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CHASING *ECHINOCOCCUS MULTILOCULARIS* IN WILD CARNIVORES FROM NORTHERN TUSCANY, ITALY

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INTRODUCTION: *Echinococcus multilocularis* (Em) is a Taeniidae cestode, spread across the Northern hemisphere, circulating among carnivores as definitive hosts and voles as intermediate, respectively (Romig et al., 2017. Adv Parasitol Part A, 95:213-314). Moreover, following egg ingestions humans can develop alveolar echinococcosis (Conraths et al., 2017. PLOS NTD, 11:1-15). In Italy, the first Em-positive foxes were found about 25 years ago in the Trentino Alto Adige region (Manfredi et al., 2002. Vet Rec, 150:757) in an autochthonous and highly endemic focus (Casulli et al., 2005. Int J Par, 35:1079-1083; Obber et al., 2022. PLOS ONE, 17:e0268045). Recently, Em eggs were extracted from shepherd dog and wolf faeces in the Ligurian Alps (Massolo et al., 2018. IJP-PAW, 7:309-316), suggesting a southern expansion of Em distribution. We aimed to investigate the Em presence in the Apuan Alps, an Apennine protected area close to the Ligurian Alps.

MATERIALS AND METHODS: Faeces of wild carnivores (wolves, foxes and mustelids) were collected from 2020 to 2023 on a quarterly basis along 52 fixed pathways, and stored at -80°C for at least five days for safety (Veit et al., 1995. Parasitology, 110:79-86) and then at -20°C until analysis. A total of 148 scats (from 10 mustelids, 58 foxes and 80 wolves) were processed by two distinct procedures. First, flotation and sieving technique (FST) with ZnCl2 solution (Mathis et al., 1996. J Helminthol, 70:219-22) for taeniid egg harvest was implemented. DNA extraction, nested PCR and sequencing of portions of nad1 and cox1 genes were conducted on individual eggs (Hüttner et al., 2008. IJP, 38:861-68; Štefanić et al., 2004. Parasitol Res, 92:347-51). Secondly, two Em-specific copro-qPCRs were then used directly on each fecal sample. The former followed Knapp et al.'s (2014. Vet Parasitol, 201:40-7) with minor modifications (Obber et al., 2022) targeting the mtDNA marker rrnL; the latter targeted primers Nad234_F and Nad234_R (Santa et al., 2018. IJP-PAW, 7:111-15).

RESULTS AND CONCLUSIONS: Cestode eggs were successfully detected by FST and sequenced from 1/9 mustelids, 4/41 foxes and 16/60 of wolves. Em DNA was detected in 1 fox and 3 wolf samples. Nonetheless, the modified Knapp et al.'s copro-qPCR on the same samples did not yield any positive result, whereas Santa et al.'s qPCR is yet to be carried out. *Taenia hydatigena* and *Taenia krabbei* were identified in wolves, whereas *Taenia polyacantha*, *Mesocestoides litteratus*, *Mesocestoides* sp. and *Dipylidium caninum* occurred in foxes. One mustelid harboured *M. litteratus* and *T. polyacantha*. If furtherly confirmed by qPCR, these findings would open for a new scenarios for Em expansion to the Apennines, which were so far considered Em-free (Crotti et al., 2023. IJP-PAW, 21:11-16). Different timelines, sample sizes and techniques specificity might have contributed to negative results.