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Estimation of abomasum strongyle nematode infections in sheep at necropsy: Tentative proposals for a simplified technique

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Abstract

Several necropsy techniques are available for estimating the abundance of gastro-intestinal nematodes in abomasum of ruminants. Standardization of techniques is needed to allow accurate comparisons between laboratories. Here we propose a standardized technique for estimating the abundance of worms. We intend to compare the worms' number estimations in lambs and ewes based on contents and washings, to determine the uniformity of worm counts in aliquots, and to estimate the total worm number from washings. The digesta (or ''contents'') and the washings of the abomasum are treated separately. The worms of each subsample are diluted with water and the total number of worms is estimated on a small volume (aliquots) of these subsamples. The use of aliquots assumes that the worms are uniformly distributed in the whole volume of each subsample. We first confirmed that the use of aliquots is appropriate in most cases. We then show that the use of the washings alone allows a faster and a suitable estimation of the total worm burden for all strongyle species of the abomasum in both ewes and lambs. The evaluation of our necropsy procedure is a first step to a standardized technique which should be improved by validation in other laboratories.

Keywords: Sheep; Nematode; Sampling technique; Statistical estimation

1. Introduction

Gastro-intestinal nematode identification is a necessity for establishing diagnostic and understanding of epidemiology of infection in ruminants. Several necropsy techniques are available for gastro-intestinal nematodes (MAFF, 1986; Vignau et al., 1999; Eysker and Kooyman, 1993) and important differences are recorded between them. The first difference is that digesta (or ''contents'') is (Eysker and Kooyman, 1993; MAFF, 1986) or not (Hansen and Perry, 1994; Vignau et al., 1999) treated separately from the

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''washings'' of the organ. The second difference is that the organ is soaked or not in tap water or saline at 37 °C for several hours (1.5 h in Clark et al., 1971 or 4–6 h in Reinecke, 1967); the reason for soaking during several hours the abomasum is that inhibited larvae can be found more easily in the soakings (Downey, 1981; Kassai, 1999). The last difference concerns the proportion of the total volume examined and the number of aliquots. Several aliquots of the pooled ''contents and washings'' are generally used to estimate of the whole worm population (Clark et al., 1971). The basic assumption of using aliquots is that worms are uniformly and independently distributed between the aliquots. Observed variation is due to random variation and accurate estimation of worm burden is based upon this assumption.

Eysker and Kooyman (1993) proposed their technique used at the University of Utrecht in the Netherlands as a standardised technique to allow comparison between laboratories. Three fractions (contents, immediate water wash of the organ and saline wash after 5 h) of the abomasum are examined. The distinct advantage was that most worms will be separated from the large bulk of the contents. The precision of their method was tested on both calves and lambs but on relatively small sample size (12 animals for each host species). It was although clear that most worms were recovered in the washings (79 and 83%, for *Haemonchus contortus* and *Teladorsagia circumcincta* in sheep, respectively). The saline wash in adult and juvenile-not L4 *Ostertagia* of calves represented 22% (Downey, 1981) or 38% (Eysker and Kooyman, 1993). Since that, no attempt was made to standardise worm recovery and counts: the last World Association for the Advancement of Veterinary Parasitology recommendations for assessing anthelmintic efficacy do not provide detailed information on worm counts at necropsy (Coles et al., 2006).

The worm counts in the contents are time-consuming due to the presence of many plant debris and the sole examination of the washings means a considerable reduction of the time spent for worm number evaluation. In this paper, we propose a faster and precise way to estimate worm burdens by using only the number of worms in the washings. We started from the following hypotheses: (i) the adult worms are in their large majority attached on the abomasum mucosa, and (ii) after death they progressively migrate or are found in the contents (much over 90% adult worms of *T. circumcincta* and *Trichostrongylus axei* are recovered in the contents on frozen abomasum samples: Cabaret J., 1977–1979. Unpublished 90 ewes data). We first described a technique based on the examination of two fractions of the abomasum: contents and washings (it includes immediate water wash and saline wash of Eysker and Kooyman, 1993). Then, we tested the uniformity of worm distribution between aliquots of experimental *T. circumcincta* and *H. contortus* infections in lambs. Finally, we checked whether the washings gave a sufficient estimation of the total worm burdens of lambs or ewes infected by either *T. circumcincta* or *H. contortus* or *Trichostrongylus axei* in abomasum.

2. Materials and methods

2.1. Parasitological data

All lambs and ewes that are studied in the present work, were primarily part of other experiments or epidemiological investigations. The main characteristics of their strongyle infections are presented in Table 1. The necropsies were mostly processed by two laboratory technicians and no significant difference in their results was demonstrated, and thus the results are presented independently from the operators that performed the necropsies and counts.

2.2. Estimating worm burdens

The abomasum are removed from the animal less than 15 min after slaughter and immediately processed. The abomasum is cut open over a tray in which the contents are caught, and then placed into a flask. The abomasum wall is washed thoroughly under a stream of water from a tap and the washings are placed into another flask (immediate water wash or washing 1). The abomasal mucosa (after being washed) is retained into a third flask for further processing. In the laboratory, the abomasal mucosa is immersed in warm tap water for 4–5 h (37 °C) to recover inhibited or not larvae, and remaining juvenile and adult worms (the washing 2 corresponds to the saline wash of Eysker and Kooyman, 1993). Up to 1 or 2 l of water are added to contents, washing 1 or 2, each. This volume may