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Short Note

Anti-Morbillivirus Antibodies in Stranded Striped Dolphins (*Stenella coeruleoalba*): Time and Temperature Dependent Fluctuations

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Morbilliviruses have emerged over the last 20 y as a group of pathogens which have dramatically impacted the health and conservation status of several free-ranging aquatic mammal species and populations worldwide (Kennedy, 1998; Di Guardo et al., 2005; Di Guardo, 2008; Van Bressem et al., 2009). Therefore, it is vital to accurately monitor the presence of morbilliviruses in stranded pinnipeds and cetaceans. Indirect evidence of (recent or past) exposure to these highly pathogenic agents can be achieved through the determination of specific anti-morbillivirus antibodies (Abs) in sera obtained from stranded animals (Duignan et al., 1994, 1995, 1997; Barrett et al., 1995; Di Guardo et al., 1995, 2010). Stranded animals provide a unique opportunity for monitoring the health status of free-ranging populations (Di Guardo et al., 1995; Cornaglia et al., 2000); however, carcasses are often found in a compromised preservation condition with advanced autolysis, making histopathological and immunohistochemical investigations for morbilliviruses and other viral and non-viral pathogens difficult. Despite these difficulties, morbillivirus infections have been diagnosed by means of a reverse transcriptase polymerase chain reaction (RT-PCR) technique in heavily autolyzed stranded bottlenose dolphins (*Tursiops truncatus*) (Krafft et al., 1995). Taking into account the limited amount of published work addressing this issue, we evaluated the time and temperature dependent variations of anti-morbillivirus antibody titres in a series of serum samples from three striped dolphins (*Stenella coeruleoalba*) stranded along the Italian coastline in 2007.

The sera were obtained, after centrifugation at 1,000 to 1,500 revolutions/min for 15 min, from blood clots collected from the heart chambers and/or large vessels of three striped dolphins (one male calf, one subadult female, and one adult female, classified as such on the basis of their body length and weight). The specimens were found stranded in a good state (condition 2; Geraci & Lounsbury, 2005) on the Ligurian coast (Imperia province, northwestern Italy, 22 August, 10 September, and 25 September, respectively). These dates coincided with the morbilliviral epidemic affecting both pilot whales (*Globicephala melas*) near Gibraltar (Fernández et al., 2008) and striped dolphins along the coast of Spain (Raga et al., 2008).

At post-mortem examination, all three dolphins exhibited severe bilateral pneumonia, characterized by bronchointerstitial and parasitic bronchopneumonia, the latter due to *Halocerchus lagenorhynchi*.

Three days after necropsy, anti-morbillivirus Abs were detected in the sera, at titres of 1:10 (calf), 1:20 (subadult), and 1:40 (adult). A serum neutralization (SN) test was employed, using the "Onderstepoort strain" of Canine Distemper Virus (CDV) as antigen (Di Guardo et al., 2010).

After being stored for 8 to 9 mo at -20° C, Ab titres were reassessed in the same sera using an identical SN test conducted 24 h before the start of the experiment (defined as *time 0* for the purposes of this study). In order to re-create a scenario mimicking "field" conditions (i.e., varying temperatures associated with season conditions and elapsed time before carcass recovery), single aliquots (20 µl) from each of the three sera were stored at constant temperature values of 10° C, 20° C, 28° C, and 37° C for 24 h, 48 h, 7 d, 14 d, and 21 d, respectively. Temperatures of 10° C and 20° C were chosen since they reflect the average temperatures occurring throughout fall-winter-spring in the Mediterranean basin,

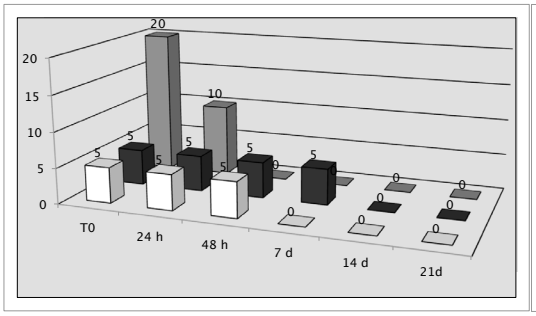

Figure 1

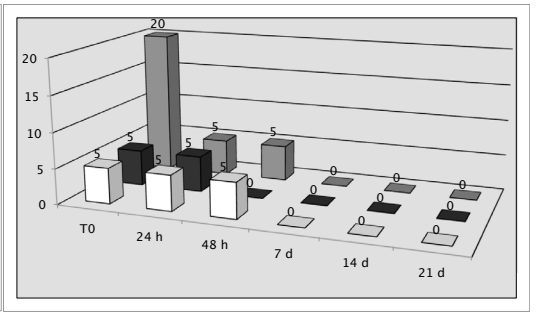

Figure 2

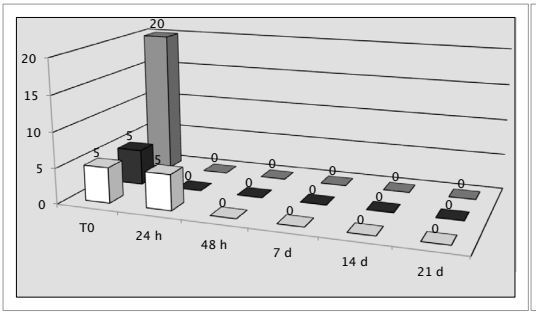

Figure 3

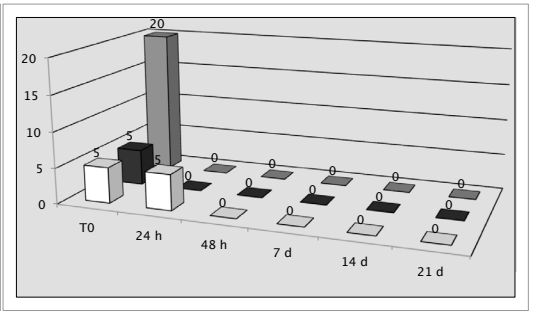

Figure 4

Histogram representation of neutralizing antibody titres against morbillivirus (Canine Distemper Virus [CDV], "Onderstepoort strain") determined at time 0 and after application of 10° C (Figure 1), 20° C (Figure 2), 28° C (Figure 3), and 37° C (Figure 4) temperatures for 24 h, 48 h, 7 d, 14 d, and 21 d, respectively, on the sera of three striped dolphins (*Stenella coeruleoalba*) found stranded in 2007 along the Ligurian Sea coast of Italy.

whereas 28° C and 37° C values reflect average temperatures during summer months.

Following storage at the defined temperatures for the aforementioned time intervals, anti-morbillivirus (CDV) neutralizing Ab titres were re-determined in the sera. At the beginning of the experiment (time 0), anti-morbillivirus (CDV) titres in the three samples were 1:5 (calf, subadult) and 1:20 (adult) (Figures 1 through 4). Anti-morbillivirus (CDV) neutralizing immunoglobulin titres were not found in the sera from two dolphins (subadult and adult, having pre-existing titres of 1:5 and 1:20, respectively) after 24 h for samples stored at both 28° C and 37° C, disappearing after 48 h in the calf's serum (having a pre-existing titre of 1:5). A less conspicuous reduction of anti-morbillivirus (CDV) neutralizing Ab titres was documented when the serum samples were exposed to 20° C and 10° C, with immunoglobulin titres at time 0 unchanged after 48 h and 7 d of exposure to 20° C (two samples) and 10° C (one sample), respectively. These results are illustrated in the form of histograms in Figures 1 through 4.

Dolphin morbillivirus (DMV) or porpoise morbillivirus (PMV), which are almost identical from a genomic and antigenic standpoint (Kennedy, 1998; Di Guardo et al., 2005; Van Bressem et al., 2009), would have represented a more suitable option than CDV as the laboratory antigen(s) to be utilized in the SN reactions for this study. As a consequence, we would have most likely detected higher immunoglobulin titres in the sera. Nevertheless, it should be pointed out that CDV antigen has been widely used in sero-epidemiological surveys aimed at estimating the prevalence of morbilliviral infections in several free-ranging pinniped (Duignan et al., 1994, 1995, 1997; Barrett et al., 1995) and cetacean (Barrett et al., 1995; Di Guardo et al., 1995, 2010) species and populations. The phrase *anti-morbillivirus (CDV) Abs/immunoglobulins* was used in place of *anti-DMV (or anti-PMV) Abs/immunoglobulins* so as to clarify the antigen used in this study. The use of CDV antigen is also justified by the fact that morbilliviruses, especially wild types, are difficult to isolate and propagate in cell culture systems (Appel & Jones, 1967; Nielsen et al., 2008).

Post-mortem autolytic changes are known to be strongly influenced by a number of variables, with environmental temperature and time playing a major role (Myers & McGavin, 2007). Consequently, the time dependent reduction of

anti-morbillivirus (CDV) neutralizing Ab titres, which was less consistent when the serum samples were exposed to 20° C and 10° C as compared to 28° C and 37° C, was not an unexpected finding. As far as immunoglobulin degradation is concerned, it should be emphasized that proteolytic digestion, a key process during post-mortem autolysis, has been shown to dramatically affect serum Abs (IgG) more than exocrine/mucosal immunoglobulins (IgA) in humans (*Homo sapiens*) (Brown et al., 1970). Furthermore, a number of degradation sites (Kroon et al., 1992) and chemical degradation pathways (Ionescu & Vlasak, 2010) have been reported in monoclonal Abs following storage at different (temperature) conditions. No previously published reports concerning this issue are available for stranded cetaceans, which are often discarded when heavily autolyzed, with virtually no biological specimens being collected for *ad hoc* laboratory investigations (e.g., sero-epidemiological surveys). Consequently, it is imperative to establish a set of "controlled data" involving time and temperature-related persistence of serum Abs for pathogens (e.g., morbillivirus, *Toxoplasma gondii*, *Brucella* spp.) that have the potential to seriously impact the health and conservation status of cetaceans (Van Bressem et al., 2009).

The results presented here address a relevant issue for both the health and the conservation of free-ranging cetaceans (and, potentially, free-ranging pinnipeds), providing a preliminary basis for future studies sharing similar objectives. These results provide further justification for preserving blood sera from stranded animals, which, if stored properly, could be available for an unlimited length of time and used for retrospectively monitoring the presence of several biologic disease agents among free-ranging aquatic mammal species.

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