



Identification of effective treatment criteria for use in targeted selective treatment programs to control haemonchosis in periparturient ewes in Ontario, Canada

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ABSTRACT

Haemonchosis is often associated with late gestation and parturition in ewes in Canada. Due to widespread concerns about development of anthelmintic resistance (AR), targeted selective treatment (TST), where individual animals are treated with an anthelmintic rather than the entire flock, is a possible strategy to control clinical signs in recently lambed ewes while still maintaining parasite refugia. Performing fecal egg counts (FEC) on individual animals is often cost-prohibitive, so indicators that identify ewes with high FEC are essential for TST programs. The study objectives were to: a) evaluate the ability of four TST indicators to identify periparturient ewes with high *Haemonchus* sp. FEC and b) determine appropriate treatment thresholds for statistically-significant indicators. A field study was conducted during the 2013 and 2014 lambing seasons (February–May) on three client-owned farms in Ontario with documented AR and problems with haemonchosis in ewes. Ewes were examined within three days of lambing and selected for treatment with oral closantel (10 mg/kg body weight), a novel anthelmintic to Canada, if they met at least one of four criteria: a) the last grazing season was their first grazing season; b) body condition score ≤ 2 ; c) Faffa Malan Chart (FAMACHA[®]) score ≥ 3 ; and/or d) three or more nursing lambs. Fecal samples were collected *per rectum* on the treatment day from each of 20 randomly selected treated and untreated ewes on each farm. *Haemonchus* sp. percentages on each farm, as determined by coproculture, ranged from 53% to 92% of total fecal trichostrongyle-type egg counts. Mean *Haemonchus* sp. FECs were significantly higher in treated ewes ($n = 136$) than in untreated ewes ($n = 103$) on the day of treatment in both years ($p = 0.001$), suggesting the indicators were suitable for identifying animals with high *Haemonchus* sp. FEC. A linear mixed model was fit with logarithmic-transformed *Haemonchus* sp. FEC as the outcome variable, the four indicators and year as fixed effects, and farm as a random effect. FAMACHA[®] score was the sole indicator to remain significantly associated with FEC ($p = 0.002$). A receiver-operator curve determined that test sensitivity was maximized (92.4%) with FAMACHA[®] score ≥ 3 as the sole indicator. FAMACHA[®] score should therefore be included in TST programs to identify ewes requiring treatment at lambing due to *Haemonchus* sp.

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1. Introduction

Haemonchus contortus, a highly pathogenic gastrointestinal nematode (GIN) parasite of sheep, attaches in the abomasum and ingests blood, causing hypoproteinemia, anemia, and potentially

death in the host (Taylor et al., 2007). While significant morbidity and mortality from *H. contortus* in grazing lambs is well-recognized and described throughout the world, severe haemonchosis outbreaks in periparturient ewes have also been reported in several countries (Allonby and Urquhart, 1975; Darzi et al., 2004; Van Dijk and Morgan, 2006; Sargison et al., 2007). Haemonchosis outbreaks in ewes in Ontario, Canada are also associated with the peripartum period.

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In temperate northern climates, such as Canada, nearly all fourth-stage larvae undergo hypobiosis (i.e., arrested development) when conditions are not ideal for survival on pasture (e.g., late autumn at winter housing; Ayalew and Gibbs, 1973; Waller et al., 2004). Haemonchosis in periparturient ewes likely develops as result of a down regulation in the ewes' immunity at this time, facilitating the resumed development of hypobiotic larvae acquired during the previous grazing season (Blitz and Gibbs, 1972; Falzon et al., 2014). Anthelmintic treatment of entire flocks has traditionally been used in Ontario to control haemonchosis and egg shedding in periparturient ewes prior to spring turnout (Falzon et al., 2013a); however, when combined with low levels of pasture contamination (i.e., poor larval survival over the winter; Falzon et al., 2014), whole-flock treatment has been associated with development of anthelmintic resistance (Besier, 2001; Van Wyk, 2001; Leathwick et al., 2006a).

Targeted selective treatment (TST), in which only animals with significant parasite burdens are treated, may potentially delay the development of anthelmintic resistance by maintaining populations of parasites that have not been exposed to the anthelmintic (i.e., refugia) while limiting disease within the flock (Leathwick et al., 2008; Kenyon et al., 2009; Kenyon and Jackson, 2012). Parasite populations within sheep flocks are highly aggregated, such that most pasture contamination comes from a small proportion of individuals (Barger, 1985; Streeter et al., 1994). If only those individuals with parasite burdens above a critical threshold can be identified for treatment, the remaining animals can remain untreated, thus contributing to refugia and delaying the onset of anthelmintic resistance.

Identifying highly-burdened ewes is often a challenge. A parasite diagnostic test should be highly sensitive (i.e., accurately identify animals with high parasite burdens), particularly when *Haemonchus* sp. is the dominant parasite. If animals with high *Haemonchus* sp. burdens are not treated (i.e., false negatives), they will contaminate the pasture and/or may die. Currently, the gold-standard test is the fecal egg count (FEC); however, it is impractical to perform individual FECs on all individuals in a flock due to the cost and labor involved.

Previous research has investigated both pathophysiological and production-based indicators in attempts to identify animals with high FECs (Bath and van Wyk, 2009; Kenyon et al., 2009; Bentounsi et al., 2012; Kenyon and Jackson, 2012; Leathwick and Besier, 2014). These indicators have been investigated primarily in lambs on pasture, and their utility depends on the predominant parasite genera. The FAMACHA® score, which compares the color of the conjunctival membrane to a reference card, has been validated for identifying anemic animals and is a useful predictor of *Haemonchus* sp. parasitism (Malan et al., 2001; Kaplan et al., 2004; Bath and van Wyk, 2009). Other indicators, such as diarrhea score (Ouzir et al., 2011; Bentounsi et al., 2012), body condition score (BCS) (Bath and van Wyk, 2009; Gallidis et al., 2009; Ouzir et al., 2011), and weight gain (Leathwick et al., 2006b; Stafford et al., 2009; Gaba et al., 2010), have had varying success in identifying animals with high FEC. Additional TST indicators specific to periparturient ewes have also been investigated. There is some evidence that ewes raising multiple lambs, or having completed only one grazing season, may have poorer immunity to parasites and higher fecal egg shedding (Mahieu and Aumont, 2007; Saddiqi et al., 2011; Jones et al., 2012).

Few studies have examined the utility of TST indicators for determining which periparturient ewes should be treated, either as individual or combined indicators. The objectives of this study were therefore to: a) evaluate four TST indicators for identifying recently-lambd ewes with high *Haemonchus*-specific FEC; and b) determine appropriate treatment thresholds for the statistically-

significant indicators to maximize sensitivity while allowing some animals to remain untreated in order to maintain refugia.

2. Materials and methods

2.1. Farm and animal enrollment

Three sheep farms across southern Ontario, Canada were enrolled in the study for two consecutive lambing seasons (February 2013–May 2014). These farms also participated in a concurrent study on the efficacy of closantel, a novel anthelmintic to Canada, against anthelmintic-resistant *Haemonchus* infections (Westers et al., 2016). The farms were purposively selected from a list of Ontario sheep farms that had fecal egg count reduction tests performed as part of a previous study (Falzon et al., 2013b). Inclusion criteria were: a) documented GIN resistance to ivermectin (Falzon et al., 2013b); b) losses due to haemonchosis in both periparturient ewes and grazing lambs during the previous year; c) the farm would be pasturing ewes and lambs together during the grazing season for both years of the study; d) the animals had not previously been treated with closantel; e) farms were located within 250 km of the research laboratory; f) farms had at least 100 ewes; and g) producers were willing to allow multiple visits on farms by researchers over the two-year study period. Ewes were excluded from the study if they did not give birth to a live lamb, or if they were treated with any medications within four weeks of the treatment date. A questionnaire (available upon request) was administered to all three farms in February 2013 to gain an understanding of farm management practices prior to the study. Approval for this study was obtained from the University of Guelph's Animal Care Committee (Animal Use Protocol Number: 1427) and by the Research Ethics Board for the Ethical Acceptability of Research Involving Human Participants (Certification Number: 13FE002).

2.2. Selection of individual ewes for treatment

A flow chart of the total number of ewes used in the study is presented in Fig. 1. Parallel interpretation of the following four indicators was used to determine whether to treat an individual periparturient ewe: a) BCS ≤ 2 (measured on a five-point scale where 1 = extremely thin and 5 = over-conditioned, with half scores permitted; Thompson and Meyer, 1994); b) FAMACHA® score ≥ 4 (measured on a five-point scale where 1 = red or normal and 5 = white or severely anemic; Malan et al., 2001); c) three or more nursing lambs at the time of treatment; and/or d) if the previous grazing season was their first grazing season these treatment criteria were intended to target animals that would typically display the highest levels of fecal egg shedding (Van Wyk and Bath, 2002; Mahieu and Aumont, 2007; Bath and van Wyk, 2009; Kenyon et al., 2009; Stafford et al., 2009; Jones et al., 2012).

During the first year of the study (2013), ewes on Farm 2 were assessed for treatment within three days of lambing. Lambing on the other farms had already finished by the time closantel arrived, and ewes were assessed between 5 and 76 days of lambing. Ewes on all farms were assessed for treatment within three days of lambing during the second year of the study (2014). To ensure consistency, BCS and FAMACHA® scores were always assigned by the same individual. Producers were present for all farm visits and informed the research team regarding which animals had grazed pasture for only one season either ahead of time or on the day of assessment. The number of nursing lambs for each ewe was determined by the research team on the day ewes were assessed. It was decided *a priori* that the number of lambs would include those in the lambing pen with the ewe, rather than the number of lambs the ewe birthed,

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