Veterinary Parasitology 217 (2016) 21–24

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Effect of vacuum packing and temperature on survival and hatching of strongyle eggs in faecal samples

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ARTICLE INFO

Article history:
Received 26 April 2015
Received in revised form
30 November 2015
Accepted 14 December 2015

Keywords:
Strongyle eggs
Vacuum
Temperature
Horse
Pig
Cattle

ABSTRACT

Strongyle eggs of helminths of livestock usually hatch within a few hours or days after deposition with faeces. This poses a problem when faecal sampling is performed in the field. As oxygen is needed for embryonic development, it is recommended to reduce air supply during transport and refrigerate. The present study therefore investigated the combined effect of vacuum packing and temperature on survival of strongyle eggs and their subsequent ability to hatch and develop into L3. Fresh faecal samples were collected from calves infected with *Cooperia oncophora*, pigs infected with *Oesophagostomum dentatum*, and horses infected with *Strongylus vulgaris* and cyathostomins. The samples were allocated into four treatments: vacuum packing and storage at 5 °C or 20 °C (5 V and 20 V); normal packing in plastic gloves closed with a loose knot and storage at 5 °C or 20 °C (5 N and 20 N). The number of eggs per gram faeces (EPG) was estimated every fourth day until day 28 post set up (p.s.) by a concentration McMaster-method. Larval cultures were prepared on day 0, 12 and 28 p.s. and the larval yield determined. For *C. oncophora*, the EPG was significantly higher in vacuum packed samples after 28 days as compared to normal storage, regardless of temperature. However, *O. dentatum* EPG was significantly higher in samples kept at 5 °C as compared to 20 °C, irrespective of packing. For the horse strongyles, vacuum packed samples at 5 °C had a significantly higher EPG compared to the other treatments after 28 days. The highest larval yield of *O. dentatum* and horse strongyles were obtained from fresh faecal samples, however, if storage is necessary prior to setting up larval cultures *O. dentatum* should be kept at room temperature (aerobic or anaerobic). However, horse strongyle coprocultures should ideally be set up on the day of collection to ensure maximum yield. Eggs of *C. oncophora* should be kept vacuum packed at room temperature for the highest larval yield.

1. Introduction

Gastrointestinal helminths of domestic animals are usually diagnosed by microscopic techniques detecting eggs in faeces. These techniques are easy to perform, cheap and have been available for many years. However, the accuracy of these techniques is very much dependent on the freshness of the faecal samples and the species of the parasite of interest (Roepstorff and Nansen, 1998; Cringoli et al., 2010). Especially, strongyles are problematic when it comes to transport or storage of faecal samples as the eggs are not very resistant to environmental factors. Strongyle eggs usually hatch within hours (e.g. horse strongyles) or a couple of days (e.g. pig and ruminant strongyles), if not refrigerated (Mfitilodze and Hutchinson, 1987: Roepstorff and Nansen, 1998; Nielsen et al., 2007). This poses a problem if samples are collected in the field where cold storage is not available, or during long distance posting. For strongyles, the first stage larva (L1) develops quickly inside the egg and is released upon hatching, then develops into L2 and L3, the latter being the infective stage. As embryonation is an aerobic process (Brown, 1928), a new method of removing air from the faecal sample by vacuum packing before storing or posting is gaining recognition. The manuals for the FLOTAC technique (quantification of parasite eggs in faeces) thus mention vacuum packing as an important step for storing faecal samples of herbivores, carnivores, omnivores and humans (Cringoli et al., 2010). Additionally, the American Association of Equine Practitioners' Parasite Control Guidelines (Nielsen et al., 2013) recommends vacuum packing of horse faecal samples for storage, or at least refrigeration for no more than 7 days before estimation of faecal egg counts. However,

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http://dx.doi.org/10.1016/j.vetpar.2015.12.014
0304-4017/

the effect of vacuum packing on survival of strongyle eggs has so far only been reported for the sheep strongyle (species not stated) in a study by Rinaldi et al. (2011). As most strongyle eggs have overlapping morphology, copromicroscopy does not allow for species differentiation of mixed natural infections. For this, eggs need to hatch and subsequently develop to the L3 stage, but it is not known how vacuum packing may affect this process. The present study therefore aimed to investigate the effect of vacuum packing and temperature on survival of strongyle eggs and the subsequent ability of the eggs to hatch and develop into L3.

2. Materials and methods

2.1. Faecal material

Fresh faecal samples were collected from calves experimentally infected with *Cooperia oncophora* pigs experimentally infected with *Oesophagostomum dentatum,* and horses naturally infected with *Strongylus vulgaris* and cyathostomins, as determined by larval culture (Roepstorff and Nansen, 1998). Samples were primarily collected from the rectum. However, to get enough horse samples a few droppings were collected from the pen immediately after deposition (visually confirmed; only the top portion of the sample collected) which is acceptable according to Nielsen et al. (2010).

2.2. Sample preparation and experimental design

Faecal samples from the same animal host (calf, pig or horse) were pooled and thoroughly homogenized for 20 min by hand. Each faecal mixture was divided into 84 samples of 20 g each and placed in individual thin plastic gloves (Polythene Disposable Gloves, Tradesource, Denmark). These were then randomly allocated into four different treatment groups: vacuum packing and storage at 5 °C (5 V), normal packing and storage at 5 °C (5 N), vacuum packing and storage at 20 °C (20 V), and normal packing and storage at 20 °C (20 N). On day 0 post setup (p.s.) the number of strongyle eggs were estimated in five replicates for every group where after each group was sampled in triplicates every fourth day until day 28 p.s. For the normal packing we used our standard procedure; faeces were obtained used a polystyrene glove, the glove was everted and the opening folded while pressing out air and all samples placed in the same plastic bag. The vacuum packing was done by folding the glove tightly around the sample and placing it inside a vacuum bag from which the air was evacuated and sealed using a domestic appliance vacuum machine (Freshield Touch, CSE Co., Korea). The sample preparation was done within 5 h at room temperature after collection of the faeces from all three animal hosts.

2.3. Faecal egg counts

A concentration McMaster technique (Roepstorff and Nansen, 1998) was used for the faecal egg counts using a NaCl-glucose monohydrate flotation fluid with a specific gravity of 1.27 g/ml and a detection limit of 20 eggs per g faeces (EPG). Additionally, the developmental stage of the embryo was determined for the first ten eggs encountered in each sample.

2.4. Larval cultures

Triplicate larval cultures were prepared for each treatment on day 0, 12 and 28 using 5 g faeces mixed with vermiculite (Roepstorff and Nansen, 1998), and left in an aerated cupboard in darkness at 20–22 °C for 14 days to ensure L3 development. All samples were then baermannized by placing the larval culture directly in conical sedimentation glasses filled with tap water and left overnight at

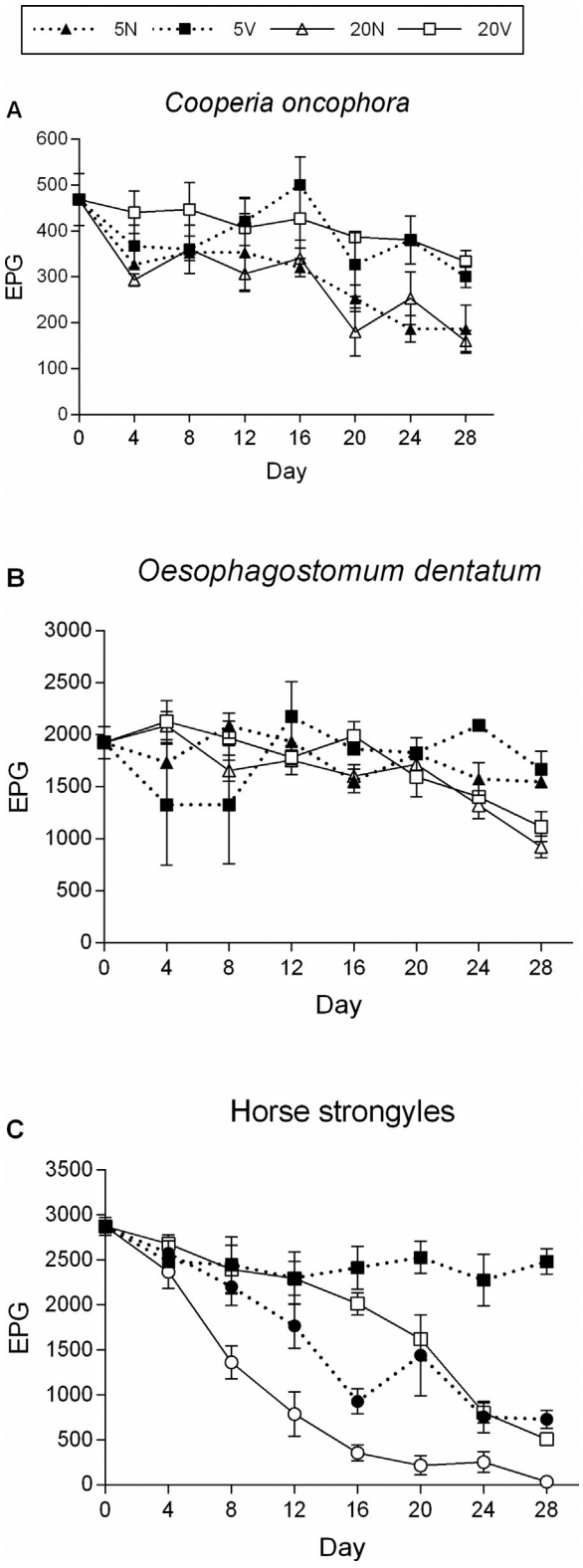

Fig. 1. Mean number of strongyle eggs per gram faeces (EPG ± S.E.) of calves (A), pigs (B), and horses (C) in four treatment groups (5 V, 5 N, 20 V and 20 N) at day 0 ($n = 5$) to 28 post set up (at day 4–28, $n = 3$). Note the different scales on the *Y*-axis.

20–22 °C (Roepstorff and Nansen, 1998). The next day, the sediment in the conical glasses was harvested using a glass pipette and the total number of L3 was counted in each sample after addition of Lugol's iodine (10%) to calculate the larvae per gram faeces (LPG). To evaluate the inadvertent occurrence of egg hatching for the horse strongyles, an additional direct Baermann analysis (without prior