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An evidence-based approach to the evaluation of ethnoveterinary medicines against strongyle nematodes of equids

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ABSTRACT

Cyathostomins are the most important gastrointestinal nematode infecting equids. Their effective control is currently under threat due to widespread resistance to the broad spectrum anthelmintics licenced for use in equids. In response to similar resistance issues in other helminths, there has been increasing interest in alternative control strategies, such as bioactive plant compounds derived from traditional ethnoveterinary treatments. This study used an evidence-based approach to evaluate the potential use of plant extracts from the UK and Ethiopia to treat cyathostomins. Plants were shortlisted based on findings from a literature review and additionally, in Ethiopia, the results of a participatory rural appraisal (PRA) in the Oromia region of the country. Systematic selection criteria were applied to both groups to identify five Ethiopian and four UK plants for *in vitro* screening. These included *Acacia nilotica* (L.) Delile, *Cucumis prophetarum* L., *Rumex abyssinicus* Jacq., *Vernonia amygdalina* Delile. and *Withania somnifera* (L.) Dunal from Ethiopia and *Allium sativum* L. (garlic), *Artemisia absinthium* L., *Chenopodium album* L. and *Zingiber officinale* Roscoe. (ginger) from the UK. Plant material was collected, dried and milled prior to hydro-alcoholic extraction. Crude extracts were dissolved in distilled water (dH_2O) and dimethyl sulfoxide (DMSO), serially diluted and screened for anthelmintic activity in the larval migration inhibition test (LMIT) and the egg hatch test (EHT). Repeated measures ANOVA was used to identify extracts that had a significant effect on larval migration and/or egg hatch, compared to non-treated controls. The median effective concentration (EC-50) for each extract was calculated using PROBIT analysis. Of the Ethiopian extracts *A. nilotica*, *R. abyssinicus* and *C. prophetarum* showed significant anthelmintic activity. Their lowest EC-50 values were 0.18 (confidence interval (CI): 0.1–0.3), 1.1 (CI 0.2–2.2) and 1.1 (CI 0.9–1.4) mg/ml, respectively. All four UK extracts, *A. sativum, C. album, Z. officinale* and *A. absinthium*, showed significant anthelmintic activity. Their lowest EC-50 values were 1.1 (CI 0.9–1.3), 2.3 (CI 1.9–2.7) and 0.3 (CI 0.2–0.4) mg/ml, respectively. Extract of *A. absinthium* had a relatively low efficacy and the data did not accurately fit a PROBIT model for the dose response relationship, thus an EC-50 value was not calculated. Differences in efficacy for each extract were noted,

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dependent on the assay and solvent used, highlighting the need for a systematic approach to the evaluation of bioactive plant compounds. This study has identified bioactive plant extracts from the UK and Ethiopia which have potential as anthelmintic forages or feed supplements in equids.

1. Introduction

Cyathostomins are clade V gastrointestinal (GI) nematodes and are the most abundant parasitic nematodes of equids worldwide (Kaplan and Vidyashankar, 2012; Love et al., 1999). Their pathogenic effect ranges from non-specific weight loss to colic and larval cyathostominosis. The latter is a potentially fatal condition caused by the mass emergence of fourth stage larvae from the large intestinal wall (Lyons et al., 2000; Murphy and Love, 1997; Peregrine et al., 2006; Uhlinger, 1991). Their pathogenic effect in developing countries is not well defined due to a lack of research. However, cases of larval cyathostominosis have been reported in developing regions (Oryan et al., 2013) and there is evidence to suggest that high strongyle burdens are negatively associated with body condition in working equids (Yoseph et al., 2005).

Effective control of cyathostomin infection is threatened by the widespread prevalence of anthelmintic resistance. The three broad-spectrum classes of anthelmintic that are licensed to control cyathostomins are the benzimidazoles (BZs, fenbendazole), the tetrahydropyrimidines (pyrantel) and the macrocylic lactones (MLs, ivermectin and moxidectin). The MLs are the most potent and widely used anthelmintics (Allison et al., 2011; Nielsen et al., 2006; Reinemeyer and Rohrbach, 1990). In developed countries, there is widespread resistance in cyathostomin populations to BZs (Kaplan et al., 2004; Lester et al., 2013; Lind et al., 2007; Traversa et al., 2007, 2009b, 2012) and resistance to pyrantel is common in some regions (Lyons et al., 2001; Nielsen et al., 2013; Stratford et al., 2014; Traversa et al., 2007, 2009b, 2012). More recently, ML resistance, measured primarily as a reduction in strongyle egg reappearance period, has been reported in several regions (Canever et al., 2013; Milillo et al., 2009; Molento et al., 2008; Nareaho et al., 2011; Relf et al., 2014; Traversa et al., 2009b; Trawford and Burden, 2009). None of the new anthelmintic classes that have been licensed for use in small ruminants are likely to be licensed in equids (Epe and Kaminsky, 2013). There have been no published reports of anthelmintic resistance in cyathostomins in developing countries, however resistance has been demonstrated in helminths of small ruminants (Eguale et al., 2009; Kumsa and Abebe, 2009; Sissay et al., 2006a,b). The development of anthelmintic resistance could be accelerated by the variable quality of anthelmintics available in these environments (Monteiro et al., 1998; Shakoor et al., 1997). Another impediment to effective parasite control in such regions is a lack of available treatments in poor rural communities.

The challenges of continued effective helminth control using conventional anthelmintics have led to a growing interest in alternative control strategies. One method, which may be applicable in developed and developing countries, is the use of plants with anthelmintic activity. This practice has been employed across the globe for many centuries. In developed countries, it has largely ceased due to the widespread availability of chemical anthelmintics, but in developing countries these practices remain (Githiori et al., 2006). Plants with proven efficacy against nematode infections are a potentially rich source of anthelmintic compounds that could be developed into a novel therapeutics (Geary et al., 2012). A recently published study by Payne et al. (2013) was the first to demonstrate the efficacy of some Australian plant extracts against cyathostomins *in vitro*. Scientific evidence supporting the anti-parasitic properties of plants comes mostly from *in vitro* studies (Athanasiadou and Kyriazakis, 2004), the advantage being that large numbers of plant extracts can be tested at relatively low cost. The most commonly used assays in the screening process are the egg hatch test (EHT) (Coles et al., 1992), the larval development test (LDT) (Gill et al., 1995), the larval migration inhibition test (LMIT) (Demeler et al., 2010; Matthews et al., 2008) and the adult worm motility assay (AWMA) (Stepek et al., 2005).

Here, we used an evidence-based systematic approach to evaluate the effect of several plant extracts, derived from the UK and Ethiopia, against cyathostomin eggs and third stage larvae (L3).

2. Materials and methods

2.1. Plant identification

Ethiopia: The results of a participatory rural appraisal (PRA) in the Oromia region of Ethiopia (Scantlebury et al., 2013), were used to rank local plants according to the frequency with which they were used and their reported efficacy. Interviews were also held with traditional healers, identified as key informants in the PRA, in order to identify additional plants used as anthelmintics in the region. Where possible plants of interest were collected from the Eastern Shewa and Arsi zones of the Oromia region in July 2011 and identified to species level according to the international plant names index (IPNI) at the University of Addis Ababa, National Herbarium, Ethiopia. A literature review was used to identify supportive evidence of anthelmintic activity in these plant species. Five plants were shortlisted based on the following criteria: identification in the PRA, ranking in the PRA, supportive evidence of *in vitro* or *in vivo* efficacy in the literature, a lack of reported severe side effects and availability. *UK*: plants were shortlisted by means of a literature review using the PubMed search engine (http://www.ncbi.nlm.nih.gov>NCBI>Literature). The search criteria entered were 'plant' and 'anthelmintic'. This yielded 2480 results, 139 of which reported the *in vitro* or *in vivo* effect of plants on GI nematodes. The data