The effects of Benzimidazoles on the larval stage of Toxocara cati in experimentally infected chickens

Oryan1, A., Sadjjadi, S.M.2 and Azizi, S.1
1 Department of Veterinary Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. P. O. Box 71345-1731.
2 Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
E-mail address: oryan@shirazu.ac.ir
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Abstract. Toxocara cati (T. cati) and Toxocara canis (T. canis), roundworms of cats and dogs, are zoonotic parasites that cause visceral and ocular larval migrans in human beings. Humans and other paratenic hosts are infected by ingesting the infective Toxocara eggs from contaminated soil, unwashed hands, contaminated raw vegetables or ingestion of under-cooked organs and muscle tissues of infected paratenic hosts such as chickens, cattle and sheep. It has been shown that the seroprevalence of toxocariasis in the rural and urban children of southern Iran is high and more than 50% of cats of this area are also infected with T. cati. It is stated that consumption of raw chicken meat resulted in visceral toxocariasis. It is possible that poultry reared outdoors and feeding in open range system, gain Toxocara eggs from soil and or by eating infected earthworms as paratenic host. The aim of the present study was to investigate the effects of albendazole and febendazole in experimentally infected chickens with eggs of T. cati by histopathological and digestive methods. Pathologic lesions were observed only in the untreated group and larvae were detected in brain of 3 chickens of this group by squash method. No larva was observed at histopathological level in liver, lungs, brain, cardiac and skeletal muscles and other examined organs of either treated or untreated animals. No lesion was seen in other tissues of the infected untreated chickens. Treatment resulted in disappearance of the larvae and disappearance of the gross and histopathologic abnormalities from their organs. No detectable difference was observed in chemosusceptibility of the two drugs.

INTRODUCTION

Toxocara cati and Toxocara canis are cosmopolitan zoonotic nematodes of the family Ascaridae, whose adult forms inhabit the proximal small intestine of their mammalian definitive hosts of canides and felids (Sadjjadi et al., 2001; Mehrabani et al., 2002). They have a wide range of paratenic hosts, including birds, rodents, human beings and other mammals. Human beings are infected by ingesting the infective Toxocara eggs from contaminated soil, unwashed hands, contaminated raw vegetables and fruits (Glickman & Schantz, 1981; Glickman & Shofer, 1987), or ingestion of under-cooked organs and muscle tissues of infected paratenic hosts such as chicken, cattle and sheep (Ito et al., 1986; Nagakura et al., 1989; Salem & Schantz, 1992; Aragane et al., 1999). While the prevalence rate of infection of dogs of Shiraz area, southern Iran, with T. canis was reported to be 1.95% (Mehrabani et al., 2002), the prevalence of T. cati in stray cats of this area was estimated to be 52.8% and it seems that T. cati plays a more important role in infecting human beings than T. canis in this area. Seroprevalence rates of toxocariasis in urban and rural children of southern Iran are reported to be 30.15%
and 20.2%, respectively (Sadjjadi et al., 2000). Gethings et al. (1987) described prevalence of *T. cati* as high as 63% in cats of Bristol area in United Kingdom and similarly Dubinský et al. (1995) found that the prevalence rate of *T. cati* in cats to be about 60-70% in cities, in the rural and mountainous areas of Slovakia. Compared to *T. cati* most of the previous experimental studies were conducted on the *T. canis* migration pattern. However, the presence of *T. cati* larvae in the tissues of mice, sheep and chicken, have been reported earlier (Mossalam et al., 1971; Taira et al., 2003, 2004; Azizi et al., 2007). There is only little information about the migration pattern and the consequences of this infection for the paratenic host when compared to *T. canis* larval migration. Those very rare studies conducted previously using chickens as experimental models showed that chicken can be a paratenic host for *T. cati* since its larval forms was found in various organs of these hosts and consumption of infected meat of such hosts presumably spreads the disease among human beings as paratenic hosts and cats as final hosts (Taira et al., 2004; Azizi et al., 2007). It is possible that poultries reared outside and feed in free system, gain *T. cati* eggs from soil and, or by eating infected earthworms (Pahari & Sasmal, 1991).

To date, there is no experimental evidence from pharmacological studies in chickens and human beings about susceptibility of *T. cati* larvae to benzimidazole carbamates as the most effective anti-toxocaral drug. However, among benzimidazole carbamates, the effects of albendazole (ABZ), fenbendazole (FBZ) and mebendazole (MBZ) on *T. canis* larval recovery are the most extensively studied (Nicholas & Stewart 1979; Abo-Shehada & Herbert, 1984). The references concerning the successful medication of human visceral larval migrans (VLM) are contradictory. In order to decrease clinical symptoms in human toxocariasis, corticosteroids are generally used. Majority of the experiences concerning the effectiveness of various anthelminthics are published following the treatment of experimental animals, in particular mice infected with *T. canis*, shortly after infection (acute phase). Those very few studies that are conducted to clarify the chemosusceptibility of *Toxocara* larvae in chronic phase of infection in paratenic host are performed on *T. canis* (Fok & Kassai 1998) and there are no investigations showing the effect of benzimidazoles in the chronic stage of *T. cati* infection. There is also no report as yet to show the effect of benzimidazoles on *T. cati* larvae either in acute or chronic phase in chicken as a paratenic host. Chemosusceptibility of *Toxocara* larvae may be different during their parasitism of the paratenic host. The present experiment was designed to get further information on the histopathological changes and larval distribution pattern of *T. cati* and to investigate the effects of benzimidazoles on the chronic stage of infection in chickens.

**MATERIALS AND METHODS**

**Recovery of embryonated eggs from infected cats**

Stray cats were captured with a cage from different areas of Shiraz city in Fars Province, southern Iran and were autopsied after they were euthanized by standard methods of Iranian Veterinary Organization Rule at the autopsy room of the Pathology Department of Shiraz Veterinary School for collecting *T. cati* from their small intestines. Eggs of *T. cati* were collected by dissecting the adult female worms. The eggs were then transferred into 2% neutral formalin saline and kept at 27°C for 2-4 months until they were embryonated (Zibaei et al., 2007).

**Inoculation of *T. cati* eggs to chickens**

The *T. cati* eggs were washed three times with normal saline before inoculation. 0.5 ml normal saline was added to each 1000 embryonated eggs. Before inoculation of the embryonated eggs, the chickens were checked to be free of any parasitic or
microbial infections. Thirty, four day old chicks were randomly divided to 3 equal groups (A, B, C), each group having 10 chickens, with each chick infected orally with 1000 embryonated eggs of *T. cati*. Another 5 chickens of the control group (Group D) received no egg and were left untreated during the course of experiment. Subsequently, 123 days after eggs inoculation, treatment was started and continued for 30 days before the chickens were sacrificed. Groups A and B were treated daily for 30 days with albendazole and fenbendazole respectively at 1.6 g kg\(^{-1}\) and 6 g kg\(^{-1}\) food. Group C were inoculated only with embryonated eggs and received no treatment. The chickens of group D (n=5) did not receive *T. cati* eggs or treatment.

Each chicken of the experimental and control groups was housed individually in a clean cage with nipple drinker throughout the experiment. The chickens were free of any clinical symptoms during the course of this study. Each chicken of the experimental group was inoculated, using plastic pipette with 1000 embryonated *T. cati* eggs. After inoculation, all experimental and control chickens were kept under standard temperature, food and water. At the end of the experiment the chickens were euthanized humanely 3 days after finishing treatment by standard methods of Iranian Veterinary Organization Rule.

**Gross pathology and histopathology**

The lungs, liver, brain, kidneys, spleen, heart and skeletal muscles from the infected and control groups were grossly inspected. Then, 1x1x1 cm samples of these organs were fixed in 10% neutral buffered formalin, dehydrated with graded ethanol and embedded in paraffin. Tissue sections of 5 µm in thickness were stained with haematoxylin and eosin and studied with a light microscope.

**Digestive method for recovery of *T. cati* larvae**

The liver, lungs, kidneys, spleen, striated muscles of limbs, chest and intercostal areas of infected and non-infected chickens were finely minced; then were added to a solution containing 0.5% pepsin (1:10000), 0.7% HCl (37%) in distilled water and were incubated at 37°C for 18 hrs with constant stirring (Bardón *et al*., 1994). After incubation, the digests were filtered through a system of sieves with numbers 70, 200, 400 and 500. The sedimental liquids then were centrifuged for 2 min at 1,500 rpm. The sediments were then collected, transferred to a Petri dish, and viewed under a stereoscopic microscope (Wang *et al*., 1983; Taira *et al*., 2004). Squash method was used for brain using fresh unstained samples.

**RESULTS**

**Larval recovery results**

Larvae were recovered only from the brain of three of the untreated chickens (group C) by squash method. No larvae were recovered from other organs of the treated chickens (Groups A, B), untreated (Group C) and uninfected–untreated (Group D) chickens.

**Pathologic findings**

Remarkable pathologic lesions were observed in the parenchyma of liver and lungs of the untreated chickens. At macroscopic level the liver showed fine white multifocal nodules and hemorrhagic tracts at the surface and depth of the parenchyma (Fig. 1). At histopathologic level, large necrotic areas and hemorrhagic foci (Figs. 2 and 3) that in some cases were surrounded by fibrous connective tissue were evident in the liver parenchyma. Other lesions in the liver included chronic portal hepatitis with infiltration of lymphocytes, plasma cells and a few eosinophils. Many lymphocytes and plasma cells and fewer eosinophils were also infiltrated around vessels and produced conspicuous perivascularitis.

In the lungs, lesions were observed mostly at histopathologic level. Chronic peribronchiolitis with infiltration of lymphocytes and hyperplasia of
bronchiolar associated lymphatic tissues (BALT) and goblet cells were evident in the lungs of the infected untreated chickens. Alveolitis with diffuse infiltration of eosinophils were also seen in these chickens. Interstitial pneumonia with infiltration of lymphocytes, plasma cells and a few eosinophils in the lungs.

Figure 1. Hemorrhages and necrosis of the liver parenchyma in an untreated infected chicken with 1000 *T. cati* eggs.

Figure 2. Liver parenchyma of an untreated chicken received 3000 embryonated eggs. The parenchyma of the liver is destructed due to recent larval migration. Severe haemorrhages and necrosis is seen in this section (H and E, x85).
Figure 3. Severe linear hemorrhages and necrosis in the parenchyma of an untreated chicken infected with 1000 embryonated eggs of *T. cati* (H and E, x85).

Figure 4. Chronic myocarditis in an untreated chicken infected with 1000 embryonated eggs of *T. cati*. The inflammatory cells infiltrated between muscle fibers are mostly lymphocytes and plasma cells with few macrophages and eosinophils (H and E, x85).

Parenchyma was another criterion in most infected untreated chickens. Mild hemorrhages and infiltration of lymphocytes and a few eosinophils were observed in the meninges especially over the cerebellum of the infected untreated animals. Mild hemorrhages were also seen around some of the vessels in the cerebral
parenchyma of most of the infected chickens of this group. Mild focal infiltration of lymphocytes was present between cardiac muscle fibers of the infected untreated chickens (Fig. 4). No larva was observed at histopathological level in liver, lungs, brain, cardiac and skeletal muscles and other examined organs of either treated or untreated animals. No lesion was seen in other tissues of the infected untreated chickens.

Two chickens of Group A and one chicken of Group B showed a small increase in the fibrous connective tissue around the sinusoids and periportal areas of the livers. There was mild interstitial pneumonia with increase of collagen fibers in the septum of the alveoli of one chicken of each treated group (Groups A and B), but no other gross or histopathologic lesion was present in these animals. No abnormality was observed at gross and histopathological level in the liver, lungs, kidneys, heart, brain and other examined tissues of the uninfected-untreated control animals (Group D).

DISCUSSION

The embryonated eggs of Toxocara are infective for different paratenic hosts including invertebrate, rodents, birds and human beings. It is showed that in the paratenic host the larvae of Toxocara will not develop but migrate through the viscera and survive for a long time (Tsvetaeva et al., 1979; Maruyama et al., 1994; Azizi et al., 2007). Human infection can occur as a result of ingesting embryonated eggs from the environment such as contaminated vegetables and fruits (Dubinský et al., 1995; Magnaval et al., 2001; Talvik et al., 2006). The possibility of ingestion of larvae within small paratenic hosts, such as beetles, or in uncooked meat, cannot be excluded (Fisher, 2003). Toxocariasis causes two important syndromes of Visceral larval migrance (VLM) and Ocular larval migrance (OLM) in human.

The diagnosis of the early stage of toxocariasis in infected human beings is not feasible and majority of the cases are recognized when patients show clinical symptoms during the chronic stage of toxocariasis. However in most of the experimentally infected animals, the treatment is conducted during the acute phase and there are very few reports of application of chemotherapy in the chronic infected animals. The second problem is that majority of studies is conducted with T. canis infection and mice have been used as an experimental model for examining the migratory larval behaviour.

In the present examination the effects of albendazole and fenbendazole on the larval stages of T. cati in the chicken in chronic phase of infection is investigated. This is the first experimental study with T. cati in chicken as a paratenic host for evaluating the effect of benzimidazoles on larval recovery and histopathologic changes.

Hrčkova et al (2001), studied pathomorphological changes in mice infected with T. cati following administration of fenbendazole and glucan. They showed that treatment resulted in the reduction of larvae with the highest effect after co-administration of fenbendazole and liposomized glucan. They also studied the effects of liposomized albendazole and fenbendazole co-administered with liposomized immunomodulator glucan in mice infected with T. canis eggs and showed that the highest efficacy of both drugs was recorded after their subcutaneous administration. In their study, fenbendazole was more effective in the brain at 28 dpi. Abo-Shehada & Herbert (1984), studied the anthelmintic effects of levamisole, ivermectin, albendazole on larval Toxocara canis infection in mice 2 to 7 dpi. The result of their anthelmintic treatment was retention of larvae in the liver, and fewer larval migration to the brain and musculature of treated mice compared to untreated controls. Most of the larvae in the liver died there and were not recovered by 35 dpi. Fok & Kassai (1998), studied multidose treatments of
fenbendazole (FBZ), albendazole (ABZ), flubendazole (FUBZ), oxibendazole (OBZ) and ivermectin at days 2, 24, 81, 87 and 123 days post infections of mice with *T. canis*. They observed reduction of 84.2 to 99.7, or 88.8 to 100% of group mean larval counts after 20-30 day courses of feeding pellets containing FBZ at 6 g/kg⁻¹ or ABZ at 1.6 g/kg⁻¹ food, respectively. Ivermectin at various dosing regiments showed only moderate larvicidal potential. Their results indicated no detectable difference in chemosusceptibility of the migrating early infection larvae and the resting hypobiotic chronic infection larvae. They suggested that dormant larvae also remain susceptible to anthelmintics during their period of quiescence.

In our study, the most prominent pathologic lesions due to migration of larvae were observed in liver and to a lesser extent in lungs and brain. It is reported that in chickens the predilection site of *T. canis* larvae is the liver, where the larvae may survive for up to 3.5 years (Galvin, 1964; Tsvetaeva *et al*., 1979; Agnihotri *et al*., 1987; Maruyama *et al*., 1994). Perivascular infiltration of lymphocytes and eosinophils in liver may be related to migration of larvae via blood circulation. Hemorrhages in the liver after 8 months suggest that larvae may return back to this organ via circulation system. Similar to these results, it is previously reported that the percentage of total larval recoveries are widely diverse among various organs of chickens, irrespective of the date post infection or egg dose levels (Taira *et al*., 2003). Taira *et al*., (2003) infected chicken with *T. canis* eggs and described that larvae may migrate back to the liver by 6 dpi. This phenomenon could be true in chronic toxocariasis of the paratenic hosts too. It is showed that the dormant larvae may become reactive years later due to immune suppression (Fok, 2002). Pathologic findings in lungs were similar to a study that was described by Pinelli *et al*., (2001). It is stated that because of their mobility and the brief persistence in tissues (Buiks *et al*., 1994) or migration of larvae in formalin fixed tissues (Prociv, 1989) the larvae are rarely found at histopathologic level (Fenoy *et al*., 2001). Previous studies with *T. canis* reported presence of a few larvae in the brain and other organs such as spleen, kidneys, heart and muscles on 6 dpi and afterwards (Galvin, 1964; Okoshi & Usui, 1968; Maruyama *et al*., 1994; Gargili *et al*., 1999). Skerrett & Holland (1997) showed that the larvae of *T. canis* migrated through different organs of the infected mice and then made their way to the brain where their numbers increased up over time. Bardon *et al*., (1994) studied the larval distribution of *T. canis* in BALB/c mice one year post-inoculation and demonstrated that skeletal muscles and the brain were the principal sites of larval localization. Hrčková *et al*., (2001) showed that migratory pattern of *T. cati* larvae in mice is different from that of *T. canis* and larvae accumulated in the muscles after first month post infection. Schön & Stoye (1986) found the larvae of the *T. cati* in musculature and brain of the experimentally infected mice.

There are very few reports on the distribution of *T. canis* larvae in different organs of birds (Agnihotri *et al*., 1987; Gargili *et al*., 1999; Taira *et al*., 2003) but there are only three reports showing the experimental infection and tissue distribution of *T. cati* in chickens (Okoshi & Usui, 1968; Mossalam *et al*., 1971; Azizi *et al*., 2007). Gargili *et al*., (1999) infected chicken with *T. canis* eggs and no larvae was detected in skeletal muscles between 2nd and 12th dpi but Maruyama *et al*., (1994) suggested that the striated muscles were the 2nd organ for selection and larval accumulation.

Oral administration of drugs was used, as benzimidazoles are commonly administered in this way. Use of these drugs in dry feed increases their bioavailability (Mc Kellar *et al*., 1990). The anthelmintic activity of benzimidazoles appears to be the result of the length of time during which the parasite is exposed to the drug rather than the peak concentration to which the parasite is exposed. Results of this study showed that
the larvae can be killed in the brain of infected chickens even at chronic phase. Lack of active inflammatory response in the liver, lungs and brain of the treated chicken indicate that possibly these drugs caused elimination of dead larvae without initiating much inflammatory responses. It can be concluded that administration of benizimidazoles to experimentally infected chickens was highly effective in arresting the progression of larvae to the neurotropic-myotropic phase of infection.

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REFERENCES


