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Original Research

Preliminary Observations of the Effect of Garlic on Egg Shedding in Horses Naturally Infected by Intestinal Strongyles

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A B S T R A C T

Intestinal strongyles are the most common endoparasites of horses, and anthelmintic treatments are the main strategy to control these nematodes. However, the development of anthelmintic resistance has led to a decreased efficacy of synthetic drugs, and for this reason, there is a growing interest in alternative control strategies as the use of medicinal plants. The aim of the present study was to determine the in vivo efficacy of garlic (*Allium sativum*) in horses naturally infected by intestinal strongyles. The field trial was conducted in a horse trotter farm in Southern Italy. Fifteen mares were selected based on fecal egg count >200 eggs per gram and allocated into three groups of five animals: fresh garlic group (FG group), animals received 40 g of fresh crushed garlic once daily for 15 days; dry garlic group (DG group), animals received 40 g of commercial dry garlic flakes food supplement once daily for 15 days; and control group (C group), not treated. Two weeks after the first administration of garlic, fecal egg count reduction test showed failure of garlic to reduce intestinal strongyles egg shedding (−11.7% and −19.4% for FG and DG groups, respectively). Red blood cell count values were in the normal ranges over the entire period of garlic administration. In our study model, the oral administration of garlic formulations has no effect on reducing the egg shedding of intestinal strongyles, and the garlic supplementation over a short period of time is not responsible for hematological changes in horses.

1. Introduction

Intestinal strongyles, mainly Cyathostominae, are the most common horse endoparasites; they often represent the 99% to 100% of the total worm burden in equine populations [1], and the infection can be considered virtually inevitable [2]. At present, three anthelmintic drug classes are registered to be used against endoparasites in horses: tetrahydropyrimidines, benzimidazoles, and macrocyclic lactones [3]. Although anthelmintic treatments represent the main strategy to control equine endoparasitosis [4], their overuse has determined the development of anthelmintic resistance worldwide [5]. Moreover, new anthelmintic compounds are unlikely to be approved for use in equids in the near future [6].

The onset of drug resistance in nematode populations has led to an increasing interest in alternative control strategies, such as the use of bioactive plant compounds [7–9]. Hoste et al [10] reviewed the potential beneficial effects of some plant components on gastrointestinal nematodes in small ruminants. For parasite control programs in horses, although there is an evidence of activity of plant-derived bioactive components in vitro models [11], there are very few studies confirming their therapeutic efficacy in vivo.

Animal welfare/ethical statement: This study has been reviewed by the Ethical Animal Care and Use Committee of the University of Naples Federico II on June 26, 2014, and on the basis of the Italian Legislative Decree 26/2014, article 2, has received institutional approval.

Conflict of interest statement: None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the article.

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Garlic is a bulbous plant belonging to the onion genus, *Allium sativum* (family Liliaceae). It is one of the most common medicinal plant used worldwide, and it has parasiticide, amebicide, larvicide, fungicide, immune-stimulant, anticancer, hypoglycemic, cardioprotective, antiatherosclerotic and antihypertensive-recognized properties [12]. Its use as a medicinal plant dates back to ancient times. In Egypt, garlic was a part of the daily diet of the people involved in the construction of the pyramids. Hippocrates used garlic in lung diseases as cleansing or in abdominal growths as purgative agent [13]. Pliny the Elder, in his *Historia Naturalis*, reported 23 uses of garlic against different diseases as hepatic disorders and infections [14]. In Europe, garlic was introduced when Roman legions moved toward the North, and it was used by the legionaries to alleviate constipation when consumed with beverages [15]. During the Renaissance, physic gardens were established at universities to grow plants of medical interest, and garlic was one of the main plant grown for this purpose. Pietro Mattioli, an Italian physician of the 16th century, administered garlic to his patients for digestive and renal disorders, worm infestations, and to help mothers during difficult childbirth [13].

In veterinary practice, the use of plants is widespread in Europe and Italy [16]; more than 280 plants are used for therapeutic issues especially in cattle, sheep, horses, pigs, dogs, rabbits, and poultry [17]. *A. sativum* has been used in chicken and cattle as a healing agent in parasitic diseases and disorders of gastrointestinal system [16]. In Italian folk veterinary medicine, in dogs and cats affected by endoparasites, a small raw meatball containing a large amount of garlic was administered for some days [18].

In equid management, several owners consider herbal products, such as garlic, as a valid alternative to synthetic anthelmintics. Furthermore, garlic formulations are marketed by the equine food industry as adjuvants in endogenous toxicosis, intestinal infections, and as vasodilator and fly repellent agents; these formulations are used in prevention and treatment of intestinal parasites in the routine practice [19]. Allicin is the most important thiosulfinate compound present in garlic, and it has been associated with antibacterial, antifungal, antiviral, and antiprotozoal activities [20]. Recently, *A. sativum* showed a positive effect in larval migration inhibition test and egg hatch test against cyathostomin third-stage larvae (L_3) and eggs, respectively [9]. To the best author's knowledge in equine practice, there are no data supporting the real deworming effectiveness of garlic based on field trials. In literature is present a unique in vivo study performed on a small number of donkeys infected by strongyles, showing no anthelmintic efficacy of garlic after a single oral administration [21].

The aim of the present study was to evaluate the field efficacy of a commercial garlic flakes food supplement and crushed fresh garlic to control adult stages of intestinal strongyles in naturally infected horses.

2. Materials and Methods

2.1. Farm and Study Animals

The study was conducted in an Italian Trotter horse breeding farm located in Southern Italy and consisted of 25 mares. Fecal examinations (individual fecal egg counts [FECs] and pooled fecal cultures) were performed before the beginning of the trial (Day 3), showing high individual FEC and high prevalence of intestinal nematodes (Cyathostominae and *Strongylus vulgaris*) in all animals. During the experimental period, horses were housed together in an outdoor paddock (1 hectare); supplement feedings such as hay and oat were given, and water was provided *ad libitum*. In the farm, all horses have not been treated with any anthelmintic drugs during the previous 6 months, and they did not show any clinical signs. The investigation was approved by the Ethical Animal Care and Use Committee of University of Naples Federico II.

2.2. Experimental Groups and Treatment Procedures

Based on horse selective therapy cutoff (FEC >200 eggs per gram [EPG]) [22], 15 mares, aging from 2 to 22 years (mean 11 ± 5.4 years), were selected and allocated in three treatment groups of five animals each.

The animals were ranked from lowest to highest EPG counts. Based on increasing EPG counts, replicates of three animals were formed. Within each replicate, animals were randomly assigned to treatment. The 15 selected horses were assigned consecutively to the following treatment groups of five animals each: fresh garlic group (FG group), animals received 40 g of fresh crushed garlic once daily for 15 days; dry garlic Group (DG group) animals received 40 g of a commercial dry garlic flakes supplement (garlic flakes; FM Italia) once daily for 15 days; and untreated control group (C group). For both garlic group, the dose was established according to the manufacturer's instructions for the dry commercial formulation that suggest to administer 40 g per animal per day regardless of horse weight; moreover, both garlic formulations have been administered for 15 days considering the practices commonly adopted by horse breeders [23].

At each study time points (from Day 1 to Day 15) in the morning (7 AM–8 PM), both garlic formulations were mixed with 200 g of oat and administered individually to horses. The three experimental groups were maintained under the same conditions throughout the course of the study because the prepatent period of intestinal strongyles ranges from about 2 months (small strongyles) to about 11 months (large strongyles), the sharing of the pasture of the horses belonging to the three groups cannot influence the evaluation of the effectiveness of the treatment.

2.3. Clinical Examination and Red Blood Cell Count

During the study period, all animals were subjected daily to a clinical examination to verify potential adverse reactions to garlic until Day 15, and the other two additional examinations were performed at Days 21 and 28. Blood samples (5 mL) were collected by jugular venipuncture into K3-EDTA anticoagulant tubes at the first day of the experimental period (Day 1) before garlic administration, and at Day 15, last day of garlic treatment. A red blood cell count (RBC) was performed using a semiautomatic cell counter (HM5; Abaxis, Union City, CA). The body condition score (BCS) was assessed using a five-point system [24] by the same investigator at Days 1, 15, and 28.

2.4. Coprological Examinations and Fecal Egg Count Reduction Test

According to recommendations proposed by Nielsen et al. [25], fecal samples were taken directly from the rectum of each animal. The specimens were stored in a refrigerator (4°C) and examined within 5 hours to reduce the effect of egg hatch. Individual FECs were performed daily from Day 1 until Day 21, and then at Day 28 using a special modification of McMaster method, with a detection limit of 10 EPG and a Sheather's saturated sugar solution with a specific gravity of 1.250 [26].

To determine the efficacy of the two garlic formulations, the arithmetic mean (AM) of EPG was calculated at each sampling day, and the percent efficacy (%) for each group was considered in terms of Fecal Egg Count Reduction Test (FECRT) using the formula: $FECRT = ([AM\ FEC_{pretreatment} - AM\ FEC_{posttreatment}]/AM\ FEC_{pretreatment}) \times 100$, according to the American Association of Equine Practitioners (AAEP) guidelines [22].

For the interpretation of results were considered the cutoff values used for pyrantel proposed in AAEP guidelines: FECRT >90% (susceptible), 85%–90% (suspected resistance), and <85% (resistant) [22].

Furthermore, over the entire study period, group pooled fecal samples were incubated for each time points at 27°C for 7 to 10 days for larval development. Third-stage larvae (L_3) were recovered using the Baermann technique and identified according to the keys proposed by Cernea et al [27]. When a coprocolture had 100 or less L_3, all were identified; when a coprocolture had more than 100 L_3, only 100 were identified.

2.5. Statistical Analyses

Microsoft Office Excel 2016 software was used for data recording, and FECR, expressed as percentage with 95% confidence intervals, was calculated using the RESO FECRT analysis program, version 4 (http://sydney.edu.au/vetscience/sheepwormcontrol/).

The resistance was indicated if the lower confidence limit (LCL) was below 90% [28], and the mean percentage of FECRT was below 85%.

Because of nonnormal distribution of data, logarithmic transformation was performed, and subsequently, *t*-test was applied. *P* values < .05 were considered to be significant.

3. Results

At clinical examination, no adverse reactions were observed in any of the horses treated, and the BCS values were in the normal, physiological range for horses during the study. RBC values were in the normal ranges over the entire period of garlic administration [29] (Table 1).

Regarding the parasitological examinations, 3 days before the start of the study, the mean EPG was 1,374, 1,074, and 1,126 for the C, FG, and DG groups, respectively. No significant differences were observed regarding the posttreatment FEC between the three groups from Day 1 until Day 28 (Table 2). Both garlic formulations were not effective (FECRT < 85%) throughout the study period. Specifically, FEC percentage reductions for the FG group were −40.6% (LCL −306.7%) on Day 7, −11.7% (LCL −253.1%) on Day 14, −16.2% (LCL −271.2%) on Day 21, and −28.3% (LCL −310.3%) on Day 28. The FEC percentage reductions for the DG group were −48.5% (LCL −354.4%) on Day 7, −19.4% (LCL −210.9%) on Day 14, −0.5% (LCL −139.2%) on Day 21, and −13.7% (LCL −247.7%) on Day 28 (Table 2). On all sampling days, in both FG and DG groups, FEC was upper to 200 EPG, considered the cutoff values for anthelmintic treatment [22]. There was no significant difference in FEC values between the control and two garlic-treated groups. In the experimental groups, fecal cultures revealed the presence of Cyathostominae (*Cyathostomum sensu lato*, *Poteriostomum*, *and Oesophagodontus*) and *S. vulgaris* larvae for all sampling days.

4. Discussion

The effectiveness of plant active components can be attributed to two modes of action: their immunomodulatory properties and antiparasitic effects [30]. The medical properties of garlic are attributed to the presence of cysteine sulfoxides (allicin, cycloalliin, methiin, and isoalliin) and γ-glutamylcysteins (γ-glutamyl-S-allylcysteine, γ-glutamyl-S-methylcysteine, and γ-glutamyl-S-t-propenylcysteine); of these molecules, allicin is the most abundant and with the more important biological activity [31], produced when the enzyme alliinase reacts with its substrate alliin in crushed garlic. Allicin is also responsible for the characteristic garlic smell [21].

Table 1
Hematological values of horses treated with fresh garlic, dry garlic, and control groups.

Parameter	Day 1			Day 15			Reference Ranges [28] (Min to Max)
	FGG Mean + SD (Min to Max)	DGG Mean + SD (Min to Max)	CG Mean + SD (Min to Max)	FGG Mean + SD (Min to Max)	DGG Mean + SD (Min to Max)	CG Mean + SD (Min to Max)	
RBC (10^{12}/L)	8.11 ± 0.80 (7.05–9.24)	8.58 ± 1.49 (7.04–10.79)	7.99 ± 0.67 (7.27–8.76)	8.47 ± 1.02 (6.81–9.30)	8.14 ± 0.87 (7.04–9.06)	8.15 ± 0.66 (7.47–9.20)	6.0–10.0
Hgb (g/dL)	12.5 ± 0.8 (12.0–13.9)	13.6 ± 1.8 (12.0–16.4)	12.8 ± 0.8 (12.0–14.0)	13.2 ± 1.0 (12.0–14.2)	13.2 ± 1.0 (12.0–14.5)	13.1 ± 0.8 (12.2–14.2)	12.0–17.0
HCT (%)	37.6 ± 3.1 (35.0–42.7)	41.1 ± 7.1 (32.6–51.3)	39.5 ± 3.0 (35.4–43.7)	38.4 ± 3.3 (33.3–42.4)	39.5 ± 2.8 (36.5–42.8)	38.7 ± 3.3 (35.3–43.9)	32–50
MCV (fL)	46.5 ± 1.9 (44.5–49.6)	47.9 ± 2.7 (45.7–52.6)	49.6 ± 4.2 (45.3–54.8)	45.5 ± 2.5 (42.1–48.8)	48.9 ± 4.5 (43.1–53.8)	47.5 ± 1.0 (45.9–48.8)	42–58
MCH (pg)	15.5 ± 0.9 (15.0–17.0)	16.0 ± 0.9 (15.1–17.0)	16.1 ± 1.3 (14.7–17.6)	15.6 ± 1.1 (15.0–17.6)	16.3 ± 0.9 (15.1–17.2)	16.1 ± 0.7 (15.4–17.2)	15–20
MCHC (g/dL)	33.4 ± 0.9 (32.3–34.3)	33.3 ± 2.0 (32.0–36.8)	32.1 ± 0.8 (31.9–33.9)	34.4 ± 1.9 (32.4–36.6)	33.4 ± 2.3 (32.0–37.4)	34.0 ± 1.2 (32.3–35.3)	32–38

Abbreviations: CG, control group; DGG, dry garlic group; FGG, fresh garlic group; Hgb, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cells; SD, standard deviation.

Table 2
Strongyle egg counts in eggs per gram and percentage reductions in fecal egg counts in horses treated with fresh garlic and dry garlic, compared with untreated control group at each study time points.

Day	C Group		Fresh Garlic Group		FECR (%)	*P* Value	Dry Garlic Group		FECR (%)	*P* Value
	EPG AM	EPG Range	EPG AM	EPG Range			EPG AM	EPG Range		
−3	1,374	600–2,000	1,074	260–3,060	—	.2709	1,126	510–1,940	—	.5004
1	1,448	650–2,100	1,418	500–2,970	−32.0	.7423	1,582	700–3,220	−40.5	.9761
2	1,616	880–2,670	1,166	460–2,190	−8.6	.2689	1,514	280–3,690	−34.5	.4818
3	1,630	640–2,800	1,550	600–2,700	−44.3	.7983	1,512	250–2,760	−34.3	.6359
4	1,458	800–2,130	1,224	650–1,890	−14.0	.4643	1,284	540–2,400	−14.0	.4203
5	1,462	690–2,140	1,364	440–2,700	−27.0	.6556	1,706	590–4,940	−51.5	.7329
6	1,688	990–2,450	1,606	920–2,120	−49.5	.8102	1,680	610–4,180	−49.2	.5209
7	1,606	780–2,230	1,510	660–2,070	−40.6	.7988	1,672	780–2,230	−48.5	.5185
8	1,562	620–2,180	1,576	520–2,530	−46.7	.9495	1,236	310–3,270	−9.8	.3080
9	1,592	800–2,500	1,802	700–2,730	−67.8	.7714	1,456	730–3,370	−29.3	.5755
10	1,486	660–2,100	1,436	750–2,720	−33.7	.8589	1,778	580–3,830	−57.9	.9696
11	1,236	700–1,980	1,116	550–1,650	−3.9	.6357	1,164	600–2,000	−3.4	.6921
12	1,464	730–2,100	1,418	780–2,150	−32.0	.8637	1,644	450–3,880	−46.0	.8367
13	1,488	720–2,210	1,246	610–2,500	−16.0	.4683	1,270	330–2,170	−12.8	.4942
14	1,420	710–2,250	1,200	440–2,200	−11.7	.4840	1,344	320–3,310	−19.4	.5049
15	1,568	680–2,400	1,312	590–2,580	−22.2	.5037	1,228	240–2,860	−9.1	.3441
16	1,520	600–2,350	1,420	690–2,650	−32.2	.8028	1,322	320–3,360	−17.4	.4556
17	1,432	650–1,900	1,364	660–2,330	−27.0	.7783	1,280	370–3,260	−13.7	.4321
18	1,532	680–2,300	1,296	540–2,580	−20.7	.5046	1,450	570–3,150	−28.8	.6386
19	1,506	730–2,130	1,500	680–2,130	−39.7	.9825	1,418	550–3,500	−25.9	.5401
20	1,464	650–2,010	1,326	730–2,400	−23.5	.6622	1,348	450–3,140	−19.7	.5327
21	1,586	620–2,560	1,248	380–2,290	−16.2	.4415	1,132	470–2,650	−0.5	.2830
28	1,612	650–2,580	1,378	340–2,550	−28.3	.5421	1,280	290–3,740	−13.7	.3086

Abbreviations: AM, arithmetic mean; FECR, fecal egg count reduction; EPG, egg per gram.
Parametric *t* test (garlic groups vs. C group) after logarithmic transformation of data was used, and *P* value <.05 was considered to be significant.

Several experimental studies demonstrate the garlic antiprotozoal activity. Allicin and its condensation products, ajoene and diallyl trisulphide, act interacting with thiol-containing enzymes and inhibiting the growth of different protozoan parasites [31]. Allicin reduces the development of several *Babesia* parasites in vitro, and it shows inhibitory effect on the growth of *B. microti* in mice experimental models. Significant inhibition of the growth of *Babesia caballi* and *Theleria equi* occurs at concentration of 10 and 100 μM of allicin, respectively, probably at the level of erythrocyte invasion stage [32].

The action of garlic on helminths is less known than that on protozoa. Recently, garlic suspended in distilled water and dimethyl sulfoxide showed a strong inhibitory effect (maximum 100%) on egg hatch (egg hatch test) of Cyathostominae eggs collected from donkeys living in the Donkey Sanctuary in the United Kingdom; moreover, a garlic suspension exhibited a moderate inhibiting activity on larval (L_3) migration (larval migration inhibition test) of Cyathostominae, with a maximum percentage reduction in migration of 53.3% ± 7.9 [9]. A recent in vitro study reported a mortality percentage of horse intestinal strongyle larvae ranging from 82.75% to 100% after an incubation period of 2 days, depending on concentration of hydroalcoholic extracts of garlic used [33]. An in vivo study was performed in a commercial goat farm from the United States to evaluate the efficacy of garlic juice and garlic bulbs against gastrointestinal nematodes (mainly *Haemonchus contortus*), showing that garlic is not recommended to control gastrointestinal nematodes in small ruminants [7]. In adult Boer goats, similar results were obtained in the control of *H. contortus* infection using increasing concentrations of garlic juice, obtained by dilution with distilled water [34].

In horses, there are no in vivo studies that demonstrate the anthelmintic efficacy of garlic, whereas in donkeys, Sutton and Haik [21] reported that garlic was not effective to control intestinal strongyles after oral administration of a head of garlic boiled in 300 mL of water. However, it is important to underline that allicin is not very stable, and most likely it is destroyed by boiling [35].

In our study, the administration for 15 days of fresh and dry garlic flakes once daily was not effective to reduce intestinal strongyles egg shedding in horses naturally infected. In this study, we evaluated the efficacy of garlic using the method proposed by Nielsen et al. [22] for the synthetic anthelmintic drugs, based on the percentage reduction of the number of eggs in fecal samples before and after treatment. In absence of parameters in the literature to evaluate the anthelmintic efficacy of medicinal plant components, the effectiveness of garlic has been compared with that of pyrantel, that is, the anthelmintic drug having the lowest efficacy cutoff value (FECR > 90%), compared with benzimidazoles (FECR > 95%) and macrocyclic lactones (FECR > 98%) [22].

Probably, if the mode of action of garlic is not to kill the nematodes but to stimulate the host's immune system against the parasites, we can hypothesize that in our study, the immune response was too weak to reduce the worm parasitic load in relation to the dose and the time of garlic administration. However, further studies are needed to explore the mode of action and the potential efficacy of garlic on horse intestinal nematodes.

The onset of acute and chronic toxicity, because of consumption of garlic, has been described in dogs [36]. The toxicological mechanism of garlic sulfur compounds is oxidative hemolysis with the development of methemoglobinemia and formation of Heinz bodies in the red blood cells. In dogs, symptoms may appear one day or several days after consumption depending on the amounts ingested [37]. The Heinz bodies precipitate out of the cytosol forming a projection at the erythrocyte membrane, and they are efficiently removed by the spleen; a low prevalence of Heinz bodies does not influence the number of red blood cells, but if there are too many injured cells, these are removed from circulation with resulting erythropenia and hematocrit reduction [38]. In horses, chronic administration of garlic at a maximum intake, with a progressive increasing dosage from 0.05 to 0.25 g/kg twice daily for 71 days, caused anemia because of an oxidative effect on erythrocytes similarly to dogs [38]. In our study, the garlic administration was not associated with hematological changes in horses, as reduction of red blood cells and increase of MCV previously described by Pearson et al. [38] probably because it was used for a short period of time and at a lower dosage.

5. Conclusions

In equine practice, there is a traditional use to treat horses that are affected by internal parasites with garlic, but our study showed that this common practice has no effect on reducing the strongyle egg shedding. However, our results should be interpreted with caution because of small size of study animals, and it is crucial to perform a large-scale survey to assess the effectiveness of garlic formulation to control equine endoparasites.

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