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Eimeria species in wild rabbits (*Oryctolagus cuniculus*) in Fars province, Iran

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Abstract. This investigation was accomplished during February to November 2008. A total of 71 wild rabbits (*Oryctolagus cuniculus*) of about 5 to 12 months age were collected alive from different parts of Fars province, south of Iran. Faecal sampling was carried out directly from recti and the oocysts were isolated using sedimentation and floatation techniques and the sporulated oocyst were identified based on morphological and biological characteristics. All the rabbits were apparently healthy and showed no clinical symptoms. Twenty two rabbits (31.0%) were positive for infection with *Eimeria* and six species including *Eimeria perforans* (18.3%), *Eimeria magna* (16.9%), *Eimeria media* (14.1), *Eimeria irresidua* (11.2%), *Eimeria flavescens* (4.2%), and *Eimeria coecicola* (2.8%) were identified. Eighty six percent of the infected rabbits showed mixed infections with two or three *Eimeria* species. Lack of clinical signs could be due to the agro ecological and environmental conditions of rabbit habitats specifically dry and hot climatic features in recent years. In addition, immunity induced by long term exposure to low doses of oocysts shedded by the carrier animals probably have pivotal role in impairing parasitic developmental cycles and preventing acute coccidiosis.

INTRODUCTION

Coccidioses of rabbits are ubiquitous infections caused by obligatory intracellular protozoan parasites belonging to the genus *Eimeria* and considered as major causes of significant morbidity and mortality (Lebas *et al*., 1986; Wang & Tsai, 1991; Coudert *et al*., 1995; Bhat *et al*., 1996). Except *Eimeria stidae* that invades the hepatobiliary epithelial cells and is the causative agent of hepatic lesions, fourteen other species of *Eimeria* have been found to develop in the intestinal tract of rabbit (Bhat *et al*., 1996; Taylor *et al*., 2007). Mixed infections are common and generally more than one species of *Eimeria* often parasitize the intestinal epithelium of rabbits (Toula & Ramadan, 1998). The consequences of these conditions could result in overwhelming diseases that may coincide with economic losses in rabbit rearing industries. As a result, regional identification of different *Eimeria* species may enhance control and preventive strategies.

To date, very few parasitological and epidemiological studies have been conducted to identify different *Eimeria* species in wild rabbits (*Oryctolagus cuniculus*). Thus, the purpose of the present study is to investigate the prevalence of different *Eimeria* species in the southern region of Iran. Undoubtedly, the results will increase existing knowledge on the host-parasite relationship and help future studies on the life cycle and detailed structural analysis of the changes evinced by the pathogenicity of *Eimeria* species in rabbit.

MATERIALS AND METHODS

Animals

A total of 71 adult (approximately 5-12 months old) wild rabbits (*O. cuniculus*), were collected alive from crop (particularly alfalfa) fields, in different parts of Fars province, south of Iran during February to November 2008. The average annual relative temperature of Fars Province, southern Iran was 22.2°C, its average annual relative humidity was 38.2%, and annual rain fall was 185 mm (Iran Meteorological Organization. www.irimo.ir). The animals had not been treated by any anti parasitic or anti coccidian drugs (such as coccidiostats) prior to sampling.

Faecal examination

Faecal samples were collected directly from the recti and transferred into parasitology laboratory. A suspension of each sample (3 gr per 30 ml water) was strained through a sieve and resulted filtrate subjected to the centrifugal sedimentation (Bhat & Jithendran, 1995). To isolate the oocysts of *Eimeria*, the sediments were examined by centrifugal flotation with saturated sugar solution (Ming-Hsien & Hong-Kean, 2008). The collected oocysts were transferred into 2.5% aqueous potassium dichromate solution (w/v) and incubated at 25-28°C for 168 hours to allow the oocysts to sporulate and examined periodically to determine mean sporulation time. The morphological features of sporulated oocysts including shape, shape index, size, inner and outer wall, micropyle and residium were measured and the identity of the parasites was determined by the keys previously described by Pellerdy (1974), Catchpole & Norton (1979) and Levine (1985).

RESULTS AND DISCUSSION

The morphological characteristics of sporulated oocysts isolated from a total of 71 collected faecal samples are presented in Table 1.

The present study revealed that 22 rabbits (31.0%) including 8 males (36.3% of infected animals) and 14 females (63.7% of infected animals) were infected with *Eimeria* oocysts. Six species of *Eimeria* including *Eimeria perforans*, *Eimeria magna*, *Eimeria media*, *Eimeria irresidua*, *Eimeria flavescens*, and *Eimeria coecicola* (Fig. 1) were isolated from the examined rabbits. The prevalence rate of different *Eimeria* species is showed in Table 2. *Eimeria perforans*, *E. magna* and *E. media* generally showed the higher prevalence rates, respectively, whereas *E. irresidua* had less prevalence and *E. flavescens* and *E. coecicola* showed relatively low percentage of infection. Furthermore, mixed infection with two or three species was the most frequent criteria and occurred in 86% of the infected samples.

Eimerial infections have a worldwide distribution and are prevalent in a wide range of animals (Levine, 1988). Coccidiosis in rabbits is highly contagious sporozoal infection that occurs by oral ingestion of oocysts along with infected food and water (Pellardy, 1974; Bhat *et al*., 1996). Fifteen specific rabbit-infecting *Eimeria* species have been demonstrated as yet (Bhat *et al*., 1996; Taylor *et al*., 2007) that are usually found in different anatomical regions of the intestinal epithelium of rabbit (Ming-Hsien & Hong-Kean, 2009). Concurrent or mixed infections by several *Eimeria* species often occur in rabbit intestine and is regarded as a usual finding (Levine, 1985). Present study showed a high percentage (86%) of mixed infection that is in accordance with those of the previous studies (Catchpole & Norton, 1979; Levine, 1985).

There are very few published data on the prevalence rate of *Eimeria* infection in wild or domestic rabbits. The present study demonstrated a significantly lower prevalence rate of infection (31%) in south of Iran compared to the results of similar studies in India, France and New Zealand in which a higher prevalence rate (more than 50%) were reported (Bhat *et al*., 1996;

Table 1. Morphologic characteristics of six *Eimeria* species identified in wild rabbits (*Oryctolagus cuniculus*) from south of Iran

oocyst							
Shape	Size (µm)	Shape index	Wall color	Micropyle	Residuum (µm)	Sporozoite size (µm)	Mean sporulation time (h)
Ovoid	14.4 – 28.8 x 12 – 21.6 (21.3 x 14.1)	1.21 – 1.79 (1.56)	Colorless to pink	–	3.6 – 4.8 (4.2)	4.8 – 9.6 x 3.8 – 6 (8.5 x 4.7)	36
Ovoid	28.8 – 38.4 x 16.8 – 28.8 (35.1 x 23.9)	1.34 – 1.52 (1.46)	Yellowish brown	+	9.6 – 14.4 (11.6)	12 – 16.8 x 6 – 9.6 (15.3 x 8.6)	52
Ovoid to ellipsoidal	19.2 – 36 x 14.4 – 21.6 (29.7 x 18.3)	1.45 – 1.71 (1.63)	Light pink	+	4.8 – 7.2 (6.2)	9.5 – 16.8 x 4.8 – 7.2 (13.1 x 6.9)	36
Ovoid	26.4 – 36 x 14.4 – 22.8 (31.6 x 18.9)	1.46 – 1.92 (1.53)	Yellow	+	–	12.8 – 16.8 x 7.2 – 10.8 (15.1 x 9.3)	48
Ellipsoidal	34.8 – 40.8 x 19.2 – 26.4 (37.2 x 23.8)	1.42 – 1.93 (1.54)	Yellowish brown	+	–	14.4 – 21.6 x 7.3 – 10.6 (17.9 x 8.7)	56
Ellipsoidal (cylindrical) or ovoid	25 – 38.4 x 15.6 – 21.6 (34.1 x 18.7)	1.46 – 2.12 (1.77)	Yellowish brown	+	3.6 – 6 (5.3)	15.6 – 16.2 x 2.8 – 8.2 (16.3 x 6.8)	62

Table 2. Prevalence rates of six *Eimeria* species (%) identified in faecal samples of infected wild rabbits (*Oryctolagus cuniculus*) from south of Iran

Eimeria species	No. of infected animals	Prevalence (% of total samples)
E. perforans	13	18.3
E. magna	12	16.9
E. media	10	14.1
E. flavescens	8	11.2
E. irresidua	3	4.2
E. coecicola	2	2.8

Gurpata & Khahra,1997; Gres *et al.*, 2003; Yakhchali & Tehrani, 2007). Thus, it can be stated that some limiting factors are responsible for the lower prevalence rate of coccidiosis among the rabbits of this country compared to those of other countries. This difference is likely attributed to the variations in agro-ecology, meteorology, and environmental conditions prevailing in each region. Harcourt-Brown

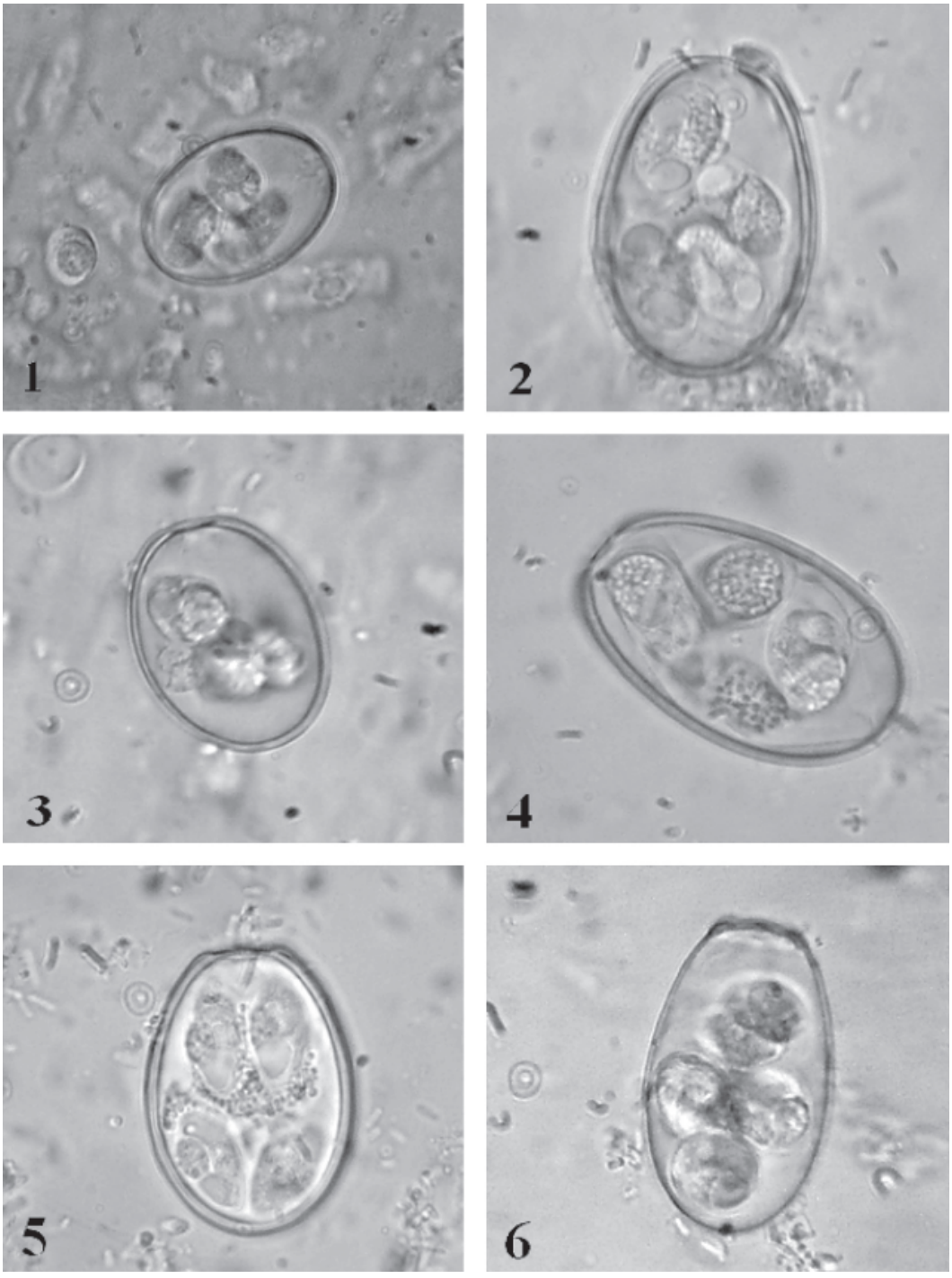

Figure 1. Sporulated oocysts of *Eimeria* isolated from infected samples of wild rabbits (*Oryctolagus cuniculus*) from south of Iran. (**1**) *E. perforans*, (**2**) *E. magna*, (**3**) *E. media*, (**4**) *E. irresidua*, (**5**) *E. flavescens*, and (**6**) *E. coecicola* (**x** 1500 except for No 2, **x**1000)

(2004) stated that oocysts can survive for a long time in the humid environment but they are susceptible to dry conditions. The average annual relative temperature of Fars Province, southern Iran is 22.2ºC, its average annual relative humidity is 38.2%, and annual rain fall is 185 mm (Iran Meteorological Organization. www.irimo.ir). These dry and warm meteorological conditions are not suitable for supporting the *Eimeria* life cycle in wild rabbits of this area. Similarly, a lower prevalence of coccidiosis in dry conditions has been reported for ruminant coccidiosis (Regassa *et al*., 2006). Therefore, it can be speculated that the dry conditions of the study area (drought), particularly in recent years with a low rain fall, is an important factor for not only reducing the infection rate but also preventing acute coccidiosis in wild rabbits. However, cecotrophy or coprophagia, that is a peculiar habit of rabbits to gain access to vitamins and proteins synthesized in the large intestine, could keep infection for a longer time in the flock and could promote spread of coccidiosis toward the population and it is considered a possible important route in the establishment of cocciosis in healthy rabbit from the infected ones (Pellerdy, 1974; Harcourt-Brown, 2004). In addition, the adult infected rabbits, which are usually symptomless, act as potential carriers within the free environment and transmit a continuous low-grade dose of oocysts to other rabbits particularly the younger ones (Wang & Tsai, 1991; Bhat *et al*., 1996). The low dose of contaminated oocysts similar to an attenuated organism in the vaccines activates the immune system of the newly infected animals and consequently they become protected by this acquired immunity. Thus, it seems rabbit acquires a durable and effective mucosal rather than systemic immunity to intestinal coccidiosis that protect animal to new infections prior to a severe infection (Pellerdy, 1974; Bhat *et al*., 1996).

From different *Eimeria* species present in the rabbits of the present study, some species including *E. flavescens* and *E. intestinalis* are regarded as highly pathogenic organisms and may have lead to haemorrhagic enteritis in rabbits (Catchpole & Norton, 1979; Bhat *et al*., 1996; Ming-Hsien & Hong-Kean, 2009). The results of the present study showed that *E. stiedae*, the causative agent of hepatic coccidiosis and one of the most important and well-known species of *Eimeria* in rabbits (Wang & Tsai, 1991; Bhat *et al*., 1996; Al-Rukibat *et al*., 2001; Gres *et al*., 2003; Al- Mathal, 2008) was not found in this area.

Although the rabbits in the present study had no clinical manifestations, presence of highly pathogenic species indicates that any weather alterations such as sudden heavy rain fall or occurrence of immune deficient diseases may act as risk factors for establishing rabbit coccidiosis in this area. Furthermore, any national program for saving the generation of these animals and keeping them under intensive rearing system needs more attention to hygienic measures for controlling rabbit coccidiosis.

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