Annabi, 1980) also called the Norman type of cerebellar ataxia (or recently called the Norman-Jaeken type (Pascual-Castroviejo *et al.*, 2006)). Summers and coworkers (1995) suggest that the histopathological lesions found in cases of CCA are usually progressive, with a reduction of granule cell neurons following the Purkinje cell loss.

However, on the basis of the current literature, it is still difficult to make a clear-cut differentiation between cerebellar abiotrophy and hypoplasia. Maxie and Youssef (2007) describe cases of cerebellar hypoplasia in which the cerebellum appears grossly normal and the hypoplastic defects are only detectable on microscopic examination. In such cases, the defects are irregular in distribution, although there is a more or less severe loss of Purkinje cells, and the granular layer is here and there narrowed and deficient in cells. Such cases are very difficult to differentiate from cerebellar abiotrophy, in which the cerebellum is normal in size and gross form and the histopathological changes affect primarily the Purkinje and the Golgi cells, especially in the median lobe, leaving empty baskets and a replacement astrogliosis. Additionally, there is a simultaneous diminution in the population of granule cells. Both pathological entities can be associated with genetic and inherited factors, with BVD being one of the most prevalent causes of cerebellar hypoplasia in cattle (Maxie and Youssef, 2007). The outcome of a BVD infection of the fetal calf is related to gestational age, advancing fetal maturity being associated with increased resistance to the virus. The teratogenic effects (see hypoplasia) are manifest during the 100-170 day gestation period.

The present case exhibits no prominent macroscopical cerebellar hypoplasia associated with multiple sites of severe loss of the granular layer. The Purkinje cells were dislocated, but did not really show degenerative lesions leaving empty baskets and gliosis. On the basis of these findings, the authors could presume that the present case was similar to those of cerebellar hypoplasia without any apparent macroscopical defect. The authors could not really link the lesions to a presence of the BVD virus in the brain, even though histopathological features described in specific cases are comparable to those described in this case (Maxie and Youssef, 2007). However, only an analysis of a precolostral sample on BVD antibodies and antigens could exclude this possibility with 100% certainty (Done *et al*., 1980). The authors could also find no additional familial cases linking the lesions to an inherited or genetic factor.

The lesions noted in the present case were comparable to what was described in cases of Norman-Jaeken ataxia or cerebellar cortical atrophy, indicating that, as mentioned by Lapresle and Annabi (1979), in human patients suffering from this disorder and in whom no real familial link can be found, one should still consider an infectious (viral) cause of this disease.

Finally, due to the older age of the animal and the comparable symptoms, this report also indicates that cerebellar hypoplasia should be added to the list of differential diagnoses of bovine spongiform encephalopathy used by veterinary inspectors in slaughterhouses.

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