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Prevalence of Intestinal Parasite Infections in Stray Dogs in Taif Region, KSA

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ABSTRACT

Background: The study of intestinal parasites that occur in stray dogs in the Taif region, KSA, is most important since there is little data available on this subject.

Methods: Dog fecal samples were collected from 129 dogs in three different areas. Fecal samples were processed and analyzed by different mount techniques including, parasitological examination, wet-mount preparation and modified Kinyoun's acid-fast technique.

Result: The total prevalence of enteric parasites was 115 positive cases; for helminths was (62.7%) and for protozoa (26.3%). Mixed infection was higher than single infection in three areas. The total prevalence of helminths eggs by different mount preparation recovered from fecal sample examination from single or mixed form *E. granulosus*, *T. canis* and *T. vulpis* was of high infection rate (15.5%), (10.07%), (10.07%), followed by *A. lumbercoides* (7.7%). Finally, *T. leonine* (6.9%), both *A. caninum* and *A. braziliense* are of the same percentage (6.2%). Concerning single and mixed protozoa infection, *Giardia* was of a high prevalence (16.2%), followed by *Cryptosporidium* (8.5%) and finally *E. histolytica* (1.5%) in fecal samples of doges. These data revealed that infection levels of parasites in stray dogs in the studied areas were high, knowing that this is the first study for mixed infections between dogs and humans in KSA.

Key words: Dogs, *Giardia*, Intestinal parasites, Toxocara.

INTRODUCTION

Taif city is situated at an elevation of 1,879 m above mean sea level. The city's modern economy is still mostly dependent on agriculture (Prothero 1920). Dogs that spend their lives in rural areas are primarily "sentinels" of animal herds and homes. The dogs' close contact with humans and other animals obligates their caregivers to systematic deworming (Michalczyk *et al.*, 2019). Dogs play a potential role as reservoirs for zoonotic parasites, being especially problematic uncontrolled dog populations such as stray dogs with access to populated areas (Regidor-Cerrillo *et al.*, 2020). Dogs have close contact with humans and this represents a serious route of transmission of parasites from dogs to man (Martínez-Carrasco, 2007; Zanzani *et al.*, 2014). Dogs act as reservoirs of many parasites such as *Taenia* sp., hydatid tapeworm (*Echinococcus* sp.), dog roundworm (*Toxocara canis*), dog hookworm (*Ancylostoma* sp.), *Giardia* sp. and *Cryptosporidium* sp. (Soriano *et al.*, 2010; Weese *et al.*, 2011; Jacob and Lorber, 2015). Therefore, food and water contaminated with dog feces serve as the major sources involved in the intake of intestinal zoonotic parasites to humans (Younes *et al.* 2021). With an emphasis on parasite life cycle, transmission, pathogenicity, prevention and identification of knowledge gaps (Dantas-Torres and Otranto, 2014; Baneth *et al.*, 2016). Many researchers indicate the presence of intestinal parasites in numerous kinds of dogs in the world, but no available data about the parasites in stray dogs in the Taif region. Microscopic observation of wet mounts remains the most widely used method for identifying ova and cyst of parasites from stool specimens (Myers and Koshi, 2011). Some problems and art facts made the diagnosis of parasites difficult in fecal specimens (Zaman *et al.*, 2018). So, the present study was carried out to determine the prevalence, intensity and types of intestinal parasites in stray dogs in the Taif region in Saudi Arabia and the detection of parasites with the aid of different mount stain techniques.

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MATERIALS AND METHODS

Study area

This study was conducted at Taif University Central Laboratory, during the period from June 2020 to December 2020 in three regions, Area 1 (21°20′58.4″N 40°25′49.9″E), Area 2 (21°14′00.9″N 40°27′23.3″E) and Area 3 (21°13′17.4″N 40°22′07.3″E) at the Taif region (Fig 1).

Animals

One hundred and twenty-nine stray dogs live in three different regions at Taif. These dogs were free secured outdoors, fed on garbage, or scavenged on rodents and dead animals.

Fecal samples

Fresh fecal samples were collected from each dog population by the responsible person from the ground in the morning after voiding by dogs and individually labeled in a plastic container. The distance between the three areas was at least 5 km so that a sample from the same dog was not collected twice. The number of each dog population, each sample was preserved in formalin (10%) until examined.

Parasitological examination

Each fecal sample was examined macroscopically for adult nematodes and tapeworm proglottids. Each sample was subjected to examination by centrifugal fecal floatation technique using zinc sulphate solution (Dryden *et al.*, 2005). Furthermore, the formol ether sedimentation technique (Cornell *et al.*, 2017) was applied for each sample. Parasites were identified based on morphology to the family, genus, or, when possible, species level (Salvador *et al.*, 2014).

Wet mount preparation

Saline-based

Dilution using a normal saline (0.09%) for a minute portion of the feces was performed on a microscopic glass slide, then a coverslip was gently put over it to spread out the emulsion to obtain a thin transparent layer. Observation for stool preparation of three different wet mounts was performed in three different microscopes and examined initially under low power (10X) objective, then again under a high-power objective (40X) to confirm presumptive and positive findings (Parija and Srinivasa, 1999).

Iodine-based

Saline-based wet mounts were prepared by adding a drop of Lugols iodine to a small volume of stool, then mixing both on the glass slide that contains saline, followed by placing a coverslip on it. The usage of iodine solution aimed to make protozoan and cyst identification easier (Zajac and Conboy, 2012).

Modified trichrome (MT) stain

Fixation of the obtained concentrated pellet was performed using absolute methyl alcohol (Merck) on a clean glass slide, then stained with modified trichrome (MT) stain (Weber *et al.*, 1992).

Modified Kinyoun's acid-fast technique

After preparing modified Kinyoun's acid-fast stain, fecal smears were dried on a warmer slide at 60°C, fixed with absolute methanol for 30 seconds, then stained with Kinyoun's carbol fuchsin for 5 minutes. De-staining for the preparations was performed using acid alcohol for 2 min, then Malachite green was used to perform counterstaining for another 2 min. Slides were then rinsed with distilled water, dried on a warmer slide at 60°C for about 5 min and then mounted and examined for the detection of oocysts of coccidian (Wood *et al.*, 1982; Ash and Orihel, 1987).

Statistical analysis

Data collection was tabulated and calculated. The tabulation process was performed manually, as well as using SPSS software. An electronic calculator was used for the calculation. Using a microscope in the parasitological laboratory at the faculty of science, Taif University was used to photograph and produce pictures of the protozoan cysts and eggs (ova) of the helminth. Identification of the trophozoite, cyst, oocysts, eggs observed under the microscope was performed following descriptions and pictures published by WHO (2019).

RESULTS AND DISCUSSION

The total prevalence of enteric parasites in the fecal sample showed 115 positive cases, where helminths were 81 (62.7%) and protozoa were 34 (26.3%) out of 129 examined

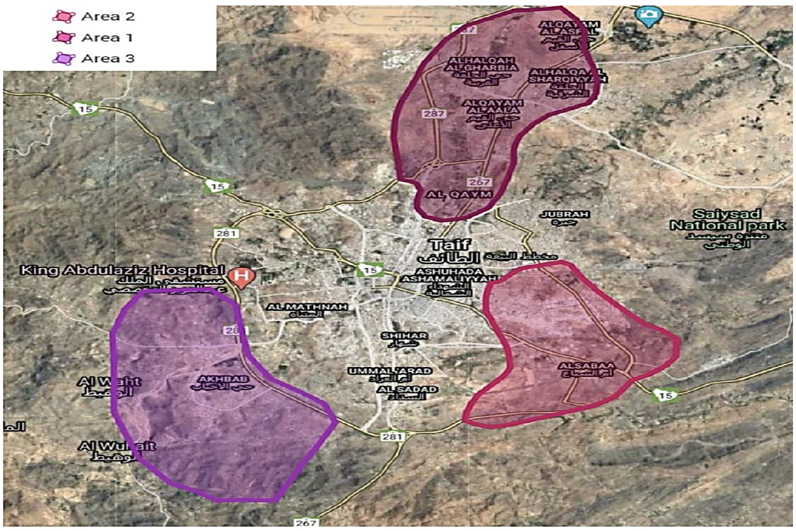

Fig 1: Three areas of Taif region where dog samples were collected.

dogs (Fig 2). Infection rates were high in the fecal samples collected from dogs in area 2, the percentages of helminths and protozoa were (27.1%), (10.07%) respectively followed by area (3) (20.1%), (9.3%) and finally area (1) (15.5%), (6.9%) in Taif region, the difference was statistically significant (p=<0.0001). The prevalence of mixed infection of enteric helminthic and protozoa in fecal samples of stray dogs in three areas was higher than single infection (Table 1, 2 and 3).

Regarding infection with helminths eggs recovered from fecal sample examination from single or mixed form, *Echinococcus granulosus*, *Toxocara canis*, *Trichuris vulpis* were of high infection rate (15.5%), (10.07%), (10.07%) followed by *Ascaris lumbercoides* (7.7%), then *Toxocara leonine* (6.9%), while both *Ancylostoma caninum* and *Ancylostoma braziliense* are of the same percentage (6.2%).

Concerning single and mixed protozoa infection, *Giardia* was of a high prevalence (16.2%) followed by *Cryptosporidium* (8.5%) and finally *Entamoeba histolytica* (1.5%) in fecal samples of stray dogs (Fig 3).

The total prevalence of helminths eggs by three different mount preparations showed *Toxocara canis* and *Toxocara leonine* eggs were (10.07%), (6.9%) respectively confirmed by the trichrome stain. At the same time, the percentage with the saline-based wet mount was (6.9%), (5.4%) and (8.5%), (6.2%) by the iodine-based wet mount. The prevalence of *Trichuris vulpis* was (10.07%) confirmed by trichrome wet mount, while the percentage (6.9%) by saline wet method and wet iodine mount preparation. *Echinoccous granulose* prevalence was (15.5%) detected by trichrome wet mount and (11.6%) by saline wet method and (13.1%) by iodine wet mount. The prevalence of *Ancylostom caninum*

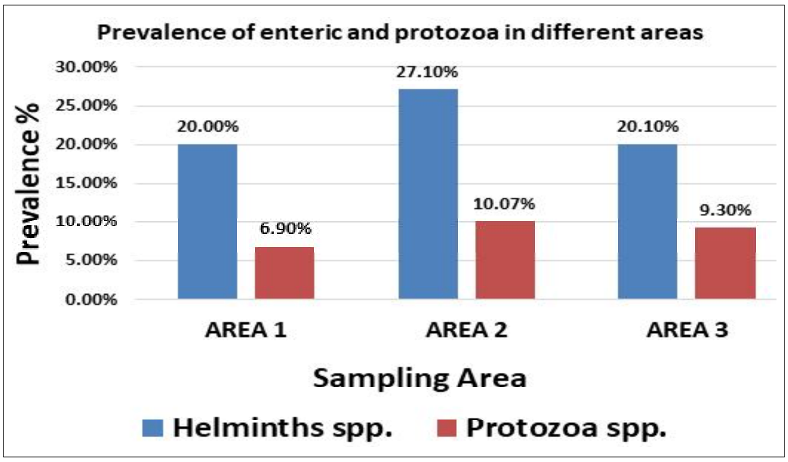

Fig 2: Prevalence of enteric helminths and protozoa in the examined dogs.

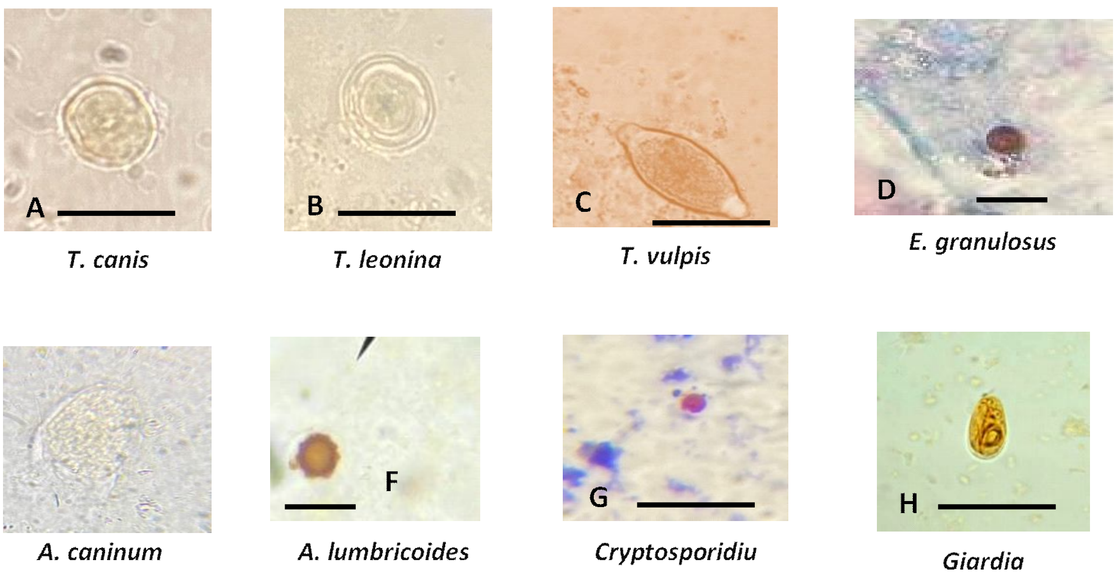

Fig 3: Showing A- *T. canis* egg; B- *T. leonine* egg; C- *T. Vulpis* egg. Scale-bar= 100 µm; D- *E. granulosus* egg. Scale-bar= 50 µm; E- *A. caninum* egg. Scale-bar= 100 µm; F- *A. Lumbricoides* egg. Scale-bar= 50 µm; G- *Cryptosporidium* oocyst; H- *Giardia* cyst. Scale-bar= 100 µm.

and *Ancylostoma braziliense* was (6.2%) detected by saline wet mount preparation, and trichrome wet mount prevalence with iodine wet mount was (4.6%) and (3.8%), respectively. A prevalence of (7.7%) for *Ascaris lumbricoides* was detected by trichrome mount wet, while prevalence with both saline wet and iodine wet mounts was (5.4%) (Table 4).

Protozoa recovered from the fecal samples and the total prevalence of *Cryptosporidium* species was (8.5%) detected by Kinyoun's acid-fast stain, while the prevalence with saline wet mount and iodine were (7.7%), (6.9%) respectively. The prevalence of *Giardia* was (16.2%) confirmed with iodine wet mount method while, with saline wet mount and Kinyoun's acid-fast were (12.4%), (13.9%). *Entamoeba histolytica* incidence was (1.5%) confirmed with iodine wet mount method and Kinyoun's acid-fast while, with saline wet mount was (0.8%) (Fig 4).

As per our knowledge, this is the first study addressing mixed infections between dogs and humans in Saudi Arabia. Public health authorities and research bodies all over the world are especially and highly interested in the intestinal parasites found in dogs. This huge attention was taken because dogs are reservoirs for these zoonotic parasites, carry and transmit them (Gracenea *et al.*, 2009). Our study showed a total prevalence (115 out of 129 cases) for enteric parasites in the fecal samples, where it was for helminths 81 (62.7%) and for protozoa 34 (26.3%). This result was recorded for the first time in Taif, KSA. As stray dogs have free access to potentially infectious; synchronized with the lack of anti-parasitic treatments, dogs are heavily parasitized animals. Humans in Taif are at high risk for being subjected to different gastrointestinal parasites of zoonotic potential, which can be spread easily via stray dogs, a fact that is relevant to what is aforementioned. Similar results were reordered in other countries in the world, such as in the Philippines and Canada (Villeneuve *et al.*, 2015; Urgel *et al.*, 2019).

Table 1: Prevalence of single infection enteric helminthic species in stray dogs in three areas.

Helminths spp.	Total positive samples	Area 1	Area 2	Area 3
T. canis	13 (10.07%)	3 (23.1%)	7 (53.9%)	3 (23.1%)
T. leonine	9 (6.9%)	2 (22.2%)	4 (44.5%)	3 (33.3%)
T. vulpis	13 (10.07%)	3 (23.1%)	6 (46.2%)	4 (30.8%)
E. granulosus	20 (15.5%)	4 (20%)	8 (40%)	8 (40%)
A. caninum	8 (6.2%)	2 (25%)	3 (37.5%)	3 (37.5%)
A. braziliense	8 (6.2%)	3 (37.5%)	3 (37.5%)	2 (25%)
A. Lumbercoides	10 (7.7%)	3 (30%)	4 (40%)	3 (30%)
Total	81 (62.7%)	20 (15.5%)	35 (27.1%)	26 (20.1%)

Table 2: Prevalence of single infection enteric protozoa species in stray dogs in three areas.

Protozoa spp.	Positive samples	Area 1	Area 2	Area 3
Giardia sp.	21 (16.2%)	6 (28.6%)	7 (33.3%)	8 (38.1%)
Cryptosporidium sp.	11 (8.5%)	3 (27.3%)	5 (45.5%)	3 (27.3%)
E. histolytica	2 (1.5%)	0 (0%)	1 (50%)	1 (50%)
Total 14 (10.8%)	34 (26.3%)	9 (6.9%)	13 (10.07%)	12(9.3%)

Table 3: Prevalence of mixed enteric parasites species infection in three areas in stray dogs.

Parasites	Area 1	Area 2	Area3
T. canis + E. granulosus	1 (1.5%)	5 (3.8%)	3 (2.3%)
T. leonine + E. granulosus	0 (0%)	4 (3.1%)	2 (1.5%)
A. caninum + Giardia	1 (0.8%)	4 (3.1%)	1 (0.8%)
A. braziliense + T. vulpis + Cryptosprodia	1 (0.8%)	4 (3.1%)	2 (1.5%)
A. Lumbercoides + Giardia	0 (0%)	5 (3.8%)	2 (1.5%)

Table 4: Comparative prevalence of different helminths by different types of wet mount preparations.

Helminths spp.	Saline mount	Iodine stain	Trichrome stain
T. canis	9 (6.9%)	11 (8.5%)	13 (10.07%)
T. leonine	7 (5.4%)	8 (6.2%)	9 (6.9%)
T. Vulpis	9 (6.9%)	9 (6.9%)	13 (10.07%)
E. granulosus	15 (11.6%)	17 (13.1%)	20 (15.5%)
A. caninum,	5 (3.8%)	6 (4.6%)	8 (6.2%)
A. braziliense	4 (3.1%)	5 (3.8%)	8 (6.2%)
A. Lumbercoides	7 (5.4%)	7 (5.4%)	10 (7.7%)

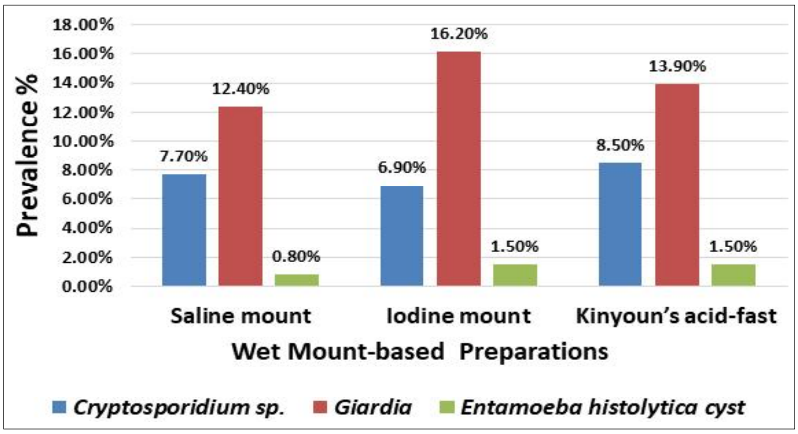

Fig 4: Comparative prevalence of different protozoa using different types of wet mount-based preparations.

Infection rates in fecal samples collected from three different areas in Taif showed higher rates of infection with helminths and protozoa in the area (2) (27.1%), (10.07%) followed by area (3) (20.1%), (9.3%) and finally area (1) (15.5%), (6.9%), respectively. The difference was statistically significant (p=<0.001). A possible explanation for these findings is that areas (2 and 3) are characterized by dogs living in close contact with livestock besides the presence of animal slaughterhouses. These animal species are intermediary hosts essentially required for many parasites to complete their life cycle (Weese *et al*., 2011). Prevalence of intestinal parasites (helminths and protozoa) in the fecal samples of dogs in three areas showed a higher rate of mixed infection rather than of mono-infection. This is attributed to the fact that the presence of one parasite species may lessen the immunity of the dogs with higher parasite intensity, which in turn enhances the presence of the other parasitic species, causing these mixed infection cases (Fontanarrosa *et al*., 2006). The present study showed types of helminth and protozoan species like those reported in earlier surveys of dogs (De Alwis, 2000).

It was found, after observing various helminths eggs by different mounting techniques, that *E. granulosus*, *T. canis*, and *T. vulpis* were the helminths in single and mixed infection and resembled high rates of infection (15.5%), (10.07%) and (10.07%), respectively followed by *A. lumbercoides* (7.7%), then *T. leonine* (6.9%), while both *A. caninum* and *A. braziliense* are of the same percentage (6.2%). These results were confirmed with the trichrome wet mount method. The trichrome stain has been considered as a helpful stain in the detection of helminths ova (Wood *et al*., 1982; Hale *et al*., 1996). They described the appearance of the most common helminth ova that was easily detected using the trichrome stain than other mount preparation.

In the present work, we found *E. granulosus* prevalence was high in the areas 2 and 3 (40%) in either single infection or mixed infection with *T. canis* and *T. leonine*. This finding is particularly important because there are many large feedlots for cattle, sheep, goats and camels in areas 2 and 3. These animals are of high infection risk by contamination of food or water supplies with eggs carried by dogs, which could result in the spread of this zoonotic infection to local stray dogs. Consequently, human and animal infection with an intermediate stage (hydatid cyst) may arise in KSA (Deplazes *et al*., 2011; Shalaby *et al*., 2011).

T. canis prevalence in the current study was (10.07%). It has been documented that *T. canis* ova were very resistant to extreme weather conditions and chemical agents (Torres-Chablé *et al*., 2015; Regidor-Cerrillo *et al*., 2020). Also, it is well known that routes of infection of *T. canis* include oral, transmammary, and transplacental and that it can be transmitted by paratenic hosts, which let the parasite everlasting in our ecosystem (Corrales *et al*., 1999). *T. leonine* percentage was (6.9%), the latter one of the more frequent parasites detected in shelter dogs in several countries (Villeneuve *et al*., 2015). The region is a shared habitat with dogs; this interaction may explain the presence of *T. leonina* in stray dogs (Beiromvand *et al*., 2013).

The prevalence of *T. vulpis* was (10.07%). The zoonotic potential of *T. vulpis* was supported by Dunn *et al.* (2002), who recorded human infection with this worm due to close contact with dogs. *Trichuris* spp. is considered to be soil-transmitted helminths (Liberato *et al*., 2018). The prevalence of *A. caninum* and *A. braziliense* was (6.2%) in this study. Transmission of this parasite can occur through penetration of skin at hair follicles or sweat glands or through direct ingestion of *A. caninum* (Wojnarowicz and Smith, 2007; Scaramozzino *et al.*, 2018). However, recorded mixed infection with *A. braziliense* and *T. vulpis* similar result by Villeneuve *et al*. (2015) has reported a strong positive association between *Ancylostoma* sp. and *T. vulpis* in North America. The *A. lumbricoides* has a prevalence of (7.7%), where the present study suggested that dogs could act as reservoir hosts of *A. lumbricoides* and environmental contaminators that increase the risk of infection in humans (Zaman *et al*., 2018).

Concerning single and mixed protozoa infection, *Giardia* was of high prevalence (16.2%), followed by *Cryptosporidium*

(8.5%) and finally *E. histolytica* (1.5%) in fecal samples of dogs. Protozoa appeared to have less shrinkage and a more distinct internal structure with the saline concentration than other mount preparations (Zaman *et al.*, 2018). The present work recorded that the prevalence of *Giardia* was (16.2%), which was confirmed by the iodine wet mount method, where internal structures of trophozoites, cysts were stained well by iodine, the method that made their recognition and identification easier in the specimens. *Cryptosporidium* species was (8.5%) detected by Kinyoun's acid-fast stain (Elsafi *et al.*, 2014). Those protozoa represent zoonotic risk factors for man, the zoonotic potentiality supported by (Robertson and Thompson, 2002; Stafford *et al.*, 2020). Finally, the higher prevalence of *Giardia* sp. and *Cryptosporidium* sp. in stray dogs may be due to dogs drinking water from small rivers and lakes that are frequently more contaminated with parasites (Frizzo *et al.*, 2016).

CONCLUSION

In conclusion, the present results clearly showed significant results of intestinal helminths and protozoa in stray dogs we have examined, as those act as hosts for several species of enteric parasites. Therefore, we should take preventive measures to avoid the environmental contamination and infection of both man and animals in the Taif region.

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Declarations

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Competing interests: The authors declare that they have no competing interests.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Code availability: Not applicable.

Authors' contributions

Jamila S. Al Malki has designed the study, collected samples and performed parasitological assays. Also, Jamila S. Al Malki performed data analysis and wrote the article draft and conducted the required revision to reach the readable and publishable approved final manuscript.

Ethics approval

Not applicable. Samples were feces collected from stray dogs, no sacrificing for animals nor tissue samples were collected that require ethical approval.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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