Occurrence and diversity of *Eimeria* species in cattle in Hamedan province, Iran

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ABSTRACT: Information on the occurrence, diversity and economic losses due to *Eimeria* infection on cattle farms is lacking in Western Iran. The principal aim of this study was to determine the prevalence and diversity of *Eimeria* species in cattle in Hamedan province, Western Iran. In a cross-sectional study performed from June 2010 to April 2013, 400 faecal samples were collected randomly from animals without clinical signs from different cattle farms of Hamedan province and examined for *Eimeria* species infection using parasitology methods. The overall infection rate of *Eimeria* species was 8.25%. Nine species of *Eimeria* were identified including: *E. bovis* (23.7%), *E. zuernii* (19.2%), *E. canadensis* (12.6%), *E. ellipsodalis* (11.4%), *E. alabamensis* (10.4%), *E. pellita* (9.1%), *E. auburnensis* (6.8%), *E. cylindrica* (4.6%) and *E. bukidnonensis* (2.3%). There were statistically significant differences with respect to *Eimeria* infection and age group ($P < 0.0001$, OR = 6), although no statistically significant relationships were found for sex ($P = 0.35$) or faecal consistency ($P = 0.587$). In conclusion, this study is the first to report the prevalence and diversity of *Eimeria* species in cattle in Hamedan province. Infection with pathogenic *Eimeria* spp. was asymptomatic in all animals; this is the reason for the transmission of infection by carriers in this region. Therefore, integrated strategies should be utilised to prevent and control *Eimeria* spp. infection on cattle farms.

Keywords: *Eimeria* spp.; OPG; risk factors; faecal samples; calf age; Iran

Bovine coccidiosis is a protozoan disease caused by various species of *Eimeria* (Almeida et al. 2011). Coccidiosis is responsible for major economic losses in animal husbandry worldwide (Nisar-Khan et al. 2013). Adult animals are usually asymptomatic carriers that often serve as a source of infection for juvenile animals, which are more susceptible to infection (Faber et al. 2002; Abebe et al. 2008).

Coccidiosis is commonly a self-limiting disease; most signs of bovine coccidiosis are chronic or subclinical (Nalbantoglu et al. 2008). The clinical picture of coccidiosis depends on the innate pathogencity of different *Eimeria* species. More than 20 *Eimeria* spp. have been identified in cattle worldwide (Daugschies and Najdrowski 2005). *E. zuernii* and *E. bovis* are known to be highly pathogenic, causing morbidity and even mortality associated with diarrhoea, mucus and blood stains. The other species have been shown experimentally to be mildly or moderately pathogenic, but they are not considered important pathogens (Lucas et al. 2006). *Eimeria* spp. develop only in the intestinal epithelial cells, leading to mucosa damage and the appearance of clinical signs, malnutrition, weakness, anaemia, diarrhoea and haemorrhagic faeces (Yu et al. 2011; Nisar-Khan et al. 2013).

Diagnosis of coccidiosis depends on the discovery of oocysts on faecal examination using direct smear, flotation or McMaster's techniques. The number of oocysts per gram of faeces (OPG) is helpful in confirming coccidiosis as a cause of clinical disease (Almeida et al. 2011; Nisar-Khan et al. 2013).

Studies on bovine coccidiosis are limited to a few studies carried out in Iran (Yakhchali and Gholami 2008; Yakhchali and Zarei 2008; Davoudi et al. 2011). However, there are no published data on coccidiosis in cattle in the Hamedan province. The main aim of the current study was to determine the prevalence and diversity of *Eimeria* species in cattle in Hamedan province, Western Iran.

MATERIAL AND METHODS

Study area. Hamedan province, a mountainous region with a mild climate, is located in the western part of Iran (34.77 °N and 48.58 °E). It covers an area of 19 546 km^2 and average annual temperature is 11.3 °C. This province is economically important for crops and animal husbandry such as cattle.

Sample collection. In a cross-sectional study from June 2012 to April 2013, 400 faecal samples were collected randomly from different cattle farms (n = 17) in Hamedan province (Thrusfield 1997). None of the animals showed clinical signs of disease. Faecal samples were removed directly from the rectum of each animal using disposable examination gloves. The samples were collected into plastic bags, labelled and stored at 4 °C until processing. Information about age (≤ 1 and > 1 years old) and sex of the animals was obtained at physical examination (Table 1).

Parasitology examination. The presence of oocysts in faecal samples was examined with a flotation method using saturated sodium chloride solution (Yu et al. 2011). The modified McMaster technique was used to quantify the OPG. For sporulation, positive samples were placed in Petri dishes, conditioned with a solution of 2.5% potassium dichromate at room temperature and aired daily for up to two weeks.

The *Eimeria* species were determined based on the morphology of oocysts and sporocysts (shape, colour, form index, micropyle and its cap, presence or absence of residual, polar granule) and time of sporulation (Soulsby 1986; Eckert et al. 1995; Yu et al. 2011). For identification, at least 10 oocysts of each species were morphologically characterised (Almeida et al. 2011).

Statistical analysis. Statistical analysis was performed using the SPSS software package version 16.0 for Windows. The differences between infection rate and sex, age groups and faecal consistency were evaluated using the Chi-square test. A P-value ≤ 0.05 was considered statistically significant.

RESULTS

Oocysts of *Eimeria* spp. were found in 33 of 400 faecal samples (8.25%, CI 0.95: 6.35–10.15). Among cattle farms, 70.5% (12/17) were found positive. In descending order of discovery rate the *Eimeria* spp. were *E. bovis* (23.7%), followed by *E. zuernii* (19.2%), *E. canadensis* (12.6%), *E. ellipsodalis* (11.4%), *E. alabamensis* (10.4%), *E. pellita* (9.1%), *E. auburnensis* (6.8%), *E. cylindrica* (4.6%) and *E. bukidnonensis* (2.3%). Mixed infections with two or more *Eimeria* spp. were observed in all animals.

The following OPG counts were determined: 100 in 42.2% of samples, 200 (24.2%), 300 (9.1%), 1000 (9.1%), 5000 (6.1%), 400 (3%), 500 (3%) and 600 (3%).

The infection rate was recorded as 14.7% and 2.7% in ≤ 1 and > 1 year age groups, respectively, (χ^2 = 18.574, P< 0.0001, Odds ratio = 6). There was no significant correlation between infection rate and sex (Table 1; χ^2 = 0.097, P = 0.754) or between infection rate and faecal consistency (χ^2 = 0.293, P = 0.587).

DISCUSSION

Coccidiosis is a common problem in cattle worldwide (Davoudi et al. 2011). Studies have shown prevalences of *Eimeria* oocyst excretion of up to 8% to 100% at farms in general (Bangoura et al. 2012). Research performed in other countries has revealed prevalence rates of *Eimeria* spp. varying from 17.9% to 93% in Poland (Pilarczyk et al. 2000; Klockiewicz et al. 2007; Pilarczyk et al. 2009), 33% in Hungary (Farkas et al. 2007), 22.6% and 33.3% in Brazil (Almeida et al. 2011; Poscoti-Bruhn et al. 2011), 35% to 47.1% in China (Yu et al. 2011; Dong et al. 2012), 50% in Pakistan (Nisar-Khan et al. 2013), 22.7% and 68% in Ethiopia (Abebe et al. 2008; Dawid et al. 2012), 20%, 68% and 75% in Turkey (Arslan and Tuzer 1998; Cicek et al. 2007; Nalbantoglu et al. 2008), 29%, 50% and 52% in

Table 1. Prevalence of *Eimeria* species according to age group, sex and faecal consistency in cattle in Hamedan province, Iran

	Sex		Age group (year)		Faecal consistency		Total
	male	female	≤ 1	> 1	normal	soft	
Number of samples	131	269	184	216	380	20	400
Number of positive samples	10	23	27	6	32	1	33
(%)	(7.6)	(8.5)	(14.7)	(2.7)	(8.4)	(5)	(8.25)

South Africa (Matjila and Penzhorn 2002), and 35% in Tanzania (Chibunda et al. 1997). The rate was reported to be 21.3% and 18% to 50% in the Kurdistan and East-Azerbaijan provinces of Iran (Yakhchali and Gholami 2008; Yakhchali and Zarei 2008; Davoudi et al. 2011).

In our study, the infection rate (8.25%) was lower than that reported in other investigations; infection was asymptomatic in all animals similar to studies in Brazil, China and Turkey (Cicek et al. 2007; Almeida et al. 2011; Yu et al. 2011).

Many factors such as the number of ingested oocysts, the presence of a concurrent microbial infection, weather conditions (ambient temperatures and moisture), management in the farms and the functional level of protective immunity may be decisive in whether clinical disease occurs or not (Parker and Jones 1987; Warui et al. 2000). The prevalence of *Eimeria* spp. in healthy animals implies that they can serve as reservoirs of infection (Almeida et al. 2011).

During sampling, faecal consistency was normal to soft; there was no statistically significant relationship between increase in OPG and faecal consistency (Table 1, $P = 0.587$). In studies from Iran and Brazil, all faecal samples were normal to soft and animals were asymptomatic despite infection with pathogenic species (Yakhchali and Gholami 2008; Yakhchali and Zarei 2008; Almeida et al. 2011).

Age is one of the major risk factors in the spread of coccidiosis; morbidity and risk of infection are greater in calves (Abebe et al. 2008). In this study, there was a 6-fold increase in *Eimeria* spp. infection in ≤ 1 year animals (14.7%, Table 1) compared to >1 year old animals (2.7%); this is consistent with the data of other researchers reporting a strong correlation ($P < 0.05$) between age groups and infection (Chibunda et al. 1997; Abebe et al. 2008; Nalbantoglu et al. 2008; Almeida et al. 2011; Yu et al. 2011; Dawid et al. 2012; Dong et al. 2012; Nisar-Khan et al. 2013). All these results run counter to the study of Abebe et al. (2008) who reported that risk of infection by *Eimeria* species appeared to increase with the age of the examined calves.

Coccidiosis is a self-limiting disease and spontaneous recovery without specific treatment is common when the multiplication stage of the coccidia has passed. This could suggest that previous exposure might have contributed to the development of a certain level of immunity in older calves as compared to the younger ones that did not experience previous exposure (Dawid et al. 2012). Chibunda et al. (1997) and Faber et al. (2002) also pointed to the presence of an immature immune system in younger calves resulting in their higher susceptibility to coccidiosis. In contrast, older calves can develop immunity in response to previous exposure, and hence be more resistant to subsequent reinfections.

In this study, the infection rate was 7.6% in male and 8.5% in female calves (Table 1, $P = 0.035$). Similarly to this finding, Dawid et al. (2012) did not find a significant association with sex. The absence of a significant correlation between infection and animal sex might suggest that both male and female animals have an almost equal likelihood of being infected with coccidia. Yet, female calves harbour more coccidia than male calves; this could be attributed to the greater physiological stress experienced by female animals in relation to pregnancies and giving birth (Dawid et al. 2012). Research on the impact and role of different breeds and sex on infection prevalence is highly recommended. Further, elucidating the effects of interracial breeding and the selection of resistant breeds will be very helpful in reducing economic losses.

In our study, two pathogenic species of *Eimeria* spp. were identified: *E. bovis* (23.7%) and *E. zuernii* (19.2%), while seven non-pathogenic species were detected: *E. canadensis* (12.6%), *E. ellipsodalis* (11.4%), *E. alabamensis* (10.4%), *E. pellita* (9.1%), *E. auburnensis* (6.8%), *E. cylindrica* (4.6%) and *E. bukidnonensis* (2.3%). This is in agreement with data reported by other researchers (Ernst et al. 1984; Kasiman and Al-Shawa 1985; Cicek et al. 2007; Farkas at al. 2007; Almeida et al. 2011), and indicates that *Eimeria* spp. have a significant pathogenic potential in cattle in Hamedan province.

A number of authors have reported that *E. zuernii* and *E. bovis* were the most prevalent species in cattle, but clinical coccidiosis was not observed in those animals (Ernst et al. 1984; Kennedy and Kralka 1987; Cornelissen et al. 1995). This finding is in close agreement with the results of our study.

The OPG count in this study shows a medium variation. The numbers of oocysts found in calf faeces ranged between 100 and 5000. The maximum OPG levels observed thus far were 267 000 in Ethiopia (Abebe et al. 2008), 109 000 in Canada (Kennedy and Kralka 1987), 30 600 in Kenya (Munyua and Ngotho 1990), and 52 000 in Turkey (Arslan and Tuzer 1998). OPG values over 5000 may indicate a clinical case (Arslan and Tuzer 1998).

Different study designs and methods, hygiene conditions and management in farms, poor nutrition and sanitation, change of diet, breeding of animals, stress factors such as weaning, high animal density, climate and different geographical regions, may all contribute to the variation in results (Yu et al. 2011; Dawid et al. 2012).

In conclusion, this study is the first to report the prevalence and diversity of *Eimeria* species in cattle in Hamedan province, Western Iran. Infection with pathogenic *Eimeria* spp. was asymptomatic in all animals; this is the reason for the transmission of infection by carriers in this region. Therefore, integrated strategies should be put into practice to prevent and control *Eimeria* spp. infection on cattle farms.

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