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Isolation of a virus with rhabdovirus morphology from a white-beaked dolphin (*Lagenorhynchus albirostris*)

Brief Report

A. D. M. E. Osterhaus[1], H. W. J. Broeders[2], J. S. Teppema[3], T. Kuiken[4], J. A. House[5], H. W. Vos[6], and I. K. G. Visser[6]

Laboratories of [1] Immunobiology, [2] Control Biological Products and [3] Pathology, National Institute of Public Health and Environmental Protection (RIVM) Bilthoven, The Netherlands
[4] The Zoological Society of London, Institute of Zoology, Regent's Park, London, U.K.
[5] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratory, Foreign Animal Disease Diagnostic Laboratory, Greenport, New York, U.S.A.
[6] Seal Rehabilitation and Research Centre, Pieterburen, The Netherlands

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Summary. A virus with rhabdovirus morphology which proved to be antigenically distinct from rabies virus and vesicular stomatitis virus was isolated from a dolphin that had beached on the Dutch coast. Neutralizing antibodies to this virus were found in several European marine mammal species.

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Infections with members of the *Rhabdoviridae* family cause serious disease in several animal species, the most extreme being rabies virus, which causes a fatal neurological disease in many susceptible animal species including humans (for review see [2, 4, 5]). Here we describe the identification of a virus with rhabdovirus morphology in a white-beaked dolphin (*Lagenorhynchus albirostris*). This dolphin had beached in a poor condition on the Dutch island of Schiermonnikoog in the spring of 1992. It died with signs of severe dyspnea after two days of intensive care in a dolphinarium.

In an attempt to isolate a virus from this animal, 10% (w/v) tissue homogenates from lungs, kidneys, brains and lymphoid organs were used to inoculate monolayer Vero cell cultures, using standard techniques [3]. Seven days after inoculation, the cultures inoculated with the lung and kidney homogenates

exhibited focal cytopathic changes (not shown), which were not observed in the cultures inoculated with the brain and lymphoid organ homogenates. By negative contrast electron microscopy, typical rhabdovirus-like bullet-shaped particles (diameter 75–85 nm, length 125–145 nm) were observed in lysates of affected cells (Fig. 1), using standard techniques [3]. The virus, tentatively named dolphin rhabdovirus-like virus (DRV), could be passaged serially at least five times with culture fluids, in which infectivity titers of $\geqslant 10^{5.0}$ $TCID_{50}$/ml proved to be present after the second passage. DRV was shown to replicate and cause cytopathic changes in primary cell cultures derived from several animal species, including dolphins, seals, pigs, rabbits, guinea pigs and cats (not shown).

Cerebral inoculation of eight one-day-old suckling mice with $10^{4.0}$ $TCID_{50}$ DRV propagated in Vero cells, resulted in the death of these animals within five days. Subsequently, DRV could be passaged in the brains of suckling mice at least five times from which it could be re-isolated in Vero cells (not shown). Inoculation of laboratory rabbits via the respiratory route, and adult mice via the respiratory, peritoneal or cerebral routes with the same inoculum used for the suckling mice, did not cause any clinical signs, but these animals did develop DRV-specific serum antibodies within eight weeks after inoculation. These antibodies were demonstrated in a virus neutralization (VN) assay and in an immunofluorescence (IF) assay. The VN assay was carried out in a microtiter system with Vero cells, using twofold dilutions of heat-inactivated serum samples with 100 $TCID_{50}$ DRV. VN titers were defined as the reciprocals of serum dilutions giving complete inhibition of cytopathic changes. The IF assay was carried out essentially as described previously [3] using the same serum dilutions on acetone fixed DRV-infected Vero cell cultures. DRV-infected mice developed serum antibody titers of 128 to 512 and of 256 to 1024 in VN and IF assays, respectively (Table 1). These antibodies did not cross-react with rabies virus when tested in VN [6] or IF [1] assays (Table 1). Also reference sera of different

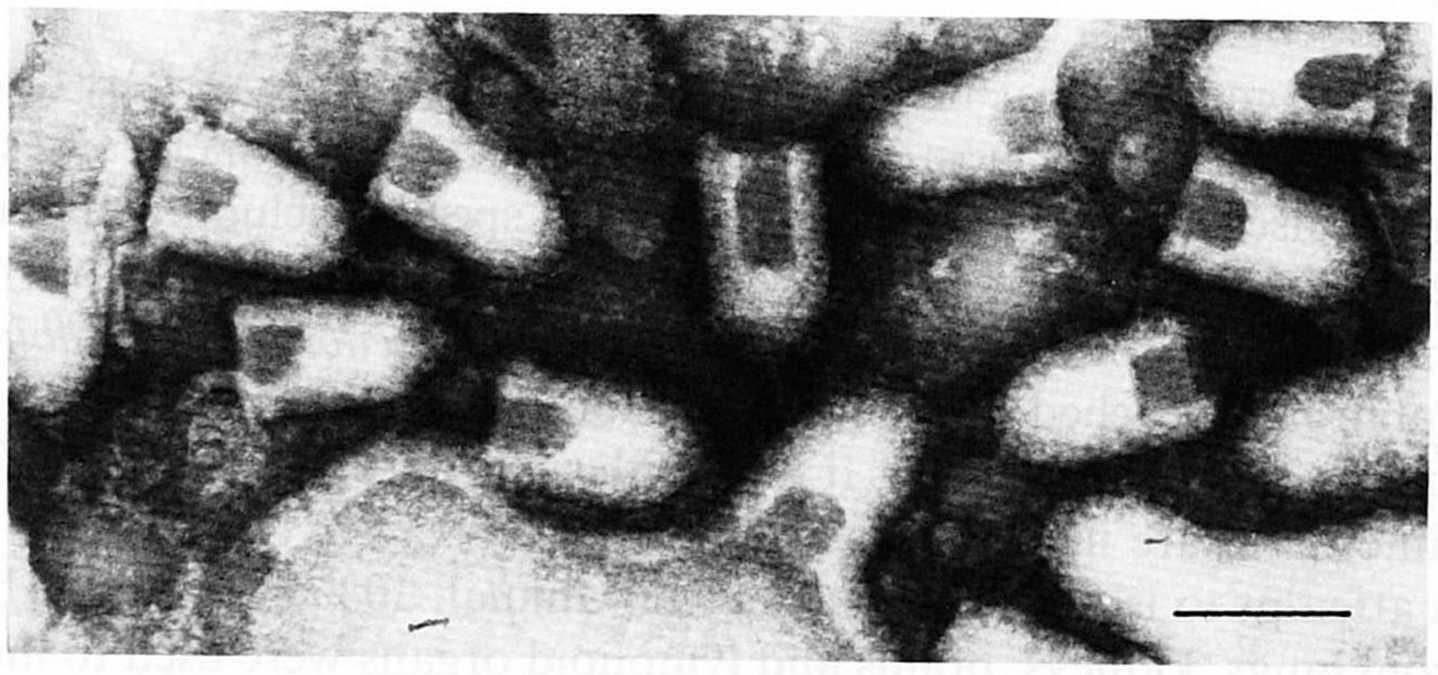

Fig. 1. Negative contrast (PTA) electron micrograph of Vero cell lysate showing rhabdovirus particles. Bar: 100 nm

Table 1. Antibody titers to DRV and RV, in sera from experimentally infected or immunized animals, measured in VN and IF assays

Rhabdovirus-specific antisera	DRV		Rabies virus	
	VN assay[d]	IF assay[d]	VN assay[d]	IF assay[d]
DRV (mouse) i.n.[a] infected (pool of 5 sera)	512	256	–[e]	–
DRV (mouse) i.p.[a] infected (pool of 5 sera)	128	512	–	–
DRV (mouse) i.c.[a] infected (pool of 5 sera)	256	1 024	–	–
DRV (rabbit) i.n. infected (pool of 2 sera)	128	64	–	–
DRV (rabbit) i.n. infected (pool of 2 sera)	128	128	–	–
Rabies virus (rabbit)[b]	–	–	> 512	256
Rabies virus (monkey)[b]	–	–	> 512	256
VSV FEV (cow)[c]	–	–	–	–
VSV Chandipura (rabbit)[c]	–	–	–	–
VSV Isfahan (rabbit)[c]	–	–	–	–
VSV Piry (rabbit)[c]	–	–	–	–
VSV Indiana 1 (guinea pig)[c]	–	–	–	–
VSV Indiana 2 (guinea pig)[c]	–	–	–	–
VSV Indiana 3 (guinea pig)[c]	–	–	–	–
VSV New Jersey (guinea pig)[c]	–	–	–	–
BEFV (cow)[c, e]	–	–	–	–

[a] *i.n.* Intranasally; *i.p.* intraperitoneally; *i.c.* intracerebrally
[b] Reference serum RIVM, Bilthoven, The Netherlands
[c] Reference serum USDA, Plum Island, U.S.A. (Dr. J. House)
[d] VN assay in Vero cells using 100 $TCID_{50}$ DRV; IF assay on acetone fixed DRV infected Vero cell cultures; rabies virus VN assay [see 5]; rabies virus IF assay [see 1]
[e] – < 8

species raised against other rhabdoviruses, like rabies virus (RIVM reference serum), different serotypes of vesicular stomatitis virus (VSV) and bovine ephemeral fever virus (BEFV) (kindly provided by Dr. J. House, USDA, Plum Island), did not cross-react with DRV in the VN and IF assays (Table 1).

A serological survey carried out amongst cetaceans and pinnipeds, which had beached or died on the coasts of northwest Europe or on the northern coasts of the Mediterranean Sea during the last five years, indicated that DRV neutralizing serum antibodies are commonly present in different species of stranded cetaceans (27 out of 64 tested; VN titers 4-256) and may also be found in seals (4 out of 83 tested; VN titers 4-32) (Table 2). In the sera of two veterinarians and two animal attendants, who had been in close contact with the stranded white-beaked dolphin from which DRV was isolated, no DRV neutralizing antibodies could be demonstrated six weeks after the animal had died.

Although an anecdotal case of rabies in a seal has been described [7], this is the first identification of a rhabdovirus that commonly infects marine mammals. The present data do not allow conclusions about the pathogenicity of

Table 2. DRV neutralizing serum antibodies in cetaceans and pinnipeds, stranded on the coasts of northwest Europe or the Mediterranean Sea, during the period 1988–1992

Species	Total number of sera	Number of sera positive (%)	Positive titer range
Cetaceans			
Stenella coeruleoalba	3	3 (100)	4–32
Delphinus delphis	19	6 (32)	8–64
Phocoena phocoena	24	7 (29)	4–32
Tursiops truncatus	6	3 (50)	4–32
Pseudorca crassidens	2	2 (100)	64–256
Globicephala melas	2	2 (100)	8
Lagenorhynchus acutus	2	0 (0)	< 4
Lagenorhynchus albirostris	6	4 (67)	8–64
Pinnipeds			
Phoca vitulina	72	1 (1)	32
Halichoerus grypus	11	3 (27)	4–8

DRV for dolphins or other marine mammals. In the light of the serious and often fatal diseases caused by rhabdoviruses in general, further studies concerning phylogenetic relationship, natural host range, mode of transmission and pathogenesis of DRV should be initiated.

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Authors' address: Dr. A. D. M. E. Osterhaus, Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, P. O Box 1, 3720 BA Bilthoven, The Netherlands.

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