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Cryptosporidium and *Giardia* Infection in Pet Animals: A review Study in Iraq

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Cryptosporidium parvum and *Giardia lamblia* are the most common zoonotic intestinal protozoan parasites causing diarrheal diseases Cryptosporidiosis and Giardiasis are significant health concerns in both humans and animals; for this reason, these parasites acquired public and veterinary importance. Dogs and cats are susceptible to infection and play an important role in transmitting these protozoans to human. Recently in Iraq, there is an interest in keeping pet animals and an increase of stray cats and dogs which accompany livestock. Some people started to attract stray cats through feeding and became like house animals. This literature review focused on the results of Iraqi researchers to overview the importance prevalence of these parasites on both human and animals. This literature review recommended to educate pet owners especially children and follow veterinarians' instructions, to control and prevent transmission of these zoonotic diseases and future researches should focus and investigate from these finding and highlight the role of domestic animals in transmission of these diseases.

Keywords: *Cryptosporidium*, *Giardia*, dogs, cats, zoonosis, protozoa, Iraq.

Introduction

Companion pets or pet animals especially cats and dogs infected with parasites have a significant risk to transfer 60% of most infectious diseases in humans [1]. Companion dogs have been domesticated 12 to 15 thousand years ago and since then became popular pet animals [2]. The role of this relation between dogs and humans has changed from the normal use of dogs for hunting and guarding a more intimate relation as their chaperons. Due to rapid pace of modern life, dogs have become beloved pets. Dogs' owners take their pets to the parks, children's playgrounds and walk with their dogs in green areas [3,4]. This relationship decreases stress levels and improve physical health in spite of the fact dogs may transfer significant parasitic diseases [5, 6].

Interaction and physical contact between dogs and their owners may pose a risk for acquiring antimicrobial resistance [7]. Rural areas are more infected with several parasitic diseases than urban environment [8]. Cats are more domesticated pets worldwide than dogs [9]. The zoonotic diseases that can be transmitted by cats may occur through contaminated water or food, especially through infected saliva or other excretions [10].

Pet animals have a role for transmitting several zoonotic parasites including *Cryptosporidium parvum, Giardia duodenalis, Echinococcus granulosus, Echinococcus multilocularis, Toxocara sp.* and hook worms of different species [1,8,11]. Roaming cats have a defecation behavior which is considered a source of zoonotic diseases [12]. In developing countries, inadequate sanitary conditions and the insufficient resources of education and medical resources increase the opportunity of exposure to the parasitic stages that eliminated from feces of infected dogs [13].

The biology, epidemiology and zoonotic significance are similar in both *Cryptosporidium* and *Giardia* which are considered distant relatives among parasitic protozoan. *Cryptosporidium*

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is a coccidian protozoan while *Giardia* is a flagellated parasite, both of them parasitize in the intestine of the most mammals, birds and reptiles. *Cryptosporidium* may be found also in other sites of the gastrointestinal tract for example: respiratory urogenital tract as well as bile and pancreatic tracts. Cryptosporidiosis traditionally considered a veterinary disease and recently acquired as a potential waterborne disease in human [14]. Giardiasis is considered as most prominent intestinal parasitic infection among companion pets (dogs and cats) in the developing countries. *Giardia* species belong to phylum Fornicata class Trepomonadea family Giardidae; can be classified according to host origin and morphology of the trophozoites basically by the appearance of the median bodies [13,14].

Depending on the origin of the host, three prominent morphological trophozoites were identified named: *Giardia lambilia* also known *Giardia duodenalis* parasites on both birds and mammals, *Giardia muris* parasites on rodents, and *Giardia aglis* toads [15]. *Cryptosporidium* and *Giardia* are two of the most widespread parasites across the world, these pathogens are considered endemic worldwide. There are ten species of Giardia on the basis of light microscopic characteristics depending on shape of the trophozoites and medium bodies [14]. Recently depending on molecular methods that groups the species into assemblages: *Giardia duodenalis* (Assemblage A) parasites on humans, cats and dogs, farm animals, primates, rodents and other wild mammals. *Giardia interica* (Assemblage B) parasites on humans, dogs and cats, primates and some wild mammals. *Giardia canis* (Assemblages C,D) parasites on dogs and other canides, *Giardia bovis* (Assemblage E) parasites on cattle and other ungulates. *Giardia cati* (Assemblage F) parasites on cats. *Giardia simond* (Assemblage G) parasites on rats. *Giardia spp.* (Assemblage H) parasites on pinnipeds. *Giardia muris* and *Giardia microti* parasite on rodents, finally *Giardia psittaci* and *Giardia adreae* parasite on birds [16,17]. Assemblages A, B are tend to infect humans and have broad host specificity and have the ability to be transmitted zoonotically [18].

The *Cryptosporidium* parasite has at least 27 species, and numerous genotypes [19]. The distinct species include *Cryptosporidium hominis* human genotype1, *Cryptosporidium parvum* also named bovine genotype or genotype2. These two species are the most commonly detected species in man, while *C.canis, C.felis*, and *C. melaegridis* were also detected in man [20]; other genotypes of *Cryptosporidium* were associated with mice, bears, deer, monkeys and cattle. Most of these parasites are considered independent species [17]. Cats are exposed to *C. fellis*, *C. murois, C. ryumae* ; and *C. parvum* while dogs are exposed to *C. canis, C. parvua, C. ubiquitum* and *C.anderson* [21]. Humans infected with Cryptosporidiosis, are usually caused by *C. homonisis, C. meleagridis, C. parvum, C. felis* and *C. canis* [22].

Within the host, the lifestyle of *Giardia* is simple compared with the complicated lifecycle of *Cryptosporidium*. *Giardia* infection occurs by ingestion of the cysts and or oocysts in *Cryptosporidium* after infection with these infection stages (cysts or oocysts) within the intestine releasing trophozoites of *Giardia* or sporozoites of *Cryptosporidium*. After ingestion the infective stage, the (cysts) of *Giardia* entre the stomach by the action of the digestive juices, especially gastric acid, which stimulates the cysts to exist in the duodenum. The resulting trophozoites attach on the surface of the intestinal epithelia without invading the mucosal tissue and within the same place multiply longitudinal binary fission every eight hours. These trophozoites stimulate to encystation when changes occur due to an unacceptable environment for trophozoites multiplication. The migration of the trophozoites into the ileum and large intestine, then cysts pass through with feces [23]. Infective stage of *Cryptosporidium* genus does not multiply outside the host, the lifecycle of this parasite involves sexual (gamogony) cycle and asexual (schizogony and sporogony) cycle. These two cycles occur in association with a parasitophorous vacuoles restricted to the microvillus regions of enterocytes of the host. The infected host eliminates two kinds of oocysts (thick and thin-walled oocysts). Thick-walled oocysts eliminated into the environment (outside the infected host) thus infected cycle is completed through the fecal oral route compared with the thin-walled oocysts which also produced, but the infective cycle is autoinfection [24,25]. The main route of infection with the *Cryptosporidium* occurs when (oocysts) of *Cryptosporidium* taken by fecal oral route mainly regarding waterborne transmission [26]. Finally, both infected cysts and oocysts shedding in feces in large numbers resistible and infective for several months in a watery, damp and cool environment [23].

Prevalence of Cryptosporidiosis and Giardiasis in dogs and cats in Iraqi provinces

Several epidemiological studies revealed the distribution of *Cryptosporidium sp.* and *Giardia intestinalis* parasite between cats and dogs worldwide [18]. In Iraq the prevalence is highly endemic due to the unsanitary conditions and favorable climates and it varies among studies depending on geographical areas, habitat of local animals' populations and seasons of the year. It is noted that prevalence of these protozoans' parasites is higher in stray cats and dogs than in pets [11, 27].

In Basrah province, the relation between animal handlers and non-animal handlers in infections with enteric parasites, revealed an increasing rate from 14.8% up to 50% in non-animal handlers and animal handlers respectively. *Cryptosporidium* oocysts were 5% in animal handlers where it was 1.14% in non-handlers [28]. In Babil province, in a case report study on Giardiasis in local breed queen found numerous cysts by centrifugal flotation method and magnesium sulfate with 1.20 specific gravity. The cat suffered from mild diarrhea and steatorrhea [29]. In Mosul province, the infection with *Cryptosporidium* and *Giardia* species in dogs and cats were 42%, 26%; 52%, 16% respectively [30].

In Baghdad, *Cryptosporidium parvum*, *Cryptosporidium muris* and *Giardia sp*. were 3.75%, 6.25% and 2.5% infection rates in domesticated cats respectively [31]. However, *Cryptosporidium spp*. was 3.73% for police and house dogs [32]. In Diwaniya province, four genera of protozoa cysts reported 9.3% of *Giardia sp*. and 6.97% of *Cryptosporidium parvium* in domestic cats (*Felis catus*) [33]. In Baghdad, two protozoa which are: *Giardia sp.* 24.1% and *Cryptosporidium sp*. 20.8% reported in stray dogs [34].

In Saladin province, 6.09% infection rate of *Giardia* species reported in a study about intestinal parasites by histopathological method [35]. In Duhok, 5.2% of Giardia reported in dogs (sheep-keeper, owned, pet and stray dogs) [36]. In Al-Mussaiab, in a checklist of parasites in stray cats (*Felies cats)*, 15 protozoa, *Cryptosporidium* and *Giardia* species were included [37]. In Basrah, 4.3% infection with *Giardia* reported only in gastrointestinal parasites [38]. In Basrah, 75.55% infection rate with Giardia by ELISA in dogs [39]. In Kirkuk province, the infection rates of *Cryptosporidium* species were 6.49%, 27.08% in stray dogs and cats respectively [40].

In Baghdad, 13.35% infection rate with *Cryptosporidium* species by ELISA in domestic mixed breed dogs [41]. In Baghdad, six genera species of gastrointestinal in domestic dogs of which *Giardia spp*. The total infection rate in dogs was 32.22% [42]. In Baghdad, Faraj reported 15% of *Cryptosporidium* infection by microscopic examination and 28.6% using PCR in domestic dogs [43]. In Baghdad, Swadi and Zenad worked on non-zoonotic *Giardia duodenalis* assemblages C and D reported variable infection rates with *Giardia duodenalis* with different methods; 9%, 5%, 5% infection rates by PCR, direct smear examination and immune chromatography assay methods respectively [44]. Other studies also reported a significantly higher infection rate 10.86% was recorded among males. Furthermore, the rate of infection was increased among younger dogs and a higher infection rate 25% was observed in stray dogs as compared to domesticated dogs 7.14%. In Baghdad Latif *et al.,* reported the rates with *Cryptosporidium* species and *Giardia intestinalis* infection were 12.9%, 9.67% in dogs respectively. While in cats the *Cryptosporidium sp*. was 31.57% in cats **[**45].

Public Health importance of Cryptosporidiosis and Giardiasis

In Iraq gastrointestinal parasitic protozoal infections are commonly and widely distributed because of the low level of environmental and personal hygiene due to improper disposal of humans and animals excreta. The infections with *Giardia* and *Cryptosporidium* occur by the fecal-oral route [46,47]. Cryptosporidium is food and waterborne disease-causing diarrhea in both humans and animals; children are highly exposed to infection due to their direct contact with pets and with insufficient hygiene habits; and may lead to death of small children, adults and immunocompromised people (AIDS patients) [48,49]. Risk factors of high prevalence rate infection in children are social and economic conditions [49]. At the level of molecular of *Giardia intestinalis* in cats, the researchers confirmed potential zoonotic genotypes A and F (feline specific as genotype) [50].

In Duhok, northern Iraq; *G. lamblia* infection was 38.5% in children [51], 50% by direct examination and 65.5% by ELISA in children [52]. In Baghdad the researcher reported the prevalence rate of humans infected with *Giardia*

reported 11.66% infections, the researcher also reported that there is a strong relation between presence of domestic animals and prevalence of Giardiasis where the rate significantly increased to 23.25% infections in humans who owned domestic animals in their household [53]. In rural villages in Basrah marshes Jarallah, 2012 reported the prevalence of *Giardia intestinalis* was 45.16% in a study conducted 294 patients (54). In the northern part of Iraq, Al-Naemy et al., 2012reported the prevalence of *Giardia intestinalis* was 37% (55). In Baghdad [56] out of 2033 children in Baghdad province, the infection rate with *Giardia intestinalis* was 45.5% and 8.75% for *Cryptosporidium* parasite.

In Kut, by microscopic examination 100% while 72.5% by ELISA of *Cryptosporidium parvium* in humans [57]. Other researchers in Kut worked on *Cryptosporidium parvium* using different methods: Direct Florescent Antibody (DFA), Modified Acid Fast Stain and Immune-Card methods 34%, 30% and 28 % respectively [58]. In Diyala, 40% of *Giardia lamblia*, 4.21% of *Cryptosporidium parvium* microscopically, but by using immunochromatography assay recorded 40% and 7.36% positive for *Giardia lamblia* and *Cryptosporidium parvium* respectively [59]. While RT-PCR recorded 47.36% for *Giardia lamblia* while 7.36% positive for *Cryptosporidium parvium*. In Baghdad, 59.52% among 25/42 of *Giardia lamblia* by multiplex real time PCR, the results showed that 7(28%) genotype A and 18 (72%) genotype B which is more prevalent than A (in males 61.11%) and (in females 38.89%). Genotype B is most frequent in children with diarrhea [60].

In Al-Qadisiya, the infection rate with *Giardia lamblia* was 54%. The researcher showed infection with *Giardia lamblia* in rural locations was 66.6% higher than in urban locations in humans [61]. In Kirkuk, three different microscopic methods: direct double wet preparation, formal ether and fecal stained smears and reported 10.31% with Giardia 1.43% *Cryptosporidium parvium* [62]. In Baghdad, the highest rate of infection was 85.57% in patients who drank from contaminated water sources whereas 79.3% humans suffering from chronic infection Giardiasis [63]. In some Iraqi provinces, reported 1.77% of *Giardia lamblia* [64]. In Maysan governorate, 40% by microscopic examination reported in women and children [65]. In Wasit province, 72% positive by Nested PCR technique [66].

In Baghdad, the infection with *Giardia intestinalis* was 33.33% in children [67]. In Najaf, by phylogenetic analysis the highest prevalence was 52.5% genotype B, 22.5% genotype A and 15% genotype F while 10% mixed subtypes AB [68].

Epidemiology

Increasing levels of infection with Giardiasis have several possible reasons, the routine and use of anthelminthic drugs, especially in small dogs decreases the prevalence of helminth parasites in contrast, intestinal protozoa are widely unaffected by the anthelminthic [69] and also poor standards sanitations, poor hygiene and large population numbers of stray cats and dogs [70]. The population of domesticated and stray companion animals is increasing annually in Mosul/Iraq. Companion animals represent a significant problem worldwide due to the harbouring of many intestinal protozoan parasites threatening human life [71]. There are multiple routes of transmission of intestinal protozoan (water and foodborne transmission, human-human transmission, zoonotic transmission and sexual transmission), also the resistance of the cysts in the external environment and the low infective dose reveals the frequency of and existence of outbreaks, Giardia plays as major outbreaks being waterborne [72,73].

Intestinal parasitic diseases cause a significant morbidity and mortality worldwide. *Giardia duodenalis* parasite is considered as one of the most common intestinal protozoans. Urban locations became a habitat for animals including livestock and companion animals which carry zoonotic pathogens [17]. The risk increasing especially in crowded cities due to bad sanitation with the disposal of manure which attracts disease vectors and scavengers. This may act as reservoirs for parasites and therefore increases risk transmission of zoonosis especially in dry season [74].

In cats and dogs infected with *Cryptosporidium* and *Giardia* species higher in stray dogs and cats less than one year. High infection rate with Giardia species found in female cats and dogs 28.12%, 17.5% compared with oocysts of *Cryptosporidium* found in dogs and cats 50%, 60% respectively [30]. A study in Basra province found the high infection rate with *Giardia lamblia* 43.75% detected in March compared with 34.78% in July and also found the infection was more common in one day to six month of age. Furthermore females showed more infection than males [39]. A

study on domestic mixed breed dogs with ELISA recorded no significant difference in age and sex [41]. In Baghdad , female rate 32.6% was higher than male 22.44% in dogs and the age group less than one year was 63.15% [32]. In another study, the infection with *Cryptosporidium* in domestic dogs by microscopic examination in males and females were 8.5%, 21.42% respectively [43]. On domestic dogs the results revealed that infection with intestinal parasite including *Giardia* in females was 33.33% higher than males 31.11%. The prevalence between dogs age less than six months 44.44% while age group more than six months was 20% [42].

Pathogenesis and clinical signs of Cryptosporidiosis

Cryptosporidium parasite develops in the epithelial cells inside parasitophorous vacuoles (PV) within the microvillus layers of small intestines while in immunodeficient host it is recognized throughout the gut, biliary and respiratory tracts and villous atrophy, crypt hyperplasia and mild lymphocytes increase in lamina propria are associated with persistent cryptosporidiosis [75]. *Cryptosporidium* infection is characterized by moderate to severe diarrhea due to increase of intestinal permeability, sodium malabsorption and secretion of electrogenic chloride. The infection of epithelial cell with Cryptosporidiosis leads to activation of a nuclear factor [76], which accelerates antiapoptotic mechanisms, but also upregulation of a pro-inflammatory cascade increasing expression of pro-inflammatory cytokines and inflammation markers are lactoferrin, tumor necrosis factor and interleukin (IL) 1, IL-8 [77]. Infected epithelial cells release chemokines: IL-8 and IP-10 [78]. Infection with cryptosporidiosis also causes increasing of intestinal permeability and decreasing fluids absorbing, electrolytes and fluxes of solutes into the gut. Clinical signs of the *Cryptosporidium* infection appear after an incubation period of about one week. One of the most prominent clinical signs is diarrhea but found substantial varieties in the clinical signs depending on the population of the host [79].

Pathogenesis and clinical signs of Giardiasis

Giardiasis causes direct damage to brush border and mucosa of the intestine by trophozoites as well as inducting response of the host immune resulting in secreting fluids as well as damaging of the intestine, affecting of duodenal flora or bile content and also apoptosis in the epithelial cells of the small intestine [80]. The increased permeability in the epithelial cells of the small intestine by myosin light chain kinase–dependent phosphorylation of F-actin [81]. Giardiasis causes a symptomatic infection, malabsorption and acute or chronic diarrhea. The degree of malabsorption is associated with many factors: host susceptibility and ferocity of genotype of parasite [82]. Infection occurs with oral ingestion from 10 - 25 cysts. 5% - 15% of *Giardia* hosts excrete cysts with no symptoms, while acute self-limiting diarrhea developed 25% - 50% and no evidence of infection in the percentage 35% -70%. The period of the disease from 7 to 14 days. Clinical signs for more than 7 to 10 days including steatorrhea, diarrhea, malaise, flatulence, foul smelling, greasy stools, abdominal cramps, malabsorption of vitamin A and B12, iron, D-xylose, bloating, nausea, anorexia and weight loss [83]. The other clinical signs less commonly reported include urticaria, constipation, reactive arthritis and biliary tract illness. Grossly pus, blood, and mucus are absent in feces. Chronic or persistent diarrhea up to 14 days may develop into weight loss, headache, profound malaise, diffuse abdominal and epigastric disturbance that is aggravated by eating. The stools from patients are often greasy, foul smelling, yellowish and repeatedly occur in small volumes [84].

Conclusion

Many researchers presented useful and valuable scientific results to design prevention programs for decreasing the risk of zoonotic parasite diseases. The host parasite relationships included considered as one health aim to reinforce knowledge of clinical and public health management to zoonotic diseases. Veterinarians play an important role in transmitting infection of parasitic intestinal zoonotic protozoan such as *Giardia duod*enalis due to direct contact to dog feces. We also have to support prevention control for these infections in pets and also to apply strategies to control population and ownership of stray dogs and cats. It is recommended that pet owners visiting dog parks should follow veterinarians' instructions and guidance in dealing with pet animals to reduce parasite infection. There is a need to find effective and wide interventions to residents and their cats counterparts.

Researchers in Iraq recommended using ELISA for being a useful test for epidemiological studies and to support diagnosing humans with clinical signs of *Giardia* infection with negative results by direct microscopic test. More efforts are required to prepare an ELISA test from a local strain of *Giardia lamblia* in order to compare it with commercially viable kit.

More studies are needed on epidemiology of parasitic infection in dogs and cats population which are useful for developing the control strategies and frequently decreasing the danger of transmitting zoonotic diseases to man. Thus, collecting more data about the spreading of the parasite infecting dogs is very important to develop veterinary and public health programs for treatment and control.

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الاصابة بطفيلي الابواغ الخبيئة و الجياردیا في الحيوانات الاليفة (الكلاب و القطط) : دراسة مرجعية في العراق

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يعتبر طفيلي الجياردیا لامبليا وطفيلي الابواغ الخبيئة من الاوالي المعوية المشتركة والشائعة المسببة للاسهال والذي يطلق عليه بمرض Giardiasis , Cryptosporidiosis في الانسان والحيوان ولهذا السبب اكتسبت هذه الطفيليات اهمية صحية و بيطرية في الانسان والحيوان. تعد الكلاب والقطط من المضائف الحساسة والمهمة للاصابة بالاضافة لكونها مضائف ناقلة لهذه الاوالي للانسان. مؤخرا في العراق، زاد الاهتمام بتربية الكلاب والقطط المدللة، وزيادة في اعداد الكلاب والقطط السائبة المرافقة لقطعان الحيوانات الحقلية ، واهتمام البعض من الناس في رعاية القطط السائبة واعتبارها كقطط منزلية اليفة. وتم التركيز في استعراض المراجع هذا على جمع ومراجعة نتائج عمل الباحثين العراقيين على مدى انتشار واهمية هذه الاوالي المعوية في الانسان والحيوان. حيث توصي هذه المراجعة على تثقيف اصحاب الحيوانات الاليفة وخاصة الاطفال وارشادهم عن طريق اتباعهم لتوصيات و تعليمات الاطباء البيطريين للسيطرة ومنع انتقال هذه الامراض المشتركة كما يجب ان تركز الابحاث المستقبلية على تسليط الضوء بعمل مسوحات وبائية واسعة حول دور الحيوانات الاليفة في نقل هذه الامراض.