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Morbillivirus threat to Mediterranean monk seals?

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LAST year several hundreds of striped dolphins *(Stenella coeruleoalba)* died during an outbreak of an infectious disease in the Mediterranean Sea. Based on virus isolations (Domingo and others 1990, Van Bressem and others 1991) and on post mortem findings, which were similar to those observed in seals infected with phocid distemper viruses (PDV-1 and PDV-2) (Osterhaus and others 1990), the primary cause of the disease in *S coeruleoalba* was supposed to be infection with a morbillivirus. It was widely speculated that transmission of PDV-1 from seals to the dolphins had taken place and that this virus would constitute a serious risk to the highly endangered species of Mediterranean monk seal *(Monachus monachus)* (Domingo and others 1990). This fear was even more substantiated when the present authors demonstrated morbillivirus antigen and nucleic acid in organs from two hooded seals *(Cystophora cristata)* found dead on the coasts near the Spanish cities of Huelva and Tarifa in the same period (unpublished observation).

To evaluate biological differences between the morbilliviruses of aquatic mammals, the authors first studied the antigenic relationship between PDV-1, PDV-2 and two morbilliviruses which were isolated from striped dolphins that died during the outbreak. To this end they used an enzyme-linked immunosorbent assay with a selected panel of morbillivirus-specific monoclonal antibodies raised against canine distemper virus (CDV) and PDV-1 (Örvell and others 1990). Lysates of infected Vero cell cultures were coated on microtitre plates and the mouse monoclonal antibodies tested in a standard dilution for their reactivities with the viral antigens, using an anti-Ig conjugate. OD450 nm values exceeding twice the mean background obtained with non-infected culture lysates were considered positive. In this analysis the authors also included two viruses which were isolated from two harbour porpoises *(Phocoena phocoena)* stranded on the Dutch coast before the Mediterranean outbreak among *S coeruleoalba* took place (unpublished observation). It has been reported that a morbillivirus infection was present in porpoises during the PDV-1 outbreak among seals in north west Europe (Kennedy and others 1988). The present authors provisionally named the virus isolated from *S coeruleoalba*, dolphin morbillivirus (DMV) and the virus isolated from the *P phocoena*, porpoise morbillivirus (PMV). As shown in Table 1, several of the epitopes studied in this way were shared between the five morbilliviruses. From these data it was concluded that the DMV and the PMV isolates are quite similar if not identical viruses, distinct from PDV-1 and PDV-2. These findings also indicate that different clusters of morbilliviruses were responsible for the infections of the seals and of the cetacean species.

In order to assess the risk of transmission of DMV and other morbilliviruses to *M monachus*, the present authors analysed the susceptibility of Concanavalin-A (Con-A) stimulated peripheral blood mononuclear cells (PBMC) from three species of aquatic mammals: the harbour seal *(Phoca vitulina)*, *M*

TABLE 1: Reactivities of canine distemper virus (CDV) specific monoclonal antibodies (MoAbs) with morbillivirus antigens in an indirect ELISA (Örvell and others 1990)

MoAb number	Raised to	Reactivity	Virus CDV	PDV-1	PDV-2	DMV (n=2)	PMV (n=2)
3·662	CDV	NP1	+	−	+	−	−
3·805	CDV	NP2	+	+	+	−	−
3·958	CDV	NP3	+	+	+	+	+
3·991	CDV	NP4	+	−	+	−	−
4·100	CDV	NP5	+	+	+	−	−
1·064C5	PDV	NP1	−	+	NT	−	−
1·069G2	PDV	NP2	−	+	NT	−	−
4·149	CDV	P1	+	+	+	+	+
3·568	CDV	P2	+	+	+	+	+
3·695	CDV	P3	+	−	−	−	−
4·051	CDV	P5	+	−	+	−	−
4·088	CDV	P6	+	+	+	+	+
1·347	CDV	H1	+	+	+	−	−
2·267	CDV	H2	+	−	+	−	−
3·734	CDV	H3	+	−	+	−	−
3·775	CDV	H4	+	−	−	−	−
4·074	CDV	H5	+	+	+	−	−
4·275	CDV	H6	+	−	−	−	−
4·941	CDV	H7	+	−	+	−	−
1·062G5	PDV	H1	−	+	NT	−	−
1·067E5	PDV	H2	−	+	NT	−	−
1·068F2	PDV	H3	−	+	NT	−	−
1·070B5	PDV	H4	−	+	NT	−	−
1·085C4	PDV	H5	−	+	NT	−	−
1·122D11	PDV	H6	+	+	NT	−	−
3·633	CDV	F1	+	+	+	+	+
3·584	CDV	F2	+	+	+	−	−
5·148	CDV	F3	+	+	+	−	−
1·062E2	PDV	F1	+	+	NT	−	−
1·068B2	PDV	F2	+	+	NT	+	+
1·092C3	PDV	F3	+	+	NT	+	+

\+ Positive reaction
− Negative reaction
PDV Phocid distemper virus
DMV Dolphin morbillivirus
PMV Porpoise morbillivirus
NT Not tested

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TABLE 2: Susceptibility of Concanavalin-A-stimulated peripheral blood mononuclear cells (PBMC) from different morbillivirus seronegative animals to infection with different morbilliviruses

Virus	Con-A stimulated PBMC from harbour seal	bottlenosed dolphin	Mediterranean monk seal
MV	+	NT	−
CDV	+	NT	+
PDV-1	+	NT	+
PDV-2	+	NT	+
DMV (n=2)	−	+	−
PMV (n=2)	+	+	+

+/− Presence/absence of morbillivirus infection in PBMC, as judged from antigen detection in immunofluorescence by fluorescence activated cell sorter analysis, with the broadly reactive F-specific monoclonal antibody F3-5 (De Vries and others 1990), seven days after inoculation with a multiplicity of infection of 10^{-2} to 10^{-3} $TCID_{50}$ per cell
MV Measles virus
CDV Canine distemper virus
PDV Phocid distemper virus
DMV Dolphin morbillivirus
PMV Porpoise morbillivirus
NT Not tested

monachus and the bottlenosed dolphin *(Tursiops truncatus)*, to infection with each of these morbilliviruses.

PBMC was chosen as the cell substrate, since these are natural target cells for the viruses in vivo. PBMC were stimulated with Con-A for two days and the cells were subsequently expanded by culturing in the presence of human interleukin-2 (IL-2). After seven to 14 days the cells were restimulated with Con-A in the presence of γ-irradiated (1800 Rad) PBMC of specific pathogen-free dogs. After three days non-vital cells were removed by density gradient centrifugation on a Ficoll-Isopaque gradient and the resulting cell populations were used for virus replication studies. For this purpose the virus suspensions were absorbed on to the cells for two hours at 37°C, which were then cultured in the presence of human IL-2. Virus replication was monitored by demonstrating viral antigen in an immunofluorescence assay using the F-specific broadly cross-reactive monoclonal antibody F3-5 raised against measles virus (MV) (De Vries and others 1990). Table 2 shows that most of the five morbilliviruses tested infected more than one of the cell substrates. However, in contrast to the two PMV isolates, the two DMV isolates could not be shown to infect PBMC from *M monachus* and *P vitulina* under these conditions.

These data suggest that *M monachus* and *P vitulina* may be less susceptible to infection with DMV than *S coeruleoalba*. However, since cells of this species can be infected by other morbilliviruses (Table 2), and the possibility of infection of this species by DMV cannot be ruled out on the basis of these data, the authors have started to assess the potential of a candidate CDV iscom vaccine, successfully used in *P vitulina* in the past (Osterhaus and others 1990), to prevent morbillivirus infections in *M monachus*.

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Welfare implications of ultrasonic flea collars

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ULTRASONIC flea collars for cats and dogs are relatively new to the pet-care market. A small sound generator on a collar is directed at the ground so that the reflected sound waves produce an ultrasonic environment around the animal. Manufacturers claim that this environment drives fleas away but is not detectable by, and is harmless to, the pets. They do not all specify the frequencies produced but one, Microtech-2, is reported to emit sound at 40 kHz (Koehler and others 1989).

There is little evidence, however, that these devices affect fleas. Oriental rat fleas (*Xenopsylla cheopsis*) and cat fleas (*Ctenocephalides felis*) are not repelled by them and mating, oviposition, development, locomotor behaviour, orientation and circadian rhythms of behaviour are apparently not affected (Koehler and others 1986, 1989, Rust and Parker 1988, Dryden and others 1989). It is probable that the pets can hear the sounds. Hearing ranges extend up to at least 70 kHz in cats (Heffner and Heffner 1985), dogs (Heffner 1983) and hamsters, gerbils, mice and rats (Fay 1988).

This paper describes a study of the acoustic characteristics of two collars, to determine their frequency and the sound field they produce around the body, and a preliminary investigation of the effects of these devices on dogs.

The acoustic characteristics of the Microtech-2 and Flearepeller collars were determined from tape-recordings made at 30 inches per second (ips) on a Racal Store 4DS recorder and replayed at 3·75 ips into a Kay Sonograph which allows the frequency and duration of the signals to be measured. Sound pressure levels (in dB re 2×10^{-5} Pa RMS) were measured using a Bruel and Kjaer 0·25 inch microphone and amplifier calibrated to give a known output in millivolts for a given sound pressure level at the microphone, together with an oscilloscope.

To avoid using live animals for measurements of the sound field, a collar was attached to a large soft toy held either 10 cm or 35 cm above a reflecting surface, to represent a cat or a dog, respectively. Measurements were taken at different points on the body and over a hard and soft surface. Measurements of sound pressure level vary with the exact positions of the sound source and microphone and depend on reflections from nearby surfaces such as furniture and walls; they would be difficult to measure on a live animal. Occasionally the reflected waves add to the emitted waves to enhance the recorded sound pressure levels, as appears to have happened in a few cases here. Measurements were made over a period of 10 seconds, and the sound pressure levels recorded for most of this time are given in Table 1. Readings around the neck were repeated at the end of the session and were within 4·5 dB of the original readings.

The Microtech-2 produced 50 kHz pulses about 12·5 ms in duration and 8 ms apart. The Flearepeller produced 1 ms pulses, alternately 6 and 8 ms apart, with the major energy at 40 kHz, but with a range from 30 to 50 kHz. The sound level produced by the Flearepeller often fell below the limits of detection of the equipment, so that readings were not always possible.

The effect of the ultrasound emissions from the Microtech-2 on the behaviour of various species was recorded when one of the collars was placed 1 m away from the animals. Rats and

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