Prevalence of Gastrointestinal Parasites of Stray Dogs with *in vivo* Assessment using the Garlic (*Allium sativum*)

[1]W.I. Felefel, [2]Kh.M. El-Dakhly [3]M.A. El-Beskawy, [4]M.F. El-Dakroury [5]M.M. Elkamshishi and [6]E.S. Mohammed

[1]*Department of Parasitology, Faculty of Veterinary Medicine, Matrouh University, Matrouh 51744, Egypt*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt*
[3]*Departement of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine Matrouh University, Matrouh 51744, Egypt*
[4]*Department of Pharmacology, Faculty of Veterinary Medicine, Matrouh University, Matrouh 51744, Egypt*
[5]*Department of Animal Hygiene and Zoonoses, Faculty of veterinary medicine, Matrouh University, Matrouh 51744, Egypt*
[6]*Department of Parasitology, Faculty of Veterinary Medicine, South Valley University, Qena 83522, Egypt*

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Corresponding Author:
W.I. Felefel
Department of Parasitology, Faculty of Veterinary Medicine, Matrouh University, Matrouh 51744, Egypt

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Abstract: A surveillance of zoonotic and other gastrointestinal parasites of stray dogs along with assessing the efficacy of garlic-based treatment was conducted in Alexandria, Egypt. Therefore, forty stray dogs were examined and divided into two groups; group 1: Dogs were given a high dose of five garlic cloves/dog twice daily, group 2: Dogs received a proposed strategy of gradual lower doses based on weight; small weight dogs (13-14 kg) received ¼ clove twice/day, medium weight (16-18 kg) dogs received ½ cloves twice/day, larger dogs (19-20 kg) received ¾ clove twice/day, heavy weight (>20 kg) dogs received one clove twice/day. The overall prevalence of gastrointestinal parasitic infections was 90.0%. Identified parasites were *Isospora* (100%), *Toxocara canis* (27.78%) and both *Taenia* spp. and *Dipylidium caninum* (5.56% each). Single infection with at least one parasite was revealed in 20 dogs and co-infections with more than one parasitic species was found in 16 dogs. Sex, age and weight of dogs were non-significant. All female dogs were infected. Upon the treatment with garlic, the coprological examination revealed a significant reduction in helminth eggs among dogs of the group 1 (79.9%; $t = -3.121$, $p = 0.006$*) rather than those of group 2 while the number of protozoaloocysts was significantly reduced in both groups ($t = -4.211$, $p = 0.001$* and $t = -6.872$, $p = 0.000$*, respectively). The mean values of most of blood parameters measured were significantly positive like

HCT, MCV, MCH, MCHC, blood platelets and neutrophils. Kidney function tests revealed that uric acid significantly increased post treatment in both groups (t = 5.257, p = 0.000* and t = 6.945, p = 0.000*, respectively) while creatinine level remained within the normal values. Moreover, liver enzymes, particularly AST and AP were significantly increased post treatment. There was a high risk of human zoonotic parasites transmission in the study area and the garlic is strongly recommended as an anthelmintic and a potential alternative to overcome rising resistance to conventional anthelmintics.

INTRODUCTION

Stray dogs are abundant carnivores distributed worldwide. Egypt has a great community of stray dogs wandering freely among cities and villages and keeping in touch with humans and residential domesticated animals[1]. Moreover, they are known to be the definitive hosts of various parasitic helminths and protozoa; some of them have a potential zoonotic importance, like *Toxocara canis*, *Dipylidium caninum* and *Taenia multiceps*[2, 3]. As they communicate with the wildlife via. the direct contact or via. the sharing of ingested food, water or even soil infected with parasitic stages, stray dogs may acquire parasitic infections as well as they infect newly introduced dogs[4-6].

In developing countries, infectious parasitic diseases are growing with the occurrence of unregulated populations of stray dogs in particular with the lack of veterinary care to improve the risk of the disease transmission[7,8]. Meanwhile, the persistent indiscriminate use of anthelmintic drugs has led to the problem of resistance of parasites to conventional treatments[9].

Garlic (*Allium sativum*) has been reported to be a parasiticide, amoebicide, larvicide as well as immunostimulant[10]. Garlic oil has a broad antimicrobial spectrum as it possesses antibacterial and anti-parasitic effects. Moreover, it influences the growth of at least 12 different human and nonhuman parasites with a potent immunomodulatory activity[11]. However, it contains 17 amino acids, like arginine, at least 33 organosulphate compounds, as alliinandallicin, eight minerals (germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc), enzymes as allinase and vitamins A, B1 and C. The efficacy of garlic against parasitic infections has been reported also in rabbits[12].

To the author's knowledge, there is a paucity data regarding the estimation of the prevalence of gastrointestinal parasites among stray dogs in Egypt, particularly zoonotic parasites. Therefore, the current work was conducted to provide recent data on the distribution of zoonotic and other gastrointestinal parasites of stray dogs in Alexandria province as well as the promising use of the garlic as a natural remedy against parasitic infections.

MATERIALS AND METHODS

Animals and husbandry: Forty stray dogs of different sexes (22 males and 18 females) and ages were collected randomly from the center and west Alexandria province, Egypt and trapped inregistered animal house with registration number 584813328 under control of Ministry of Supply and Internal Trade, Alexandria. Clinical examination of dogs including body weight, temperature and pulse was done. Infected dogs were divided into two groups; group 1 (n = 18) included dogs aged 9-36 (average 22.5) months and weighed 12-24 (average 18) kg and group 2 (n = 18) included dogs aged 12-36 (average 22) months and weighed 13-24.5 (average 17.5) kg.

Collection of fecal/blood samples: From the rectum of stray dogs, each fresh fecal sample was collected in a sterile glass bottle labeled with serial number, age and sex. Visual examinationwas done to identify adult nematodes and cestode proglottids. Then, the direct microscopy was applied using concentration and flotation techniques[13]. Identification of parasitic stages was based on morphological characteristics using ×40 and ×100 magnifications[14]. Moreover, blood samples were collected for Complete Blood Count (CBC) analysis, kidney and liver function tests. The determination of the intensity of infection also estimated through counting protozoan cysts and helminth eggs in 0.1 mL of sediment then multiplying by 10 to obtain the number of cysts or eggs per one gram stool[15].

Protocol of treatment: Coprologically, enteroparasites-infected dogs (protozoan cysts/helminth eggs) were allocated into two groups receiving the dose of garlic for ten days. Group 1 received a high dose of garlic (five cloves per dog twice daily) while group 2 received a proposed strategy of gradual lower doses based on weight; small weight dogs (13-14 kg) received ¼ clove twice a day, medium weight (16-18 kg) dogs received ½ cloves twice a day, larger dogs (19-20 kg) received ¾ clove twice a day, heavy weight (>20 kg) dogs received one clove twice a day. Garlic clove can weigh up to 5 g administered as minced raw garlic mixed the drinking water.

Assessment of treatment: The treatment assesses by comparing findings of fecal examination both macroscopically and microscopically prior to and post treatment. Blood samples were collected on the third day after the end of the administration of garlic for all treated dogs to perform the CBC and biochemical analyses comparing results prior to and post treatment.

Statistical analysis: Data were statistically analyzed using a Microsoft Excel spreadsheet and descriptive statistics. The prevalence of infection was calculated for all data as the number of infected divided by the number of individuals and multiplied by 100. Chi-square was used to assess the association of risk factors on the prevalence of parasites. T-Independent Samples Test (Levene's Test) was used to compare the normal distribution quantitative data and z-Mann-Whitney U for non-normal distribution quantitative data between two groups and t-Paired Samples Test for normal distribution quantitative data, z-wilcoxon for non-parametric quantitative data comparing two related samples. SPSS was used to analyze the data statistically. The significance level was considered at p<0.05.

RESULTS

The present study revealed that 36 (90.0%) out of 40 surveyed stray dogs exhibited endoparasitism by the use of coprological examination. Among infected dogs, 20 (55.56%) animals had a single infection and 16 (44.44%) dogs were co-infected with helminth eggs/protozoaloocysts. The identified parasites species were *Isospora* sp. (100%), *Toxocara canis* (27.78%), *Taenia* spp. and *Dipylidium caninum* (5.56% each) (Table 1).

Clinically, it has been found that there was non-significant differences among infected and uninfected dogs relative sex, age and weight. Dogs aged >18 months were highly infected with a considerable risk factor of 95% Cl 1.2(1.003-1.435). All female dogs were infected (Table 2).

Concerning the counting of helminth eggs, there was a significant reduction in number of eggs by -79.9% (t =-3.121, p = 0.006*) among dogs of group 1. On the other hand, group 2 showed no significant reduction (t = -1.031, p = 0.317). Regarding the counting of protozoaloocysts, both groups exhibited a significant reduction (t = -4.211, p = 0.001* and t = -6.872, p = 0.000*, respectively). The percentage of reduction among dogs of group 1 was higher (-62.8%) than that of group 2 (-62.8%) (Table 3).

Furthermore, findings of the blood analysis showed that in both groups, there was a significant decrease in the RBCs count post treatment (t = -14.875, p = 0.000* and t = -9.386, p = 0.000*, respectively). On the other hand, blood platelets significantly increased post treatment (t = 3.629, p = 0.002* and t = 3.056, p = 0.007*, respectively). Hemoglobin percent was significantly higher in group 2 (1.81%). The mean hematocrit (HCT) values were significantly decreased in both groups (-26.67%; -30.08%, respectively). The mean values of MCV, MCH, MCHC, platelets, RDW-SD and RDW-CV were significantly increased in dogs of group 1 compared to those of group 2 with the exception of the mean corpuscular hemoglobin

Table 1: The prevalence of parasitic gastrointestinal infections among examined dogs

	Number		Percentage	
Dog fecal samples	Single infection	Mixed infection	Single infection	Mixed infection
Infected	20	16	55.56	44.44
Isospora spp.	36		100	
Taenia spp.	2		5.56	
Toxocara canis	10		27.78	
Dipylidium caninum	2		5.56	
Uninfected	4		10.0	
Total	40		100	

Table 2: The clinical examination of examined dogs with determining associated risk factors

	Infected dogs (n = 36)		Uninfected dogs				
Risk factors	No.	Percentage	No.	Percentage	x2/t	p-values	95% Cl
Sex							
Males	18	50	4	100	0.114^		0.818(0.672-0.996)^{P}
Females	18	50	0	0			
Age							
<18 months	16	44.4	0	0	0.136^		1.2(1.003-1.435)^{R}
>18 months	20	55.6	4	100			
Mean age	22.33±10.696		24±0.0		-0.935	0.356^{t}	1.068(0.922-1.237)^{R}
Mean body weight	16.639±3.1884		16.875±2.7195		-0.142	0.888^{t}	0.941(0.590-1.501)^{P}
Mean temperature	39.233± 0.7657		40±1.1547		-1.811	0.078^{t}	2.95(0.746-11.671)^{R}
Mean pulse	82.22±8.656		87.5±8.66		-1.302	0.193^{Z}	1.079(0.928-1.255)^{R}

No = Number of examined dogs; % = Percentage of infected/uninfected dogs; ^ = Fisher's Exact Test; R = Risk factors; P = Protective factors; Z = Mann-Whitney U; t = Independent Samples Test (Levene's Test)

(MCHC) which was higher in dogs of group 2. The platelet distribution width (PDW) was significantly decreased (-54.17%; -49.315) in both groups.

Non-significantly, the Mean Platelet Volume (MPV) values were 1.92 and 0.93% in both groups. A significant reduction in the mean value of WBCs in dogs of group 1 (-6.27%) compared to that of group 2 (29.45%).

Neutrophils count was significantly higher (11.53%) in dogs of group 1 than those of group 2 (-4.58%). Significantly, lymphocytes percent increased in group 1 (260.71%) compared to group 2 (114.20%). The number of eosinophils significantly increased in group 2 (137.5%) than in group 1 (48.38%). Finally, the number of monocytes was significantly decreased in group 1 (-65.33%) and group 2 (-51.60%) (Table 4). Concerning the safety margin of the garlic, kidney function tests revealed that among both groups, uric acid significantly increased post treatment (t = 5.257, p = 0.000* and t =

Table 3: The efficacy of the administration of various doses of garlic against parasitic infections

	Infected dogs					
	Group 1 (n = 18)			Group 2 (n = 18)		
Coprological examination	Pre-treatment	Post-treatment	Percentage	Pre-treatment	Post-treatment	Percentage
Helminth eggs						
Mean±SD	27.78±35.572	5.56±7.048	-79.9	7.78±19.268	4.44±8.556	-42.93
t	-3.121			-1.031		
p	0.006*			0.317		
Protozoalo ocysts						
Mean±SD	68.89±37.083	25.56±17.564	-62.8	68.89±24.944	35.56±15.424	-48.38
t	-4.211			-6.872		
p	0.001*			0.000*		

*Significance at the level p≤0.05

Table 4: The Complete Blood Count (CBC) of infected dogs prior to and post treatment with garlic

	Infected dogs					
	Group 1 (n = 18)			Group 2 (n = 18)		
CBC findings	Pre-treatment	Post-treatment	Percentage	Pre-treatment	Post-treatment	Percentage
RBCs						
Mean±SD	7.027±0.6225	4.51±0.4126	-35.81	7.05±0.9596	4.822±0.5247	-31.60
t	-14.875			-9.386		
p	0.000*			0.000*		
HB						
Mean±SD	15.367±1.2127	15.389±1.3394	0.14	15.311±1.9944	15.589±1.410	1.81
t	0.058			0.533		
p	0.954			0.601		
HCT						
Mean±SD	47.400±12.407	34.756±2.8126	-26.67	50.856±5.7996	35.556±3.962	-30.08
t	-2.376a			-10.104		
p	0.018*			0.000*		
MCV						
Mean±SD	72.867±1.8337	77.111±2.3492	5.82	64.178±20.976	73.767±2.209	14.94
t	8.811			-2.684^{z}		
p	0.000*			0.007*		
MCH						
Mean±SD	22.022±0.9188	34.144±1.6614	55.04	29.144±16.01	33.156±2.311	13.76
t	38.929			-2.071^{z}		
p	0.000*			0.038*		
MCHC						
Mean±SD	31.122±2.9775	43.911±2.8419	41.09	30.633±2.1321	43.956±1.544	43.49
z	22.745			33.391		
p	0.000*			0.000*		
Platelets						
Mean±SD	334.89±128.84	480±113.2421	43.33	382.89±108.26	476.222±50.2	24.37
t	3.629			3.056		
p	0.002*			0.007*		
RDW_SD						
Mean±SD	13.9±0.63	38.6±3.07	177.69	16.01±4.7	37.3±1.8	132.97
t	40.608			-3.728a		
p	0.000*			0.000*		
RDW_CV						
Mean±SD	1.8±0.2	11.9±0.6	561.11	3.8±4.5	12.05±0.2	217.10
t	73.557			-3.597a		
p	0000*			0.000*		

Table 4: Continue

	Infected dogs					
	Group 1 (n = 18)			Group 2 (n = 18)		
CBC findings	Pre-treatment	Post-treatment	Percentage	Pre-treatment	Post-treatment	Percentage
PDW						
Mean±SD	49.1±5.1	22.5±1.5	-54.17	45.4±12.4	23.01±1.5	-49.31
t	-21.391			-3.597b		
p	0.000*			0.000*		
MPV						
Mean±SD	10.4±0.3	10.6±0.44	1.92	10.7±0.97	10.6±0.46	-0.93
t	1.759			-0.291		
p	0.097			0.774		
WBCs						
Mean±SD	16.076±3.3	15.067±3.7	-6.27	12.9±2.8	16.7±3.0	29.45
t	-1.417[z]			4.831		
p	0.157			0.000*		
Neutrophils (%)						
Mean±SD	31.778±18.4	35.444±8.2	11.53	32.7±15.0	31.2±5.7	-4.58
t	-1.025[z]			-0.719[z]		
p	0.306			0.472		
Lymphocytes (%)						
Mean±SD	11.2±4.4	40.4±5.1	260.71	18.3±19.1	39.2±5.9	114.20
t	15.991			-2.725[z]		
p	0.000*			0.006*		
Monocytes (%)						
Mean±SD	42.7±20.2	14.8±5.02	-65.33	37.4±23.4	18.1±4.7	-51.60
t	-6.294			-3.397		
p	0.000*			0.003*		
Eosinophils (%)						
Mean±SD	6.2±3.4	9.2±3.3	48.38	4.8±3.6	11.4±1.8	137.5
t	2.442			6.597		
p	0.026*			0.000*		
Basophils (%)						
Mean±SD	0.089±.0583	0.0±0.0	-100	0.244±0.3	0.0±0.0	-100

Table 5: Findings of kidney and liver function tests among infected dogs prior to and post treatment with garlic

	Infected dogs					
	Group 1 (n = 18)			Group 2 (n = 18)		
Kidney/liver function tests	Pre-treatment	Post-treatment	Percentage	Pre-treatment	Post-treatment	Percentage
Uric acid						
Mean±SD	1.7±0.5	2.9±1.1	70.58	1.7±0.5	3.49±0.93	105.29
t	5.257			6.945		
p	0.000*			0.000*		
Creatinine						
Mean±SD	0.86±0.13	1.19±0.17	38.37	1.02±0.24	1.1±0.12	7.8400
t	5.907			2.826		
p	0.000*			0.012*		
AST (SGOT)						
Mean±SD	55.33±14.6	150.93±28.02	172.78	65.11±23.7	151.07±39.6	132.02
t	24.372			6.904		
p	0.000*			0.000*		
ALT (SGPT)						
Mean±SD	65.22±63.7	45.28±31.128	-30.57	53.22±34.2	50.6±23.4	-49.22
t	-1.243w			-0.283b		
p	0.214		0.777			
ALP						
Mean±SD	52.44±13.9	69.52±33.2	32.57	44.7±31.7	77.52±40.6035	73.420
t	2.75			2.297		
p	0.014*			0.035*		

*Significance at the level $p \leq 0.05$

6.945, p = 0.000*, respectively) but the creatinine level remained within the normal values. Meanwhile, liver enzymes, particularly Aspartate Aminotransferase (AST) and Alkaline Phosphatase (AP) were significantly increased post treatment with the garlic (Table 5).

DISCUSSION

Canine gastrointestinal parasitism is common with a worldwide distribution affecting various species of dogs, particularly stray dogs[16-18]. The condition is frequent in

Egypt, since, it causes harmful impacts on the health and productivity of dogs[19-22]. The results of the current study revealed a high infection rate (90.0%) of gastrointestinal parasites of zoonotic potential and a public health hazard, like *Taenia* spp., *Toxocara canis*, *Dipylidium caninum* and *Isospora* spp. The current finding was almost similar to that reported in Tunisia (98·89%)[23] but it was higher than thatgiven in Ethiopia (78.8%)[24], Tanzania (67.2%)[25], Nigeria (72.5%)[26], Ethiopia (89.3%)[27], Turkey (34.68%)[28], Pakistan (26.8%)[2] and Ghana (62.6%)[29]. The widespread of gastrointestinal parasites revealed the lack of preventive control measures of those examined dogs and highlighted the existence of risk of zoonotic potentials from dogs in the study areas.

Otherwise, the present study showed that both single and mixed infections with helminth eggs/protozoanoocysts were revealed in 55.56 and 44.4% of surveyed dogs. Such finding coincided with that reported by Trasvina-Munoz *et al*.[30] in Mexico, whereas, in Ethiopia, Abere *et al*.[24] detected that the mixed infection with two and more parasite species was common rather than the single infection. The most frequently occurred parasites in the infected dogs was *Isospora* spp. (100%), although, absence of zoonotic importance, it might be the causative agent of evidential intestinal epithelium damage. Moreover, the common canid arrow-headed worm, *Toxocara canis* was recovered in an infection rate of 27.78%. The percentage was higher than that recordedin Turkey (12.9%)[28] and in Pakistan (10.5%)[2]. In Ethiopia, Abere *et al*.[24] showed a higher percentage (53.8%) than revealed in this study. Regarding *Dipylidium caninum* and *Taenia* spp., the current study revealed a prevalence of 5.56% for each. Concerning *Dipylidium*, the finding of the present work was higher than that recorded in Turkey (3.22%)[28] and lower than that determined in Pakistan (11.8%)[2] and in Mexico (16.50%)[30]. *Dipylidiasis* is an important helminthic infection for the public health that occasionally occursin young children causing various gastrointestinal troubles[31]. Children are noted more likely to be infected with *Dipylidium caninum* rather than adults[32]. Conversely, the infection rate of *Taenia* spp. was higher than that reported in Turkey (4.84%)[28] and lower than the finding recorded in Pakistan (6.57%)[2] and in Mexico (6.79%)[30].

Concerning sexes and ages of stray dogs, the prevalence of canine GIT parasitic infection wasnon-significant ($p>0.05$). Meanwhile, the elderly stray dogs (>18 months) were more infectedrather than young dogs with a non-significant difference ($p>0.05$). Such finding agreed with that reported in Ghana by Johnson *et al*.[33] who reported that 61.0% of dogs aged >12 months more infected.

The effect of curative efficacy of garlic with various doses on the parasitic intensity showed that administration of garlic reduced the intensity of protozoan cysts and helminth eggs with highly significant differences ($p\leq 0.05$). Based on the complete blood count analyses, not all dogs had anemia and this suggests that the routine clinical examination might be insufficient to diagnose anemia. Currently, the count of white blood cells increased. There was a non-significant difference in Hb estimation while neutrophils had no significant difference ($p>0.05$)[34]. Regarding the prevalence of kidney and liver function tests, it has been found the concentration of uric acid and creatinine significantly increased post administration with garlic, moreover, the levels of the liver AP and AST were significantly increased ($p\leq 0.05$). Thus, confirmatory, the current study revealed a clear therapeutic efficacy against the gastrointestinal parasites including helminths and protozoa, of stray dogs. Furthermore, Shenaway *et al*.[29] reported an increased level of natural killer cells activity that promotes the immune system function and streng then the body's defense mechanism duringthe duration of treatment by garlic.

CONCLUSION

The present study showed a higher prevalence of the gastrointestinal parasites in stray dogs in Alexandria, Egypt with various species of intestinal parasites, somewitha potential zoonotic importance. The administration with garlic could reduce the density of protozoan cysts and helminth eggs. There was a high risk of human zoonotic parasites transmission in the study area and the garlic is strongly recommended as an anthelmintic and a potential alternative to overcome rising resistance to conventional anthelmintics

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Author's contribution: All authors organized the research, conducted the experiments and created the manuscript.

Compliance with ethical standards: Dogs were collected randomly from the center and west Alexandria province, Egypt and trapped in registered animal house with registration number 584813328 under control of Ministry of Supply and Internal Trade, Alexandria, Egypt.

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