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Captive Dogs as Reservoirs of Some Zoonotic Parasites

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Abstract: Groups of stray dogs were captivated for a special drug trial. After 3 months, dog's attendants complained from gastrointestinal disturbances, headache and fever. No special symptoms were observed on dogs except diarrhea in some animals. Parasitological examination of faecal swabs showed infection by five zoonotic parasites diagnosed in both dogs and their attendants. *Entamoeba histolytica, Giardia* (trophozoites and cysts) and *Isospora* species oocysts were detected in faeces of 20, 20 and 26.6%, respectively of attendants in close contact with the dogs. Anti-*Toxocara canis*, anti-*Sarcocystis* and anti-hydatid cysts antibodies were diagnosed in 20, 33.3 and 46.66% of attendants' sera, respectively using EL1SA. Puppies and dogs were infected with *Toxascaris leonina* (20 and 16.66%), *Dipylidium caninum* (53.33 and 66.66%) and *Taenia* species eggs (26.67 and 50%). *Giardia* (53.33%), *E. histolytica* (13.33%), *Toxocara canis* (33.3%), *Isospora* (53.3%) and *Cryptosporidium* oocysts (20%) were diagnosed in puppies only. Two adult dogs (16.66%) shed *Sarcocystis* oocysts and *Ancylostoma caninum* eggs in their faeces. Some precautions were recommended to minimize the role of dogs as a source of zoonotic pathogens.

Key words: Dogs, attendants, zoonotic, parasites

INTRODUCTION

Throughout their long history of domestication, dogs have been a source of zoonotic parasites and have served as a link for parasite transmission among livestock, wildlife and humans. Globally, dogs remain an important source of emerging diseases in humans (e.g., eosinophilic enteritis by *Ancylostoma caninum*), a bridge for re-emerging infections (*Echinococcus granulosus*) and a source of parasites for immunocompromised persons (Aguilar *et al.*, 2005).

Dogs act as a source for a large number of zoonotic parasitic infections that pose a significant threat to human health all over the world. Seven helminth species have been recorded in dogs examined in Nigeria. These included *Toxocara canis* 33.8%, *Ancylostoma* sp. 34.6%, *Toxascaris leonina* 3.3%, *Trichuris vulpis* 3.7%, *Dipylidium caninum* 4.1%, *Uncinaria stenocephala* 0.7% and *Taenia* sp. 1.1%. The prevalence of infection was significantly higher in the age group 0-6 months than in older dogs (Sowemimo, 2009). *Giardia* was the most prevalent parasite in dogs (9.3%) followed by hookworms (6.7%), *Isospora* (5.6%) and *Toxocara* (3.2%) in pet dogs and cats in Australia (Palmer *et al.*, 2008).

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In Spain, the prevalence of intestinal parasitic infection in dogs was high reaching (71.33%). Parasites included *Isospora canis* (22%), *Isospora* (*Cystoisospora*) sp. (10.22%), *Sarcocystis* (2.5%), *Hammondia/Neospora* (1.94%), *Giardia canis* (1%), *Dipylidium caninum* (13.2%), *Taenia hydatigena* (7.66%), *Taenia pisiformis* (4%), *Uncinaria stenocephala* (33.27%), *Toxascaris leonina* (14.94%), *Toxocara canis* (17.72%) and *Trichuris vulpis* (1.66%). The distribution of these parasites suggested the existence of real risk for humans (Moreno *et al.*, 2007). In Northern Greece, the overall prevalence of parasites in shepherd and hunting dogs was 26%. The diagnosed parasites included, *Toxocara canis* (12.8%), *Trichuris vulpis* (9.6%), *Giardia* sp. (4.3%), *Isospora* sp. (3.9%), *Ancylostoma/Uncinaria* sp. (2.8%), *Cryptosporidium* sp. (2.8%), *Toxascaris leonina* (0.7%) and *Dipylidium caninum* (0.3%). The prevalence of *T. canis* and *Isospora* sp. was significantly higher in young compared with adult dogs. (Papazahariadou *et al.*, 2007).

In Egypt, there is no available recent data about the incidence of different parasitic infections in stray dogs. A total of 210 patients with gastrointestinal troubles selected from the outpatient's clinics of Al-Azhar University Hospitals were examined (El-Kadi *et al.*, 2006). From these patients, 54.76% had dysentery, 14% suffered from flatulence, 9.52% had epigastric pain and 9.05% had vague abdominal pain, vomiting was in 5.2 and 4.9% had fever with more than one symptom per patient. The incidence of *E. histolytica/dispar* reached 16.2% by stool examination. It was 15.65% using ELISA for detection of *E. histolytica* antibodies in serum samples of diarrheic patients. ELISA did not cross-react with *E. coli*, *Giardia lamblia*, *Cryptosporidium parvum*, *Endolimax nana* and *Blastocystis hominis*. So, the use of ELISA for detection of *E. histolytica* is considered more specific than microscopical techniques. Other helminths diagnosed in infected cases were, *Schistosoma mansoni* (0.95%), *Capillaria* sp. (0.95%), *Enterobius vermicularis* (1.90%), *Hymenolepis nana* (4.3%) and *Ascaris lumbricoides* (1.43%). El-Kichaoi *et al.* (2004) diagnosed nine parasite species after examination of 8417 stool samples from patients attending Gaza Hospital at North East of Egypt. The most common pathogenic parasites identified were: *Entamoeba histolytica* (70.19%), *Ascaris lumbricoides* (14.64%) and *Giardia lamblia* (10.34%). Other parasites present were, *Trichuris trichiura, Hymenolepis nana, Enterobius vermicularis, Strongyloides stercolaris, Taenia saginata* and *Echinococcus granulosus* (*Hydatidosis*). They reported that most of these parasites are of zoonotic importance.

During an experimental trial on captive stray dogs, an infection problem emerged in dog attendants after 3 months of contact with these dogs. The attendants complained from digestive disturbances and diarrhea accompanied with different degrees of fever. The captive dogs were incriminated as the cause of these disturbances. The current study is a result of an investigation carried out on these attendants as well as on the captive dogs.

MATERIALS AND METHODS

The current study was carried out during the period from October to December 2007. Identified blood and fresh faecal samples were collected from the target attendants and dogs. Samples were transferred for examination at the Department of Zoonoses, College of Veterinary Medicine Cairo University, Egypt.

Humans
Two groups of male animal attendants were investigated. The first group comprised five attendants who were in close contact with the captive dogs as they were responsible for their feeding, cleaning and collecting samples from them. The second group consisted of

10 general animal workers and assistants who were not in direct contact with the captive dogs but they worked in the same area of the study.

Dogs

Stray dogs (12 adult females and 15 puppies 3-5 months old) were kept captive during the period of study in local private isolation pens at Abo-Rawash locality, El-Giza, Egypt. The animals were allocated in pens with concrete floor and wire doors. The pens were 1.5 m in length, 1.0 m in width and 2.0 m in height. The animals were distributed in groups of 4 adults, or 5 puppies per pen. Dogs were fed ready prepared dog food supplemented with cooked chicken bone, while puppies were fed bread soaked in milk as well as cooked chicken bone.

Examination of Samples
Parasitological Examination

Stool and faecal samples were examined macroscopically for detection of whole worms or parts of worms. The suspected trophozoites, large size eggs and larvae were diagnosed using direct smear method (Solusby, 1986). Concentration flotation technique was adopted for diagnosis of different nematode eggs, cysts and oocysts (Pullola *et al.*, 2006). Five grams of faecal samples were mixed with 70 mL of concentrated $MgSO_4$ and the mixture was sieved (mesh size 0.9 mm) into 50 mL flask. The flask was filled to the rim, cover slip was placed on the top, left for 30 min and then transferred carefully for examination under the microscope. The total number of different eggs, cysts and oocysts per gram of faeces was calculated using Mc-Master technique (Solusby, 1986).

Diagnosis of *Giardia* and *Cryptosporidium* Infection

Two Copro-ELISA test RIDASCREEN[R] kits (R-Biopharm AG, Landwehrstr. 54, D-64293 Darmstadt, Germany) were used for diagnosis of infection in stool and faecal samples, one for *Giardia* (A C:1101) and the other for *Cryptosporidium* (A C:1201) by sandwich or antigen capture (sandwich) ELISA.

Sero-Diagnosis of Some Tissue Parasites

ELISA test was adopted for determination of the anti- parasite antibodies in human and dogs' sera. The required antigens and reference anti-sera were prepared as follows:

Sarcocystis Antigen

Bradyzoites of *Sarcocystis* were extracted from macroscopic cysts of naturally infected bovine esophagus (identified as *Sarcocystis cruzi*) by crushing in 0.01 M phosphate buffered saline (PBS) pH 7.4. After washing by centrifugation, the bradyzoites were allowed to rupture separately in few amount of PBS by repeated freezing and thawing (3 times). The contents were sonicated using Cole Parmer Ultrasonic Homogenizer under 150 W interrupted pulse output at 50% power cycle in ice bath. The suspension was centrifuged at 10 000 rpm at 4°C for 1 h. The supernatant was collected and dialyzed overnight in refrigerator against PBS, pH 7.2 using a dialysis membrane (6000 to 8000 molecular weight cut off), its protein content was measured, allocated in 1 mL vials and stored at -70°C until use (Gasbarret *et al.*, 1984).

Toxocara canis and *Dipylidium caninum* Crude Antigen

Toxocara canis worms were collected from sacrificed naturally infected dogs. After several washings in PBS, their anterior parts were cut out, washed repeatedly then

homogenized using homogenizer (ULTRA- TURRAX Janke and Kunkel KG) with (0.01 M) PBS, pH 7.4 at 6000 rpm for 20 min in ice bath. The supernatant was separated after centrifugation (6000 rpm for 20 min in ice bath) (Mahmoud, 1999). The protein content was measured, then stored as before. By the same procedure, crude antigens were prepared from the scolex of *Dipylidium caninum*.

Fertile Hydatid Cysts Fluid Antigen (FHCFA)

Samples of the hydatid fluid were collected from fertile cysts of freshly slaughtered camel lungs (Cairo abattoir) and examined for viability. The fluid of fertile cysts was clarified by centrifugation at 5000 rpm for 15 min at 4°C, dialyzed against 5 mM Tris-HCl (pH 7.4) for 48 h at 4°C (Ito *et al.*, 1999). Their protein content was measured and stored as before.

Rabbit Hyper-Immune Sera (RHIS)

RHIS were raised against the previous prepared antigens (Langley and Hillyer, 1989). Six 2-months-old white New Zealand rabbits were bled for negative control sera and then injected with the previous antigens at a concentration of 1.2 mg protein for each antigen, mixed in an equal volume of Freund's complete adjuvant, injected subcutaneously at different places at the back of the rabbit. After 3 weeks, 3 consecutive injections of 0.4 mg protein antigen in equal volume of Freund's incomplete adjuvant were given intramuscularly at bi weekly intervals. Rabbits were bled from the ear vein for serum collection 10-14 days after the last injection. The collected sera were stored at -20°C until use.

N.B. are previous parasite antigens and RHIS are available from previous investigations (Sabry, 2007; Sabry and Reda, 2008).

Enzyme Linked Immunosorbent Assay (ELISA)

Optimization of antigen concentration, conjugate, positive and negative values were determined after checkerboard titration (Voller *et al.*, 1976). The ELISA plate was coated with antigen (4 μg/ well), sera tested at 1:100 dilution. Horse-Radish Peroxidase (HRP) conjugated rabbit anti-dogs and anti-human IgG (HandL) (P7414, Sigma), were used at 1: 2000 dilution. Optical Density (O.D.) values were measured using automatic plate reader (SLT Spectra, 812 SW 1) at 450 nm. The positive ELISA value was taken as that equal to double the mean value of the negative control sample (Zimmerman *et al.*, 1985).

RESULTS

Parasitological investigation of the selected dogs directly after collection from the field showed that they had been infected with nine parasites including 3 nematodes : *T. canis* in puppies (66.67%), *A. caninum* in adults (41.67%) and *T. leonina* in adults and puppies (20 and 50%, respectively). In addition, there were two cestodes including *D. caninum* (13.33 and 66.67%) and *Taenia* sp. eggs (20 and 50%) in puppies and adults respectively. Moreover, four protozoan parasites including, *Isospora* (26.67%), *Cryptosporidium* (33.3%) and *Sarcocystis* sp. (41.67%) as well as *Giardia* (26.67%) were diagnosed (Table 1).

As shown in Table 2, re-examination of the dogs' faeces, at the time of the attendant's illness indicated re-appearance of new nematode infection including *T. canis*, 33.3% in puppies, *A. caninum*, 16.66% in adults and *T. leonina*, 20 and 16.66% in puppies and adults, respectively. There was a relative increase in the previously recorded infection by *D. caninum* and Taenia sp. These increased to 53.33 and 26.67%, respectively. Incidence of infection by *Cryptosporidium* decreased to 20% in puppies and that of *Sarcocystis* species decreased to 16.66% in dogs. On the other hand, marked increase was recorded in the

Table 1: Natural parasitic infections diagnosed in dog faeces after collection from the field

	Infection in puppies (n = 15)		Infection in adult dogs (n = 12)	
Diagnosed parasites	No. of +ve	%	No. of +ve	%
T. canis	10	66.67	-	-
A. caninum	-	-	5	41.67
T. leonina	3	20.00	6	50.00
D. caninum	2	13.33	8	66.67
Taenia sp.	3	20.00	6	50.00
Isospora oocysts	4	26.67	-	-
Giardia sp.	4	26.67	-	-
Cryptosporidium	5	33.30	-	-
Sarcocystis oocyst	-	-	5	41.67

n: No. of examined dogs

Table 2: Parasites diagnosed in dogs after 3 months in captivity

	Infection in puppies (n = 15)		Infection in adult dogs (n = 12)	
Diagnosed parasites	No. of +ve	%	No. of +ve	%
From feaces				
T. canis	5	33.30	-	-
A. caninum	-	-	2	16.66
T. leonina	3	20.00	2	16.66
D. caninum	8	53.33	8	66.66
Taenia sp.	4	26.67	6	50.00
Isospora oocysts	8	53.33	-	-
Giardia sp.	8	53.33	-	-
Entamoeba histolytica	2	13.33	-	-
Cryptosporidium	3	20.00	-	-
Sarcocystis oocyst	-	-	2	16.66

n: Number of examined dogs

Table 3: Distribution of different parasites in the examined attendants

	Dog workers					Other workers							Total/ percentage (n = 15)
Pathogen types	1	2	3	4	5	1	2	3	4	5	6	7-10	
Parasites in stool													
Giardia	+	+		+									3/20
Entamoeba	+	+	+										3/20
Isospora		+	+	+	+								4/26.6
Anti- Parasite A.b. in serum													
Anti-*Toxocara*						+	+	+					3/20
Anti-*Sarcocystis*	+					+			+	+	+		5/33.3
Anti-Hydatid		+				+	+	+	+	+		+	7/46.6

n: Number of examined workers

incidence of infection by *Isospora* and *Giardia* to 53.33 and 53.33% in puppies. New infection by *E. histolytica* (13.33%) was recorded for the first time in puppies.

Three months after the start of the experiment, stool examination of attendants in the first and second group, revealed infection by three protozoan parasites including *Giardia* (20%), *E. histolytica* (20%) and *Isospora* oocysts (26.6%) in samples from the first group. Attendants in the first group also complained from digestive disturbances and diarrhea.

On the other hand, examination of these attendants sera using ELISA technique, revealed presence of antibodies (Ab) against *T. canis* (20.0%), *Sarcocystis* (33.3%) and *Hydatid* cysts (46.6%) as shown in Table 3.

DISCUSSION

Zoonotic pathogens such as viruses, bacteria or parasites can cause severe diseases in both humans and animals. Free-ranging animals with sporadic or indirect contact with

domestic livestock and humans may serve as reservoirs or sentinels for diseases (Aschfalk and Holler, 2006). Hence, continuous contact between diseased or carrier dogs and their attendants under improper hygienic measures initiate development of endemic foci for spreading of different pathogens, specially zoonotic ones with direct life cycle (Pullola *et al.*, 2006).

In the current study, three months contact between field collected stray dogs and their attendants under moderate hygienic conditions led to health complaints in these attendants. They suffered from variable degrees of weakness, diarrhea, abdominal pain and fever. These observations indicated the dogs as a potential source for this problem. Parasitological investigation of these dogs' faecal samples revealed infection by nine parasites including *T. canis* in puppies, *A. caninum* in adults and *T. leonina* in puppies and adults. *D. caninum* and *Taenia* sp. eggs in puppies and adults. Moreover, *Isospora*, *Cryptosporidium* and *Sarcocystis* sp. as well as *Giardia* cysts were diagnosed. The diagnosed parasites are commonly reported in stray dogs and most of them pose a significant threat to human health. The diagnosed species of parasites, in the present study, are in agreement with those mentioned by Sowemimo (2009) in Nigeria; Palmer *et al.* (2008) in Australia and Moreno *et al.* (2007) in Spain, but the recorded percentages were on the contrary with that mentioned by these authors. This difference in incidences (add percentage) may be related to the locality as well as the number of the examined dogs. Similar results have been reported in Egypt (El-Kichaoi *et al.*, 2004; El-Kadi *et al.*, 2006). The high prevalence of parasitic infections in young puppies in the present investigation confirm similar findings previously reported by Sowemimo (2009) and Papazahariadou *et al.* (2007).

Re-examination of the dogs' faeces, at the time of attendants illness, indicated re-appearance of new nematodal infections including *T. canis* in puppies, *A. caninum*, in adults and *T. leonina* in puppies and adults. This might have been due to some migrating larvae which escaped from the applied experimental treatment. The results showed a relative increase in the previously recorded *D. caninum* and *Taenia* sp. infection in puppies. The increase in *D. caninum* infections might have been due to indirect transmission of this parasite via fleas of wild cats and rodents which have easy access to dog's boxes as previously indicated by Aschfalk and Holler (2006).

Incidences of infection by *Cryptosporidium* and *Sarcocystis* species decreased. This may be attributed to the nature of these parasites life cycles, as they shed during early infection resulting in an acute stage, then the animal keeps the infection and becomes a carrier thereafter (Levine *et al.*, 1980). On the other hand, the increase in the rate of infection by *Giardia* and *Isospora*, may be due to presence of the parasite infective stages which contaminate the environment through faecal shedding around the animals. Also transmission of these parasites can occur directly from a reservoir to a susceptible animal or human being (Solusby, 1986). The diagnosed *E. histolytica* infection in puppies in the present investigation may be considered to be of human origin as this parasite was not detected in the examined dogs faecal samples at the beginning of the exposure period.

Examination of stool samples collected from attendants in the first and second groups, revealed infection by *Giardia*, *E. histolytica* and *Isospora* oocysts. All of these parasites were diagnosed in attendants from the first group i.e., those who were in close contact with the dogs and who complained from digestive disturbances and diarrhea. The zoonotic importance of these parasites have previously been emphasized by Acha and Szyfres (1991).

Giardia and *Isospora* infection were recorded only in attendants in close contact with the dogs. As these parasites were previously diagnosed in the same dogs, they may be considered as the main source of the attendants' parasitic infection. This confirms previous

findings by Acha and Szyfres (1991) that dogs, cats and humans share the role of being final hosts for *E. histolytica, Giardia* and *Isospora* with high degree of adaptation between the three species.

Examination of sera of these attendants using ELISA revealed presence of antibodies (Ab) against *T. canis, Sarcocystis* and *Hydatid* cysts. Presence of anti-parasite IgG antibodies in sera means chronic infection (Sadjjadi *et al.*, 2007). This may be due to infection transmitted from the infected dogs in the surroundings or it may be due to an old infection.

On the other hand, the diagnosed anti-*Toxocara* antibodies detected in workers not in close contact with the captive dogs, may be due to infection transmitted from other dogs. The detected anti-*Sarcocystis* antibodies in sera of examined workers may be due to transmission of infection from the vital oocysts present in feaces of these contact dogs.

The captivity conditions have probably acted as stress factor that depressed the hosts resistance and increased their susceptibility to infection. Hence, the captive dogs in the present investigation may have constituted an endemic focus from which infection is disseminated under suitable environmental conditions to human beings in the surroundings.

It is thus concluded that, captive dogs that are going to be used for experimental studies must be thoroughly examined for all suspected pathogens. They must also be kept under strict hygienic conditions and continuous close observation. Animal attendants must be exposed to extension lessons on transmission of different disease as well as the ways and means by which they may protect themselves from infection. Animal boxes must be regularly cleaned and disinfected and measures to protect dogs from rodents and other vectors must be taken.

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