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PRE-ANALYTICAL BIAS MAY SEVERELY IMPACT ON THE RELIABILITY OF COPROMICROSCOPY DATA IN CHAMOIS AND OTHER MOUNTAIN DWELLING UNGULATES

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INTRODUCTION:
Use of non invasive parasitological data (eg., copromicroscopic data) as hallmarks of individual and herd fitness in free-ranging wildlife is tempting due to low cost, ease of the techniques and the possibility to obtain repeated samples and/or a large sample size. With the increased emphasis on faecal egg counts analysis, there is a need to revisit the methodology and identify procedures that can be implemented as good management practices. Yet, little attention has been paid to evaluating pre-analytical factors such as collection and storage of faecal samples prior to analysis. The aim of this contribution is showing to which extent desiccation and freezing of faecal samples from Southern chamois (Rupicapra pyrenaica ornata) and Barbary sheep (Ammotragus lervia) may mirror negatively on the accuracy of parasitological results, hence on reliability of the associated conclusions.
MATERIALS AND METHODS:
Two experiments (A and B) were carried out on the effects of desiccation on fecal egg counts in two study locations: the Abruzzo, Lazio and Molise National Park, Italy (R. pyrenaica ornata) and the Sierra Espuna National Reserve, Spain (A. lervia). Fresh faeces (20 and 10 in experiments A and B, respectively) were collected from soil and refrigerated (4°C) during transport. Samples were subdivided in two aliquots: the first one (meant as the "gold standard" for eggs of gastro-intestinal nematodes and oocysts of Coccidia) was analysed in default of any additional treatment, whereas the second was incubated at 30°C 40% RU for 48 hours prior to analysis. In experiment A, "old" (partially dried) faeces collected from soil on the same date as fresh ones were used for further comparison. Counts were carried out of the eggs of gastro-intestinal nematodes (EPG), oocysts of Coccidia (OPG) and larvae of pulmonary nematodes (LPG), in Mc Master chambers (6 chambers/sample). In a third experiment (C), counts were similarly carried out of feacal samples of R. pyrenaica ornata (N=20) before and after one-month storage at -18°C. When relevant, data were analysed with non parametric statistical tests.
RESULTS:
Experiment A: incubator desiccation resulted in lower EPGs and OPGs (85 and 84% decrease, respectively), higher prevalence of larvae of pulmonary nematodes (50 vs 15%) and much higher LPGs (135 vs 4). Differences were even larger in naturally desiccated "old" samples from the same herd. Experiment B: incubator desiccation in a different model resulted in lower EPGs and OPGs (95 and 99% decrease, respectively) and lower prevalence of gastrointestinal nematodes (20 vs 80%) and Coccidia (60 vs 100%). Experiment C: one-month freezing resulted in lower prevalence of gastro-intestinal nematodes (40 vs 90%) and remarkably lower EPGs (98% decrease). Coccidia and larvae of pulmonary nematodes were not or mildly affected.
CONCLUSIONS:
Results show that pre-analytical physical factors have a significant negative effect on the detectability of parasitic eggs and oocysts and, eventually, the opposite is true for larvae of pulmonary nematodes. Noteworthy, interpretation of our parasitological data in terms of herd fitness would have been severely biased by field selection and/or storage modalities of faecal samples, eg., the chamois herd investigated in (A) would have appeared either extensively and heavily challenged by gastro-intestinal nematodes and little challenged by pulmonary nematodes based on fresh samples analysis, and just the opposite based on "old" samples.
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