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Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in North West Europe and Siberia

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Summary. Recently morbilliviruses were isolated from harbour seals (*Phoca vitulina*) in North West Europe (phocid distemper virus-1: PDV-1) and from Baikal seals (*Phoca sibirica*) in Siberia (phocid distemper virus-2: PDV-2) during outbreaks of severe disease which resembled distemper in dogs. PDV-1 and PDV-2 were passaged in SPF dogs, in which they caused distemper-like disease symptoms, and were subsequently passaged in Vero cells in which they caused cytopathic changes. PDV-1, PDV-2, and canine distemper virus (CDV) were compared with respect to their biological, morphological, physical, protein chemical, and antigenic properties. It was concluded that PDV-1 should be considered a newly recognized member of the genus *Morbillivirus*, whereas PDV-2 proved to be quite similar if not identical to CDV.

Introduction

To date four distinct members of the genus *Morbillivirus* have been recognized: measles virus (MV), canine distemper virus (CDV), rinderpest virus (RPV), and peste des petits ruminants virus (PPRV), which all may cause serious disease in their respective host species [for review, see 2, 8, 19]. Two severe disease outbreaks, closely resembling distemper in dogs, were observed among harbour seals (*Phoca vitulina*) in North West Europe starting in 1988 and among Baikal seals (*Phoca sibirica*) in the Siberian Lake Baikal starting in 1987. From these harbour and Baikal seals two morbilliviruses were isolated [25, 29]. These viruses, phocid distemper virus-1 (PDV-1) and phocid distemper virus-2 (PDV-2) respectively, were identified as the primary cause of the observed outbreaks

[3, 5, 9, 10–12, 15–17, 24–27, 35]. We speculated, that an epizootological link might have existed between both outbreaks [29]. Therefore we have now compared PDV-1, PDV-2, and CDV with respect to their biological, morphological, physical, protein chemical, and antigenic properties. These data showed that PDV-1 was quite distinct from CDV, whereas PDV-2 could not be distinguished from this virus. On the basis of these and other data [5, 16, 17, 30] it was concluded that PDV-1 should be considered a newly recognized member of the genus *Morbillivirus* and that no epizootological link has existed between both recent disease outbreaks in seals.

Materials and methods

Viruses and infectious tissues

CDV strain Bussel [4] was used in virus neutralization (VN) tests and cell susceptibility studies, CDV strains Bussel and Convac [4, 21] were used in immunofluorescence and antibody enzyme linked immunosorbent assay (ELISA) and CDV strain Rockborn [32] was used in Western blot assay.

A 10% suspension of spleen, lung and intestinal lymph node cells was prepared from three harbour seals that had died during the outbreak in the Dutch Waddensea in 1988 with distemper like symptoms [25]. A 10% homogenate was prepared of spleen and liver material from a Baikal seal that had also died with distemper like symptoms in Siberia in 1988 [29].

Antibody preparations

Serum samples were taken from specified pathogen free (SPF) Beagle dogs at daily intervals during a period of 12 days after experimental infection with organ material isolated from seals from either the Dutch Waddensea or the Siberian Lake Baikal as described below. During and after the outbreaks of distemper among seals in North West Europe starting in 1988, and in Siberia starting in 1987, serum samples were collected from healthy and moribund harbour seals and Baikal seals respectively. Mouse monoclonal antibody (MoAb) preparations raised against the Convac strain of CDV were described previously [21, 33]. Measles virus fusion (F) protein-specific rabbit serum was obtained by repeated immunization of SPF rabbits with measles virus F protein purified by immuno affinity chromatography [6].

Hyperimmune dog anti-CDV serum was obtained after infection of SPF Beagle dogs with the Schneider Hill strain of CDV [7].

Cells

The following cell substrates were used for passaging of viruses using techniques previously described [1]: African green monkey cell line (Vero), primary seal kidney cells (SeKC) [22], subcultured cells derived from seal skin explants (SeSkC), and Madin Darby canine kidney (MDCK) cell line. Cells were monitored for cytopathic changes and immunofluorescence (see below) seven to ten days after passage. If no changes or immunofluorescence were observed, cells were subcultured and examined in the same way once more.

Virus passage in dogs

Four SPF Beagle dogs, from a caesarean derived breeding colony, that by regular serological screening was shown to be free of the viruses that are known to regularly infect dogs

(unpubl. obs.), were used for infection experiments. During the experiments the SPF dogs were housed in two pressurised glove boxes. Two SPF dogs, housed in one glove box, were infected by the oculonasal and intraperitoneal routes with 2 ml volumes each of the cell suspension from the seals that died in the Dutch Waddensea [25]. The other two SPF dogs, housed in the other glove box, were inoculated in the same way with 2 ml of the tissue homogenate from the Baikal seal [29]. Clinical symptoms which occurred during a period of 12 days after inoculation, including rise in body temperature, excessive nasal discharge and lymphopenia were recorded. During this period heparinized blood samples were collected at daily intervals for counting of peripheral blood lymphocytes, isolation of peripheral blood mononuclear cells (PBMC) and serological studies.

Virus isolation in vitro

PBMC were isolated from the SPF dogs by centrifugation on Ficoll-Isopaque and were used for virus isolation by co-cultivation of PBMC with primary lung macrophage cultures as previously described [7]. During an incubation period of 7 days these cultures were monitored daily for cytopathic changes, including syncytia formation, the presence of morbillivirus antigen by immunofluorescence and viral structures by negative contrast electron microscopy on water disrupted cells as described below. Pools of PBMC that had been shown to contain infectious morbillivirus, were cultivated in Iscove's modified Eagle's medium (IMEM) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) (p/s), and 2% foetal calf serum (FCS) in round-bottomed 96 well microtitre culture plates (Greiner, Solingen, Federal Republic of Germany) and stimulated with 5 µg phyto haemagglutinin (PHA) (Flow Laboratories, Rickmansworth, U.K.) per 5×10^4 PBMC per well for 3 days (37 °C) in a humidified atmosphere containing 7% CO_2. After this period, the cells were resuspended, washed and 10^5 cells were co-cultivated in 75 cm^2 Falcon flasks (J. Bibby Science Products Ltd., Stone, Staffordshire, U.K.) with 10^6 Vero cells in Dulbecco's modified Eagle's medium (DMEM) supplemented with p/s and 5% FCS. Cultures were screened daily for cytopathic changes by light microscopy. Every three days culture medium was changed and every ten days one third of the cells was passaged into new flasks. The cultures were monitored for the presence of morbillivirus infection at regular intervals, as described above.

Antigen capture ELISA

Morbillivirus antigen was detected by an antigen capture ELISA, as previously described [35]. In short, ELISA-strip plates (E.I.A./R.I.A. Strip plate-8, Costar, Cambridge, U.S.A.) were coated with CDV F-specific MoAb 5086 [21], followed by an incubation with the culture supernatants or gradient fractions to be tested. After incubation with polyclonal hyperimmune dog anti-CDV serum and HRPO-conjugated goat anti-dog immunoglobulin (Cappel, Cooper Biomedical, Inc., Malvern, U.S.A.), $OD_{450\,nm}$ values were measured. $OD_{450\,nm}$ exceeding 2.0 times the mean background value obtained with a Vero cell control sample were considered to contain morbillivirus antigen.

Electron microscopy

Small drops of PDV-1 and PDV-2 infected Vero cell lysates, obtained by osmotic shock, were placed on formvar/carbon coated grids, negative-contrasted with 2% phosphotungstic acid (BDH) (pH 6.0, adjusted with 1 M KOH) and examined by electron microscopy (Philips 400 T, 80 kV).

Immunofluorescence assay (IFA)

Cells were trypsinized, seeded on glass slides (Labteck, Miles Scientific, Naperville, U.S.A.), incubated overnight in Eagle's medium supplemented with p/s and 2% FCS, washed with

phosphate buffered saline (pH 7.4) (PBS) and fixed for 3 min at 20 °C with 3% (w/v) paraformaldehyde in PBS solution. After incubation with PBS containing 10% FCS, morbillivirus infected cells were detected with a mixture of MoAbs directed to the fusion (F) protein, the haemagglutinin (H) protein, the nucleoprotein (NP), and the polymerase (P) protein of CDV [21, 33] and a goat anti-mouse fluorescein isothiocyanate (FITC) conjugate (Dakopatts, Glastrup, Denmark) diluted in PBS containing 10% FCS.

Virus production and purification

Vero cells were seeded into roller bottles (Corning, Corning, N.Y., U.S.A.) at a concentration of 10^7 cells per 100 ml DMEM supplemented with p/s and 2% FCS and directly infected at an m.o.i. of 10^{-4}. Seven days after infection, when 90% of the cells showed cytopathic changes, culture supernatants were harvested and after clarification (20 min, 2,400 rpm), virus particles were pelleted through a 20% sucrose cushion in TN buffer (10 mM Tris-HCl, 0.9% NaCl (w/v), pH 7.6) by ultra-centrifugation for 4 h at 18,000 rpm using an SW 28 rotor (Beckman Instruments, Inc., Palo Alto, U.S.A.). The pellet was resuspended in TN buffer and layered on top of a linear 10–60% (w/v) sucrose gradient in TN buffer and centrifuged for 18 h at 18,000 rpm at 4 °C in an SW 40 rotor (Beckman Instruments). Gradient fractions were collected by bottom unloading and buoyant densities of each fraction were calculated on the basis of their refractive indices and infectivity titres. Morbillivirus antigen in the fractions was quantified by antigen capture ELISA and by Western blot analysis as previously described [35]. The latter technique was also used to compare relative mobilities (M_r) of virus-specific proteins.

Identification of virus-specific proteins

The proteins of the sucrose gradient purified virus CDV (strain Rockborn), PDV-1 and PDV-2, were detected in Western blot assay [34]. Viral proteins were separated on a 12.5% SDS-polyacrylamide gel [14], electrophoretically transferred to nitrocellulose sheets (Schleicher & Schuell, Dassel, Federal Republic of Germany) and stained with monoclonal or polyclonal antibody preparations raised against CDV. The H, NP, and P proteins were stained with TMB/DONS as described [35], after incubation with MoAbs, 1.347 (H1), 3.805 (NP3), and a mixture of MoAb 3.780 (P4), 3.788 (P), and 4.174 (P1) respectively [21, 33], followed by an incubation with HRPO-labelled goat anti-mouse IgA, M, G, (Cappel, Cooper Biochemical, Inc., Malvern, U.S.A.). The F_1 protein was stained after incubation with a MV F protein-specific rabbit serum, followed by incubation with a HRPO-labelled swine anti-rabbit conjugate (Dakopatts, Glostrup, Denmark).

Infectivity test

Vero cells were seeded at a density of 10^4 cells per 50 µl in DMEM supplemented with p/s and 2% FCS into 96-well microtiter flat-bottomed culture plates (Greiner, Solingen, Federal Republic of Germany) and were inoculated with serial ten fold dilutions of each sucrose gradient fraction in 100 µl. The plates were sealed and after an incubation period of 7 days at 37 °C in a humidified 7% CO_2 atmosphere, $TCID_{50}$ values were determined microscopically on the basis of cytopathic changes.

Antibody ELISA

Reactivities of CDV-specific MoAbs with CDV (Convac strain), with PDV-1 and PDV-2 were tested in an indirect ELISA, as previously described [21]. $OD_{450\,nm}$ values exceeding 2.0 times the mean background value obtained by Vero cell lysate coated plates were considered positive.

Virus neutralization (VN) assay

VN tests were carried out in 96 well microtitre plates. Serial two-fold dilutions of serum samples from the experimentally infected SPF dogs, three-fold dilutions of serum samples from seals from North West Europe and from the Lake Baikal, were tested for their ability to neutralize 10–30 $TCID_{50}$ of CDV (Bussel strain), PDV-1 and PDV-2. These virus preparations were obtained by clarifying (20 min, 2,400 rpm) Vero cell lysates obtained by one cycle of freeze-thawing. Serum dilutions (50 µl) were pre-incubated with 50 µl volumes of the respective virus preparations for 1 h at 37 °C, in microtitre plates, before addition of 10^4 Vero cells (in 50 µl) to each well. After incubation for seven days in DMEM supplemented with p/s and 2% FCS in a humidified 7% CO_2 atmosphere, virus neutralizing (VN) antibody titres were determined microscopically on the basis of cytopathic changes and expressed as reciprocal of the highest serum dilution still giving 100% reduction of cytopathic changes.

Results

Passage and isolation of PDV-1 and PDV-2

Within 2 weeks after inoculation the two SPF dogs (#1 and #2) inoculated with cells from seals that had died in the European outbreak, and the two SPF dogs (#3 and #4) inoculated with the organ material from the Siberian outbreak

Table 1. Clinical signs developed by SPF dogs and virus isolation from these dogs upon inoculation with seal organ material, containing PDV-1 or PDV-2

Time after inoculation (days)	PDV-1								PDV-2							
	Dog 1				Dog 2				Dog 3				Dog 4			
	N	T	L	V	N	T	L	V	N	T	L	V	N	T	L	V
1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
4	−	+	+	−	−	−	+	+	−	−	+	−	−	+	−	−
5	−	+	+	+	−	−	+	+	−	−	+	−	+	−	−	−
6	−	−	+	+	−	−	+	+	−	−	NT	NT	−	−	NT	NT
7	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+
8	−	−	−	−	+	−	−	−	−	−	−	NT	−	−	−	NT
9	−	−	−	−	+	−	−	−	−	−	−	+	−	+	−	+
10	−	−	−	−	+	−	−	−	−	−	−	NT	−	−	−	NT
11	+	−	−	−	−	−	−	−	−	−	−	NT	−	−	−	NT
12	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+

N Nasal discharge (+)
T Body temperature $\geqslant 39$ °C (+)
L lymphopenia $\leqslant 1.5 \times 10^6$ lymphocytes/ml (+)
V Virus isolation from PBMC (+)
− No clinical signs or virus isolation
NT Not tested

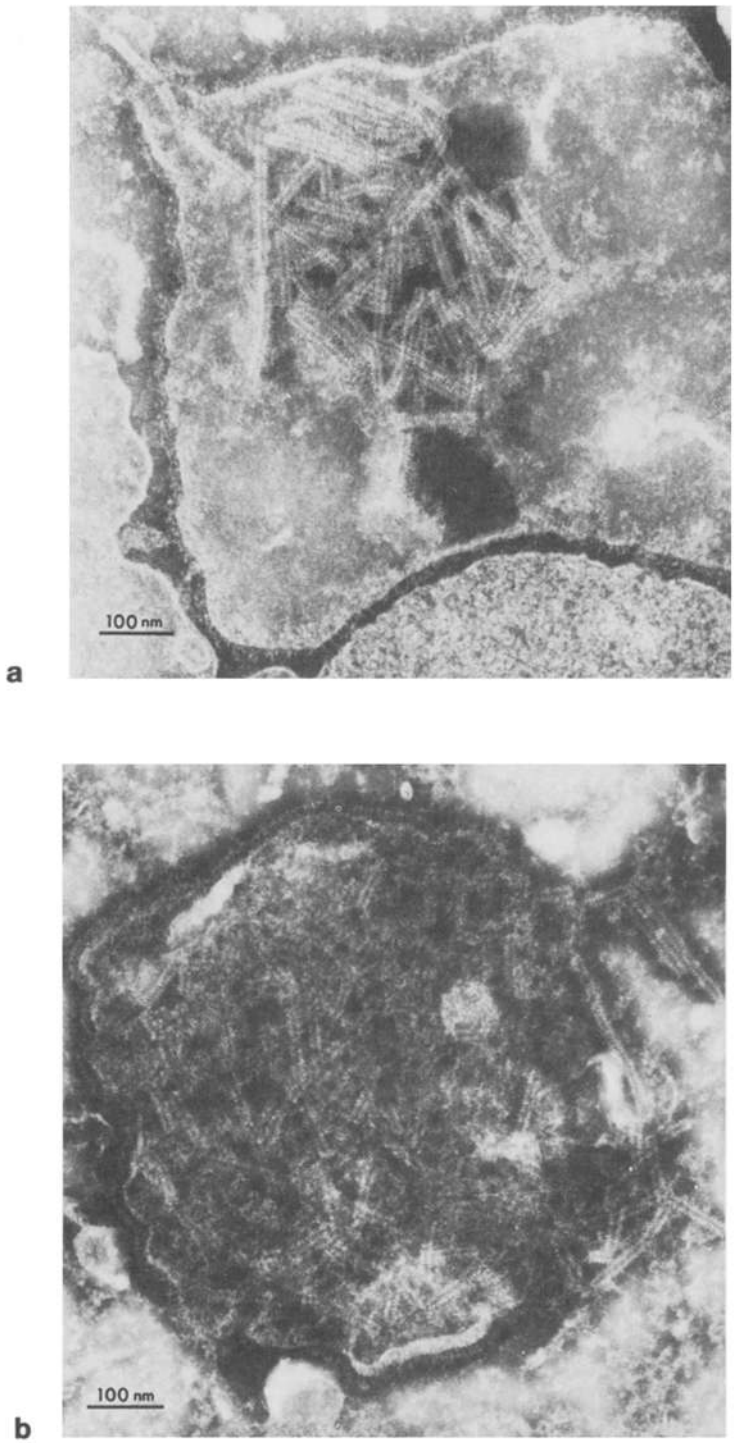

Fig. 1. Negative contrast electron micrograph of Vero cell lysates showing PDV-1 (**a**) and PDV-2 (**b**) nucleocapsids and membranes with viral glycoproteins

Table 2. Cythopathic changes and immunofluorescence observed in different cell substrates infected with PDV-1, PDV-2, or CDV strain Bussel

Cell line	Virus strain	Cythopathic changes	Immuno-fluorescence
Vero	PDV-1	+	+
	PDV-2	+	+
	CDV	+	+
SeSk	PDV-1	−	+
	PDV-2	+	+
	CDV	+	+
SeK	PDV-1	+	+
	PDV-2	+	+
	CDV	+	+
MDCK	PDV-1	−	−
	PDV-2	−	+
	CDV	−	+

+ Cythopathic changes or immunofluorescence
− No cythopathic changes or immunofluorescence

developed mild clinical symptoms as previously described [25, 29]. These included fever, lymphopenia, and nasal discharge as indicated in Table 1. Morbilliviruses were isolated in dog macrophages from dogs #1 and #2 on days 4, 5, and 6 and from dogs #3 and #4 on days 7, 9, and 12 after inoculation, as was shown by the formation of syncytia and confirmed by immunofluorescence and by negative contrast electron microscopy as previously described [25, 29]. Virus isolates from dog #2 (PDV-1) and from dog #4 (PDV-2) were also obtained by co-cultivating their PBMC with Vero cells and further passaging of these Vero cells three or four times respectively. The presence of PDV-1 or PDV-2 in Vero cells was demonstrated by showing formation of syncytia and CDV-specific immunofluorescence and by showing disrupted virus particles and/or nucleocapsids by negative contrast electron microscopy in cell lysates (Fig. 1). The nucleocapsids of PDV-1 and PDV-2 measured about 17 nm in diameter, which is characteristic for paramyxoviruses. Spike projections characteristic for paramyxoviruses were also observed. Both viruses were further passaged in Vero cells with infected cells or with cell free preparations. Susceptibilities of seal and canine cells for infection with the PDV-1 and PDV-2 isolates and CDV, was subsequently evaluated by passaging infected Vero cell lysates in these cells and monitoring them for cytopathic changes and CDV-specific immunofluorescence (Table 2). All the three viruses were shown to replicate in Vero, SeK, and SeSk cells. In contrast to PDV-1, PDV-2 and CDV

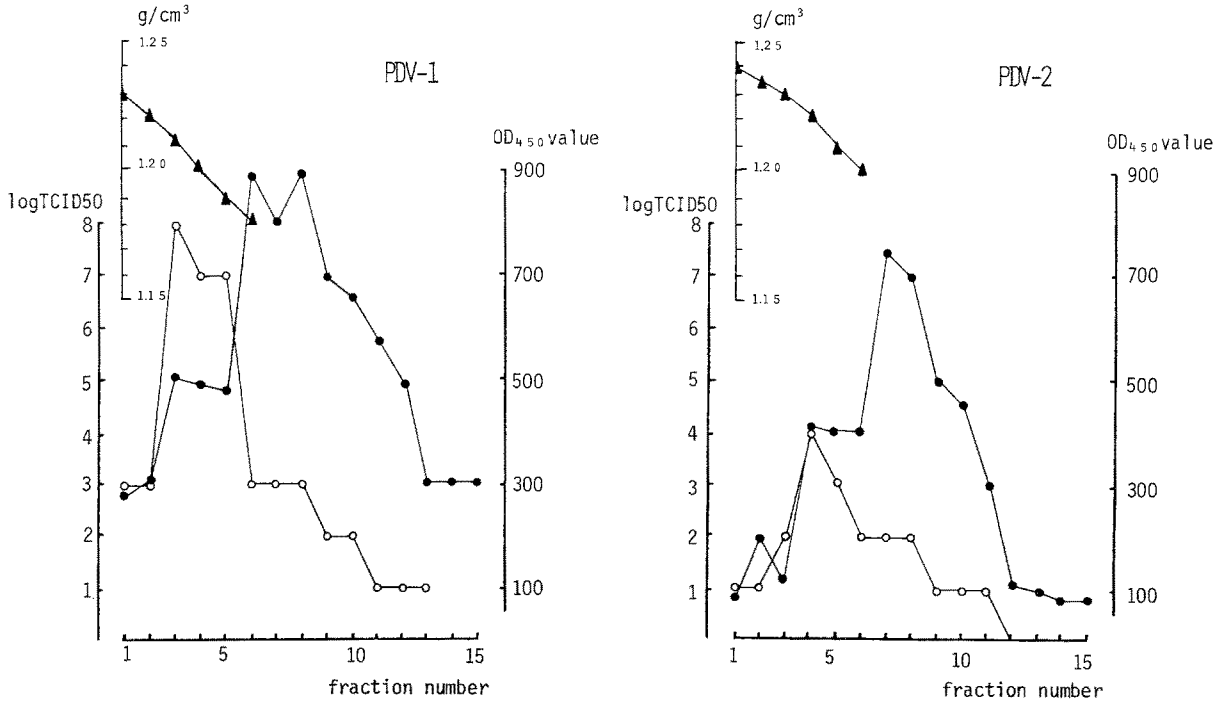

Fig. 2. Buoyant densities (▲, g/cm³), morbillivirus antigen contents (●, ELISA-$OD_{450\,nm}$), and infectivity titres (○, log$TCID_{50}$) of sucrose gradient fractions prepared during purification of PDV-1 and PDV-2

were shown to replicate in MDCK cells as well, without showing cytopathic changes.

Buoyant densities of PDV-1 and PDV-2

Buoyant densities of PDV-1 and PDV-2 in sucrose were estimated by determining infectivity titres, antigen content in ELISA and refractive indices of sucrose gradient fractions obtained during purification of these viruses. For both viruses a buoyant density of 1.21–1.22 g/cm³ was calculated on the basis of the refractive indices of the fractions with the highest infectivity titres (Fig. 2), which is within the range of buoyant densities found for the other morbilliviruses [13, 18].

Virus-specific proteins of PDV-1 and PDV-2

The M_r of the H-, F_1-, NP-, and P-proteins of sucrose gradient purified PDV-1 and PDV-2 were compared to those of CDV by Western blot analysis (Fig. 3). The P protein of PDV-1 was shown to migrate faster than the P protein of CDV, whereas the P protein of PDV-2 migrated slower than the P protein of CDV. The H proteins of both PDV-1 and PDV-2 migrated slightly slower than

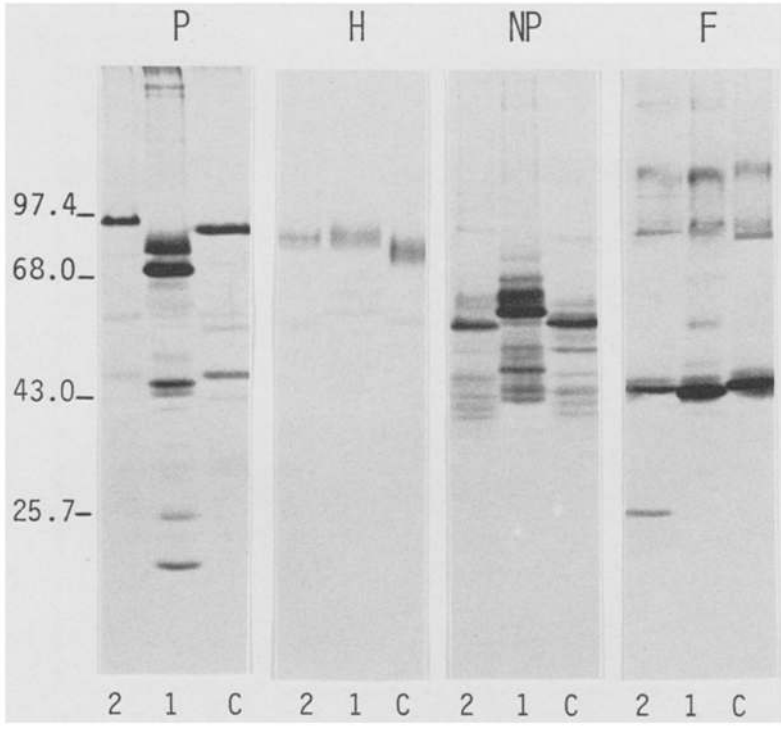

Fig. 3. Comparison of M_r of viral proteins of PDV-1 (*1*), PDV-2 (*2*), and CDV strain Rockborn (*C*) by Western blot analysis

the H protein of CDV. The NP protein of PDV-1 migrated slightly slower than the NP proteins of the two other viruses. Little or no difference was found in the mobilities of the three F_1 proteins. However, the differences observed, are within the ranges observed between different morbilliviruses [8, 20, 31, 32].

Antigenic properties of PDV-1 and PDV-2

Antigenic differences between PDV-1 and PDV-2 were first studied by comparing the reactivities of serum samples of dogs and seals infected with either of these viruses in VN assays, using PDV-1, PDV-2, and CDV. Dogs #1 and #2, experimentally infected with PDV-1 [25], developed VN serum antibodies against PDV-1 within five days, which reached titres $>1{,}280$ within 12 days. VN serum antibodies against PDV-2 and CDV did not develop before eight and eleven days and reached titres up to 1,280 and 40 respectively within 12 days (Fig. 4a). Dogs #3 and #4, experimentally infected with PDV-2 [29], developed VN serum antibody titres against all the three viruses within seven and nine days, which reached levels of 40 and 160 within twelve days (Fig. 4b).

The majority of serum samples collected from seals during the outbreak in North West Europe, and serum samples collected from seals during the outbreak in the Lake Baikal showed VN antibody titres against all the three viruses. The

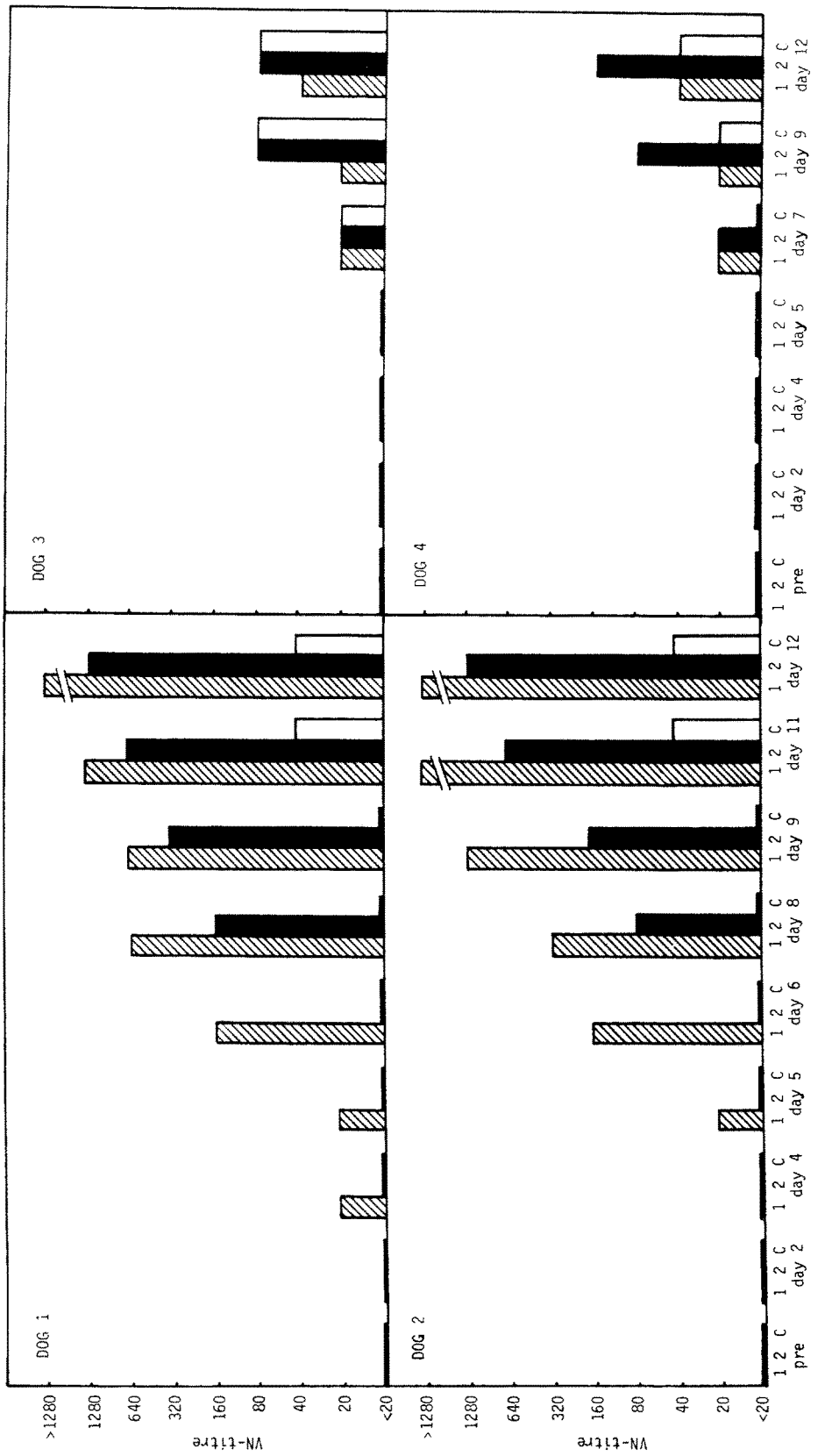

Fig. 4. Development of VN antibody titres directed against PDV-1 (▨), PDV-2 (■) and CDV strain Bussel (□) in SPF dogs #1 and #2 (**a**), and SPF dogs #3 and #4 (**b**), inoculated with organ materials from Dutch and Siberian seals respectively

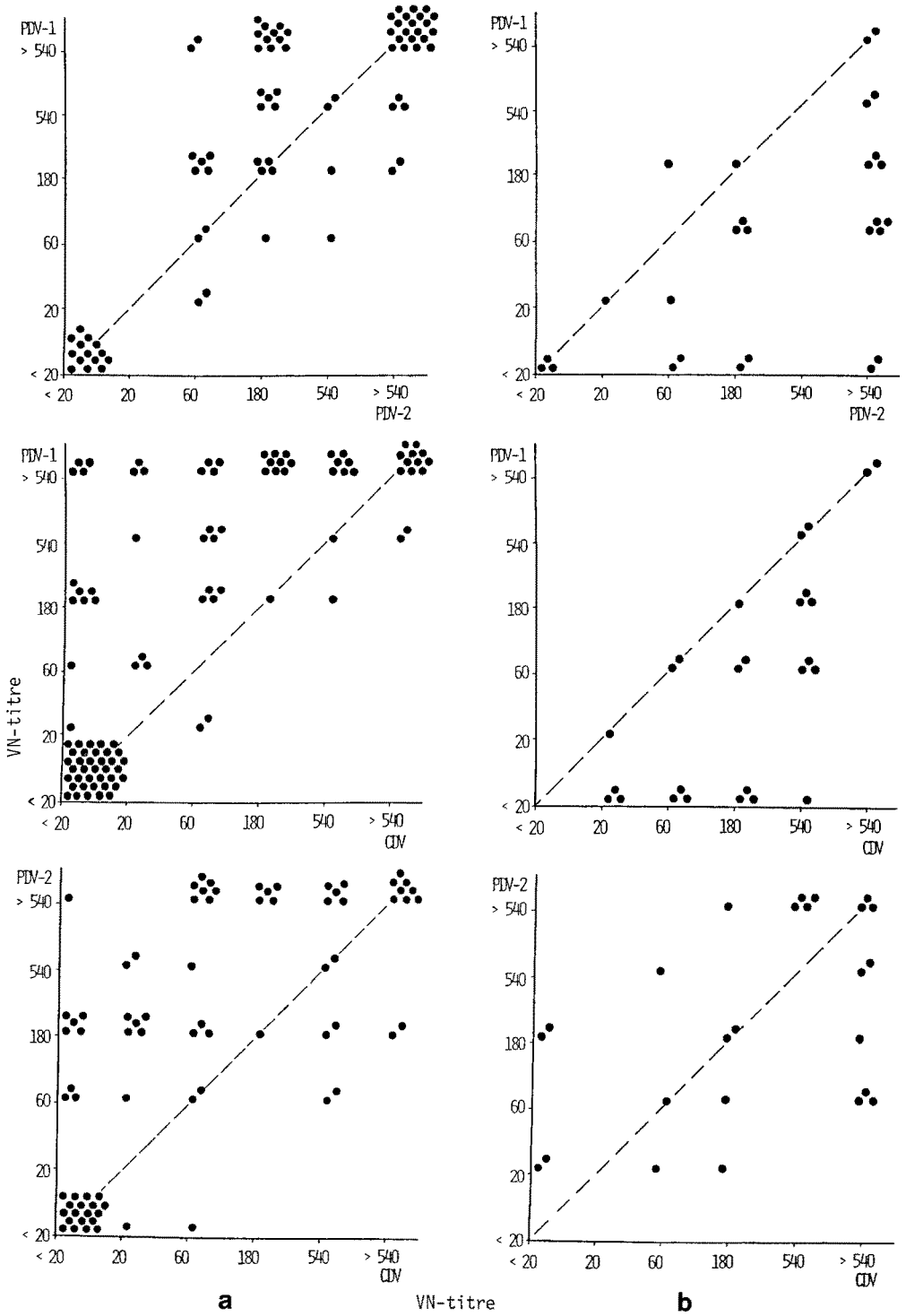

Fig. 5. Comparison of VN antibody titres of serum samples of seals from **a** North West Europe and from **b** the Siberian Lake Baikal against PDV-1, PDV-2, and CDV strain Bussel

Table 3. Reactivities of MoAbs, raised against CDV strain Convac, in antibody ELISA with CDV, PDV-1 or PDV-2 infected Vero cells

MoAbs	Specificity	CDV	PDV-1	PDV-2
3.564	NP	+	−	+
3.662	NP1	+	−	+
3.721	NP1	+	−	+
3.755	NP	+	+	+
3.805	NP3	+	+	+
3.851	NP	+	+	+
3.958	NP4	+	+	+
3.991	NP5	+	−	+
4.100	NP7	+	+	+
4.271	NP	+	+	+
3.568	P2	+	+	+
3.630	P	+	−	+
3.695	P3	+	−	−
3.768	P	+	+	+
3.780	P4	+	+	+
3.788	P	+	+	+
4.051	P5	+	−	+
4.088	P6	+	+	+
4.149	P	+	+	+
4.174	P1	+	+	+
3.551	F2	+	+	+
3.584	F2	+	+	+
3.633	F1	+	+	+
3.697	F2	+	+	+
4.068	F2	+	+	+
4.985	F3	+	−	+
5.086	F1	+	+	+
5.148	F3	+	+	+
1.347	H1	+	+	+
2.267	H2	+	−	+
3.734	H3	+	−	+
3.775	H4	+	−	−
3.900	H3	+	−	+
4.074	H5	+	+	+
4.275	H6	+	−	−
4.941	H7	+	−	+

\+ Reactivity
− No reactivity

serum antibody titres of the European seals were generally higher against PDV-1 than against PDV-2 and CDV [16], whereas the titres in these sera against PDV-2 were generally higher than those against CDV (Fig. 5a). The antibody titres in the sera from Siberian seals were generally higher against PDV-2 and CDV, than those against PDV-1 (Fig. 5b). Antigenic differences between PDV-1 and PDV-2 were further studied by comparing their reactivities in indirect ELISA with a panel of CDV-reactive monoclonal antibodies (Table 3). All the 18 MoAbs raised against the NP and the F proteins of CDV cross-reacted with PDV-2. One out of the ten P-specific and two out of the eight H-specific MoAbs did not react with this virus. Three out of the ten NP-specific, three out of the ten P-specific, one out of the eight F-specific, and six out of the eight H-specific MoAbs did not react with PDV-1.

Discussion

In the present paper we have compared several properties of PDV-1 and PDV-2, which we had recently identified as the primary causes of severe outbreaks of distemper in seals in North West Europe and Siberia respectively [3, 5, 9–12, 15–17, 23–27, 29, 30, 35]. Upon experimental infection with organ material from European or Siberian seals, SPF dogs developed CDV neutralizing antibodies and clinical symptoms similar to those observed in SPF dogs infected with the pathogenic Schneider Hill strain of CDV [7]. Since in the SPF dogs no antibodies were found against other viruses isolated from seals during the outbreaks (unpubl. obs.). PDV-1 and PDV-2 were indeed considered to have caused the symptoms observed in the SPF dogs [25, 29]. In vitro isolation of PDV-1 and PDV-2 in canine macrophages and subsequently in Vero, canine, and seal cells, enabled us to further characterize and compare the two viruses. On the basis of serological cross-reactivities with CDV in VN tests, IFAs and ELISAs, electron microscopical analysis, buoyant densities of 1.21–1.22 g/cm^3, and cythopathic changes caused in several cell culture systems, both viruses could be identified initially as morbilliviruses closely related to CDV [1, 2, 8, 13, 18, 20, 31, 32]. Also the electrophoretic mobilities of four of the viral proteins of PDV-1 and PDV-2 were within the range observed with other morbilliviruses. The multiple bands observed in the lanes identifying the M_r of NP and P proteins can be explained by their susceptibility to proteolysis as described [31]. More detailed analysis of antigenic properties of PDV-1 and PDV-2 was carried out by comparing serological responses of experimentally infected SPF dogs and naturally infected seals in VN assays with these two viruses and CDV. The observation that the dogs infected with PDV-1 showed faster and higher antibody responses to PDV-1 than against the two other viruses and the dogs infected with PDV-2 developed similar responses to all the three viruses, was the first indication for biological differences between PDV-1 and PDV-2. Similar observations were made in naturally infected seals: seropositive European seals had generally developed higher VN titres to PDV-1 than to

PDV-2 and CDV, whereas seropositive Siberian seals had generally developed higher titres to PDV-2 and CDV than to PDV-1. This closer relatedness of PDV-2 to CDV was further demonstrated by the cross-reactivities of MoAbs raised against four CDV proteins, with the respective proteins of PDV-2 in ELISA. Only one out of the ten P-specific and two out of the eight H-specific MoAbs did not react in ELISA with PDV-2, whereas all the other 33 MoAbs against the different proteins of CDV did recognize PDV-2. On the basis of these data and observations with a similar panel of MoAbs in IFA [30], it may be concluded that PDV-2 is closely related if not identical to CDV. Also different CDV strains show different patterns in the reactivities of their proteins with these H- and P-specific MoAbs [32]. The differences observed in the polyclonal antibody responses of dogs and seals against PDV-1 on the one hand and PDV-2 and CDV on the other hand, was in agreement with the finding that fewer of the MoAbs reacted with PDV-1 than with the other two viruses. The conclusion that PDV-2 is closely related if not identical to CDV, is in line with the observation at the Lake Baikal that the disease outbreak in seals coincided with a similar outbreak in dogs in the same area [9]. They also indicate that, in contrast to our previous speculations [9, 29], no epizootological link has existed between the disease outbreaks in Europe and Siberia. Further proof that the two morbilliviruses isolated from seals, and probably also a third, which we have recently demonstrated to have infected European seals before the outbreaks in Europe and Siberia took place [28], are indeed different viruses, should come from analysis of their nucleic acid sequences.

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