

Research Note

Assessing the usefulness of mineral licks containing herbal extracts with anti-parasitic properties for the control of gastrointestinal helminths in grazing sheep – a field trial

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Article info

Received May 29, 2015
Accepted November 26, 2015

Summary

One of the alternative methods of parasite control, of particular importance in sustainable farming, is the use of medicinal plants. The specific aim of the present field trial was to assess the anti-parasitic effects of herbal extracts contained in a commercially available lick formulation for sheep. At the outset of this study conducted during the grazing season, all animals were de-wormed and then randomly assigned to one of the two separately kept groups (treatment and control), each consisting of 25 animals (11 ewes and 14 lambs). The treatment group received mineral licks containing the extracts of the plants with anti-parasitic properties, while control animals received standard mineral licks *ad libitum*. Rectal fecal samples were collected monthly from all animals for the McMaster analyses. There were no significant differences in the prevalence and intensity of helminth infections between the treatment and control groups. Thus, we were not able to ascertain the efficacy of the commercial herbal de-wormer tested for the control of gastrointestinal helminths in grazing ewes and their lambs.

Keywords: sheep; gastrointestinal nematodes; *Moniezia* spp.; medicinal plants; mineral licks

Introduction

The widespread occurrence of gastrointestinal helminths has adverse effects on the productivity of commercial flocks of sheep, even in the subclinical course of parasitoses (McLeod, 1995; Perry & Randolph, 1999; Michalski, 2007). In Poland, due mainly to economic reasons, the extensive pastureland sheep farming is commonplace, but this method of animal management is invariably associated with frequent parasitic infections of which nematode infections are the most prevalent (Nowosad *et al.*, 2000). The prophylaxis of gastrointestinal helminthiasis in sheep typically entails the use of injectable anti-helminthic drugs. However, the incidents of drug-resistant populations of worms along with the emerging trend to limit the application of chemical agents, especially in organic farming, has prompted the development of

alternative approaches to anti-parasitic therapies (Cabaret *et al.*, 2002; Waller & Thamsborg, 2004; Torres-Acosta & Hoste, 2008). A broad range of ethnoveterinary therapeutic products, most of them utilizing various medicinal plants with anti-parasitic effects, are currently being evaluated (Akhtar *et al.*, 2000; Waller *et al.*, 2001; Githiori *et al.*, 2006; Athanasiadou *et al.*, 2007). However, the effectiveness of herbal formulations marketed for parasite control in small ruminants under field conditions has been scarcely documented (Bouilhol *et al.*, 2001; Luginbuhl *et al.*, 2006; Burke *et al.*, 2009a). Hence, the main objective of the present study was to determine the utility of commercially available mineral licks containing the herbal extracts with anti-parasitic properties on the level and dynamics of gastrointestinal helminth infections in previously de-wormed sheep throughout the grazing season.

Material and Methods

This study was conducted from May to October, at the Bielany Experimental Station of the Agricultural University in Krakow, Poland (latitude: 50°20'N and longitude 19°49'E), and it utilized a total of 50 Polish Longwool ewes and lambs. Animals were sheltered in a deep litter barn with straw bedding at night and were let out to graze on the good quality alfalfa grass pasture during the day. Clean water was available *ad libitum* to all animals and lambs had unlimited access to oats in the barn.

Prior to the present field trial, all animals underwent routine coproscopic analyses using the McMaster technique (Anonymous, 1986) and coprocultures were prepared from bulk fecal samples to detect the nematode larvae (van Wyk and Mayhew, 2013). No tapeworms were found in three consecutive fecal samples. However, due mainly to detection of *Haemonchus contortus* and *Nematodirus battus*, all sheep had been de-wormed (IVOMEC® Liquid for Sheep, Merial, France; 0.2 mg of ivermectin per 1 kg b.w.) with a 100 % efficacy, as confirmed by the fecal egg count reduction test (FECRT; Coles *et al.*, 1992) performed 10 days post-treatment.

Two weeks post-treatment, the animals were randomly assigned to one of the two separately kept groups of mixed age, namely the untreated control and treatment groups of 11 ewes and 14 female lambs each. Lambs (born in February) remained with their mothers for the duration of the present study. Ewes were 2.5 to 6 years of age, weighing 55 to 70 kg, and not mated throughout the entire experimental period. Control animals had unlimited access to standard mineral licks (Lisal Se; Kopalnia Soli "Kłodawa" S.A.; <http://www.sol-klodawa.com.pl>) while the treatment group received the Star Bloc Phyto Vers (Guyokrma Ltd.; <http://www.guyokrma.cz>) *ad libitum*; this commercially available product contains the extracts of the wormwood *Artemisia absinthium*, wormseed *Artemisia cina*, tansy *Tanacetum vulgare*, thyme *Thymus vulgaris*, garlic *Allium sativum*, wormmoss *Alsidium helminthochorton*, male fern *Dryopteris filix-mas*, carrot *Daucus carota*, goosefoot *Chenopodium*, and pomegranate *Punica granatum*. The mineral composition of both licks is given in Table 1.

During the entire grazing period, the sheep were not given any anti-parasitic drugs. From both groups of animals, fecal samples were collected *per rectum* each month for McMaster's analyses (starting two weeks into the grazing season); the sensitivity of the test was 20 eggs per 1 g of feces (EPG). In addition, at the end of grazing season, the sections of the entire digestive tract obtained from three adult sheep (one control and two treated ewes) that were slaughtered for the reasons independent of the present experimental procedures were used to detect gastrointestinal helminths, applying the method described by Hansen and Perry (1994).

Fecal egg counts and the percentage of animals with parasitic infections were subjected to statistical comparisons. The present data sets did not exhibit the normal distribution even after transfor-

Table 1. The mineral composition of two lick formulations used in the present field trial in grazing sheep (May – October)

Elements	Lisal Se	Star Bloc Phyto Vers
Sodium	35 %	8 %
Calcium	–	14 %
Phosphorus	–	4 %
Magnesium	2000 mg/kg	4 %
Zinc	810 mg/kg	8000 mg/kg
Manganese	830 mg/kg	6700 mg/kg
Iron	354 mg/kg	–
Iodine	100 mg/kg	80 mg/kg
Cobalt	18 mg/kg	40 mg/kg
Selenium	10 mg/kg	30 mg/kg
Copper	–	20 mg/kg

mations because of a highly aggregated, right-skewed frequency distribution of the EPG counts. Therefore, the Quantitative Parasitology 3.0 software (<http://www.zoologia.hu/qpl/>) for non-transformed data matching the negative binomial distribution (Rózsa *et al.*, 2000) was used to compare mean values in the present subsets of ewes and lambs. Due to seasonal fluctuations in the dynamics of parasitic infection, the analyses were done separately for each month. The Fisher's exact test was run to compare the prevalence of gastrointestinal parasitoses (% of infected animals), and the Bootstrap 2-sampled t-test was employed to evaluate and compare the intensity of parasitic infections (EPG counts).

Results and Discussion

Both groups of animals used the supplied mineral licks eagerly and uniformly throughout the study. No clinical signs of parasitic infections have been observed but the animals did contract gastrointestinal helminths (Figs. 1 – 3). In the post-mortem examinations of the three culled ewes, a highly pathogenic *Haemonchus contortus* was detected (a total of 630, and 26 adult parasites in the abomasums of two animals), and a few specimens of a stomach worm *Teladorsagia circumcincta* were seen in one ewe. Some other nematodes (i.e., *Chabertia ovina*, *Oesophagostomum venulosum* and *Trichuris ovis*) were present in the large intestine of all sheep examined (1 to 16 worms per ewe).

There was no difference between the treatment and control groups in the results of coproscopic examinations. Strongyle egg counts, which remained low in the early months of the trial due to initial de-worming, increased during the grazing season and reached maximum values between August and October (Figs. 1 and 2). In the month of August, the intensity of strongyle infection was greater ($P < 0.05$) in the treated compared with control ewes (772 vs. 281 EPG, respectively) but in October, control ewes exceeded ($P < 0.05$) treated animals in strongyle egg counts (217 vs. 979 EPG for treated and control ewes, respectively). With regards to the

tapeworms of *Moniezia* spp., the prevalence of infection was greater in control than in treated ewes in June (36.3 vs. 9.1 %; $P < 0.05$) whereas in July it was greater in the treated compared with control animals (18.2 vs. 54.5 %; $P < 0.05$), and thereafter there were no significant differences between the two groups (Fig. 3).

Similarly to our present results, Burke *et al.* (2009a) reported a lack of significant anti-helminthic effects of a herbal mixture containing *Artemisia absinthium*, *Allium sativum*, *Thymus vulgaris*, fennel *Foeniculum vulgare*, black walnut *Juglans nigra*, pumpkin *Cucurbita pepo*, hyssop *Hyssopus officinalis*, and sweetleaf *Stevia rebaudiana* in goats. Furthermore, in the trial conducted by Luginbuhl *et al.* (2006), another commercially available herbal de-wormer was ineffective in reducing EPG counts in goats, and in the study by Bouilhol *et al.* (2001) there were no apparent anti-parasitic effects of five different herbal preparations on gastrointestinal helminths in grazing sheep.

Contrary to the aforementioned field studies, a number of *in vivo* and *in vitro* experiments have been supportive of the efficacy of using plant extracts for the control of gastrointestinal parasites, especially the nematodes, in small ruminants (Githiori *et al.*, 2006; Athanasiadou *et al.*, 2007) and others reported inconsistent outcomes (Torres-Acosta & Hoste, 2008). A high dose of *A. sativum* extracts – one of the plants used in the preparation of mineral licks tested in the present trial – reduced the nematodes' EPGs in sheep (Masamba *et al.*, 2010). However, garlic failed to decrease the fecal nematode egg counts in the study by Worku *et al.* (2009) or Burke *et al.* (2009b) in spite of the fact that its active compound (ajoene) caused *H. contortus* larval-stage mortality in an *in vitro* experiment (Ahmed *et al.*, 2013). With respect to the wormwoods, Tariq *et al.* (2009) have demonstrated that an *A. absinthium* extract containing santonin reduced the numbers of gastrointestinal nematodes in sheep, in a dose-dependent manner, and the extracts of a similar

plant, *Artemisia brevifolia*, substantially reduced *H. contortus* egg counts following an oral route of administration (Iqbal *et al.* 2004). Alternatively, Cabaret (1996) was unable to demonstrate any decrease in fecal egg output of gastrointestinal nematodes after an oral application of *Artemisia cina* at homeopathic 9 – 15 centesimal dilution in lambs. A study by Jabbar *et al.* (2007) has suggested that *Chenopodium album* and *C. ambrosioides* possess strong anti-parasitic activity against sheep gastrointestinal nematodes. Several other plants have been used as therapeutics or are thought to have some anti-helminthic properties because of a high content of various natural parasiticides (e.g., filicid acid in male fern or thujon in tansy; Waller *et al.*, 2001). The anti-helminthic effects of pomegranate containing isopelletierine have also been described in ruminants (Akhtar *et al.*, 2000). It is, therefore, feasible that the doses of extracts used by the manufacturer of the Star Bloc Phyto Vers mineral supplements are not high enough to produce therapeutic or anti-parasitic effects. On the other hand, continual oral administration of the larger amounts of some herbal extracts contained in this product could be harmful to animals because of their potential toxicity and hence relatively narrow ranges of therapeutic doses (Waller *et al.*, 2001; Athanasiadou *et al.*, 2007).

In sheep with the mixed nematode infections and presence of blood-sucking species such as *H. contortus* or *T. circumcincta* the estimated threshold levels for helminthiasis are 50 – 800 EPGs for light, 800 – 1200 EPGs for moderate, and >1200 EPGs for heavy infections (Hansen & Perry, 1994). In light of these classification, the EPG counts for nematodes recorded during the present experiment were indicative of a moderate (in ewes of the control and lambs of the treatment group) or high level of infection (in control lambs) during the last month of the grazing period.

There exists an urgent need for alternatives to chemical anti-parasitic drugs, especially in organic farming practices, where the

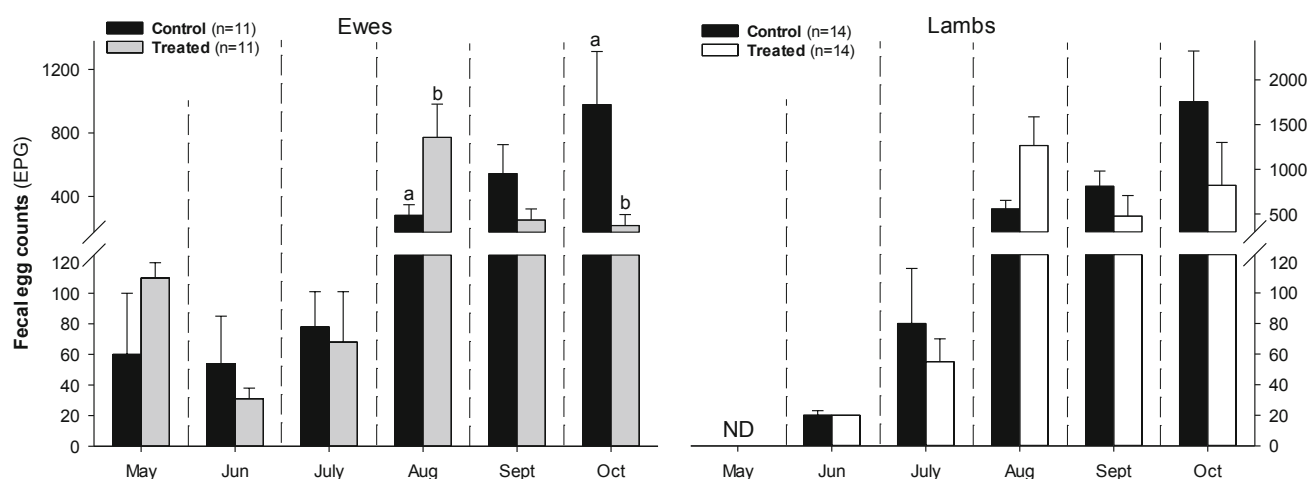


Fig. 1. Fecal egg counts (EPG, eggs per 1g of faeces; mean + SEM) of strongyle nematodes detected in ewes and their lambs throughout the grazing season (May–October). Treated animals received mineral licks containing the extracts of the plants with anti-parasitic properties (Star Bloc Phyto Vers), while their respective control animals received standard mineral licks (Lisal Se) *ad libitum*. ND - not detected. Within months, means denoted by different letters are significantly different ($P < 0.05$)

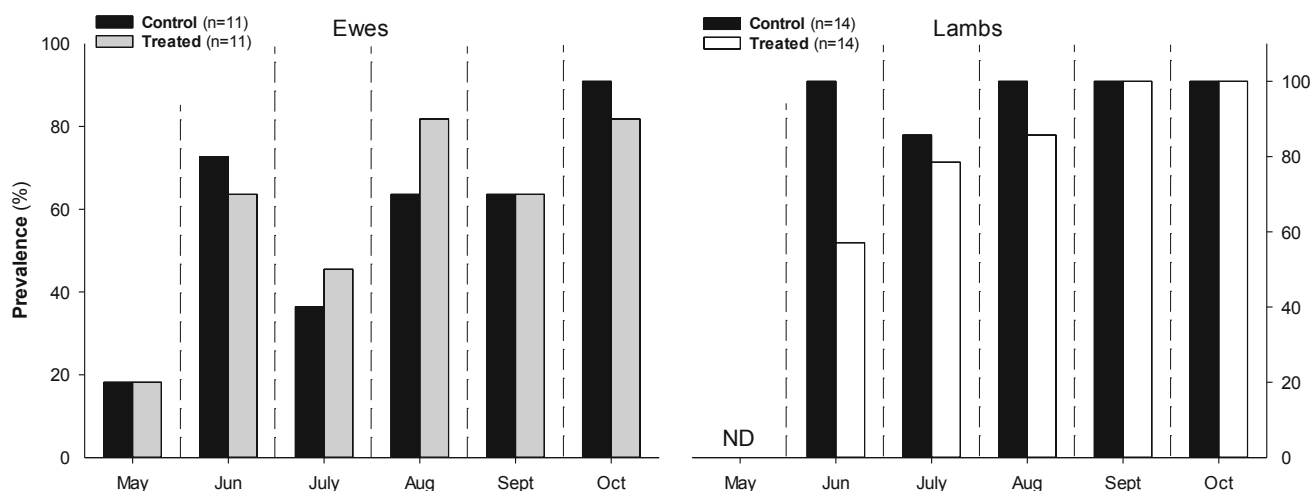


Fig. 2. Prevalence (%) of strongyle nematodes infections in ewes and their lambs determined by coproscopic examination during the grazing season (May-October). Treated animals received mineral licks containing the extracts of the plants with anti-parasitic properties (Star Bloc Phyto Vers), while their respective control animals received standard mineral licks (Lisal Se) *ad libitum*. ND - not detected

risk of contracting helminthiasis is particularly high. Pilarczyk *et al.* (2008) have shown that the prevalence of gastrointestinal nematode infections in conventional operations is more than twice as high as in intensive farming (79.0 % vs. 30.6 %, respectively). There are a few other methods of parasite control currently used in organic production systems, such as rational pasture management (Cabaret *et al.*, 2002; Torres-Acosta & Hoste, 2008), exploitation of intrinsic/genetic resistance of sheep (Nowosad *et al.*, 2003), or nutritional modulation of the host's immune responses and gut environment (Waller & Thamsborg, 2004). The latter refers to controlled grazing on bioactive forages or feeding condensed tannins as nutraceuticals (Min & Hart, 2003; Tzamaloukas

et al., 2005; Torres-Acosta & Hoste, 2008; Houdijk *et al.*, 2012), although controversy regarding the use of tannins is constantly rising as their excessive consumption has been associated with the reduction in food intake and digestibility as well as impaired rumen metabolism (Athanasidou *et al.*, 2007).

Based on several earlier studies, certain plants are more effective against specific parasites than the others (Tzamaloukas *et al.*, 2005; Athanasidou *et al.*, 2007). This variability in the therapeutic potential of medicinal plant may be related to the parasite niche or bioavailability of herbal compounds in different compartments of the gastro-intestinal tract of infected host animals. Whether the mineral licks used in the present study contain plant extracts that

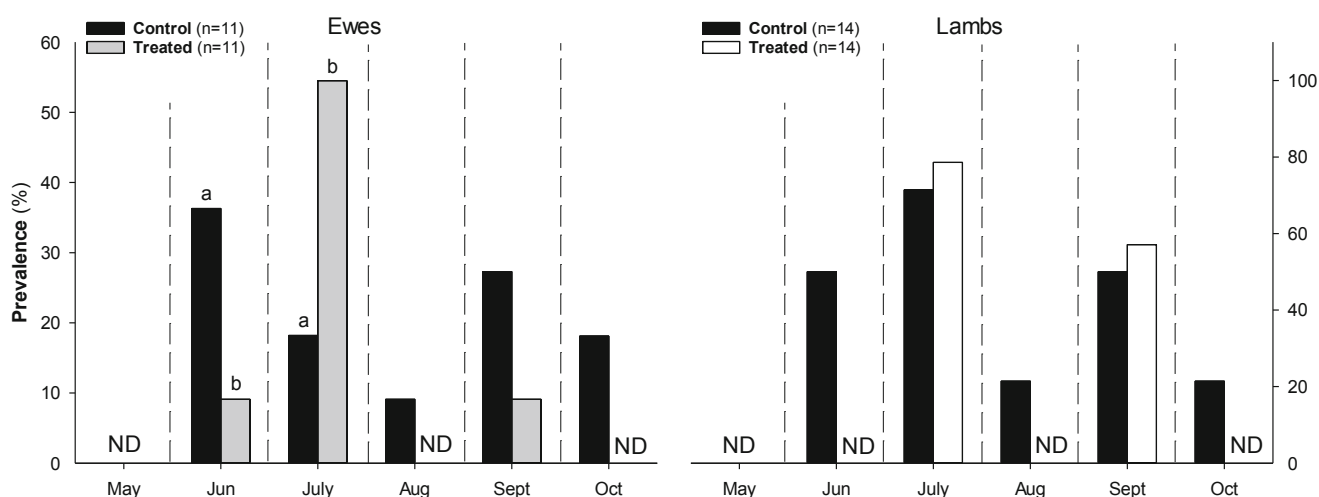


Fig. 3. Prevalence (%) of *Moniezia* spp. infections in ewes and lambs determined by coproscopic examination during the grazing season (May-October). Treated animals received mineral licks containing the extracts of the plants with anti-parasitic properties (Star Bloc Phyto Vers), while their respective control animals received standard mineral licks (Lisal Se) *ad libitum*. ND - not detected. Within months, values denoted by different letters are significantly different ($P < 0.05$)

exert direct effects on *H. contortus* or *T. circumcincta* remains to be elucidated. Moreover, some medicinal plants possess immunomodulatory effects, which may constitute additional benefit to herbivores. Athanasiadou *et al.* (2007) postulated that in addition to evaluating the direct anti-helminthic effects of plant extracts, one should always be looking for evidence of their influences on the resistance or resilience of individual animals as well as their overall well-being.

To summarize, the present findings are in agreement with the results of some previous field trials and showed a lack of effect of the commercially available Star Bloc Phyto Vers lick containing medicinal plant extracts on gastrointestinal helminths in moderately infected grazing sheep. Whether or not this outcome is due to the extract content or application regimen remains to be elucidated.

Acknowledgements

We would like to thank very much the reviewers whose comments contributed greatly to the improvement of our article.

This research was financed by the Ministry of Science and Higher Education of the Republic of Poland. Any mention of the trade names of commercial product in this manuscript is solely for the purpose of providing specific information and does not imply either the endorsement or disapproval of such products. The authors have no present conflicts of interest to declare.

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