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Pathology and molecular analysis of *Hapalotrema mistroides* (Digenea: Spirorchiidae) infecting a Mediterranean loggerhead turtle *Caretta caretta*

M. Santoro[1,*], F. Di Nocera[1], D. Iaccarino[1], S. P. Lawton[2], A. Cerrone[1], B. degli Uberti[1], M. D'Amore[1], A. Affuso[3], S. Hochscheid[3], F. Maffucci[3], G. Galiero[1]

[1]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute n. 2, 80055 Portici, Italy
[2]Molecular Parasitology Laboratory, School of Life Sciences, Pharmacy and Chemistry, Kingston University, Kingston upon Thames, Surrey KT1 2EE, UK
[3]Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

ABSTRACT: Turtle blood flukes belonging to the family Spirorchiidae (Digenea) represent a major threat for sea turtle health and are considered the most important parasitic cause of turtle stranding and mortality worldwide. Despite the large diversity of spirorchiid species found globally, there are only 2 records for free-ranging Mediterranean sea turtles that date back to the late 1800s involving just *Hapalotrema mistroides* Monticelli, 1896. This study describes the first fatal confirmed case of spirorchiidiasis in a free-ranging Mediterranean loggerhead turtle *Caretta caretta* (Linnaeus) and, owing to the complexities of taxonomic identification of these parasites, provides the first molecular characterization and phylogenetic analysis of *H. mistroides* from the Mediterranean Sea. The loggerhead turtle showed cachexia and digestive disorders associated with severe damage to the pancreas and intestinal ganglia, caused by deposition of *Hapalotrema* eggs forming granulomas. Massive *Hapalotrema* egg emboli in several tissues and organs and encephalitis were the most probable contributions to the death of the turtle. The congruence between the phylogenetic analysis of both the ITS2 and 28S rDNA resolved the Italian and USA *H. mistroides* as the same species, confirming the parasite identification. The case here described clearly indicates that the blood flukes should be considered in the differential diagnosis of Mediterranean sea turtle diseases.

KEY WORDS: Spirorchiids · Blood flukes · Pathological changes · ITS2 · 28S rDNA · Mediterranean Sea

INTRODUCTION

The Spirorchiidae Stunkard, 1921, a family of blood flukes within the superfamily Schistosomatoidea (Platyhelminthes: Digenea), parasitize the cardiovascular system of marine and freshwater turtles. The Spirorchiidae comprises 21 genera, 10 of which exclusively parasitize sea turtles (Platt 2002, Orélis-Ribeiro et al. 2014, Roberts et al. 2016a,b). Spirorchiids produce vast numbers of eggs which penetrate the gut wall, migrate into the intestinal lumen and are eliminated into the external environment with the host's feces (Smith 1972). However, many eggs become lodged in tissues, leading to a granulomatous response and resulting in a disease presentation analogous to that of schistosomiasis in mammals (Smith 1972). These infections are considered the most important parasitic cause of stranding and mortality of sea turtles worldwide; however, incidental findings of infection with no associated clinical signs are also common (Santoro

*Corresponding author: mario.santoro@izsmportici.it