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Research Note

Evaluation of a marketed polyherbal dewormer against intestinal strongyles in naturally infected donkeys

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Summary

The study evaluated the effectiveness of a commercially available polyherbal dewormer to control intestinal strongyles in naturally infected donkeys. The animals were allotted to two groups: treated with the herbal dewormer (n=8) according to manufacturer recommendations and untreated control group (n=6). Fecal samples were taken from each animal on days 0 (day of treatment), 14, 21 (day of second additional treatment), 35, and 42. Faecal egg count reduction tests showed very negligible or no reduction in number of strongyle eggs for donkeys in the phytotherapeutic treatment group compared to those in the control group. Thus, the herbal dewormer was mostly ineffective in reducing fecal egg counts in donkeys infected with intestinal strongyles. These findings can make equine practitioners aware of possible disadvantages of herbal dewormers.
Keywords: Donkeys; intestinal strongyles; herbal dewormer; phytotherapy

Introduction

Donkeys play a key role as working animals in developing countries, and they are also used for meat and milk productions, social activities, tourism and leisure (Camillo *et al*., 2018). Consequently, the interest in the welfare and diseases of this species is constantly increasing (Bonelli *et al*., 2016; Sgorbini *et al*., 2017, 2018), including parasitic diseases and strategies for their control.

The high occurrence of large and small strongyles in donkeys has been widely reported (Matthee *et al*., 2000; Getachew *et al*., 2010; Matthews *et al*., 2013; Ismail *et al*., 2016; Jajere *et al*., 2016; Dibaba *et al*., 2017). Intestinal strongyles may negatively affect body conditions, live weight and haematological parameters of donkeys (Matthee *et al*., 2002; Yoseph *et al*., 2005) and their control is typically performed by the administration of anti-helminthic drugs licensed for use in horses (Matthee *et al*., 2002). However, the development of drug-resistant populations of small strongyles have been reported in donkeys (Matthee *et al*., 2002; Lawson *et al*., 2015). This factor and the growing consumer trend to ask for food products free of chemical residues, including milk from donkeys raised according to organic farming methods (Camillo *et al*., 2018), prompt researchers, practising veterinarians, and organic donkey farmers to search for sustainable alternatives to control gastrointestinal parasites in this host species. Among the sustainable alternatives, there are various medicinal plants that are reported to have anthelmintic properties in donkeys (Scantlebury *et al*., 2013). However, to the best of the authors' knowledge, the effectiveness of herbal formulations marketed for parasite control in donkeys under field conditions has not been yet investigated so far. Hence, the aim of the present study was to determine the effectiveness of a commercially available polyherbal dewormer, marketed for use in equines, to reduce the faecal output of strongyle eggs in donkeys.

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Materials and Methods

Study animals, sample collection, and laboratory procedures

The study was carried out at the facilities of the veterinary teaching hospital "Mario Modenato" (University of Pisa, Italy) between January and February 2019. The study period was chosen for practical reasons. Indeed, female donkeys selected for the study were going to start the reproductive season in the following months (late winter/early spring). So, we wanted to avoid using the animals for the study purposes during the heat period, mating, and pregnancy. Fourteen asymptomatic Amiata donkeys (*Equus asinus*) of both sexes (10 unpregnant females and 4 intact males) were enrolled in the study irrespective of the parasite burden. They were aged 7 – 13 years and none of them had received any anthelmintic treatment for at least 12 months before the beginning of the study. The donkeys were naturally infected by intestinal strongyles. Infections were confirmed prior to the beginning of the study by the Mini-FLOTAC technique in combination with Fill-FLOTAC. A commercial sodium nitrate solution with specific gravity of 1.200 (Coprosol®, Candioli Farmaceutici S.p.A., Beinasco (TO), Italy) was used as flotation solution. The Mini-FLOTAC technique in combination with Fill-FLOTAC has a reported analytic sensitivity of five eggs per gram (EPG) of faeces and it was performed according to the detailed instructions provided at the web site of the supplier (https://www.parassitologia.unina.it/wp-content/uploads/2015/07/001-Erbivori-fresh_Layout-1-1_cut.pdf). This tool was chosen for copromicroscopic diagnosis and EPG counts because of the excellent performance previously shown in various studies to assess gastrointestinal helminth infections and anthelmintic drug efficacy in veterinary medicine (Rinaldi *et al*., 2014; Bellaw *et al*., 2018).

Study design

Each of the 14 donkeys was allotted to one of two groups: treated group (n=8) and untreated control group (n=6). Because there is no indication in previous studies that the response to anthelmintic treatment is different between sexes in donkeys, animals were allotted as follows. Females were randomly assigned to either treated or control group and males were assigned to control group. Thus, the treated group contained 8 females whilst the control group contained 2 females and 4 males. After allotments, the two groups were kept in separate paddocks as follows. Eight unpregnant females in the treatment group were kept all together in the same paddock. Due to management reasons, the 6 donkeys in the control group were separated by sex into five paddocks, that is 2 unpregnant females were kept together in the same paddock whilst the 4 intact males were kept separately in individual paddocks. All the donkeys allotted to each group were fed with hay from mixed-grass meadows and water ad libitum during the entire study period. On day 0, faecal samples were individually collected from the rectal ampulla of each donkey into plastic containers. Samples were appropriately labelled and brought to the laboratory where individual EPG counts were performed by using the Mini-FLOTAC technique in combination with Fill-FLOTAC, as above reported. The same day the bodyweight of each donkey in the treated group (n=8) was estimated by a weight tape, ranging from 280 to 393 kg. Thereafter, these animals were treated by oral administration of a marketed polyherbal dewormer at a dose rate of 8.3 gr per 100 kg bodyweight. The product is commercialized in the form of paste and is licensed for use in equines in Italy. It contains extracts of *Mallotus philippinensis*, *Carduus marianus*, *Urtica urens*, *Genziana lutea*, and *Eucalyptus globulus*. It is considered atoxic, can be used in pregnant and young animals, and has no withdrawal time. According to the dose suggested in the drug package, a 50 gr syringe is useful for treatment of horses of weight up to 600 kg. Thus, the dose used for treatment of donkeys in the present study was extrapolated from the dose recommended for horses by the manufacturer. During the oral administration of the herbal dewormer, all donkeys in the treated group showed that they enjoyed the taste very much. The remaining 6 animals, assigned to the control group, were kept untreated. Subsequently, faecal sample collections and EPG counts were individually performed as above reported in both groups on days 14, 21, 35, and 42. Furthermore, on day 21 all the donkeys in the treated group were administered again an additional administration of a 50 gr syringe of the paste used for the initial treatment, as adjunct treatment irrespective of bodyweight. After treatment, they were observed for possible adverse reactions for about 1 hour.

Data analysis

The faecal egg count reduction (FECR) test was used to determine the anthelmintic efficacy of the tested polyherbal dewormer (Kaplan & Nielsen, 2010). Arithmetic means (AMs) of the EPG counts were calculated to determine the mean percentage reductions on day 14 and on day 35, according to the following formula:

$$\text{FECR } \% = \frac{\text{Pre-treatment EPG - Post-treatment EPG}}{\text{Pre-treatment EPG}} \times 100$$

Egg shedding reduction can vary based on the drug tested and the number of horses investigated (Kaplan and Nielsen, 2010) but there are currently no available indications for phytotherapeutic products in literature. Thus results were interpreted following the classification of Kaplan and Nielsen (2010) and we arbitrarily assumed that the cut-off value for appropriate efficacy of the tested polyherbal dewormer against intestinal strongyles in donkeys was FECR at least ≥90 %.

AMs ± standard error of the mean (SEM) of the EPG counts were determined on each sampling day after treatment. AMs of the EPG counts were compared between treated and untreated groups by Student's t test. *P* values ≤0.05 and ≤0.01 were considered statistically significant or highly significant, respectively.

Ethical Approval and/or Informed Consent

For this study, formal consent is not required. The research related to animals complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results and Discussion

On day 0, EPG counts ranged from 180 to 700 and from 100 to 770 in faeces of treated and untreated donkeys, respectively. No side effects were observed after treatment. FECR test showed that the polyherbal dewormer produced a very negligible reduction (5 %) of strongyle eggs on day 0 onwards and no reduction or lower reduction (2 %) on day 21 till the end of the study, even though donkeys were treated again with a much higher dose, as adjunct treatment, on day 21. The values of AM±SEM of the EPG counts on day 0 to day 42 in treated and untreated donkeys are presented in Table 1. Unexpectedly, the AMs of the EPG counts were higher in the treated group both on day 14 and on day 21, reaching highly significant differences (P=0.0033 and P=0.0029, respectively). The values of the AMs in the treated donkeys became lower in comparison with their untreated counterpart on day 35 and on day 42 but differences did not reach statistically significant values (P=0.6530 and P=0.1796, respectively).

Results of the FECR test in the present study were compared to cut-off limits proposed by Kaplan and Nielsen (2010) as general guidelines to assess the occurrence of anthelmintic resistance after treatment with drugs currently used in horses, when a recommended range of at least 5 – 10 horses on each farm is included. Reductions >90 % for benzimidazoles and pyrantel or >95 % for ivermectin and moxidectin suggest that the strongyle population is susceptible to the drug tested. Lack of effectiveness is suspected when reductions <80 % for benzimidazoles and pyrantel or <90 % for macrocyclic lactones are found. In this study, only a very negligible reduction (5 %) was seen on day 14. In general, the anthelmintic activity of plants has been reported to be lower than that found for synthetic anthelmintic drugs (Macedo *et al*., 2010). In addition, Iqbal *et al*. (2004) reported that an increase in EPG reduction was noted with an increase in the dose of *Artemisia brevifolia* administered as crude powder, crude aqueous extract, and crude methanol extract against mixed infection of gastrointestinal nematodes in sheep. Similarly, Zajac and Gipson (2000) reported that a single treatment with fenbendazole was able to reduce EPG counts by only 50 % but 2 doses administered in a 12 h interval increased efficacy to 92 % in a goat herd. Thus, it is possible that the dose rate (8 gr of paste/100 kg bodyweight) recommended by the manufacturer for the polyherbal dewormer tested in our study may not be high enough to produce anthelmintic effects in donkeys. For this reason, a second treatment with a much higher dose of the product (50 gr of paste irrespective of the bodyweight) was additionally administered as adjunct treatment on day 21. Despite of this, no egg reduction could be demonstrated on day 35. Moreover, no important differences in individual FECR tests were observed among donkeys treated with the herbal dewormer. Finally, the occurrence of anthelmintic resistance can be excluded since the phytotherapeutic product tested had never been used before in the donkey population of the study. Therefore, our results show that the polyherbal dewormer administered in the present study failed to produce any suitable anthelmintic efficacy against intestinal strongyle infections in donkeys after both the first and mostly the second administration.

Our findings are in agreement with those of other authors. Lugin-

Table 1. Individual counts and arithmetic means ± standard error of the mean (AM±SEM) of eggs per gram (EPG) of faeces from day 0 to day 42 in donkeys treated with a commercial polyherbal dewormer on day 0 and on day 21 (I to VIII), and in donkeys of the control group kept untreated (IX to XIV).

Groups	Donkeys	EPG counts				
		Day 0	Day 14	Day 21	Day 35	Day 42
Treated	I	380	610	325	1000	230
	II	180	120	230	315	180
	III	240	440	570	305	495
	IV	580	480	480	745	160
	V	700	325	230	490	395
	VI	630	600	555	865	440
	VII	200	190	175	275	140
	VIII	370	350	325	510	260
	AM ± SEM	410 ± 71.9	389.4 ± 62.9[a]	361.2 ± 54.6[b]	563.1 ± 97.7	287.5 ± 48.4
Control	IX	770	215	185	285	215
	X	410	115	100	1115	400
	XI	280	170	150	835	585
	XII	110	45	225	1005	640
	XIII	100	60	150	195	285
	XIV	200	15	95	435	305
	AM ± SEM	311.6 ± 103	103.3 ± 31.7[a]	150.8 ± 20.3[b]	645 ± 159.5	405 ± 70.3

[a, b]highly significant differences

buhl *et al*. (2006) reported the lack of anthelminthic effects of a commercially available herbal dewormer to reduce EPG counts in meat goats. Burke *et al*. (2009a) showed that another marketed herbal dewormer, involving the use of two different formulations, was ineffective to control gastrointestinal nematodes in dairy goats. The two herbal mixtures contained *Artemisia absinthium*, *Allium sativum*, *Foeniculum vulgare*, *Juglans nigra*, and *Stevia rebaudiana* as well as *Cucurbita pepo*, *Hyssopus officinalis*, and *Thymus vulgaris*. Similarly, a commercially available certified organic garlic product, fresh garlic juice, and garlic bulbs or papaya seeds failed to control gastrointestinal strongyles in goats and lambs (Burke *et al*., 2009b). In a field trial to assess the usefulness of a mineral lick containing herbal extracts with anti-parasitic properties for the control of gastrointestinal helminths in grazing sheep, there were no significant differences in the prevalence and intensity of helminth infections between the treated and control groups (Nosal *et al*., 2016). The commercially available product consisted of the extracts of *A. absinthium*, *Artemisia cina*, *Tanacetum vulgare*, *T. vulgaris*, *A. sativum*, *Alsidium helminthochorton*, *Dryopteris* filix-mas, *Daucus carota*, *Chenopodium*, and *Punica granatum*. However, contrary to the aforementioned studies, other in vivo investigations have reported the efficacy of some medicinal plants against gastrointestinal strongyles. Iqbal *et al*. (2004), Masamha *et al*. (2010), Jabbar *et al*. (2007), and Tariq *et al*. (2009) reported the anthelmintic activity of *A. brevifolia*, *A. sativum*, *Chenopodium album* and *Caesalpinia crista*, or *A. absintium* in sheep, respectively.

To conclude, the phytotherapeutic potential and pattern of anthelmintic effectiveness of plant extracts vary widely from study to study. Consequently, though phytotherapy has become more and more popular with both donkey breeders and veterinarians, phytotherapic compounds marketed as herbal anthelmintics need evidence-based validation. For this reason, our trial tested the effectiveness of an herbal dewormer against intestinal strongyles in naturally infected donkeys. Based on the FECR test and current criteria used to evaluate the efficacy of anthelmintic drugs, the marketed polyherbal product tested in this study cannot be recommended as an effective alternative or complementary treatment for the control of intestinal strongylosis in donkeys. The findings of our study can help make practitioners aware of the possible disadvantages of natural herbal medicines for the treatment of parasitoses. In our opinion, monitoring FECR test results after treatment with herbal medicines is strongly recommended to provide the equine practitioner with the information necessary to administer extracts of anthelmintic plants consciously. Further in vivo trials focusing on suitable plants with promising potential anthelmintic properties are advisable for the development and the release of commercially available alternative and complementary anthelmintics to be used in donkeys.

Conflict of Interest

Authors state no conflict of interest.

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