Veterinary Parasitology 193 (2013) 150–162

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

Anthelmintic resistance in sheep flocks in Ontario, Canada

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ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form 7 November 2012
Accepted 8 November 2012

Keywords:
Gastro-intestinal nematodes
Ivermectin drench failure
Fecal Egg Count Reduction Test
Anthelmintic resistance
Larval Development Assays
Post-treatment larval cultures

ABSTRACT

Gastrointestinal nematodes (GIN) are a significant constraint to pasture-based sheep production worldwide. Anthelmintic resistance (AR) has been reported in most sheep-raising areas in the world, yet little is known about the AR status in Canada. This study was conducted to determine the frequency of AR in GIN in sheep flocks in Ontario, Canada. Forty-seven sheep flocks were enrolled in the study, and their level of parasitism was monitored monthly throughout a grazing season by analyzing owner-acquired fecal samples from 15 grazing lambs per flock. When the mean GIN fecal egg count (FEC) reached a threshold of 200 eggs per gram (epg), oral ivermectin was supplied to producers to check ivermectin efficacy; the reduction in mean FEC 14 days after ivermectin treatment was calculated. 'Drench failure' was defined as a reduction in mean FEC of <95%. In those flocks with apparent drench failure, researchers performed a Fecal Egg Count Reduction Test (FECRT), dividing sheep into 4 treatment groups (n = 10–15): control (i.e. untreated), ivermectin, and, if sufficient numbers of animals – fenbendazole and levamisole. AR was defined as a reduction in mean FEC <95% and a lower 95% confidence interval <90%. Larval cultures were performed on pooled post-treatment FECRT samples. Larval Development Assays (LDAs) to detect the presence of resistance to thiabendazole and levamisole were performed prior to the ivermectin drench check on pooled owner-acquired fecal samples that reached the 200 epg threshold. Approximately 89% (42/47) of the farms reached the FEC threshold of 200 epg; 93% (39/42) of these farms performed an ivermectin drench check, and 88% (34/39) of these farms had drench failure. The FECRT was performed on 29 of the 34 farms. Resistance to ivermectin, fenbendazole and levamisole was demonstrated on 97% (28/29), 95% (19/20) and 6% (1/17) of the farms tested, respectively, with considerable variability in resistance levels among farms. *Haemonchus* sp. was the most commonly cultured parasite from post-treatment fecal samples. LDA results for 21 farms were available; of these, 14% (3/21) and 62% (13/21) had low and high levels of thiabendazole resistance, respectively, while none of the farms exhibited resistance to levamisole. Amongst these tested farms, resistance to both ivermectin and benzimidazoles was very common. These findings strongly suggest that AR, particularly in *Haemonchus* sp., is a serious problem in these sheep flocks. Thus, marked changes in GIN management need to be instituted immediately to mitigate a worsening situation.

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http://dx.doi.org/10.1016/j.vetpar.2012.11.014

1. Introduction

Parasitic gastroenteritis caused by gastrointestinal nematodes (GINs) is widely considered the most important disease of grazing sheep worldwide, causing weight loss, diarrhea and death (Sutherland and Scott, 2010). Gastrointestinal nematode infections are typically controlled with anthelmintic drugs, and sheep producers worldwide have customarily relied heavily on such drugs to maintain sheep health and productivity, while improving the overall profitability of the sheep industry (Sargison, 2008).

In North America, three broad-spectrum anthelmintic drug classes are most commonly used in sheep: macrocyclic lactones (e.g. ivermectin and moxidectin), benzimidazoles (e.g. thiabendazole, fenbendazole and albendazole) and imidazothiazoles (e.g. levamisole) (Adams, 2001). In Canada, only ivermectin is licensed for use in sheep (Compendium of Veterinary Products, Canada, 2012). Thiabendazole was the first benzimidazole to be marketed in Canada in the early 1960s (Adams, 2001), but was subsequently replaced with other structurally similar, but improved drugs, such as fenbendazole and albendazole. Fenbendazole and albendazole are licensed for use in Canada in cattle (Compendium of Veterinary Products, Canada, 2012), but are often used in sheep in an extra-label manner. Levamisole has not been licensed for use in sheep in Canada for the past 10 years (Health Canada – Drug Product Database Online Query, 2012).

Anthelmintic resistance (AR) is defined as the "heritable ability of the parasite to tolerate a normally effective dose of the anthelmintic" (Abbott et al., 2009), and if sufficiently prevalent in a parasite population, results in treatment failure. However, treatment failure may also be caused by other confounding factors (McKenna, 1990), such as underdosing or incorrect administration of anthelmintic drugs (El-Abdellati et al., 2010).

Anthelmintic resistance is an escalating problem in most sheep-rearing countries worldwide (Papadopoulos, 2008), and is a threat to both agricultural income and sheep welfare (Wolstenholme et al., 2004). It is widespread in New Zealand (Waghorn et al., 2006), Australia (Love et al., 1992; Besier and Love, 2004), and in many South American countries, such as Brazil and Uruguay (Waller et al., 1996; Cezar et al., 2010). In recent years, AR has also been described in the United States (Kaplan and Vidyashankar, 2012) and in several European countries including Greece (Gallidis et al., 2009), Italy (Cringoli et al., 2009) and the United Kingdom (Jackson and Coop, 2000). In 2007, the first case of AR in Canada was described in a sheep flock in Ontario (Glauser et al., 2007). Ontario is considered to have a humid continental climate, with cold snowy winters and warm to hot summers (World Maps of Köppen-Geiger Climate Classification, 2012). While recent studies have investigated how this climate affects the epidemiology of GIN infections in sheep (Mederos et al., 2010), no surveys have been published on how widespread the problem of treatment failure and AR is in sheep flocks in Canada and, in particular, Ontario.

The Fecal Egg Count Reduction Test (FECRT) is the standard test for determining AR under field conditions (Coles et al., 1992), and provides an indirect measurement of anthelmintic efficacy by determining the reduction in fecal egg counts (FECs) after treatment (McKenna, 2006). Several authors have suggested different threshold values for defining the presence of AR (McKenna, 1990; Wood et al., 1995; Smart, 2009), but the most commonly accepted threshold is that endorsed by the World Association for the Advancement of Veterinary Parasitology (WAAVP), which defines AR as a Fecal Egg Count Reduction (FECR) of <95% and a lower 95% confidence interval (CI) of <90%; if only one of these two factors is present, the farm is defined as being 'suspected' of having resistance (Coles et al., 1992).

Despite being the standard test for AR determination, the FECRT is laborious, expensive and time-consuming (Craven et al., 1999; El-Abdellati et al., 2010). As a result, various alternative diagnostic tests have been suggested for the determination of anthelmintic susceptibility (Coles et al., 2006). The Larval Development Assay (LDA) described by Taylor (1990) is based on culturing a known number of GIN eggs in the presence of different anthelmintics. It is reported to be relatively easy to perform, more sensitive than the FECRT, and allows for the identification of parasite larvae to the genus level (Taylor, 1990). However, some LDAs are unable to reliably detect resistance to avermectins (Grimshaw et al., 1994), and the methodology is considered by some to require a high level of technical expertise, thus limiting its use outside of research laboratories (Kaplan and Vidyashankar, 2012).

The objectives of this study in Ontario sheep flocks were: (i) to determine the frequency of ivermectin treatment failure; (ii) to determine the frequency of resistance to ivermectin, fenbendazole and levamisole using a FECRT; and (iii) to assess the frequency of resistance to thiabendazole and levamisole using the LDA.

2. Materials and methods

2.1. Number and selection of sheep farms

The study was conducted in Ontario, Canada, for two consecutive grazing seasons (May to November 2010 and May to November 2011). The target population was sheep farms in Ontario, while the study population was eligible and willing sheep producers (as defined below) that were members of the Ontario Sheep Marketing Agency (OSMA) – a producer-operated agency formed under the Ontario Farm Products Marketing Act that represents all producers that raise and sell market lambs. All registered producers ($n = 3600$) receive the magazine 'Ontario Sheep News', with over 70% of these producers also receiving emails from the OSMA email list-serve (OSMA office, personal communication).

Forty-seven sheep farms were recruited during the summers of 2010 or 2011. This sample size is associated with a precision of 14%, using a 95% level of confidence and an estimated ivermectin drench failure prevalence of 60% (latter estimate based on an unpublished pilot study conducted on Ontario sheep farms in 2009, in which 8 of 13 farms tested had ivermectin drench failure).

Recruitment required volunteer participation and was carried out through talks given at various OSMA sheep producer meetings held across the province and letters posted