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SHORT ORIGINAL COMMUNICATION

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Deposition patterns of disease-associated prion protein in captive mule deer brains with chronic wasting disease

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Abstract Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) in captive and free-ranging cervids in the USA; its origin is obscure. Archival formalin-fixed and paraffin-embedded specimens of 16 captive mule deer brains with CWD were analyzed using immunocytochemistry for the disease-associated prion protein (PrP). The most prominent pattern of PrP deposition were plaque-like structures, a substantial proportion of which were florid plaques surrounded by a rim of spongiform vacuoles. The percentage of florid plaques was highly variable according to region, ranging from 0% to 52.7%. The highest percentage was observed in the medulla and basal ganglia, the lowest in the cerebral cortex. Only three brains contained no florid plaques. There were also punctate synaptic-type and perivascular deposits, particularly in areas of severe spongiform change, and subpial and subependymal plaque-like deposits, whereas cerebellar involvement was mild. Thus, CWD brain pathology prominently features florid PrP plaques, as does variant Creutzfeldt-Jakob disease (vCJD), but differs in other characteristics from vCJD.

Keywords Chronic wasting disease · Immunocytochemistry · Mule deer · Prion diseases · Transmissible spongiform encephalopathies

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Introduction

In March 1996, a variant (v) of Creutzfeldt-Jakob disease (CJD) was reported as a possible consequence of bovine spongiform encephalopathy (BSE) transmission from cattle to humans [32]. The latter hypothesis was strengthened by the same lesion profile of mice infected with either BSE or vCJD [4] and by the same glycosylation pattern of the disease-associated prion protein (PrP) in both BSE and vCJD [18]. vCJD is characterized by a peculiar neuropathology, which includes the presence of so-called "florid plaques": an amyloid core surrounded by a corona of spongiform vacuoles [19, 32]. While florid plaques were previously observed in mice infected with Icelandic scrapie (rida) [7], they were subsequently found in macaques infected with BSE [22]. They are regarded as the neuropathological hallmark of vCJD.

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) in free-ranging mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*) in Colorado and Wyoming, USA [30], that was originally described in captive cervids [33, 34]. Its origin is obscure. Neuropathologically, CWD is characterized by the presence of vacuolated neurons, spongiform change in the neuropil and, in mule deer, by numerous amyloid plaques [12, 13, 14, 33, 34, 35]. The spectrum of PrP deposition has been incompletely described in CWD. For the series of brains of captive mule deers with CWD that we re-examined here, some immunohistochemical results for PrP were previously published [13]. However, at that time immunohistochemistry for PrP did not include specific pretreatment of sections which has been shown to greatly enhance sensitivity [17]. We report here that CWD brain pathology prominently features florid PrP plaques, as does vCJD, but differs in other characteristics from vCJD.

Material and methods

Archival formalin-fixed and paraffin-embedded specimens of 16 captive mule deer brains with CWD were studied. From each animal, between 1 and 21 blocks were available; sampling was not uniform between cases (Table 1). In addition, 10 normal mule deer brains were studied as control.

For anti-PrP immunocytochemistry, sections were pretreated with a three-tiered protocol of hydrated autoclaving, concentrated formic acid, and guanidine isothiocyanate [15, 17]. We used the polyclonal rabbit anti-PrP antiserum 6800 III (kindly provided by Dr. Heino Diringer, Berlin, Germany) at a dilution of 1:1,000. This antiserum was prepared against the human PrP sequence WGQGGGTHSQWNKPSK and was found to stain hamster and human material. In addition, we used the well-characterized 3F4 monoclonal antibody, which is known to strongly decorate human and hamster PrP [15, 16, 25]. To calculate the proportion of florid plaques against the number of all plaques, we counted plaques using a graticule under an objective magnification ×20. Only plaque-like structures larger than an oligodendroglial nucleus within the graticule or touching the upper and right lines were counted. The area of each count field was approximately 0.25 mm^2. In each block, the number of count fields ranged from 1 to 12 (average 5.97); in blocks with few plaques only few fields were counted. The number of plaques counted in each block ranged from 1 to 92

Table 1 Distribution of spongiform change and PrP plaques in different brain regions. Score of PrP plaques: *0*, none; +, a few; ++, moderate number; +++ numerous. Spongiform change was scored according to [9]. If there was variation of spongiform change within the block, the most affected part determined the score (*PrP* prion protein, *Temp*. temporal, *Bas*. basal, *Sp*. spinal)

Case		Region	Spongiform change score	Density of plaques	Percentage of florid plaques
No.	Block code				
1	80W8.16	Cerebellum	0	+	35.2
2	80W9	Temp. cortex	0	+++	29.6
3	80W101	Medulla	0	+++	8.6
4	80W102	Cerebellum	0	+	14.6
5	80W230.18	Cerebellum	0	++	2
	80W230.2	Cortex	3	+++	6.5
	80W230.9	Bas. ganglia	2	+++	2
6	80W231.1	Cortex	1	+++	n.d.
	80W231.9	Temp. cortex	1	++	7.9
7	80W232	Medulla	3	+++	0
8	80W320.1	Cerebellum	0	+++	25
	80W320.2	Cortex	2	+	0
	80W320.3	Cerebellum	0	++	0
9	80W321	Cortex	5	++	n.d.
10	80W343.a	Cortex	3	+++	12.5
	80W343.c	Cortex	2	+++	28.5
	80W343.b	Cortex	5	+++	n.d.
	80W343.d	Medulla	2	++	20
	80W343.2	Cortex	3	+++	13
	80W343.3	Cortex	5	+++	17.5
	80W343.4	Cortex	1	+++	5.6
	80W343.5	Temp. cortex	0	+++	20
	80w343.6	Cortex	0	++	15.11
	80W343.7	Cortex	0	+++	5.8
	80W343.8	Temp. cortex	0	+++	34.4
	80W343.9	Temp. cortex	0	++	0
	80w343.10	Cortex	0	+	0
	80W343.11	Cortex	0	+++	12.5
	80W343.13	Bas. ganglia	3	+++	52.7
	80W343.14	Cerebellum	1	+++	5.4
	80W343.15	Cerebellum	1	++	13.3
	80W343.16	Bas. ganglia	5	++	0
	80W343.17	Sp. cord	1	++	20
	80W343.18	Sp. cord	2	++	40
	80W343.19	Sp. cord	1	+++	0
11	81W21	Medulla	4	+++	5.1
12	81W197	Medulla	0	+++	10
13	81W234.1	Cortex	0	+	0
	81W234.2	Medulla	0	+++	0
14	81W235.1	Cortex	5	++	4
	81W235.2	Medulla	4	++	11.4
15	81W321	Cortex	5	++	9.3
16	81W322	Cortex	5	++	0

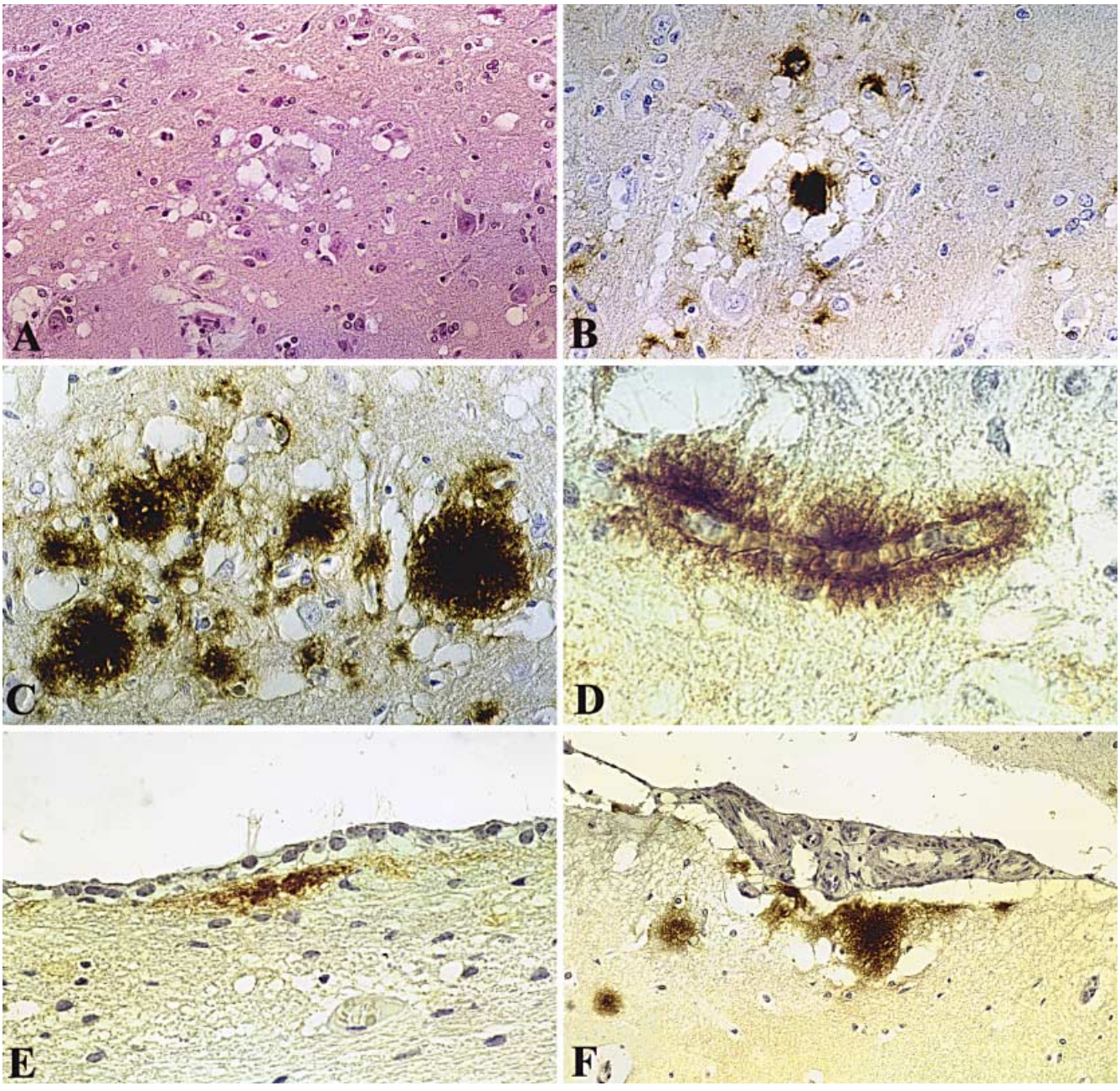

Fig. 1A–F Case 10, sections from cortex and wall of lateral ventricle. **A**, **B** Florid plaques are well visible both with H&E (**A**) and IHC for PrP (**B**). **C** Large aggregate of florid plaques; IHC for PrP, Nomarski optics. **D** Plaque-like PrP deposits radiate from a small vessel; IHC for PrP, Nomarski optics. **E**, **F** Linear, patchy and plaque-like subependymal (**E**) and subpial (**F**) PrP deposits; IHC for PrP (*IHC* immunocytochemistry, *PrP* prion protein). **A**, **F** ×180; **B**, **C** ×280; **D** ×720; **E** ×450

(average 41.5), with the number of florid plaques ranging from 0 to 37 (average 6.47).

Results

Both 6800III and 3F4 stained PrP deposits in CWD brains. However, using 6800III slightly more, and more strongly labeled, deposits were visualized than with 3F4. Thus, only slides immunostained with 6800III were used for plaque counting. Different patterns of PrP accumulation were seen: the most prominent were plaque-like structures, a substantial proportion of which were florid plaques surrounded by a prominent rim of spongiform vacuoles (Fig. 1A–C). The percentage of florid plaques was highly variable according to region, ranging from 0% to 52.7% (Table 1). The highest percentage was observed in the medulla and basal ganglia, the lowest in the cerebral cortex. Only three brains contained no florid plaques. The florid plaques were visible in routine hematoxylin and eosin (H&E)-stained sections to contain pale and delicate, radially arranged fibrils (Fig. 1A), sometimes with a slight basophilic tinge, and were occasionally clustered into large aggregates (Fig. 1C). Other plaques were typical

stellate kuru-type plaques with denser fibrillar structure, and more amorphous shapeless deposits of various sizes. There were also punctate synaptic-type PrP staining and linear, patchy or radially arranged perivascular deposits (Fig. 1D), particularly in areas of severe spongiform change, and linear or patchy subependymal (Fig. 1E) and subpial (Fig. 1F) plaque-like deposits. Some PrP was rarely deposited on the luminal surface of ependymal cells.

The distribution of PrP deposits was relatively uniform in all studied areas, in contrast to that of spongiform change (Table 1). However, the cerebellum was only slightly affected with rare plaques in both molecular and granular cell layers.

Discussion

Brain pathology of CWD does not appear to differ between captive and wild cervids [30]. We report here the prominence of florid plaques and other PrP deposits in CWD of captive mule deer. In this respect, the pathology of this disease of wild ruminants is similar to vCJD [19]. While florid plaques in CWD have been previously illustrated and described, they were not described as "florid" nor quantified at the time of investigation [13, 35]. These florid plaques are most prominently visualized by PrP immunocytochemistry, but can be seen on routine H&E sections. Another characteristic feature of vCJD is prominent involvement of the cerebellum by PrP deposition, especially in the molecular layer [19]; however, the cerebellum was only slightly affected in CWD. Thus, the topography of PrP deposits differs between vCJD and CWD.

The morphological similarity between CWD and vCJD with regard to florid plaques might prompt speculations about similar pathways of origin: similar strain properties of the original agent, e.g. from scrapie, might account for similar pathologies in new host species such as mule deer and humans (after passage through BSE). However, there are many arguments against such a speculation. First, the neuropathology of CWD in cervids is different from that of BSE in cattle, despite their relatedness as ruminants, as that of vCJD differs from BSE. Moreover, CWD is not BSE in cervids based on several grounds: (1) the lack of evidence that BSE exists in the USA; (2) the lack of an association of CWD with feeding ruminant protein; (3) the fact that BSE did not affect cervids in UK zoos; (4) the fact that CWD is transmissible to mice only at low frequency; and (5) a CWD strain-typing profile distinct from BSE [4]. Collectively, while CWD and vCJD differ in the majority of biological properties, they present similar neuropathology in respect to florid plaques.

The percentage of florid plaques is variable, from null to more than 50% among different brain regions and different individuals. On this basis, one cannot form an idea as to the nature of the disease (sporadic versus infectious). However, the epidemiology of CWD in both captive and free-ranging cervids favors interspecies lateral transmission [26, 27]. As PrP is shed from neuronal membranes [21], it is possible that the amount of amyloid reflects inherently diverse properties of neurons that are the source of PrP, as well as a given stage in the evolution of brain disease.

The PrP gene for mule deer has been cloned and sequenced [5, 28]. The 3F4 monoclonal reacts specifically with the amino acid 109–112 region of the hamster PrP sequence [5]. The complete sequence of this epitope both in hamster and man is MKHM, and that of the corresponding epitope 112–115 in mule deer, red deer and American elk, as well as in cat, is MKHV (www.expasy.ch). Thus, the sequence differs by one methionine; however, cat PrP has been shown to bind 3F4 [29] and has the same sequence as mule deer. Nevertheless, it is surprising that 3F4 also binds to a differently composed epitope.

Subependymal PrP deposits have been observed in naturally occurring and experimentally induced TSEs: natural scrapie in sheep [6, 31], mule deer with CWD [13], and in experimental scrapie [8]. We also observed subependymal deposits in hamsters infected with the Echigo-1 strain of CJD [24] and in hamsters infected with the 263K strain of scrapie [24]. By transmission electron microscopy, we readily observed PrP fibrils floating in the subependymal space [20, 23]. It seems that the subependymal localization of PrP deposits is a frequent finding of TSEs, and in this respect CWD is no exception. The significance of this phenomenon is unknown. The presence of PrP fibrils in the ventricular space may, however, contribute to the spread of infectivity within the brain. Indeed, 4 out of 27 cases of CJD were transmitted via the CSF [2].

Cerebrovascular PrP amyloid was first described in experimental scrapie [3] followed by natural scrapie in sheep [1, 11], and is the hallmark feature of the Y145s stop mutation of genetic human TSEs [10]. Whether such a location is incidental or has any pathogenetic meaning, as with the molecularly different amyloid of Alzheimer's disease in the same location, is currently unknown.

We have reported that kuru, the first human TSE of infectious origin to be described, may show a small proportion of plaques that meet criteria of florid plaques [16]. However, this proportion is so small that kuru neuropathology cannot be considered to resemble vCJD [16]. While florid plaques may very rarely also be found in sporadic CJD [15], large numbers of these structures have been confined to vCJD. Also, CWD has to be added to TSEs with prominent florid plaques.

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