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Measuring larval nematode contamination on cattle pastures: Comparing two herbage sampling methods

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ABSTRACT

Assessing levels of pasture larval contamination is frequently used to study the population dynamics of the free-living stages of parasitic nematodes of livestock. Direct quantification of infective larvae (L_3) on herbage is the most applied method to measure pasture larval contamination. However, herbage collection remains labour intensive and there is a lack of studies addressing the variation induced by the sampling method and the required sample size. The aim of this study was (1) to compare two different sampling methods in terms of pasture larval count results and time required to sample, (2) to assess the amount of variation in larval counts at the level of sample plot, pasture and season, respectively and (3) to calculate the required sample size to assess pasture larval contamination with a predefined precision using random plots across pasture. Eight young stock pastures of different commercial dairy herds were sampled in three consecutive seasons during the grazing season (spring, summer and autumn). On each pasture, herbage samples were collected through both a double-crossed W-transect with samples taken every 10 steps (method 1) and four random located plots of 0.16 m^2 with collection of all herbage within the plot (method 2). The average (±standard deviation (SD)) pasture larval contamination using sampling methods 1 and 2 was 325 (±479) and 305 (±444) L_3/kg dry herbage (DH), respectively. Large discrepancies in pasture larval counts of the same pasture and season were often seen between methods, but no significant difference ($P = 0.38$) in larval counts between methods was found. Less time was required to collect samples with method 2. This difference in collection time between methods was most pronounced for pastures with a surface area larger than 1 ha. The variation in pasture larval counts from samples generated by random plot sampling was mainly due to the repeated measurements on the same pasture in the same season (residual variance component = 6.2), rather than due to pasture (variance component = 0.55) or season (variance component = 0.15). Using the observed distribution of L_3, the required sample size (i.e. number of plots per pasture) for sampling a pasture through random plots with a particular precision was simulated. A higher relative precision was acquired when estimating PLC on pastures with a high larval contamination and a low level of aggregation compared to pastures with a low larval contamination when the same sample size was applied. In the future, herbage sampling through random plots across pasture (method 2) seems a promising method to develop further as no significant difference in counts between the methods was found and this method was less time consuming.

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1. Introduction

Gastro-intestinal nematodes are an important threat to economic livestock farming worldwide (Charlier et al., 2014). Ruminants get infected with these parasitic nematodes by ingestion of the free-living infective larvae (L_3) during grazing. Since long, assessment of the pasture larval contamination with L_3 has been used to understand the population dynamics of the free-living stages in epidemiological studies and to evaluate the effect of anthelmintic treatment programmes (Rickard et al., 1991; Satrija and Nansen, 1996; Bauer et al., 1997; Gossellin et al., 1998; Sargison et al., 2012). Pasture larval counts (PLC) will be used in the field validation of nematode vaccination strategies and targeted control programmes onwards (Le Jambre et al., 2008; Bassetto et al., 2014). As a proxy for the parasite infection risk to which animals are exposed, PLC serve both as input parameter and validation tool for the development of predictive nematode transmission models (Ward, 2006; Gaba et al., 2012; Laurenson et al., 2012a, 2012b; Fox et al., 2013; Rose et al., 2015).

Different techniques have been used to measure pasture larval contamination (Bryan and Kerr, 1988), including the use of grazing animals fistulated at the oesophagus, necropsy of tracer animals and direct quantification of L_3 on herbage. The ethical and economical aspects of using fistulated or tracer animals (Cabaret et al., 1986; Bryan and Kerr, 1988), put important limitations on the application of these techniques. These limitations do not apply to the direct quantification of L_3 on herbage. However, this technique has other important drawbacks: it is labour intensive (Boag et al., 1989; Demeler et al., 2012) and considerable variation is often seen between repeated measurements (Boag et al., 1989; Couvillion, 1993).

The process of direct quantification of L_3 on herbage consist generally of three phases; (1) herbage collection, (2) processing and (3) L_3-species identification (Couvillion, 1993). Until now, research to improve and facilitate quantification of L_3 on herbage has mainly focused on the two latter phases. Repeatability, recovery rates and speed of the processing phase have been improved during recent years (Demeler et al., 2012; Cassida et al., 2012) and also progress on molecular identification of L_3 on pasture samples has been made (Sweeny et al., 2012; Bisset et al., 2014). Despite these efforts, the herbage collection process still needs to be addressed to facilitate the use of PLC as routine diagnostic. Traditionally, herbage collection is done by walking a double-crossed W-transect across a pasture (Taylor, 1939). Throughout the years, modifications on this method have been made (e.g. Lancaster, 1970; Bryan and Kerr, 1988; Aumont and Gruner, 1989; Demeler et al., 2012), but differences in outcomes between sampling approaches remain poorly explored (Waller et al., 1981; Bryan and Kerr, 1988). The challenge is to develop a user-friendly sampling method that estimates pasture larval contamination with a precision that is acceptable in an epidemiological context. The aim of this study was (1) to compare two different sampling methods in terms of PLC and required time to sample herbage, (2) to assess the amount of variation in PLC at the level of sample plot, pasture and season, respectively and (3) to assess the adequate sample size for collecting herbage using random plots across pasture.

2. Materials and methods

2.1. Study design

In 2013, eight cattle pastures located in Flanders, Belgium were sampled in the morning during three consecutive seasons, spring (May/June), summer (August/September) and autumn (November/December). First season grazers grazed these pastures from April to November. The age of the animals at turn-out ranged from 6 to 24 months. At each sampling moment, pastures were sampled by two different methods, using both a double-crossed W-transect with samples taken every ten steps and four random located plots of 0.16 m^2 with collection of all herbage within the plot. The same protocol for L_3-recovery and L_3-identification was applied for all samples by a modified technique described by Taylor (1939) and expressed as number of L_3 per kg of dry herbage (L_3/kg DH). Climate data (precipitation (mm) and temperature (°C, minimum and maximum)) were registered daily by an automated weather station of the Royal Meteorological Institute, Belgium, located at maximum 34 km from the pastures (N 50°59′1.193″; E 3°48′43.548″).

2.2. Sampling methods

The first sampling method (method 1) was a modification of the technique described by Taylor (1939), in which

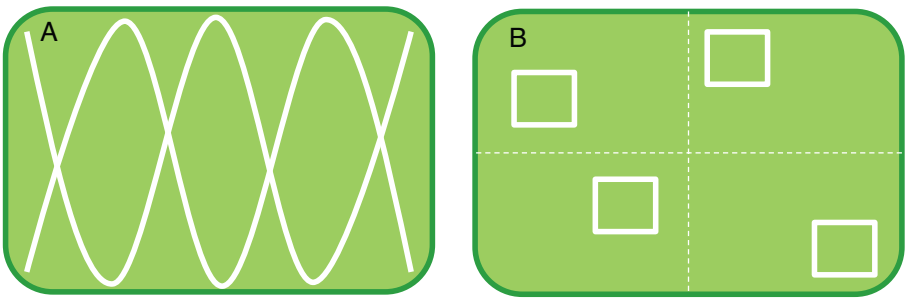

Fig. 1. The different herbage sampling methods used in this study. (A) Method 1 consists of sampling along two W-shaped transects across pasture and (B) method 2 was based on sampling of four random located plots of 0.16 m^2 in each quadrant of the pasture.