Optimizing fecal sample storage for small ruminant fecal egg counts

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Introduction

Gastrointestinal nematode parasites (GINs) significantly impact small ruminant health and productivity. One of the most common and cost-effective methods of diagnosing GINs is fecal egg counts (FECs). Short-term delays between sample collection and FECs are common (ex. shipping from farm to laboratory). Since FECs can only detect eggs, hatching can lead to underdiagnosis and animals needing treatment may be missed. Sample storage is therefore an important consideration in the accuracy of FECs. Hatching is temperature, oxygen and time-dependent, with cool temperatures and low oxygen delaying hatching. Refrigeration and suspension in water (to create a truly anaerobic environment) are proposed as the gold standard for feces storage. However, refrigeration interferes with future larval culture and advanced diagnostic tests to speciate GINs, such as nemabiome sequencing. This study sought to determine the optimal storage conditions that balance delayed hatching with advanced GIN diagnostics after FECs.

Materials and methods

Fecal samples from Ontario sheep and goats with a minimum of 500 eggs per gram (epg) were obtained from two ongoing studies. A total of 28 samples (pooled or individual depending on feces weight) were split into 4 duplicates and stored as follows: in water at 20 °C, in water at 4 °C, in an airtight bag at 20 °C, and in an airtight bag at 4 °C. At baseline (day 0) and every 24 hours for 72 hours, FECs were conducted using a modified McMaster method with an analytical sensitivity of 8.33 epg. The development stage of 100 eggs was also assessed, with eggs classified as either modulated or larvated. Data were analyzed for differences in epg and development between storage conditions and days using paired T-tests.

Results

The proportion of morulae and FECs were not normally distributed and were therefore subjected to logarithmic transformation prior to analysis. Differences in FECs across time and between storage conditions were mostly non-significant ($P > 0.05$), with few exceptions. Counts were significantly higher on day 1 in bagged samples at both temperatures and in water at 4 °C than on day 3 in bagged samples at 20 °C ($P = 0.01$-0.05). Day 1 FEC in water at 4 °Cwas also significantly higher than day 3 FEC in the same storage conditions ($P = 0.02$). Collectively, these findings suggest that the time between sample collection and FEC rather than storage condition has a greater effect on FECs. Interestingly, FEC was also significantly higher in water-suspended samples at 20 °C on day 3 than in water-suspended samples at 4 °C ($P = 0.04$) or bagged samples at 20 °C($P= 0.05$) on the same day, indicating that temperature has a lesser influence on egg preservation than oxygen exposure. Samples stored in airtight bags at 20 °C had significantly fewer morulae on day 1 than all other storage conditions and time points ($P = 0.0001$-0.002); however, differences between egg development in other storage conditions were not significant. This suggests that temperature and storage container have a negligible effect on egg development and the results of FECs for at least 3 days after packaging of samples.

Conclusion

Chilling small ruminant fecal samples does not improve the reliability of FECs provided samples are kept in airtight containers, and suspension in water is not superior to well-sealed plastic bags for short-term preservation of nematode eggs in feces. Small ruminant feces can be stored in sealed bags at room temperature for at least 3 days prior to FEC without a negative impact on diagnostic utility. Storage at room temperature offers the added benefit that samples remain suitable for later culture and advanced molecular diagnostic techniques. This confirms the reliability of FECs as a tool for the diagnosis and treatment of GINs while also allowing flexibility in sample handling depending on whether further diagnostic testing is planned.

