Canine distemper virus ISCOMs induce protection in harbour seals (*Phoca vitulina*) against phocid distemper but still allow subsequent infection with phocid distemper virus-1

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A candidate canine distemper virus (CDV) ISCOM vaccine has been shown to be effective in protecting harbour seals (Phoca vitulina) *from phocid distemper in 1988. However, of the 35 harbour seals receiving this vaccine upon admission to a seal rehabilitation and research centre (Pieterburen, The Netherlands) in 1989, six developed mild inflammatory symptoms of the respiratory tract. Phocid distemper virus-1 (PDV-1) could be isolated from three of these animals. This indicates that the vaccine affords protection from phocid distemper, but may still allow PDV-1 infection of the respiratory tract. Contacts with non-vaccinated seals should then be prevented until no more virus is excreted. It is speculated that this PDV-1 infection of the respiratory tract in CDV-ISCOM vaccinated seals is followed by a lifelong immunity.*

Keywords: Phocid distemper virus; canine distemper virus; ISCOMs; seal; vaccination

During recent disease outbreaks among seals in North West Europe and Siberia in which many thousands of harbour seals (*Phoca vitulina*) and Baikal seals (*Phoca sibirica*) died with symptoms similar to those observed in canine distemper, two different morbilliviruses – phocid distemper virus-1 (PDV-1) and phocid distemper virus-2 (PDV-2), respectively – were shown to be the primary cause of the outbreaks[1–3]. The viruses were isolated and subsequently characterized on the basis of their biological, morphological, physical, protein chemical and antigenic properties. PDV-1 proved to be a newly recognized member of the genus Morbillivirus, whereas PDV-2 was quite similar if not identical to canine distemper virus (CDV)[3].

In spite of the antigenic differences between PDV-1 and CDV, it was shown in a vaccination–challenge experiment that harbour seals could be protected from phocid distemper by vaccination with a candidate subunit ISCOM vaccine, based on CDV[4,5]. In this experiment the sham vaccinated animals developed signs of distemper, including fever, respiratory distress, weight loss and nervous symptoms and died following challenge with PDV-1.

Preventive vaccination with the CDV-ISCOM preparation was then routinely implemented in several captive seal groups including animals in the Seal Rehabilitation and Research Centre (SRRC) in Pieterburen (The Netherlands). Upon arrival at the SRRC all animals were subjected to a three dose CDV-ISCOM vaccination protocol[5]. In the 4 months before this preventive vaccination was implemented, more than 90% of all the seals admitted to the SRRC had died from phocid distemper. It was shown that virtually all animals which were CDV-seronegative at arrival had developed morbillivirus-specific virus neutralizing (VN) antibody titres after completion of the vaccination procedure. They all remained free from clinical signs of phocid distemper, indicating that the vaccination had been successful[5]. Survival rates of animals that showed serological evidence of PDV-1 infection upon admission, did not exceed 30% after using the same vaccination schedule, indicating that postexposure vaccination was far less effective[5].

In 1989, the year following the massive phocid distemper epidemic in North West Europe, 35 harbour seals were admitted to the SRRC. Each animal was

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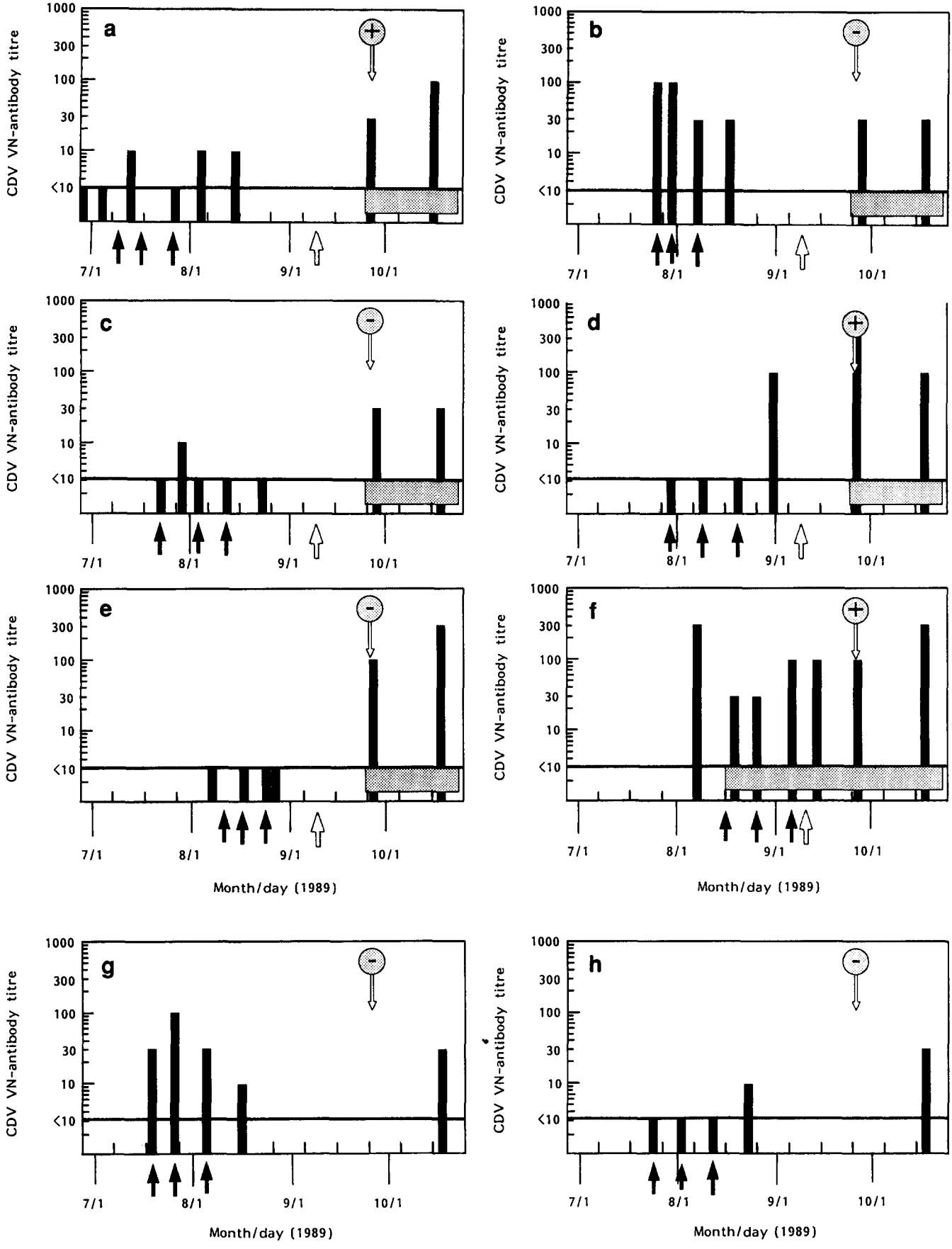

Figure 1 Levels of VN-antibody serum titres to CDV (▮) and presence of mild respiratory symptoms (▨) in seals at different times after introduction in the SRRC. Seals nos 89-21 (a), 89-27 (b), 89-28 (c), 89-30 (d), 89-33 (e) and 89-34 (f) were kept in the same basin. Seals nos 89-26 (g) and 89-29 (h) are two of the non-affected animals kept in another basin. The moment of introduction in the SRRC coincides with the first sampling for CDV serology. ↑, Vaccination with CDV-ISCOM; ⇧, introduction of seal no. 89-34 into the group of five seals developing clinical symptoms; ⊖, demonstration of PDV-1 in swab from nose and pharynx; ⊕, no demonstration of virus in swab from nose and pharynx

Table 1 Reactivity of monoclonal antibodies (mAbs) raised against canine distemper virus (CDV, Convac strain[a]) and phocid distemper virus (PDV[-1], reference strain[a]) with Vero cell lysates infected with PDV isolated from seals nos 89-21, 89-30 and 89-34 as tested in an indirect ELISA[a]

		Virus strain/isolate[b]				
mAb no.	Raised[a] against	CDV (Convac)	PDV-1 ref. strain	PDV no. 89-21	PDV no. 89-30	PDV no. 89-34
3.564	CDV NP	+	–	–	–	–
3.662	CDV NP1	+	–	–	–	–
3.721	CDV NP1	+	–	–	–	–
3.755	CDV NP	+	+	+	+	+
3.805	CDV NP2	+	+	+	+	+
3.851	CDV NP	+	+	+	+	+
3.958	CDV NP3	+	+	+	+	+
3.991	CDV NP4	+	–	–	–	–
4.100	CDV NP5	+	+	+	+	+
4.271	CDV NP	+	+	+	+	+
1.064C5	PDV NP1	–	+	+	+	+
1.069G2	PDV NP2	–	+	+	+	+
1.071E2	PDV NP1	–	+	+	+	+
1.295F5	PDV NP	+	+	–	+	–
3.568	CDV P2	+	+	+	+	+
3.630	CDV P	+	–	–	–	–
3.695	CDV P3	+	–	–	–	–
3.698	CDV P1	+	+	NT	NT	NT
3.768	CDV P	+	+	–	+	–
3.780	CDV P4	+	+	–	+	–
3.788	CDV P	+	+	+	+	+
4.051	CDV P5	+	–	–	–	–
4.088	CDV P6	+	+	–	+	–
4.149	CDV P1	+	+	+	+	+
4.415	CDV P1	+	+	+	+	+
4.174	CDV P1	+	+	+	+	+
1.347	CDV H1	+	+	+	+	+
2.267	CDV H2	+	–	–	–	–
3.734	CDV H3	+	–	–	–	–
3.775	CDV H4	+	–	–	–	–
3.900	CDV H3	+	–	–	–	–
4.043	CDV H5	+	+	+	+	+
4.074	CDV H5	+	+	+	+	+
4.275	CDV H6	+	–	–	–	–
4.941	CDV H7	+	–	–	–	–
1.062G5	PDV H1	–	+	+	+	+
1.063C3	PDV H1	–	+	+	+	+
1.063E9	PDV H1	–	+	+	+	+
1.067E5	PDV H2	–	+	+	+	+
1.068F2	PDV H3	–	+	+	+	+
1.069D9	PDV H1	+	+	+	+	+
1.070B5	PDV H4	–	+	+	+	+
1.071E5	PDV H1	–	+	+	+	+
1.072C4	PDV H4	–	+	+	+	+
1.085C4	PDV H5	–	+	+	+	+
1.122D11	PDV H6	+	+	+	+	+
3.551	CDV F2	+	+	+	+	+
3.584	CDV F2	+	+	+	+	+
3.633	CDV F1	+	+	+	+	+
3.697	CDV F2	+	+	+	+	+
4.068	CDV F2	+	+	+	+	+
4.985	CDV F3	+	–	–	–	–
5.086	CDV F1	+	+	+	+	+
5.148	CDV F3	+	+	+	+	+
1.062E2	PDV F1	+	+	+	+	+
1.067D2	PDV F2	+	+	+	+	+
1.068B2	PDV F1	+	+	+	+	+
1.062G	PDV F3	+	+	+	+	+

[a]NP1–5, nucleoprotein sites 1–5; P1–6, polymerase protein sites 1–6; H1–7, haemagglutinin protein sites 1–7; F1–3; fusion protein sites 1–3.
[b]+ Reactivity in indirect ELISA; – no reactivity in ELISA; NT, not tested

routinely checked for the presence of morbillivirus-specific antibodies upon arrival. Subsequently the animals were kept in quarantine for at least 3 weeks and both the seronegative ($n = 20$) and the seropositive ($n = 15$) seals were vaccinated thrice with the CDV-ISCOM preparation[5]. Four of the seronegative and 13 of the seropositive seals were pups born in 1989. After the quarantine period and completion of the vaccination schedule, five of these pups, one (no. 89-27) which was originally seropositive and four which were originally seronegative (nos 89-21, 89-28, 89-30, 89-33), were gathered in one basin. Seven to thirteen weeks after

admission they almost simultaneously developed mild respiratory symptoms, including a paroxysmal cough, serous nasal discharge and a conjunctivitis that persisted for more than 2 weeks. This occurred 5–8 weeks after completion of the three-dose vaccination procedure (*Figure 1*), which was about 2 weeks after the introduction of a sixth seal pup (no. 89-34) into this group of five. This animal, which had also received the standard treatment described above, had been seropositive upon admission, had shown mild respiratory symptoms from the second week onward, but in spite of this was introduced into the group of five.

Standard virus isolation procedures performed with swabs taken from the nose and pharnyx of these six affected and of five non-affected seal pups (nos 89-26, 89-29, 89-35, 89-37 and 89-44) kept in another basin were carried out in primary seal kidney cell (SeKC) cultures with subsequent passaging in Vero cells as previously described[3]. The swabs were taken between 3 and 9 days after the first appearance of clinical signs in the five affected seal pups. Virus infection was demonstrated in the Vero cells inoculated with supernatants from SeKC cultures inoculated with swab materials taken from seal no. 89-34 and from seals no. 89-21 and no. 89-30 by the appearance of typical cytopathic changes and by the presence of virus particles in negative contrast electron microscopy. The three virus isolates were identified as PDV-1 by determining their reactivities with a panel of morbillivirus-specific monoclonal antibodies in enzyme-linked immunosorbent assays (ELISA) and immuno-fluorescence assays (IFA) as shown in *Table 1*[3,5,8,9]. The presence of PDV-1 nucleic acid in RNA extracts from these cultures was shown by Southern blot analysis after amplification of part of the polymerase-associated (P) protein gene of the virus in a polymerase chain reaction using a set of PDV-1 specific primers[6,7]. No virus could be isolated using the same procedure, either from the pups in the control group, or from the three other pups which displayed mild respiratory symptoms. The development of CDV-specific VN antibodies in the six seal pups which displayed respiratory symptoms and in two of the five seal pups which remained healthy – one seropositive and one seronegative upon admission – are given in *Figure 1*. After completion of the vaccination schedule, these animals had all developed CDV-specific VN antibodies. Seals nos 89-21, 89-27, 89-28, 89-30 and 89-34 had developed a titre of 30 and seal no. 89-33 a titre of 100. After the onset of the disease the antibody titres of affected seals nos 89-27, 89-28 and 89-30 remained the same (titre = 30), whereas those of affected seals no. 89-21 and no. 89-33 showed a threefold increase. Since in the two non-affected seals (no. 89-26 and no. 89-29) a threefold increase in titre was also observed, it could not be concluded whether this titre rise in the affected animals was related to the observed infection with PDV-1.

From these and our previous data we conclude that vaccination with the candidate CDV-ISCOM vaccine does induce CDV-neutralizing antibodies and protection against the serious disease known as phocid distemper. However, this heterologous vaccination does not induce complete protection against replication of PDV-1 in the respiratory tract, which may apparently result in mild upper respiratory illness. This finding is in agreement with results obtained in previously described protection experiments using other heterologous morbillivirus vaccines. Appel *et al.*[10] demonstrated that heterologous vaccination of dogs with live attenuated measles vaccines also induces partial protection against canine distemper. Similarly, we have shown that CDV-ISCOMs induce complete protection in dogs against CDV infection and distemper, whereas measles virus (MV)-ISCOMs only induced partial protection against distemper in dogs[11]. To be able to induce complete protection against phocid distemper and PDV-1 infection by means of vaccination we have considered the development of an homologous PDV-1-ISCOM vaccine. If such a vaccine would also protect against PDV-1 infection of the respiratory tract, this would prevent the threat to non-vaccinated seals imposed by temporary spread of PDV-1. It could, however, be argued that the incomplete but effective protection against phocid distemper afforded by the heterologous CDV vaccine is preferable since it would still allow subsequent infection with PDV-1, resulting in mild upper respiratory disease followed by lifelong immunity, similar to the immunity seen after recovery from other morbillivirus infections. Contacts with non-vaccinated seals should then be prevented until no more virus is excreted.

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REFERENCES

1 Visser, I.K.G., Teppema, J.S. and Osterhaus, A.D.M.E. Virus infections of seals and other pinnipeds. *Rev. Med. Microbiol.* 1991, **2**, 105–114

2 Osterhaus, A.D.M.E., *et al.*, and Grachev, M.A. *et al.* Distemper virus in Baikal seals. *Nature* 1989, **338**, 209–210

3 Visser, I.K.G., Kumarev, V.P., Örvell, C., De Vries, P., Broeders, H.W.J., Van de Bildt, M.W.G. *et al.* Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in North West Europe and Siberia. *Arch. Virol.* 1990, **111**, 149–164

4 Osterhaus, A.D.M.E., Visser, I.K.G., Vedder, E.J., Reijnders, P.J.H., Kuiper, J. and Brugge, H.N. Seal vaccination success. *Nature* 1989, **337**, 21

5 Visser, I.K.G., Van de Bildt, M.W.G., Brugge, H.N., Reijnders, P.J.H., Vedder, E.J. *et al.* Vaccination of harbour seals (*Phoca vitulina*) against phocid distemper with two different inactivated canine distemper virus (CDV) vaccines. *Vaccine* 1989, **7**(6), 521–526

6 Barrett, T., Crowther, J., Osterhaus, A.D.M.E., Subbarao, S.M., Groen, J., Haas, L. *et al.* Molecular and serological studies on the recent seal virus epizootics in Europe and Siberia. *Sci. Tot. Envir.* in press

7 Haas, L., Subbarao, S.M., Harder, T., Liess, B. and Barrett. T. Detection of phocid distemper virus RNA in seal tissues using slot hybridisation and the polymerase chain reaction amplification assay: genetic evidence that the virus is distinct from canine distemper virus. *J. Gen. Virol.* 1991, **72**, 825–832

8 Örvell, C., Shesberadaran, H. and Norrby, E. Preparation and characterisation of monoclonal antibodies directed against four structural components of canine distemper virus. *J. Gen. Virol.* 1985, **66**, 443–456

9 Örvell, C., Blixenkrone-Möller, M., Svansson, V. and Have, P. Immunological relationships between phocid and canine distemper virus studied with monoclonal antibodies. *J. Gen. Virol.* 1990, **71**, 2085–2092

10 Appel, M.J.G., Shek, W.R., Shesberadaran, H. and Norrby, E. Measles virus and inactivated canine distemper virus induce incomplete immunity to canine distemper. *Arch. Virol.* 1984, **82**, 73–82

11 De Vries, P., UytdeHaag, F.G.C.M. and Osterhaus, A.D.M.E. Canine distemper virus (CDV) immune-stimulating complexes (iscoms), but not measles virus iscoms, protect dogs against CDV infection. *J. Gen. Virol.* 1988, **69**, 2071–2083