

Causes of Stranding in Four Risso's Dolphins (*Grampus griseus*) Found Beached Along the North Adriatic Sea Coast

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Zucca, P., Di Guardo, G., Francese, M., Scaravelli, D., Genov, T. and Mazzatenta, A., 2005. Causes of stranding in four Risso's dolphins (*Grampus griseus*) found beached along the North Adriatic sea coast. *Veterinary Research Communications*, **29**(Suppl. 2), 261–264

Keywords: Friuli Venezia Giulia, *Grampus griseus*, Italy, Slovenia, strandings

Abbreviations: CT, Computer Tomography; 3D, three dimensional; w/w, wet weight

INTRODUCTION

The Risso's dolphin (*Grampus griseus*) (Cuvier, 1812) is a medium-sized odontocete, between 2.8 and 3.5 m in length, widely distributed in the Mediterranean Sea, mainly in deep waters. This species has rarely been sighted in the North Adriatic. There have been no recent sightings or strandings of this dolphin along the Slovenian coasts while episodic sightings and strandings, have occurred along Italian coasts since the beginning of the 1900's until today (Lapini *et al.*, 1995; Notarbartolo Di Sciara and Demma, 1994). This report describes the pathological data related to the strandings of this species along the North Adriatic Sea coasts, specifically those in the Friuli Venezia Giulia area.

MATERIALS AND METHODS

Since 1993, out of a total of 11 Risso's dolphins observed in the area of study, four males were found stranded. One subject (no. 1, 22/12/96), was released into the open sea after a short surgical treatment at Grado's shore while two other live Risso's dolphins (no. 2 and no. 3, 03/07/00 Ravenna and 05/05/01 Lignano, UD, respectively) died after a recovery period in a dolphin rehabilitation facility at the Fondazione Cetacea of Riccione (Rimini, Italy). A fourth dolphin was stranded dead near Trieste. The pre-stranding behavior of Risso's dolphins 3 and 4 were recorded from a boat. A detailed necropsy of stranded dolphins was carried out according to the established protocols (Dierauf, 1990; Geraci and Lounsbury, 1993).

Routine tissue samples, as well as the nasal sinus mucosa and upper respiratory tract mucosa, were collected. These were immediately fixed in 10% neutral buffered formalin, embedded in paraffin and cut into five micron-thick sections, which were finally stained with haematoxylin and eosin (H&E) for light microscope examination. Specimens of the endoparasites were fixed in 70°C ethanol and sent to a laboratory to confirm their identity (Prof. J.A. Raga, Department of Animal Biology, University of Valencia, Spain).

Mercury and other heavy metals were measured by thermal decomposition with an automatic analyzer (AMA 254, Altec Ltd. Khodlova 1297 CZ-19300 Prague, Czech Republic) by a specialized laboratory (Centro Studi Ambientali, Rimini, Italy).

The head of a Risso's dolphin (specimen no. 3), was examined, before necropsy, using computed axial tomography (spiral CT unit, Philips AV1, Philips, Eindhoven, The Netherlands) and digital 3D models of the head were created to evaluate the pathological lesions of parasitic origin on an independent workstation (EasyVision Release 5.2 system of Philips Medical Systems, Philips, 5684 PC Best, The Netherlands).

RESULTS

The clinical examination of Risso's dolphin no. 1, stranded alive in Grado, showed a big hook in the upper jaw. The dolphin was released into the open sea after surgical treatment on the shore. Dolphins 3 and 4 were swimming actively close to the shore during the pre-stranding period, but exploring only a limited area. One dolphin (specimen no. 3), which had been sighted early in the morning, started to show signs of tiring. This animal headed toward sandy shallows and stranded later on the same evening.

The other animal (specimen no. 4) was sighted and followed for several days along a short stretch of coast. This dolphin showed the same behavioral patterns as specimen 2, "patrolling" a short area close to the coast. Two weeks after having first been sighted, it was found dead along the shallow, rocky stretch of coast which it had been exploring. Photo-identification confirmed that the animal was the same one observed over the preceding 2 weeks.

Post mortem examination of the body and internal organs of the dolphins stranded lifeless (nos. 2–4) did not show any pathognomonic lesions related to a specific disease or condition that could explain their stranding and death. The body condition of all the dolphins under study was very poor and the stomach and intestine of all three animals were completely empty, indicating that they had not eaten for some time. Signs of hepatic degeneration were also noted.

The main macroscopic lesions observed in the three dolphins were related to a heavy parasitic infection by *Crassicauda grampicola* in the nasal and pterygoid sinuses, as well as infection close to tympanic *bullae*. The nematodes showed a smooth opaque cuticle, a cephalic portion with two lateral expansions of the cuticle, four cephalic submedian *papillae* and two lateral *papillae*. Males had a mean length of 16.6 cm and thickness of 1.44 mm, 12–14 cloacal pedunculate *papillae* in two rows and a cloacal-body end distance of 783 µm. The females had a length of 18–20 cm and thickness of 1.26 mm, with the vulva opening 2.45 mm from the end of the body end and eggs of 44.2/29.7 µm (Raga, 1987).

Microscopically, the liver showed chronic circulatory disturbances, with evidence of degenerative and/or necrotic changes affecting several hepatocytes. Furthermore, dolphin no. 3 showed a moderate pulmonary *oedema*.

The air sinuses were affected by severe inflammatory lesions, with multiple and extensive necrotic foci associated with severe haemorrhage, the latter mainly showing a submucosal location. The anatomo-histomorphological changes described above, were better characterized for dolphin no. 2 by means of appropriate CT scans (Zucca *et al.*, 2004). Toxicological examinations revealed high levels of mercury in the livers of dolphins no. 3 and 4, with values of 498 and 497 mg/kg w/w, respectively.

DISCUSSION

In spite of the numerous bibliographic references concerning parasitic infection by *Crassicauda grampicola* and its possible role in the stranding of marine mammals (Geraci and Lounsbury, 1993; Raga, 1987; Raga *et al.*, 1997), very little is known about the biology, life cycle and the real pathogenicity of this parasite.

In addition, the tolerance limit for mercury in the mammalian liver seems to be in the range of 100–400 mg/kg w/w, over which hepatic damage occurs (Wageman and Muir, 1984). The mercury levels found in the liver of Risso's dolphins no. 2 and 3 may have contributed to the occurrence of the liver damage observed for both animals and may have compromised their immune response, making them more vulnerable to parasitic infections to which they may have previously been resistant.

In any case, whether *Crassicauda grampicola* is considered a primary parasitic pathogen, or whether it is correlated with the more or less severe immuno-suppressive action of heavy metals, this parasite most likely represents an important regulatory factor of marine mammal populations, especially Risso's dolphins, as it has also been reported by other authors (Raga *et al.*, 1997).

ACKNOWLEDGEMENT

We wish to thank Servizio per la Tutela degli Ambienti Naturali e della Fauna, Regione Autonoma Friuli Venezia Giulia, Italy; Prof. Juan Antonio Raga, Department De Biologia Animal, Universidad de Valencia, Spain; Prof. Giorgio Vallortigara, Laboratory of Animal Cognition and Comparative Neuroscience, University of Trieste, Italy, for their help in the field work and for the suggestions and bibliography that they kindly supplied.

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