

## HERPES-LIKE VIRUS IN DUSKY DOLPHINS, *LAGENORHYNCHUS OBSCURUS* FROM COASTAL PERU

M.-F. Van Bresseem,<sup>1,2</sup> K. Van Waerebeek,<sup>1</sup> A. Garcia-Godos,<sup>1</sup> D. Dekegel<sup>3</sup> and P.-P. Pastoret.<sup>2</sup>

<sup>1</sup> Centro Peruano de Estudios Cetológicos (CEPEC), casilla 1536, Lima 18, Peru.

<sup>2</sup> Department of Virology and Immunology, Faculty of Veterinary Medicine, University of Liège, B43, Local R80, Sart Tilman, 4000 Liège, Belgium.

<sup>3</sup> Department of Electron Microscopy, Pasteur Institute of Brabant, 642 rue Engeland, B-1180 Brussels, Belgium.

Herpesviruses have been described from a wide variety of animal species ranging from fishes to mammals, in which they can cause severe disease (for review, see Roizmann *et al.* 1992). The family Herpesviridae comprises three subfamilies: the Alpha-, Beta- and Gammaherpesvirinae (Roizmann *et al.* 1992). After infection, herpesviruses remain latent in their natural hosts. Periodically shedding can occur following reactivation, triggered by different factors including stress and viral or bacterial diseases (Thiry *et al.* 1986, Wyler *et al.* 1989).

Many herpesviruses are highly host specific, while others may affect a wide range of species (Bublott *et al.* 1991, Fenner *et al.* 1993). Among marine mammals they have been reported in pinnipeds and cetaceans (Osterhaus *et al.* 1985, Kennedy-Stoskopf *et al.* 1986, Martineau *et al.* 1988, Barr *et al.* 1989). Phocid herpesvirus-1 (PHV-1) was the etiological agent of a generalized disease resulting in severe necrosis of the liver and interstitial pneumonia among suckling North Sea harbor seals, *Phoca vitulina*, nursed in a seal orphanage (Osterhaus *et al.* 1985, Borst *et al.* 1986). Characterization of PHV-1 indicated that it is antigenically related to felid and canid herpesviruses and should be considered a member of the Alphaherpesvirinae (Osterhaus *et al.* 1985). Subsequent serological surveys showed that the infection by PHV-1, or an antigenically related herpesvirus, is common among several species of pinnipeds (Vedder *et al.* 1987, Stenvers *et al.* 1992). Herpesviruses were also isolated from harbor seals which died of phocid distemper virus infection during a 1988 epidemic in the North Sea (Horvat *et al.* 1989) and from a captive California sea lion, *Zalophus californianus*, and a grey seal, *Halichoerus grypus*, (Kennedy-Stoskopf *et al.* 1986, Kennedy-Stoskopf 1990). In the latter species the virus was associated with recurring ulcerative skin lesions (Kennedy-Stoskopf 1990).

In cetaceans herpesviruses were documented solely in free-ranging and captive beluga whales, *Delphinapterus leucas*, from the St. Lawrence and Churchill rivers, Canada (Martineau *et al.* 1988, Barr *et al.* 1989). In these animals the infection was recognizable macroscopically by circular areas of pale skin slightly in relief, eventually outlined by a dark rim. The skin lesions were either numerous and widely dispersed over the whole body (Martineau *et al.* 1988), or scarce and restricted to the trunk (Barr *et al.* 1989).

**Table 1.** Occurrence of skin lesions associated with herpesvirus-like particles in dusky dolphins, *Lagenorhynchus obscurus*, caught in Peruvian coastal waters.

Number	Sex	SL (cm)	TEM	Description
AGG-559	F	157,0	-	Few black points on the rostrum, perceptible by the touch.
AGG-561	M	157,0	+	Few black points on the rostrum, perceptible by the touch.
AGG-562	F	150,0	-	Many black points on the rostrum, around the eyes, on the fin and flippers, perceptible by the touch.
AGG-563	F	155,0	+	Few black points on the rostrum, perceptible by the touch.

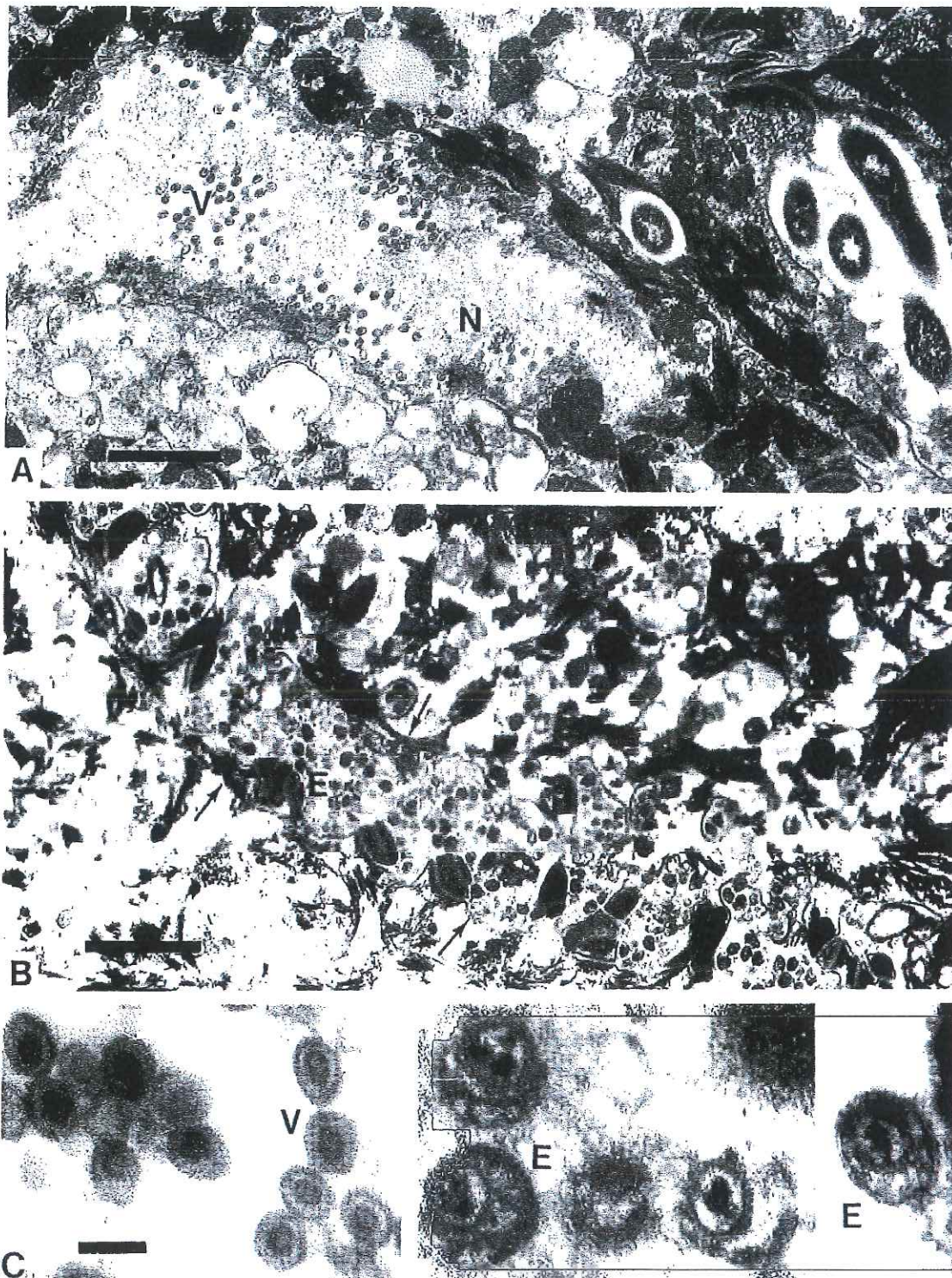
SL = standard length; TEM = examination by transmission electron microscopy; + = skinsample positive for the presence of herpesvirus-like particles; - = skinsample negative for the presence of herpesvirus-like particles.

Greenwood *et al.* (1974), Baker (1992), and Baker and Martin (1992) briefly referred to possible herpesvirus skin lesions in killer whale, *Orcinus orca*; striped dolphin, *Stenella coeruleoalba*; and harbor porpoise, *Phocoena phocoena*, the latter two species from the North Sea. However, as no attempt was made to demonstrate the presence of the virus, these observations remain to be confirmed.

Here we document skin lesions associated with herpesvirus-like particles in the dusky dolphin, *Lagenorhynchus obscurus*, from Peruvian coastal waters. Samples were collected from animals caught in the ongoing artisanal gillnet and harpoon fishery off central Peru (Van Waerebeek and Reyes 1990, in press).

While monitoring the dolphin catch at the fishing port of Ancón (11°47'S) in October 1991, skin lesions consisting of a few black dots on the beak, perceptible by the touch, were noticed in four dusky dolphins (AGG-559, -561, -562, and -563; Table 1). One of these (AGG-562), moreover, had numerous lesions dispersed all over its body, especially around the eyes, on the dorsal fin, and the flippers. The dolphins were sexually immature with standard lengths ranging between 150 and 157 cm (Table 1). It is likely that they had been caught together because they were landed by a single gillnet fishing boat. For each dusky dolphin about 1 cm<sup>2</sup> of affected skin was excised, fixed in 5% glutaraldehyde buffered with cacodylate (0.1 M, pH 6.8), and further processed for examination by transmission electron microscopy (TEM) using standard procedures (Glauert 1975). Briefly, the skin samples were postfixed with osmium tetroxide (OsO<sub>4</sub>) 1%, stained with uranyl acetate, and embedded in a mixture of Epon and Spurr resin. Semithin sections (2 μm) were examined with a Reichert anoptical phase contrast microscope to localize tissues. Ultrathin sections obtained with a Reichert ultracut microtome were stained with lead citrate and subsequently examined with a Siemens 102 transmission electron microscope (Berlin, Germany) at 100 kV.

**Figure 1.** Transmission electron micrographs of herpesvirus-like particles in ultrathin sections of epithelial cells of a dusky dolphin . A: naked virions (V) in the nucleus (N) of an epithelial cell. Bar = 1  $\mu\text{m}$ . B: accumulation of enveloped virions (E) in the intercellular space (arrows) between epithelial cells. Bar = 1  $\mu\text{m}$ . C: high magnification of intranuclear virions (V) and enveloped virions (E). Bar = 0,1  $\mu\text{m}$ .



Numerous naked capsids (diameter 90-100 nm), empty or containing a core, were observed in the nucleus of the epithelial cells of two dusky dolphins (AGG-561 and AGG-563) (Fig. 1A, C). Several of these showed a hexagonal outline. Enveloped capsids (150-250 nm) were detected in the cytoplasm and the inter- and extracellular space (Fig. 1B, C). These morphological features are compatible with herpesviruses. The nucleus of the affected cells displayed margination of the heterochromatin (see Fig. 1A). No virus was detected in the skin lesions of the two remaining dolphins (AGG-559 and AGG-562), which may be explained by a possible convalescent stage of the lesion in which few or no viruses remained. Apart from the herpes-like lesions, dolphin AGG-559 bore on its head a circular dark skin mark known as a "tattoo" (see Geraci *et al.* 1979, Flom and Houk 1979) caused by a poxvirus. The latter was demonstrated by TEM.

As in the beluga whales, the herpes-like virus infection in the dusky dolphins showed a skin tropism, although the lesions were quite different (see above). The virus did not seem to be more than mildly pathogenic since there was no apparent evidence of poor health. The clinical signs were reminiscent of infection by members of the Alphaherpesvirinae as, for example, herpesvirus simplex 1 (HSV-1) and varicella zoster (VZV) of humans, or bovine herpesvirus 2 (BHV-2). However, antigenic and molecular characterization is necessary to classify this virus.

The dusky dolphin is a highly sociable species which may form durable bonds between individuals over long periods of time and which can congregate in supergroups of 700-800 animals (Wiirsig and Wiirsig 1979, Wiirsig and Bastida 1986, Van Waerebeek 1992). Those habits make it especially vulnerable to contagious diseases. The young individuals studied presumably were members of a herd in which the herpesvirus may have been enzootic with infection occurring via an animal shedding the virus. The high levels of fishery mortality, with concomitant disruption of herd structure, is bound to create considerable stress among survivors from gillnet or harpoon encounters, which in turn may contribute to the reactivation and subsequent shedding of a latent herpesvirus. Further studies should yield more detailed information on the factors that influence the dynamics of transmission and the pathogenesis of the infection by this virus.

So far, a poxvirus and a herpes-like virus have been detected in skin lesions of free-ranging dolphins off Peru (Van Bresse *et al.* 1993, this paper). The prevalence of these and possibly other viruses in dolphins and porpoises in the South Pacific deserves further attention and is the subject of present studies.

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