Prevalence of subclinical coccidiosis in river buffalo calves of southwest of Iran

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Abstract

Despite the importance of buffalo farming in Iran, little is known in this country about the abundance and distribution of *Eimeria* spp. in the animal species. The present study was designed to investigate the prevalence and species characterization of *Eimeria* oocysts in river buffalo calves of Khuzestan province, southwest of Iran. Of the total 108 fecal samples examined for *Eimeria*, 108 (100%) were found infected with 11 species of the parasite. Among the identified species of *Eimeria*, *E. bovis* was found to be the predominant etiological agent (76.85%), followed in order by *E. canadensis* (62.96%), *E. zuernii* (47.2%), *E. ellipsoidalis* (26.85%), *E. subspherica* (25.92%), *E. brasiliensis* (19.4%), *E. auburnensis* (18.51%), *E. alabamensis* (14.81%), *E. pellita* (11.1%), *E. illinoisensis* (5.5%) and *E. bukidnonensis* (2.7%). In most calves multiple infections with three species were present. While, 20.7% of calves showed heavy infection, 50.4 and 24.8% of calves showed weak and moderate infection, respectively. There was no significant difference in the OPG values between the calves of different localities. There was also no significant difference between the prevalence rate of infection in males and females. A total of 16.6% of all faecal samples were found to be diarrheic. A highly significant relationship could be identified between the occurrence of diarrhea and the level of *E. bovis* and *E. zuernii* oocysts excretion. Considering the pervasive occurrence and negative effects of the infection on the health condition and the growth performance of buffalo calves, infections should receive increased attention by both farmers and veterinarians.

Keywords

Coccidiosis, *Eimeria*, oocyst, Buffalo calves

Introduction

Eimeria spp. is a protozoan genus that causes the world wide distributed parasitic disease known as coccidiosis. Infection by this protozoan causes economic losses due to reduced weight gain and deaths of young animals. More than twelve different species of *Eimeria* in cattle and buffalo have been documented until now. Most species are considered to have a low pathogenicity, whereas infection with *E. bovis* or *E. zuernii* may cause severe disease in calves (Daugochies and Najdrowski 2005). High infection pressure, lack of preventive and therapeutic measures when clinical disease manifests, can lead to outbreaks and high mortality among calves (Fox 1985). Coccidiosis in calves commonly occurs as subclinical, without typical signs of the disease but may though causes great economic losses due to damage in intestine lining, resulting in malabsorption. Infected calves are also more susceptible to secondary diseases, such as pneumonia, bacterial enteritis and

viral infections (Fox 1985). Nevertheless, over-reliance on these drugs is an economic burden. Additionally parasite resistance may reduce our ability to successfully control this disease (Harper and Makatouni 2002). Recent studies have shown that *Eimeria* spp. are widespread in ruminants, but these parasites appear to be of low priority when questioning the farmers and observing statistics of veterinary diagnostics. This situation has triggered efforts towards the investigation on this parasite. According to the latest available statistics, there are about 459 thousand head of buffaloes in Iran which is ranked 16th among 43 countries in the world. There is a considerable population (>138,000 head) of river buffalo (*Bubalus bubalis*) in Khuzestan province southwest region of Iran which play a significant role in rural life by producing milk and meat while tolerating the impact of harsh environmental conditions (Taheri Dezfouli *et al.* 2011). In Iran, there is no published data available on *Eimeria* infection in river buffalo. Since, the highest prevalence of oocyst shedding and dis-

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ease incidence occurs in calves less than a year of age, the objective of this study was to obtain information about the prevalence of coccidiosis amongst the river buffalo calves in farms of the region and to determine which *Eimeria* spp. are involved in natural infections.

Materials and Methods

The study was conducted in Khuzestan, southwest province of Iran from December 2011 to July 2012. It has a border of about 64236 km², between 47 degree and 41 minutes to 50 degree and 39 min of eastern longitude from prime meridian and 29 degree and 58 min to 33 degree and 4 min of northern latitude from equator (Statistical book of Khuzestan province, 2006). The province has hot and wet summers, mild spring and cold winters. The buffalo population mainly comprises local domestic species, which are well adapted to the climate of the area.

A total of 108 fecal samples (46 females and 62 males) were collected from calves (birth to 6 months of age). Samples were taken from four geographical localities of Khuzestan province where the most population of buffalo herds are raised. Samples were taken from northeast of the province (26 samples), south of the province (30 samples), east of the province (24 samples) and southwest of the province (28 samples). The farms participating in this study had been selected randomly. Most of the buffalo herds in this province have small size (less than fifty animals). Up to 7 farms in each locality were visited once during the observation period. On average, 10% of the total numbers of animals in the farms were sampled. Therefore, on each farm, individual samples were collected from three to five randomly selected calves. The age of the animals was documented according to the owner records.

Fecal samples were collected directly from rectum or immediately after defecation in a wide-mouth plastic bottle. All the samples were classified on collection according to their consistency, as normal or diarrheic feces (liquid or semi-liquid feces). Until tested for *Eimeria* spp. oocysts in the laboratory, the fecal samples were stored under refrigerated conditions. Each fecal sample was examined for coccidian oocysts using modified McMaster technique with Sheather's sugar flotation solution and light microscope (Ernst and Benz 1981) and expressed as oocysts per gram of faeces (OPG). After counting the *Eimeria* oocysts in the feces of each animal, the numbers of OPG were graded as follows: 2,500 OPG (1+, weak); 2,500 to 5,000 OPG (2+, moderate); and >5,000 OPG (3+, heavy). To reduce variation, all counts and identification of oocysts were done by one individual throughout the study. Three g of the feces were mixed thoroughly with 50 ml potassium dichromate 2.5% (w/v) solution. The mixture was strained through thick gauze to remove coarse plant matter, poured in thin layers into Petri dishes and left to sporulate for 10–15 days at room temperature. After sporulation, the potassium dichro-

mate fecal solution was centrifuged in a test tube at 3,000 rpm for 5 min. The supernatant was decanted and sediment put into a faecalysers tube. A 40% sugar solution was poured in to the faecalysers tube until a meniscus formed. A coverslip was placed on the tube, then carefully removed and placed on a microscope slide. At least 30 sporulated oocysts from each sample were measured using a research microscope with 100 × magnification. Differentiation of *Eimeria* spp. oocysts was made by measurement and based on morphological criteria according to Levine (1985), Eckert *et al.* (1995) and Soulsby (1986). Results were analysed using the One-Way ANOVA and Independent-Samples T test (SPSS 16). The significance levels are expressed at a 95% confidence level ($p \leq 0.05$) throughout.

Results and Discussion

Coccidiosis is a serious economic problem in sub-clinically infected animals because they appear normal outwardly, but developmental stages damage the absorptive surface of the intestine and weaken the immune system, leading to reduced feed consumption, poor feed conversion, slow weight gain and increased susceptibility to other infections (Fitzgerald 1980, Dauschies and Najdrowski 2005). In Khuzestan province of Iran no attention has yet been paid to this parasitic infection because of the lack of detailed information on the presence of different *Eimeria* spp. of local buffalo herds. In the present study 100% of calves were infected with *Eimeria* oocysts. The high proportion of infected calves, supports our hypothesis that probably all farms in Khuzestan province are infected with these ubiquitous parasites. The susceptibility of hosts to eimerian parasites depends on their age, genetic predisposition, innate or adaptive immunity, stress level, handling, location of the parasite in the intestinal epithelium, number and location of endogenous stages, as well as climatic and other factors (Hayat *et al.* 1994). The hot and humid climate of Khuzestan province is advantageous to both the expansion of river buffalo herds and the high prevalence of parasites in these animals. These environmental conditions may have con-

Table I. Percentage of infection in faecal samples for each coccidia species for calves

<i>Eimeria</i> species	No. of infected calves (prevalence, %)
<i>E. bovis</i>	83 (76.85)
<i>E. canadensis</i>	68 (62.96)
<i>E. zuernii</i>	51 (47.2)
<i>E. ellipsoidalis</i>	29 (26.85)
<i>E. subspherica</i>	28 (25.92)
<i>E. brasiliensis</i>	21 (19.4)
<i>E. auburnensis</i>	20 (18.51)
<i>E. alabamensis</i>	16 (14.81)
<i>E. pellita</i>	12 (11.1)
<i>E. illinoisensis</i>	6 (5.5)
<i>E. bukidnonensis</i>	3 (2.7)

tributed optimal conditions for oocyst sporulation and thus, for buffalo infection. Other factors like poor nutrition, poor sanitation, and overcrowding increased the level of infection and incidence of the disease due to stress-induced immunosuppression. Some studies showed that a strong association was observed between housing system and risk of *Eimeria* infection (Rehman *et al.* 2011). In this study animals were reared in closed houses with the non-cemented floor type and they were fed on the ground. Feeding of calves on the ground increase the chances of contamination of the feed with *Eimeria* oocysts and non-cemented floor is difficult to clean. Abebe *et al.* (2008) and Rehman *et al.* (2011) believed that grounds fed animals are at higher risk to *Eimeria* infection than trough fed calves. In this study sex has no significant effect on the prevalence or intensity of infection ($p > 0.05$). These results were in accordance with findings of Ahmed and Soad (2007), but this data is in contrast with the results obtained by Rehman *et al.* (2011) and Priti *et al.* (2008). They showed that *Eimeria* infection was found to be more prevalent in female calves. Based on our results 11.1% of infected calves were infected with only one *Eimeria* spp. Mixed infections were generally present. In most calves (62.96%) multiple infections with three species were present. The species detected and their prevalence rates are given in Table I. *E. bovis* was found to be the most prevalent species. It occurred in 76.85% of the samples. *E. canadensis* was the second frequent species (62.96%), followed by *E. zuernii* (47.2%) and *E. ellipsoidalis* (26.85%).

It was found that most infections were weak (54.5%) whereas 24.8% and 20.7% of samples contained moderate and large numbers of oocysts, respectively. There was no significant difference in the OPG values between calves of different localities. Among the six identified species of *Eimeria* in Rehman *et al.* study (2011), *E. bovis* was found to be the highest prevalent species. After sporulation of positive samples in Koutny *et al.* (2012) experiment, 11 *Eimeria* species were found. Ten different species of *Eimeria* were identified from the fecal samples of local domestic beef cattle collected from the five localities of Afyon province of Turkey (Cicek *et al.* 2007). In Hungary *Eimeria* oocysts were found in 33% of calves and seven species were identified. Among the identified species of *Eimeria*, *E. auburnensis* was found to be the highest prevalent species (Farkas *et al.* 2007). In our study 16.6% of fecal samples were found to be diarrheic. A highly significant relationship could be identified between the occurrence of diarrhea and the level of oocyst excretion of *E. bovis* and *E. zuernii*. Two bovine species (*E. bovis* and *E. zuernii*) are known pathogens causing morbidity and mortality (Niilo 1970; Friend and Stockdale 1980). On the other hand, although *E. bovis* was the most prevalent species in calves, but clinical coccidiosis was not observed. Many authors believe that the presence of a pathogenic *Eimeria* species (*E. bovis* and *E. zuernii*) does not necessarily indicate clinical disease (Parker and Jones 1987, Waruiru *et al.* 2000). In this study, species of the animals were found to be harbouring pathogenic species of coccidia and yet no clinical symptoms were being

exhibited. This indicates that other factors, the number of oocysts ingested, the presence of a concurrent microbial infection (Hoblet *et al.* 1992), weather conditions including ambient temperatures and moisture (Munyua and Ngotho 1990), the production system and management practices used by the farmer and general conditions of animal husbandry (Niilo 1970), the functional level of protective immunity and enzootic stability (Parker and Jones 1987), may be decisive in whether clinical disease is precipitated or not. Most of animals examined during the present study had low OPG, suggesting that the infections were usually sub-clinical. However, the economic consequences of the sub-clinical infections are of importance. Most animals can act as asymptomatic carriers and shed large numbers of oocysts into the environment and remain a main source of infection.

Based on these preliminary results, it can be concluded that *Eimeria* spp. are widely distributed in Iranian river buffalo farms. To minimize the impact of coccidiosis, proper hygiene measures and ensuring unfavorable conditions for oocyst survival in the environment should be of utmost importance. If necessary and possible, application of anti-coccidial compound should be also considered.

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