Molecular evidence for *Toxoplasma gondii* in the brain of striped dolphins (*Stenella coeruleoalba*) stranded along the Ligurian Sea coast of Italy

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INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite of mammals and birds. This pathogen commonly infects also marine mammals, e.g. sea otters and cetaceans (dolphins, porpoises and whales), causing central nervous system impairment, behavioural changes, abortion and foetal or neonatal death (Dubey *et al*, 2003; Miller, 2008). Although infection by *T. gondii* appears to be widespread in marine mammal species, information on epidemiology, biology, genetics and pathogenic potential is still incomplete. With the aim to enhance our knowledge on *T. gondii*, this work provides molecular evidence for toxoplasmosis in the brain tissue from five striped dolphins (*Stenella coeruleoalba*) found stranded in 2007-2008 along the Ligurian Sea coast of Italy. These animals showed a more or less severe, subacute to chronic, non purulent, multifocal meningo-encephalitis. Additionally, the cerebral parenchyma of 2 dolphins harboured protozoan cysts and zoites which were immunohistochemically linked to *T. gondii* (Di Guardo *et al*, 2010; Figure 1). Consequently, a preliminary molecular investigation was undertaken to confirm the presence of the protozoan in these animals.

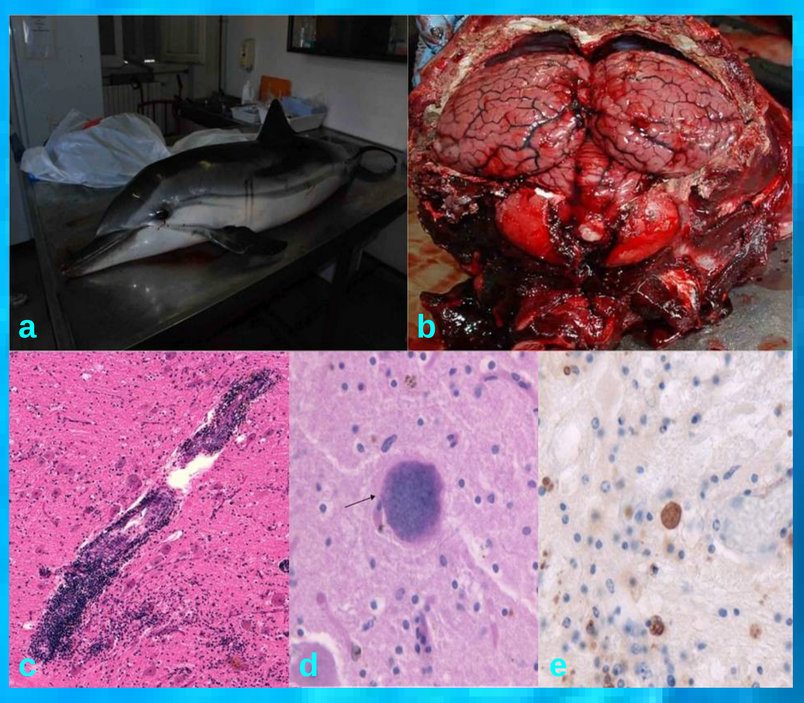

Fig. 1: Striped dolphin (*Stenella coeruleoalba*). Adult female affected with non-suppurative meningo-encephalitis and seropositive (1:320) to *T. gondii* (and negative to all direct and indirect investigations against *Morbillivirus*) **(a).** Same animal as in previous picture. Brain. Prominent meningeal hyperaemia **(b)**. Histologic evidence of non-suppurative, multifocal encephalitis involving the *pons* and the *mesencephalon*, where prominent perivascular cuffs of inflammatory mononuclear cells are observed in association with microglial nodules scattered throughout the surrounding neuropil **(c).** A single, basophilic structure compatible with a *T. gondii* cyst is shown in the thalamus from an adult *T. gondii*-seropositive femal dolphin **(d)**. Positive immunohistochemical labeling of *T. gondii* cysts and zoites in the brain tissue from the above animal **(e).**

MATERIALS & METHODS

DNA was extracted from the brain tissue of the five animals (DNEasy® Tissue Kit, Qiagen) and the extracts were examined with a nested PCR specific for the B1 gene of *T. gondii.*

PCR mixtures were prepared with the primer pair B1outF (5 -GGAACTGCATCCGTTCATGAG-3) and B1outR (5 -TCTTTAAAGCGTTCGTGGTC-3) in a first round of PCR, followed by a second round using the primer set B1intF (5 -TGCATAGGTTGCAGTCACTG-3) and B1intR (5 -GGCGACCAATCTGCGAATACACC-3). In the first step, the PCR mixture (50 l) consisted of 4 l of genomic DNA, 50 pmol of each of the primers and 25 l of Ready Mix REDTaq (Sigma, St. Louis, MO). The conditions for the second PCR step mixture were identical to those for the primary PCR with the exception of the template amount (i.e. 4 l of a 1/10 dilution of the primary PCR product, determined to be optimal). Both amplification rounds consisted of an initial step of 10 min at 95° C, 35 cycles, each of 60 sec at 94° C, 60 sec at 50° C (first step) or 54° C (second step), 60 sec at 72° C, with a final extension of 10 min at 72° C. The successful PCR reactions were sequenced and sequences analysed.

RESULTS

Three out of the 5 dolphins (2 of which were also immunohistochemically positive) scored positive upon B1-PCR. Sequences were subsequently searched against the GenBankTM Database using the Basic Local Alignment Search Tool. The three sequences were identical to each other and showed 100% homology with the corresponding B1 sequence of *T. gondii,* with no insertions/deletions nor substitutions. The other 2 dolphins were negative upon B1-specific PCRs and also upon PCRs targeting other *T. gondii* genes further performed (i.e. GRA and UPRT). To explore more in depth the genetic make-up of these isolates, complementary studies are presently ongoing on GRA and UPRT genes of the 3 animals which were molecularly confirmed (i.e. B1-PCR) to be infected by *T. gondii*.

DISCUSSION

This study confirms the presence of *T. gondii* in marine mammals and is a starting point to elucidate the pathogenic role that such protozoan can exert on free-ranging cetaceans, thereby potentially affecting the conservation status of already endangered species and populations. Additionally, at the light of the zoonotic potential expressed by this parasite, it would be desirable to conduct further studies aimed at understanding the epidemiology of the infection in aquatic mammals and, consequently, at reducing the risk of transmission to humans. These studies should be particularly focused on the pathogenicity and distribution of *T. gondii* genotypes circulating among cetaceans (with special emphasis on pelagic species such as striped dolphins), in order to monitor and prevent infection in these species, as well as in human beings.

REFERENCES: Dubey JP *et al., 2*003, Vet Parasitol, 116: 275-296; Miller MA, 2008, Zoo and Wild Animal Medicine, Current Therapy, Sixth edition, pp. 319-340; Di Guardo G *et al.,* 2010, Vet Pathol, 47: 245-253.