Morbillivirus threat to Mediterranean monk seals?


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seals (Cystophora cristata) found dead on the coasts near the Spanish cities of Huelva and Tarifa in the same period (unpublished observation).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(Phocoena phocoena) stranded on the Dutch coast before the Mediterranean outbreak among S. coeruleoalba took place (unpublished observation).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 with 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 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:                           | Reactivity                      | cDV  | cDV  | cDV  | cDV  |
| Number                         |                                 |      |      |      |      |
| 3-602                           | cDV                            | NP1  | +    | +    | -   |
| 3-605                           | cDV                            | NP2  | +    | +    | +   |
| 3-658                           | cDV                            | NP3  | +    | +    | -   |
| 4-100                           | cDV                            | NP4  | +    | +    | +   |
| 1-064C5                         | cDV                            | NP5  | +    | +    | +   |
| 3-696                           | cDV                            | NP6  | +    | +    | +   |
| 4-109                           | cDV                            | NP7  | +    | +    | +   |
| 4-149                           | cDV                            | P1   | +    | +    | +   |
| 3-698                           | cDV                            | P2   | +    | +    | -   |
| 3-699                           | cDV                            | P3   | +    | +    | +   |
| 4-051                           | cDV                            | P4   | +    | +    | +   |
| 4-088                           | cDV                            | P5   | +    | +    | +   |
| 1-384                           | cDV                            | H1   | +    | +    | +   |
| 2-397                           | cDV                            | H2   | +    | +    | +   |
| 3-734                           | cDV                            | H3   | +    | +    | +   |
| 3-775                           | cDV                            | H4   | +    | +    | +   |
| 4-074                           | cDV                            | H5   | +    | +    | +   |
| 4-275                           | cDV                            | H6   | +    | +    | +   |
| 4-090                           | cDV                            | H7   | +    | +    | +   |
| 1-056G5                         | cDV                            | H8   | +    | +    | +   |
| 1-057E5                         | cDV                            | H9   | +    | +    | +   |
| 1-058F2                         | cDV                            | H10  | +    | +    | +   |
| 1-070B9                         | cDV                            | H11  | +    | +    | +   |
| 1-052C4                         | cDV                            | H12  | +    | +    | +   |
| 1-122D11                        | cDV                            | H13  | +    | +    | +   |
| 3-693                           | cDV                            | F1   | +    | +    | +   |
| 4-584                           | cDV                            | F2   | +    | +    | +   |
| 5-148                           | cDV                            | F3   | +    | +    | +   |
| 1-082E2                         | cDV                            | F4   | +    | +    | +   |
| 1-082B2                         | cDV                            | F5   | +    | +    | +   |
| 1-082C3                         | cDV                            | F6   | +    | +    | +   |

+ Positive reaction
- Negative reaction

cDV: Phocid distemper virus
DMV: Dolphin morbillivirus
PMV: Porpoise morbillivirus

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References


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TABLE 2: Susceptibility of Concana
avin-A-stimulated peripheral blood
mononuclear cells (PBMC) from different morbillovirus seronegative
animals to infection with different morbilloviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>harbour seal</th>
<th>Con-A stimulated PBMC from bottlenosed dolphin</th>
<th>Mediterranean monk seal</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>+</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>CVV</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PPD-1</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>PPD-2</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>DMV</td>
<td>(n=2)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PMV</td>
<td>(n=2)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

n/a: Presence/absence of morbillovirus infection in PBMC as judged from antigen detec
tion 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 roncaco per cell

MV: Measles virus
CVV: Canine distemper virus
PPD: Phoxid virus
DMV: Dolphin morbillivirus
PMV: Porpoise morbillivirus
NT: Not tested

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

PBMC was chosen as the cell substrate, since these are natural
 targets 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 non-vital cells were removed by den-
sity 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
onto 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-reactive monoclonal anti-
body F3-5 raised against measles virus (MV) (De Vries and others
1990). Table 2 shows that most of the five morbillviruses
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. How-
ever, since cells of this species can be infected by other mor-
billviruses (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.

Acknowledgement.— We thank Ms C. Kruysen for help in
preparing the manuscript.

Welfare implications of ultrasonic flea collars

D. J. Roe, G. D. Sales

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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. Manufac-
turers 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 cheopis) and cat fleas
(Platynocephalus 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 sound waves.

Hearing ranges extend up to at least 70 kHz in cats
(Heffner and Heffner 1983), dogs (Heffner 1983) and hamsters,
gerbils, mice and rats (Fay 1985).

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 investiga-
tion 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 2x10^-5 Pa RMS) were measured using
a Bruel and Kjaer 025 inch microphone and amplifier cali-
brated to give a known output in millivolts for a given
sound pressure level at the microphone, together with an oscil-
oscope.

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
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 dif-
ficult 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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