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Seroepidemiological Survey of Important Parasitic Infections of Wild Carnivores

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ABSTRACT

In this study prevalence of parasites in 118 samples (62 foxes and 56 jackals) was investigated in Ilam province of Iran, in 2010-2013, situated in the western part of the national capital of Iran. The nematodes which were cleared in lactophenol, cestodes and acanthocephalans were stained using acetocarmine and haematoxylen, respectively for identification of the parasite species. Cryptosporidium spp. was detected by means of the modified Ziehl-Neelsen method. Also, 2– 4g of fecal material was suspended in 33% zinc sulfate (SG 1.18) for detection the parasite eggs, cysts, or oocysts present in samples. Evaluation of samples indicated that 100% foxes and 100% jackal were infected with at least one of the following parasites: T. canis., A. caninum., U. stenocephala., M. lineatus., D. caninum., M. hirudinaceus., D. immitis., T.hydatigena., E. granulosus., Cryptosporidium., Giardia., Isospora., Cyclospora., Ctenocephalides canis., Rhipicephalus spp., Haemaphysalis spp. or Ixodes ricinus. The most frequently detected helminthes was D. caninum (33.92%) followed by M. lineatus (30.35%) in jackals and M. lineatus (29.03%) and T. canis (27.41%) in foxes. Giardia spp and Isospora spp were the most prevalent protozoa parasite of jackals (7.14 and 7.14%) and foxes (11.29 and 9.67 %), respectively. The most frequently observed ectoparasites were Ctenocephalides canis (10.81%) in jackals and Rhipicephalus spp (12.9%) in foxes. The results of the present investigation have implications for the ongoing control of parasite infections in carnivores in Ilam province, located in Iran and Iraq border line.

Key words: Intestinal parasites, Helminth, Protozoa, Ectoparasite, Red fox, Jackal

Introduction

Endo-ectoparasites are important enteropathogens in carnivores. Many potentially zoonotic organisms of parasitic origin are associated with carnivores. Moreover, several canine gastrointestinal parasites are zoonotic and are considered important to public health, particularly ascarids (Toxocara spp), hookworms (Ancylostoma spp and Uncinaria spp), whipworms (Echinococcus spp and Dipylidium spp) and protozoan (Giardia and Cryptosporidium) are a common and important finding in carnivores presenting to veterinarians (Johnson et al., 1993 and Dubna et al., 2007 and Susan et al., 2009) These gastrointestinal parasites can cause occasional infections of humans and can even develop in to a disease, though not very frequently (Susan et al., 2009). In the life cycles of several helminth species, such as nematode like

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Toxocara canis or cestodes like Echinococcus spp. or Dipylidium caninum, humans are involved as paratenic hosts with all its disadvantages. Migrating A. caninum and T. canis larvae can cause severe damages while incysted in the spinal cord, brain or eye. Similarly, the growing Echinococcus cyst may be a reason for severe liver, brain, lung or bone damage, or even cause the death of a human host. D. caninum, which localizes in the small intestine, is a common parasite of carnivores also develops in humans after accidental consumption of the intermediate host, a flea. Some helminth eggs, e.g. T. canis, are environmentally resistant, and remain infective for a long time (Anderson, 1992). Infections with Endo-ectoparasites parasites may result in disease in carnivores such as vomiting, diarrhea, anemia, anorexia, dermatitis, and loss of condition (Anderson, 1992 and Susan et al., 2009) All these parasites have oral-fecal transmission cycle and a major component for the spread of these parasites is the shedding of oocysts or cysts into the environment (Claerebout et al., 2009) The transmission of zoonotic agents could be through indirect contact with animal secretions and excretion, infected water and food, and through direct with the animal (Bugg et al., 1999) Carnivores are associated with more zoonotic disease among which parasite in particular, helminthosis, can pose serious public health concerns worldwide (Katagiri et al., 2007 and Khante et al., 2009). In addition, some canine intestinal parasites are zoonotic. For example, hookworm larvae may penetrate the skin of a person inducing cutaneous larva migrans; in addition, Ancylostoma caninum has been reported to develop to the adult worm in the small intestine of people causing eosinophilic enteritis (Anderson, 1992 and Smith et al., 2003). Ziaie (Ziaie, 1996) reported 29 species of wild carnivores from Iran, which jackals and foxes are the two most abundant species with the ability to adopt a variety of habitats and human proximity. Zoonoses with wildlife reservoirs represent a major public health problem affecting all continents, therefore the importance and recognition of wildlife as a reservoir is increasing (Criado et al., 2000 and Eslami, 2005 and Eslami et al., 2009) A survey of red fox and jackal from Iran reported a high prevalence of gastrointestinal parasites 33.3% and 66.7% for red fox and jackal, respectively; Toxocara canis and Mesocestoides lineatus were dominant species (Eslami et al., 2009). Understanding the epidemiology of the different parasites infections in a specific carnivore's population is a useful tool for the veterinarian practitioner when he/she has to provide a clinical diagnostic. Do parasite of stray dogs and wild canids are also frequently implicated in public health problems?. Numerous studies have been conducted on the prevalence of the endoparasites of stray dogs and wild carnivores from areas all over the world including Iran. (Dalimi et al., 1992 and Deplazes et al., 1995). Results of these studies showed that the change of parasitic infection is moderate, depending on various factors (age, locality and origin of carnivores, etc.). Considering aspects related to public and animals' health, study of the prevalence of parasite infection in dogs should, therefore, be continuous task, with the most relevant aim being the establishment of control measures. The aim of the present study was to further investigate the intestinal parasites of the fox population of this region and to extend the analysis to several tissue parasites, particularly zoonotic parasites.

MATERIALS AND METHODS

Study Areas

Western Iran in general and Ilam province in particular, has unique geographical and climatic condition that supports a rich flora and fauna. Ilam province is located in western of Iran in the vicinity of Iraq country. The length of common Iran –Iraq borderline is 465 Km. Average annual precipitation of the area is less than 500 mm. The region is considered as one of the most important sheep raising areas in the country as well as one of the largest migratory sheep producer pathways. These carnivores may consume infected organs of slaughtered animals which sometimes are left behind around the non-standard abattoirs in small villages.

Samples collected

In this study prevalence of parasites in 62 foxes and 56 jackals was investigated in Ilam province of Iran, in 2010 - 2013, situated in the western part of the national capital of Iran.

Parasitological procedure

The large helminthes were collected from each part. Meanwhile the contents of each small intestine and its epithelial scraping were washed gently in a 100 mesh sieve and were investigated for parasite using a dissecting microscope. The nematodes were cleared in lactophenol and the cestodes and acanthocephalans were stained using acetocarmine and haematoxylen, respectively for identification of the species. Hearts after opening and other organs after slicing into pieces were examined macroscopically for parsitic infections. Morphological characteristics that described helminths and external parasites were used for identification of the parasites (Walker, 1994 and Yamaguti, 1961). The faecal samples were stored in plastic tubes at 4 0C for a week prior to analysis. Cryptosporidium spp. was detected by means of the modified Ziehl-Neelsen method. Faecal smears of the specimens were taken and stained by the modified Ziehl-Neelsen technique (Causape et al., 1996) Also, 2– 4 g of fecal material (2 g minimum sample size) is suspended in 33% zinc sulfate (SG 1.18), strained to remove debris, and placed in a15 mL centrifuge tube. The samples are centrifuged at 500 – 650 g rpm for 5 min to concentrate any parasite eggs, cysts, or oocysts present in the uppermost layer, removed from the centrifuge and placed in a rack, and then filled the rest of the way with 33% zinc sulfate solution to form a reverse meniscus on top and a cover slip added to the top. The tubes are left undisturbed for at least 3 additional minutes to allow the eggs to continue to rise and then the cover slip is removed and placed on a glass slide and examined by microscopy. Any parasite stages noted are identified morphologically (Mundim et al., 2007). For detecting or collecting ectoparasite the bodies of animals were combed and their skin rubbed with a piece of cotton sucked in ether to remove the ectoparasites. In the case of collecting mites we had search does part of the fox and jackle body how suffering from hair loss, alopecia or swelling with the help of scalpel contaminated with oil or glycerin, the ectoparasites were separated and baggies in 75 % ethanol.

RESULTS

The present study investigated prevalence of intestinal parasites infections in red foxes and jackals from Ilam province, Iran, from 2011 – 2012. Evaluation of samples indicated that 100% Foxes and 100% Jackal were infected with at least one of the following parasites: Toxocara canis., Ancylostoma caninum., Uncinaria stenocephala., Mesocestoides lineatus., Dipylidium caninum., Macrocanthorynchus hirudinaceus., Dirofilaria immitis., Taenia hydatigena., Echinococcus granulosus., Cryptosporidium., Giardia., Isospora., Cyclospora., Ctenocephalides canis., Rhipicephalus spp., Haemaphysalis spp., Ixodes ricinus, Otodectes cynotis (Table 1). The most frequently observed helminthes was D. caninum (33.92%) followed by M. lineatus (30.35%) in jackals and M. lineatus (29.03%) and T. canis (27.41%) in foxes (Table 1). The data presented in (Table 2) indicated that Giardia spp and Isospora spp were the most prevalent protozoa parasite of jackals (7.14 and 7.14%) and foxes (11.29 and 9.67 %), respectively. The most frequently observed ectoparasites were Ctenocephalides canis (10.81%) in jackals and 12.9% in foxes (Table 1). Agents with zoonotic potential were detected in 89.6% of samples which collected, including D. caninum., T. canis., U. stenocephala., A. caninum., E. granulosus., Mesocestoides lineatus., Macrocanthorynchus hirudinaceus., Giardia spp and Cryptosporidium spp.

Infection with cestodes alone was very common, accounting for 30.64% of all red foxes and 39.28% of all jackals examined (Table 2). As for nematodes, concurrent infections with cestodes were more common than single infections. The overall prevalence with cestodes, nematodes, acanthocephalans, protozoan and ectoparasites was 66.1%, 28.81%, 4.23%, 20.33% and 18.64% respectively (Table 3).

DISCUSSION

The present study shows that endoparasites and ectoparasites are very common in both red fox and jackal in region. According to the studies conducted in different countries worldwide, the estimate prevalence of dog's parasites has wide vaiation.(Gorski et al., 2006 and Massoud et al., 1981 and Mehrabani et al., 1999). Some factors such as geographical location, status of animal ownership, sampling protocols, demographic factors, anthelmintic usage, and diagnostic techniques are responsible for the wide range of parasites prevalence Several of the intestinal helminthic parasites of carnivores recorded here can cause significant human disease. Some of the important parasites reported in these animals are T. canis, A. caninum, U. stenocephala, T. multiceps, E. granulosus, D. caninum, M. lineatus and M. hirudinaceus. However, human infections with larval stage of T. multiceps as well as adult form of D. caninum, M. lineatus and M. hirudinaceus have never been reported in Iran (Dalimi et al., 2006) The fox and jackal represent a potential source of infection for humans, because they can acquire the tapeworm by feeding on rodents in the grassland surrounding cities and defecate within human settlements. Cestodes are highly frequent in carnivores from our study (66.1%). Most of the foxes in jackals harboured M. lineatus and D. caninum. Frequencies of other cestodes (E. granulosus, Taenia hydatigena) were always low.These are in agreement with those obtained by (Chautan et al., 2000). Echinococcus multilocularis is a small tapeworm of foxes, particularly of the red fox and more important and could have direct effect on the sanitation in the region. Cystic echinococcosis is considered endemic in the entire Mediterranean zone including all countries from the Middle East Considering the Iran as one of the endemic area of echinococcosis, three distinct cycles of E. granulosus have been suggested there: a domestic cycle between dogs and livestock, a desert cycle between dogs and camels and a sylvatic cycle between wild carnivores and wild ruminants. The overall prevalence of E. granulosus in red fox and jackal (6.45 and 3.57%) was higher than that reported by (Dalimi et al., 2006) but was lower with E. multilocularis infections of 16.8% in the city of Stuttgart, 47.3% in Zurich, 43.1% in Geneva and 1% in the area around Copenhagen (Deplazes et al., 2006 and Kapel et al., 2000) The reported infection rate in carnivore's population varied from 1 to 63.5% in East Africa, South Africa, South America, East Europe and parts of China (Macpherson et al., 1997 and Rausch, 1995) T. canis is probably the most common gastrointestinal helminth of wild carnivores worldwide. In this study, the overall frequently of T. canis obtained here is comparable to those obtained in previous. The rate of T. canis infection was relatively high in the present study. These results are in agreement with those reported (Eslami et al., 1998 and Papadopoulos et al., 1997 and Willingham et al., 1996) . T. canis should be considered the most prevalent ascarid in red fox. In different parts of the world, the reported infection rate in domestic dog populations vary from 3.5 (adults) to 79% (pups) and in foxes 70 % and up to 80% (Horn et al., 1990 and Overgaauw et al., 2000)

D. caninum, which may also infect humans, was the most predominant cestode species in jackal in this study (33.92%). Such a lower infection rate is consistent with previous observe made (Smith et al., 2003) in Great Britain and (Dalimiin et al., 2006) Iran. Ancylostoma spp has been referred to as one of the most frequent intestinal parasites of carnivores. Besides A. caninum which is one of the most pathogenic species for red foxes and jackals, larvae and adults of different Ancylostoma species are involved in human infections. Cutaneous larva migrants or creeping eruption (Vellho et al., 2003) is the most common of them.

M. lineatus has a wide distribution in Asia, Europe and Africa. The present study, jackals (30.35%) were the most infected animals with M. lineatus compared to golden red foxes (29.03%). The high infection rate of M. lineatus in carnivores can explain mites are probably the infective intermediate host, and incidental intake of infected mites has resulted in a few human cases too. (Lloyd, 1998)reported that adult worm have been recorded in humans in Japan, China and Korea. In this study, U. stenocephala and T.

hydatigena were the most prevalent. In contrast, (Ajlouni et al., 1984) reported infection rates of 46% for T. hydatigena in Jordan, but (Smith et al., 2003) reported infection rates of 41.3% for U. stenocephala Great Britain. Giardia spp was the most frequent protozoan found in foxes 11.29% and jackals 7.14%. In Australia, Giardia spp, is the most frequently intestinal parasite in carnivores. This high prevalence was attributed to the fact that Giardia spp can colonize niche previously occupied by parasites such as T. canis and D caninum, and most of the anthelmintics do not interfere in the development of Giardia spp (Bugg et al., 1999)With respect to Cryptosporidium spp., the infection rate of 3.22% in red foxes and 0.0% in jackals. Epidemiological studies on the prevalence of Cryptosporidium in other carnivores (such as dogs) showed the infection rates are variable according to geographic area and range from 1.4% in Czech 2.41 % in Brazil (Huber et al., 2005), 1.4 % Uberlandia (Mundim et al., 2007), 1.4% in Japan (Uga et al., 1989), 2% in California (El et al., 1991), 10% in france (Chermette et al., 1989) between 0.7% and 19.6% in Australia (Johnson et al., 1993) and 20% in Chilia (Araya et al., 1987) The finding a source of oocyst could explain differences in prevalence between different areas. Other researchers suggested that prevalence may be highest in dogs from rural environments, since Cryptosporidiosis is primarily associated with farm livestock (Causape et al.,1996). Another most common parasite found in the evaluated red foxes was Isospora spp. 9.67% and 7.14% in jackals, which shows that these coccidia are the main intestinal protozoa found in these carnivores, mostly in younger animals, as indicated (Ramirez et al.,2004).The present study showed that fleas were the most abundant ectoparasites. This is one species of fleas have been recognized including Centenocephalides canis, that is in agreement with study on other carnivores, Iran (Eslami et al., 2009) This flea have been found to be the predominant species parasitizing carnivores in several studies conducted in the United Kingdom (Shaw et al., 2004) and Greece (Koutinas et al., 1995). The C. canis dominant fleas reported in these studies are consistent with those who already reported in Ireland (Wall et al., 1997) and New Zealand. According to our study after fleas, tick R. sanguineus dominant in red foxs and Ixodes ricinu in jackals were second predominant ectoparasite infestations. Similar results have been found in Lima/Peru with greatest prevalence of fleas (89 %), followed by ticks (30 %) and in Pretoria/ South Africa with R. sanguineus dominant ectoparasites on dogs (Estares et al., 1999 and Horak, 1982). The infestation appears to be associated with geographical region or could be due to interrelation of rural carnivore being used as red fox and jackal with sheep and goats in this area Ilam province. Our results show that the fox in Ilam providence is a host to a wide range of parasites. Most of them are known to be present in dog, and for this reason, the possibility of foxes serving as a reservoir for all the referred parasites might represent a significant risk for dogs. In addition, the environmental contamination with ascarid eggs constitutes a threat for humans. It is concluded that a consistent programmed of sanitary education must be included public health government actions as a first step for the control of intestinal parasites in carnivores. Finally, veterinary school should emphasize the client education in training veterinarians as a means to prevent or minimize zoonotic disease transmissions.

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Table1: Prevalence of endo-ectoparasites in red fox and Jackal

Helminthe	Percentage of infection	
	Fox (n=62)	Jackal (n=56)
Dipylidium caninum	14.51% (9)	33.92% (19)
Mesocestoides lineatus	29.03% (18)	30.35%(17)
Echinococcus granulosus	6.45% (4)	3.57% (2)
Toxocara canis	27.41% (17)	12.5% (7)
Ancylostoma caninum	0.0	3.57% (2)
Taenia hydatigena	8.06% (5)	7.14% (4)
Uncinaria stenocephala	3.2% (2)	0.0
Dirofilaria immitis	8.06% (5)	1.78% (1)
Macrocanthorynchus hirudinaceus	4.83% (3)	3.57% (2)
Protozoa		
Cryptosporidium	3.22% (2)	0.0
Giardia	11.29% (7)	7.14% (4)
Isospora	9.67% (6)	7.14% (4)
Cyclospora	1.6% (1)	0.0
Ectoparasite		
Ctenocephalides canis	12.9% (8)	10.81% (6)
Rhipicephalus spp	6.45% (4)	1.78% (1)
Haemaphysalis spp	0.0	1.78% (1)
Ixodes ricinus	0.0	3.57% (2)
Otodectes cynotis	4.83% (3)	1.78% (1)

Table2: Single and concurrent infection of red fox and Jackal parasites

parasite	Percentage of infection

	Fox (n=62)	Jackal (n=56)
Cestoda alone	30.64% (19)	39.28% (22)
Cestoda and Nematoda	12.9% (8)	12.5% (7)
Cestoda and Protozoa	9.67% (6)	7.14% (4)
Cestoda and Ectoparasite	-	12.5% (7)
Cestoda and Acanthocephalan	4.83% (3)	3.57% (2)
Total	58.06% (36)	75% (42)

Table3: The overall prevalence with parasites in red fox and jackal

Parasite	Percentage of infection		Total
	Fox (n=62)	Jackal (n=56)	(n=118)
Nematoda	38.7%	17.85%	28.81%
Cestoda	58.06%	75%	66.1%
Termatoda	0.0	0.0	0.0
Protozoa	25.8%	14.28%	20.33%
Ectoparasite	19.35%	17.85%	18.64%
Acanthocephalan	4.83%	3.57%	4.23%

Fig. 1: Map of study setting in bordering regions between Iran and Iraq