

COMPARISON BETWEEN MINI-FLOTAC AND A CONVENTIONAL TECHNIQUE FOR THE DETECTION OF HELMINTH EGGS IN CETACEAN STOOL SAMPLES

Marcer F.*, Cassini R., Parisotto N., Tessarin C., Zoroaster A., Marchiori E.

Dipartimento di Medicina Animale, Produzioni e Salute, Università di Padova, Legnaro, Italy

Keywords: Mini-FLOTAC, cetaceans, helminthes

INTRODUCTION: Free ranging cetaceans are sentinels of the marine ecosystem's health, and valuable information about the population health and migratory pathways can be obtained from evaluation of their parasitic community. Non-invasive methods, set up for the collection of fecal samples from whales at sea, allow to bring together parasitological data from necropsies and from healthy, free-living animals (Flores-Cascante et al., 2019. *Acta Parasitol*, 64:625-37). To evaluate which copromicroscopic protocol describes more reliably the gastrointestinal helminthic communities in cetaceans, we studied the Mini-FLOTAC (MF) (Cringoli et al., 2017. *Nat Protoc*, 12:1723-32) and a classical sedimentation-flotation technique (SF) comparing them with helminth isolation from the digestive tract.

MATERIALS AND METHODS: Gastrointestinal content and fecal samples were collected during necropsy from 45 stranded cetaceans, including odontocetes (no.= 40) and mysticetes (no.= 5), in the period 2009-2022. Helminths were recovered through a classical filtration-sedimentation technique, preserved in ethanol 70° and morphologically identified at light microscope. Fecal samples preserved in 5% formalin were submitted to a double copromicroscopic examination using a sodium nitrate, sodium thiosulphate and sucrose solution (s.g. =1.450) for both SF and MF. Sensitivity of the copromicroscopic methods was calculated using the isolation of helminths as a reference test.

RESULTS AND CONCLUSIONS: On the whole, helminths belonging to 9 taxa (i.e., the trematodes *Synthesium tursionis*, *Synthesium delamurei*, *Campula palliata*, *Braunina cordiformis*, *Pholeter gastrophilus*, the nematode *Anisakis* sp., cestodes of the family Tetrabothriidae and the acanthocephalan *Bolbosoma* sp.) were isolated with filtration-sedimentation technique. No eggs belonging to additional taxa, nor eggs of cestodes were found at copromicroscopic analyses by any method. Sensitivity of the Mini-FLOTAC method appeared higher for all taxa with respect to SF (range 30-100%) and equal for *Anisakis* sp. (56%). Evaluation of the concordance between the two tests showed a moderate to perfect agreement (K-value = 0.42-1) for the different taxa.

Not excluding the limitations in terms of sensitivity inherent to the techniques themselves, intermittent egg shedding, prepatent infections or under threshold parasitic burdens could account for negative results at copromicroscopy. Dynamics of eggs shedding in these helminth species are largely unexplored, and this is the first study comparing data from copromicroscopy and gastrointestinal helminth isolation in cetaceans. Notwithstanding the limitations linked to all these factors, we conclude that Mini-FLOTAC protocol seems to be a useful technique for estimating the composition of gastrointestinal helminthic community of cetaceans, providing at the same time a quantitative estimation.