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In vivo and in vitro expression of canine distemper viral proteins in dogs and non-domestic carnivores

Brief Report

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Summary. The occurrence of the nucleo-, phospho-, matrix, fusion, and hemagglutinin proteins of the canine distemper virus (CDV) was investigated immunocytochemically in the brains of 3 dogs, 6 stone martens, 1 polecat, and 1 weasel. In addition, viral protein expression was studied in primary brain cell cultures of the 3 dogs after co-cultivation with Vero cells. Immunohistochemically, only minor differences, restricted to the H-4 epitope, were noted between the various species and CDV isolates. The data presented indicate that the mustelid virus is antigenically not distinct from the canine morbillivirus.

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Canine distemper virus (CDV) belongs to the genus *morbillivirus*, a member of the family *Paramyxoviridae* [14]. CDV, pathogenic for animals of the orders carnivora, suborder fissipedia, and artiodactyla, family tayassuidae, causes systemic illness with or without central nervous system (CNS) affection [3, 15]. In dogs, CNS lesions can be categorized in acute encephalopathy, acute encephalitis, subacute to chronic demyelinating encephalitis (CDE), old dog encephalitis (ODE), and post-vaccinal canine distemper encephalomyelitis [13, 15]. CDV possesses 6 structural proteins comprising 3 core proteins, consisting of the nucleo-(N), phospho-(P), and large (L) protein, and 3 envelope polypeptides represented by the matrix (M), fusion (F), and hemagglutinin (H) protein [10, 14, 17]. Antigenic variations among CDV strains occur most frequently in the H protein [7, 18].

Following the mass mortality of harbour seals in the Northern and Baltic

Seas during 1988, phocine distemper virus (PDV), a CDV-like virus, was isolated from diseased seals and has been classified as a separate member of the genus morbillivirus based on its antigenic and genomic properties [7, 9, 16]. The host spectrum of PDV comprises animals to the orders carnivora, suborder pinnipedia, and cetacea. Recently, a porpoise virus isolate was preliminary classified as "delphinoid virus" (DDV) using a panel of monoclonal antibodies (mAbs) directed against CDV, PDV, and DDV. The porpoise isolate exhibited several unique epitopes and other epitopes present on CDV and PDV were absent on the porpoise virus [21]. Repeated endemic outbreaks of distemper in the dog population have raised the question of the emergence of new field strains of CDV [1, 11]. It is well known that martens, foxes, and badgers are susceptible to CDV and they may serve as a virus reservoir [6, 20]. Furthermore, previous studies showed that mustelids are susceptible to CDV and PDV [12, 18].

The aim of this study was to investigate the expression of 5 CDV-specific proteins and their epitopes a) in the CNS of dogs and mustelids and b) in co-cultures of canine primary brain cells with Vero cells.

Three dogs with naturally occurring canine distemper were used in the study. Dog 1, a 2 month-old female mixed-breed dog, presented neurologic dysfunctions only. Dog 2, a 6 month-old female mixed-breed dog, showed purulent rhinitis and conjunctivitis, hyperkeratosis of the foot pads and snout and ataxia. The third dog, a 4 month-old male shepherd dog, originating from Turkey, exhibited signs of respiratory, gastrointestinal and neurologic disease. Nothing was known about the vaccination records of dog 1 and 3. Dog 2 had received a pentavalent vaccine (incl. distemper) once. Despite intensive supportive treatment, the condition of the animals deteriorated and they were killed. Eight CDV-positive mustelids, 6 stone martens, 1 polecat, and 1 weasel were kindly provided by Dr. M. Adami (Staatliches Institut für Gesundheit und Umwelt, Abt. Veterinärmedizin, Saarbrücken, Federal Republic of Germany). Clinical histories were only sketchy; some animals showed loss of natural shyness, others were killed by dogs or found dead near human settlements. Immediately after death, the brains of the dogs were removed aseptically and one hemisphere was processed for virus isolation. Due to advanced autolysis of the mustelid carcasses, virus isolation was not attempted in these animals. The remaining hemisphere of the canine CNS and the brains of the mustelids were fixed in 10% non-buffered formalin and embedded in paraffin, in addition, tissue samples were embedded in O.C.T. and quick frozen in liquid nitrogen as described [2].

For virus isolation, single cell suspensions and explant cultures were established from cerebrum, cerebellum, and medulla oblongata. Cells were cultured in 25 and 75 cm^2 flasks and maintained in Eagle's MEM with Earle's salts, supplemented with 10% fetal calf serum and antibiotics as described [4]. The primary brain cell cultures were co-cultivated with African green monkey kidney (Vero) cells 12 (dog 1), 13 (dog 3), and 18 (dog 2) days after seeding. Following 5 (dog 1), 6 (dog 2), and 4 (dog 3) passages, cells were grown on 4-chamber slides and confluent monolayers were fixed in acetone as described [2].

Three attenuated CDV strains, the Onderstepoort strain (CDV/Ond), kindly provided by Dr. A. E. Metzler (Institut für Virologie, Universität Zürich, Switzerland), the Convac (CDV/Con), and the CDV/R 252 (Ohio) strain were employed as reference strains [8, 17, 18]. The N, P, F, and H protein and their epitopes were recognized by 15 different monoclonal antibodies and a monospecific polyclonal antibody was used to detect the M protein (Table 1). Production, specificity, Ig-subclass, and final antibody dilution for immunocytochemistry have been described elsewhere [2, 17, 19].

Routine histology was performed on H & E-stained, 5 µm-thick sections of formalin-fixed, paraffin-embedded tissue. Selected sections were stained with luxol fast blue-cresyl echt violet. For immunocytochemistry, the avidin-biotin-peroxidase-complex (ABC) method was employed on frozen and paraffin-embedded tissues and tissue culture cells as detailed previously [2, 5]. Negative controls included non-infected Vero cells, brain sections from dogs and mustelids and canine primary brain cells from CDV-negative animals. Furthermore, sec-

Table 1. In vivo and in vitro expression of 5 different CDV-specific proteins and their epitopes in naturally infected dogs and wildlife carnivores

CDV-specific proteins (clone no.)	CDV/ Ond	CDV/ Con	CDV/ R 252	Dog 1		Dog 2		Dog 3		Mustelids B
				B	C	B	C	B	C	
N-1 (3721)	+	+	+	+	+	+	+	+	+	+
N-4 (3991)	+	+	+	+	+	+	+	+	+	+
P-1 (3698)	+	+	+	+	+	+	+	+	+	+
P-2 (3568)	+	+	+	+	+	+	+	+	+	+
P-4 (3780)	+	+	+	+	+	+	+	+	+	+
M	+	+	+	+	+	+	+	+	+	+
F (5027)	+	+	+	+	+	+	+	+	+	+
F-1 (5086)	+	+	+	+	+	+	+	+	+	+
F-2 (3584)	+	+	+	+	+	+	+	+	+	+
F-2 (3551)	+	+	+	+	+	+	+	+	+	+
F-3 (5148)	+	+	+	+	+	+	+	+	+	+
H-1 (1347)	+	+	+	+	+	+	+	+	+	+
H-2 (2267)	+	+	+	+	+	+	+	+	+	+
H-3 (3734)	+	+	+	+	+	+	+	+	+	+
H-4 (3775)	+	+	−	+	+	−	−	+	−	−
H-5 (4043)	+	+	+	+	+	+	+	+	+	+

B Brain tissue
C Brain cells co-cultivated with Vero cells
CDV/Ond Onderstepoort strain of CDV
CDV/Con Convac strain of CDV
CDV/R252 Ohio strain of CDV
+ presence of epitope
− lack of epitope

tions and cells were incubated with ascites from non-immunized Balb/cJ mice or with rabbit control serum instead of the primary antibody.

Histologically, all 3 dogs exhibited subacute demyelinating encephalitis without associated inflammation. In addition, neuronal necrosis in the cerebral cortex was a prominent finding in dog 3. Brain lesions were demonstrated in 7 of the 8 mustelids. In the martens, lesions included focal vacuolation (3 animals) and focal malacia with Gitter cells (1 animal) in the cerebellar white matter. In addition, in 1 marten with focal vacuolation, single cell necrosis of cerebellar granule cells was observed. Furthermore, moderate non-purulent meningoencephalitis was seen in 1 marten. The weasel and the polecat showed moderate non-purulent meningoencephalitis. Additionally, a single granuloma was found in the polecat's brain.

In vitro, cytopathic effects (CPE) appeared up to 7 days after co-cultivating of the primary canine brain cell cultures with Vero cells. In co-cultures, CPE was characterized by multinucleated giant cells and rounded cells.

Since the immunocytochemical findings were similar in the different mustelid species, they are condensed to one description. Intracellular distribution and staining properties of the CDV-specific proteins and their epitopes were similar in dogs and mustelids. In canine brains, expression of CDV-specific proteins was prominent in the cerebellar white matter, whereas in mustelid brains, antigen expressing cells were almost exclusively located in the grey matter (Figs. 1 and 2). The core proteins had a dark brown coarse granular inclusion body-like appearance and were located in the nucleus, cytoplasm, and cell processes. In contrast, immunoreaction of the envelope proteins was characterized by dark to light brown fine granular protein accumulations restricted to the cytoplasm. A prominent perinuclear protein concentration was frequently observed with the H protein. In all species, expression of surface proteins was slightly reduced compared to the core proteins (Fig. 3 A and B).

Immunoreactivity of the CDV polypeptides and their epitopes were similar in all cells and tissues investigated except for the H-4 epitope (Table 1). This epitope was present in Vero cells infected with CDV/Ond and CDV/Con strain, in brain tissue of dog 1 and 3 and in co-cultures from dog 1. However, the H-4 epitope was missing in CDV/R 252 infected Vero cells, co-cultures of dog 2 and 3 and brain tissues of dog 2 and all mustelid species investigated. Accordingly, CDV field isolates can be distinguished from reference vaccine strains (CDV/Ond, CDV/Con) by their lack of reactivity with the H-4 epitope [7]. Similarly, dog 2, presented with typical signs of hard pad disease, and the mustelids were devoid of this antigen. Furthermore, in a recent study, H-4 was found only in 3 out of 16 dogs with spontaneous CDV encephalitis [2]. Surprisingly in dog 3, the epitope was demonstrated in brain tissue but not in co-cultures. Mechanisms resulting in variable in vitro and in vivo expression of CDV proteins are still undetermined. In addition, the significance of this epitope for virulence and the pathogenesis of distemper-associated lesions is still open to question.

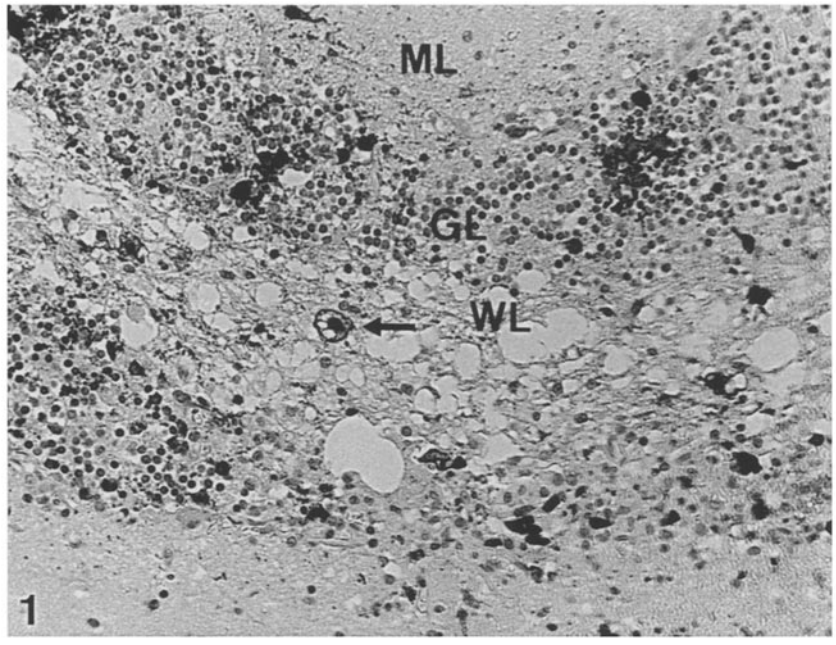

Fig. 1. Cerebellar white lamina (mustelid). Subacute non-inflammatory lesions with Gitter cells. Numerous N-4-positive cells in the granular and molecular layer; note positively stained Gitter cell (arrow). Formalin-fixed, paraffin-embedded tissue, ABC-method, slightly counterstained with hematoxylin, × 220. *WL* White lamina; *GL* granular layer; *ML* molecular layer

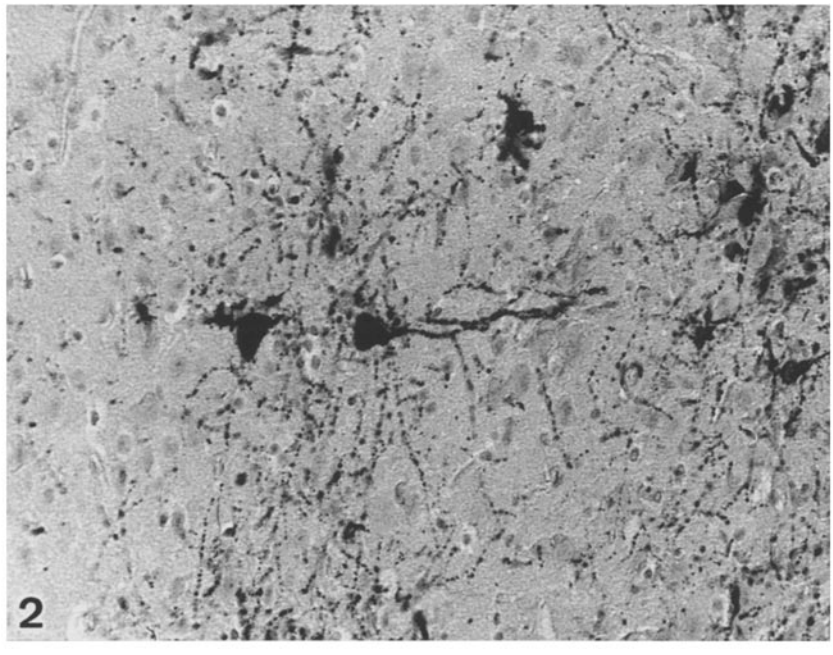

Fig. 2. Cerebral cortex (mustelid). Immunohistochemical demonstration of the N-4 epitope in nucleus, cytoplasm, and cell processes of neurons. Formalin-fixed, paraffin-embedded tissue, ABC-method, slightly counterstained with hematoxylin, × 140

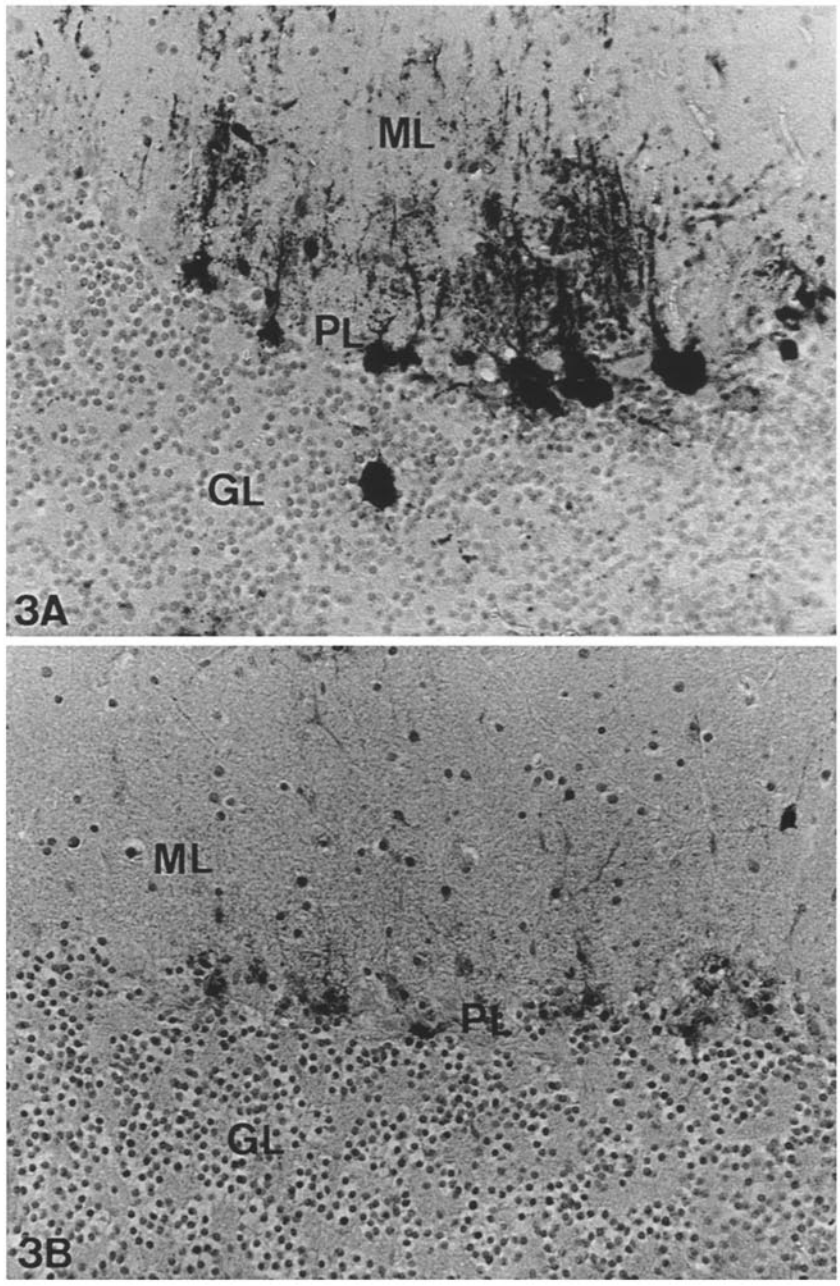

Fig. 3. Cerebellar cortex (mustelid). Strong expression of the N-4 epitope (**A**) compared to the M protein (**B**) in Purkinje cells and Bergmann glia. Formalin-fixed, paraffin-embedded tissue, ABC-method, counterstained with hematoxylin, a and b, × 220. *PL* Purkinje layer; *ML* molecular layer, *GL* granular layer

In conclusion, although it remains to be determined whether the mustelid-distemper virus is virulent for dogs and other species, the data presented indicate that the distemper virus demonstrated in various species of wild carnivores exhibits antigenic characteristics of CDV.

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