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Original Article

The Effect of Tylosin Drug on *Cryptosporidiosis* in Stray Dogs of Tabriz and Its Importance in Public Health

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Abstract

Introduction: Intestinal protozoa of dogs have a relatively high diversity, and the prevalence of infection is often observed sub-clinically and sometimes clinically. Different species of *Cryptosporidium* are observed in the dog's intestines. Due to the importance of pathogenicity of gastrointestinal protozoan parasites in dogs and also their zoonotic importance, this study was conducted on stray dogs in Tabriz.

Methods: During this study, two hundred stool samples of stray dogs from different regions of Tabriz were prepared and examined microscopically for parasitology. The samples were tested by the Formalin-Ether concentration method, and also the following Ziehl-Neelsen modified staining method was used to observe *Cryptosporidium* protozoan.

Results: Out of 200 fecal samples, 16 samples (8%) were infected with *Cryptosporidium* protozoa. Statistical analysis of the results also showed that there was a statistically significant difference in the rate of infection with protozoan parasites between dogs under one year old and dogs over one year old ($P<0.05$). Regarding the sex of dogs, there was a statistically significant difference in the prevalence of *Cryptosporidium* between male and female dogs ($P<0.05$). Sixteen dogs infected with *Cryptosporidium* parasite were also treated with tylosin for one week, and a decrease in infection with this parasite was observed in 9 treated dogs.

Conclusion: In this study, the rate of *Cryptosporidium* infection in stray dogs decreased from 8% to 3.5% after tylosin treatment, and this drug may be useful in controlling this parasitic disease in dogs, but more extensive research should be carried out to eradicate this parasite in dogs.

Keywords: *Cryptosporidium*, Tylosin drug, Stray dogs, Public health, Tabriz

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Introduction

Parasitic diseases still infect millions of people annually, especially in the tropics, and cause significant mortality. These infections cause more problems in domestic animals by reducing livestock production, meat contamination, and other economic losses. Parasitological research has helped to control these diseases with advances in medicines, vaccines, and diagnostic methods. Research on parasitic infections has generally pursued certain goals. However, the study of parasites and the relationship between parasites and hosts are still topics of interest in biological studies. Parasites are a major threat to human and animal health around the world, and their control remains an important research goal in international organizations and scientific centers around the world (1,2). Dogs play an important role in the epidemiology of zoonotic diseases and are clearly and extensively associated with humans and the human environment (3). The great intelligence and ingenuity of dogs have caused this animal to be given attention by humans and to be kept and used by humans for various purposes. Because of the importance of dogs, dog owners usually spend large sums of money on their health and well-being, and despite great efforts to control dog diseases, ranchers are always faced with a variety of diseases in dogs, and the prevalence of these intestinal protozoa in dogs is high due to the numerous methods for their transmission through water and food (4).

On the other hand, increasing the use of guard dogs has led to greater human contact and closeness with dogs; therefore, humans are exposed to zoonotic diseases caused by dogs (5).

Given the above considerations, the present study was performed on cryptosporidiosis in stray dogs in Tabriz, Iran, and the prevalence of *Cryptosporidium* in different areas and the effect of Tylosin drug on infected dogs was investigated.

Materials and Methods

This study was conducted in Tabriz and focused on the possibility of infection with protozoan parasites of the gastrointestinal tract of stray dogs to *Cryptosporidium* and

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the percentage of *Cryptosporidium* protozoan infection. Further, the following Ziehl-Neelsen modified staining method was used to study *Cryptosporidium* Oocysts.

In this study, 200 stool samples of stray male and female dogs (100 males and 100 females) with different ages were randomly prepared and tested. Stool samples were placed in special containers in 10% formalin and transferred to the parasitology laboratory of the Faculty of Veterinary Medicine. For the final diagnosis, the preparation and staining steps were performed, and the type of parasite was identified based on the morphology and size of the parasite using diagnostic keys. The samples were examined using a light microscope and the following Ziehl-Neelsen modified staining method. By observing at least 20 *Cryptosporidium* oocysts in each microscopic field of view with a magnification of 400, the sample was considered positive.

Due to the following Ziehl-Neelsen modified staining feature, the background of the sample was green, and the oocysts were red. The stained blades were easily examined with a light microscope, and the results were recorded and analyzed.

To evaluate the effect of Tylosin drug on positive samples, the available dogs were injected intramuscularly with 10% Tylosin every 24 hours twice daily for 7 days at a rate of 10 mg/kg *body weight*. Sampling was performed again after one week, and the samples were transferred to the parasitology laboratory after treatment and re-stained under Ziehl-Neelsen modified staining.

Finally, the results of contamination were recorded and statistically analyzed, then the results were descriptively reported. The chi-square test was used to compare the results between males, females, and different ages.

Results

In the present study, fecal samples of 200 stray dogs in Tabriz, Iran, were examined in 2021. Of these, 16 (8%) showed infection with *Cryptosporidium* protozoan parasite, while the number of uninfected cases was 184 (92%) as presented in Table 1.

Regarding the effect of dog sex on the prevalence of *Cryptosporidium* protozoan parasite, as Table 2 illustrates, among 200 stray dogs in Tabriz (100 females and 100 males), 10 females and 6 males exhibited *Cryptosporidium* infection.

Regarding the age of dogs and the prevalence of *Cryptosporidium* protozoan parasite, in 200 stray dogs under the age of one year in Tabriz, 10 cases were positive, and 6 cases were infected with *Cryptosporidium* in one-year-old dogs (Table 2).

Based on the results of testing 200 stools of stray dogs, 16 available dogs infected with *Cryptosporidium* parasite were treated with Tylosin for one week. After retesting the feces of the treatment group with modified Ziehl-Neelsen staining, the results were obtained (Figure 1).

According to the results in Table 3, the infected dogs (n = 16) were treated, and after treatment of 16 infected dogs, 9 dogs responded to the drug; accordingly, the number of oocysts in the microscopic test was significantly reduced.

In analyzing the data and the prevalence of infection in terms of the sex of dogs, a statistically significant difference was observed in the prevalence of *Cryptosporidium* between male and female dogs ($P < 0.05$).

Furthermore, when the overall prevalence was analyzed by age, a statistically significant difference was found in the prevalence of *Cryptosporidium* in dogs under one year of age and over one year ($P < 0.05$).

There was also a significant relationship between reducing the prevalence of *Cryptosporidium* and the use of tylosin compared to untreated dogs. The use of the drug significantly reduced the number of oocysts ($P < 0.05$).

Discussion

Cryptosporidium protozoan is an important parasitic protozoan that due to high adaptability and high vitality of its oocyst in nature can also adapt to today's conditions and exert its pathogenicity on hosts (6).

In recent years, many studies have been conducted on parasites in different parts of the world in the field

Table 1. Number and Percentage of Cases of *Cryptosporidium* Infection in 200 Stray Dogs

Parasite	Infected Dogs, No. (%)	Uninfected Dogs, No. (%)
Cryptosporidium	16 (8%)	184 (92%)
Total	200 (100%)	

Table 2. Relationship Between Age and Sex of Dogs With the Prevalence of *Cryptosporidium* Parasite in 200 Stray Dogs

Parasite	Gender		Age	
	Female, No. (%)	Male, No. (%)	Under one year, No. (%)	Over one year, No. (%)
Cryptosporidium	10 (5%)	6 (3%)	11 (5.5%)	5 (2.5%)
Total	16 (8%)		16 (8%)	

Table 3. The Rate of Parasite Infection in Stray Dogs With *Cryptosporidium*, Before and After Treatment With Tylosin Drug

Parasite	Number of Infected Dogs in the Total Population	Percentage of Infected Dogs in the Total Population
Total infected population before treatment	16	8
Total infected population after treatment	7	3.5
Total non-infected population after treatment	9	4.5

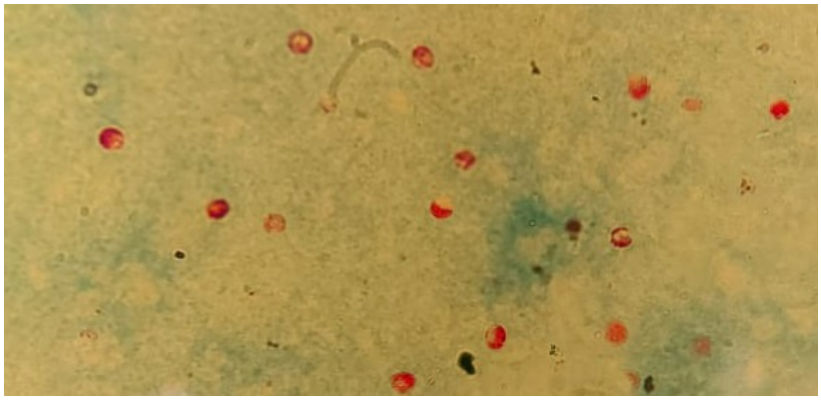

Figure 1. *Cryptosporidium* Oocytes Isolated and Stained With the Modified Ziehl-Neelsen Method.

of medicine and veterinary. The results of these studies have shown that the use of microscopic methods for *Cryptosporidium* oocyst search in feces is an accessible, easy, and reliable method due to the simplicity of the method and no need for expensive materials and equipment (7,8).

Canine intestinal protozoa are among the zoonotic parasites that are problematic for humans, and determining the level of infection, especially common protozoa such as *Cryptosporidium*, is important to minimize the risk of transmission to humans(9,10). Therefore, in line with the importance of common parasites, this study was conducted to identify the epidemiological dimensions of the parasite and to make us more aware of the status of infection of stray dogs in intestinal protozoa in Tabriz.

In this study, the intestinal protozoan in question was *Cryptosporidium* parvum, which is pathogenic to both dogs and humans (11,12). According to the results of this study in Tabriz, 8% of the tested stool samples were infected with *Cryptosporidium*.

In a study conducted by Fouladi in Kerman in 2012 on 100 domestic dogs, in general, the rate of infection with 16% of internal parasites was detected, with the highest infection being related to *Toxocara canis*, and from parasitic protozoa, *Cryptosporidium* was reported in three cases and *Isospora canis* in one case (4). The amount of *Cryptosporidium* contamination in the Fouladi's study was less than the contamination reported in the present study in Tabriz, and probably the reason for this is the difference in climate between the two regions.

In another study conducted on stray dogs by Doosti and Mirzaei in Kerman, of 98 tested dogs, 4 were infected with *Cryptosporidium* and 7 with *Giardia,* and this rate is relatively consistent with the present study conducted in Tabriz (3).

In another study, the prevalence of infection in stray dogs in Ilam province was reported to be about 7.14%, which also has a relative agreement with the present study (8%). In another study, Mosallanejad et al reported that the prevalence of *Cryptosporidium* in urban and rural dogs was 4.3%, with 2.17% and 6.4% in urban dogs and rural dogs, respectively. In Hamedan, the frequency of infection with this parasite in domestic dogs has been reported to be 3.8% (7,13). In a review of similar studies in other countries, the prevalence of *Cryptosporidium* infection in pet dogs in Tasmania was reported to be 1.8% (6). Similarly, the prevalence of *Cryptosporidium* infection in pet dogs in Brazil has been reported to be 1.4% (14,16).

In 1989, Uga et al reported the prevalence of *Cryptosporidium* infection in pet dogs in Japan to be 1.4%. In a study in South Africa, the prevalence of dogs infected with *Cryptosporidium* was 44%, with 46.2% in stray dogs and 41.7% in pet dogs (17,18). Although there are differences between reports of infection in dogs in Iran, it should be noted that infection with different species of *Cryptosporidium* is relatively common in Iran (12,19).

The reason for these differences may be related to differences in accuracy in staining and observation of parasite oocysts in extensions, sampling method, and climatic and geographical differences. However, it should be kept in mind that the existence of pollution in different parts of Iran necessitates the need for further studies in this regard (19,20).

Based on the results of this study and previous studies conducted by Garedaghi et al, the high prevalence of protozoan infection in dogs in Tabriz is significant, revealing the need for more attention to be given to the prevention and treatment of protozoan diseases in dogs. Further, the control and management of stray dogs and guard dogs, which are sometimes in contact with stray dogs, is even more important, because the access of these infected dogs to running water used by humans and agricultural fields can cause contamination of water, food, and plants for human consumption and lead to intestinal protozoan diseases in humans.

Given the relatively high level of infection in younger dogs observed in this study, it seems that the reason for this is the weakness of the immune system in puppies, which leads to more infection or possibly affects the health and environmental conditions (17). Our results in this study are consistent with the results of research conducted by Causape et al and the study of Mirzai et al (2,13).

The prevalence of *Cryptosporidium* infection in stray dogs in Ilam was 66.6% in dogs under or equal to one

year and 21.87% in dogs over one year, which indicates a significant difference (8). However, in the study by Mirzaei et al, there was no significant difference between age and the rate of *Cryptosporidium* infection (13).

Further, in another study in Shahrekord, there was no statistically significant relationship between *Cryptosporidium* infection and age (9). However, in the present study in Tabriz, a statistically significant difference ($P < 0.05$) was observed in the age prevalence of *Cryptosporidium* (under one year and over one year old). Considering the results of this study and other studies, it is suggested:

1. Health advice is provided regularly to dog owners by veterinarians, and periodic anti-parasitic treatment of dogs should be implemented.
2. Cooked food and healthy water should be considered for feeding dogs.
3. In order to prevent human infection, hygiene should be observed in dealing with stray dogs and even non-stray dogs by children and dog owners.
4. Preventing stray dogs from contacting with domestic animals, ruminants, and agricultural farms.

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Authors' Contribution

All listed author(s) have made a substantial, direct, and intellectual contribution to the workand approved it for publication.

Competing Interests

The authors declare no conflict of interests in any capacity, including competing or financial.

Ethical Approval

All ethical principles are fully observed in this study.

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