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## Research Article

# Subclinical Bovine Coccidiosis in Al-Baha Area, Saudi Arabia

### Abstract

Subclinical bovine coccidiosis is an economic problem worldwide because they appear normal outwardly, but developmental stages damage the absorptive surface of the intestine and weaken the immune system. Information on the occurrence, diversity and economic losses due to *Eimeria* infection in cattle is lacking in Al-Baha area. The objective of this work was to study prevalence of subclinical bovine coccidia, throughout Al-Baha area and to evaluate various factors that might potentially influence prevalence and rate of pathogen excretion. A cross sectional study was conducted from July 2014 up to Jun 2015. Out of the total 534 fecal samples examined for *Eimeria*, 167 (31.27%) were found infected with eight species of *Eimeria*. *Eimeria bovis* was found to be the highest frequent species (25.84%), followed in order by *E. zuernii* (20.78%), *E. ellipsoidalis* (13.11%), *E. canadensis* (10.86%), *E. alabamensis* (8.05%), *E. auburnensis* (7.78%), *E. cylindrica* (5.43%), and *E. subspherica* (2.06%). Young cattle ( $\leq 12$  month old) had significantly higher infection ( $P < 0.01$ ) of *Eimeria*. Higher infection of *Eimeria* was observed in female cattle compared to male ones. Co-infection was commonly present and declined with host age. Positive pair-wise associations were found between *Eimeria* species. In conclusion, this study was confirm that farm management frequently does not meet the requirements of effective coccidian control and the pathogenic coccidian, *E. bovis* and *E. zuernii* are the highest prevalent species in Al-Baha cattle populations. Further epidemiological investigations are required to investigate the different agro-ecological risk factor on the occurrence of bovine coccidiosis.

## Introduction

Coccidiosis is a parasitic disease of the intestinal tract caused by *Eimeria* and is one of the most common and important disease of cattle worldwide. Bovine coccidiosis is considered to be of considerable importance for the productivity and health of cattle. Coccidiosis in cattle 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 [1]. Infected calves are also more susceptible to secondary diseases, such as pneumonia, bacterial enteritis and viral infections [1-3].

Many species of *Eimeria* have been described to infect cattle and causes of coccidiosis [4]. *Eimeria bovis* and *Eimeria zuernii* are known to be highly pathogenic eimerian species in cattle worldwide, causing morbidity and even mortality associated with diarrhea, mucus and blood stains [4,5]. While most species are considered harmless, bovine coccidiosis is often associated with diarrhoeal diseases which lead to reduced weight gain and economic losses [4].

Usually infections with more than one species of *Eimeria* are found [6,7]. The co-infection is common with *Eimeria* species in cattle and in wild bovids [4,7-10]. There is evidence for interactions between parasite species, highlighting the importance of understanding these relationships before devising control strategies against specific species in livestock systems [11,12]. In assessment of the dynamics of co-infections (either naturally, or following external perturbation), measures of abundance will be more sensitive than prevalence. Furthermore, abundance will often be more informative for studies of infra community dynamics [13].

More than twelve different species *Eimeria* species have been described in cattle (Bohrman, 1991; Dausgchies and Najdrowski, 2005) [14,3], of which seven have been detected in Saudi Arabia [15]. *E. zuernii* and *E. bovis* were the most frequently and were generally the most predominant species worldwide [4,5,16]. Studies on bovine coccidiosis in Saudi Arabia are limited to one study carried out by Kasim and Al-Shawa [15]. However, there are no published data on bovine coccidiosis in cattle in Al-Baha area. Therefore, the main objective of the current study was to investigate the prevalence and diversity of *Eimeria* species in cattle and factors influencing oocyst excretion which will help in the control of bovine coccidiosis in Al-Baha area.

## Materials and Methods

### The study area

A cross sectional study was conducted from July 2014 up to Jun 2015 in Al-Baha area, west-south Saudi Arabia (20° N, 41° - 42° E). The study area is mainly hilly, with small areas of either mountainous or flat land and extends from 500 to 2500 m a.s.l. The climate in Al-Baha has two extremes. Mild winters and hot summers, with an average annual rainfall between 100 and 250 mm, prevail in the lowlands; cold winters and mild summers, with an average annual rainfall between 229 and 581 mm, prevail in the highlands [17].

### Sample collection

A total of 534 fecal samples were collected randomly from animals without clinical signs from different cattle farms and examined for *Eimeria* infection. Five grams of fecal samples were collected directly from the rectum or immediately after defecation in a wide-mouth

plastic bottle. Fecal consistency was assessed on a scale from one to five (1: normal and 2: soft).

Examined animals was categorized into two age groups as group 1 = ≤ 12 month old and group 2 = > 12 month which was determined by asking the animal owner orally [8,18]. The structure of the sampled host population of cattle is shown in Table 1. Animals were also categorized into three groups according to their body condition: good, medium and poor. This was based on different body visible bone structure and fat deposit [8,19].

### Questionnaire

A questionnaire was developed for collecting necessary information from farmers regarding associated risk factors using closed ended (dichotomous and multiple choice) questions [20]. Each farm owner was questioned about the occurrence of diarrhea in cattle in relation to their age. Season wise prevalence was noted separately. The two well-marked seasons in Al-Baha are: (i) Dry season (September to January) (ii) wet season (February to July). Additionally, they were asked about previous fecal examinations and whether the detected pathogens were known. The owners were asked if the term “coccidia” was known. Previous cases of coccidiosis and management of anticoccidial treatment were recorded.

### Parasitological examination

The collected fecal samples were stored at 4°C and analyzed within 3 days using a modified McMaster technique with a sensitivity of 5 oocysts per gram. Quantitative fecal examination was performed by McMaster technique to determine the number of oocysts per gram of feces (OPG) according to the procedures of MAFF (1986). For sporulation, positive samples were placed in Petri dishes, contained a solution of 2.5% potassium dichromate at room temperature and aired daily for up to two weeks. The *Eimeria* species were identified 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 [21]. To ensure that species identification is valid, when possible, at least 50 sporulated oocysts from each species were observed and measured [22,23]. One hundred oocysts were randomly selected and identified to determine the percentages of each *Eimeria* species present in the fecal samples and then OPG per *Eimeria* species was counted Silva et al., 2011). An estimate of intensity of each *Eimeria* species was achieved by relating their proportions to the McMaster counts [12].

### Statistical analysis

Testing of effects of both individual and interacted factors (host age, sex, season and locality) on species richness of *Eimeria* species was statistically analyzed using the General Linear Interactive Model (GLIM) after normalization of the data by log<sub>10</sub>(x+1) transformation [24,25]. Statistical analysis was performed using the SPSS software package version 17.0 for Windows. The differences between infection rate and sex, age groups and fecal consistency were evaluated using the Chi-square test. A P-value ≤ 0.05 was considered statistically significant. Correlations between host age and both prevalence and OPG number were examined by using the non-parametric, Spearman’s rank correlation coefficients (rs). Diversity of *Eimeria* species was measured using the Shannon-Weiner diversity index.

## Results

Out of the total 534 fecal samples examined for *Eimeria*, 167 (31.27%) were found infected with eight species of *Eimeria*. *Eimeria* species composition consisted of *Eimeria bovis* (25.84%), *E. zuernii* (20.78%), *E. ellipsoidalis* (13.11%), *E. canadensis* (10.86%), *E. alabamensis* (8.05%), *E. auburnensis* (7.78%), *E. cylindrica* (5.43%) and *E. subspherica* (2.06%). *E. bovis* (25.84%) and *E. zuernii* (20.78%) were the most prevalent *Eimeria* species (Table 2). The overall mean abundance and intensity of *Eimeria* species are shown in Table 3. *E. bovis* was the most abundant species.

Diversity of *Eimeria* species was measured using the Shannon-Weiner diversity index. The overall diversity of *Eimeria* community was 3.01. Diversity in terms of prevalence significantly decreased with age ( $F_{1,532}=7.76, P<0.01$ ) and showed significant differences between season ( $F_{1,532}=8.86, P<0.01$ ). The overall mean species richness of *Eimeria* species harbored per host was  $1.89 \pm 0.07$  (S.E.M.). Analysis of these data with GLIM revealed that host age and season played a significant role in determining *Eimeria* species richness ( $F=15.99, P<0.001$  and  $F=13.90, P<0.001$  respectively). A significant negative correlation between host age and no. of *Eimeria* species was found ( $rs=0.29, P<0.01$ ).

**Table 1:** The structure of the sampled host population of cattle.

Factors considered		No. of fecal samples examined
Location	Al-Baha	286
	Baljurashi	248
Sex	Male	294
	Female	240
Age categories	≤12 month old	414
	>12-18 month old	120
Season	Dry season	280
	wet season	254
Total		534

**Table 2:** Prevalence (%) of *Eimeria* species in cattle.

<i>Eimeria</i> species	No. of infected/No. of examined	Prevalence (%)
<i>Eimeria. bovis</i>	138/534	25.84
<i>E. zuernii</i>	111/534	20.78
<i>E. ellipsoidalis</i>	70/534	13.11
<i>E. canadensis</i>	58/534	10.86
<i>E. alabamensis</i>	43/534	8.05
<i>E. auburnensis</i>	41/534	7.78
<i>E. cylindrica</i>	24/534	5.43
<i>E. subspherica</i>	11/534	2.06
Overall Prevalence	167/534	31.27

**Table 3:** Prevalence (%) of *Eimeria* species in cattle. Abundance (mean ± SE) and intensity (mean ± SE) of *Eimeria* species in cattle.

<i>Eimeria</i> species	Abundance ± SE	Intensity ± SE
<i>E. bovis</i>	280.23 ±89.34 (0-1050)	307.32 ± 27.93 (50-1050)
<i>E. zuernii</i>	158.7±25.36 (0-900)	223.32 ± 24.13(50-900)
<i>E. ellipsoidalis</i>	145.77±21.09 (0-600)	180.86± 37.64 (30-600)
<i>E. canadensis</i>	43.11±9.43 (0-300)	133.66±15.58 (40-300)
<i>E. alabamensis</i>	29.76±6.92 (0-300)	80.90± 12.56(20-300)
<i>E. auburnensis</i>	20.74±11.56(0-280)	94.62±11.58 (20-280)
<i>E. cylindrica</i>	18.12±7.6 (0-100)	40.86± 19.23(20-100)
<i>E. subspherica</i>	10.54±5.63 (0-100)	30.98±17.41 (20-100)

The overall prevalence of *Eimeria* species infection was 32.51% and 29.84% in Al-Baha and Baljurashi respectively. No statistically significant difference was found in prevalence ( $X^2=0.44$ ,  $df=1$ ,  $P=0.51$ ) and OPG ( $X^2=1.55$ ,  $df=1$ ,  $P=0.09$ ) between different localities of Al-Baha area. The highest prevalence (32.51%) and OPG (245.82±37.51) were observed in cattle in Al-Baha locality (Table 4).

The fecal consistency was normal to soft; there was no statistically significant relationship between increase in OPG and fecal consistency (Figure 1,  $P = 0.503$ ).

The overall prevalence of *Eimeria* oocysts in female was significantly higher (34.16%; 82/240,  $\chi^2=5.14$ ,  $p<0.01$ ) when compared to male (28.91%; 85/294). The OPG number was higher in female (126.94±20.63) when compared to male (112.53±18.73). No Statistically significant difference found in in OPG between male and female (Table 5).

The overall prevalence of *Eimeria* species in different age categories were 36.96% (153/414), and 11.66% (14/120), in ≤ 12-month old, and >12-month old respectively. The highest prevalence (36.96%) was found in young age category (≤12-month old). OPG number was higher (343.45±43.54) in ≤12-month when compared to >12-month age category (Table 6). Significant difference was found in prevalence ( $X^2=27.63$ ,  $df=1$ ,  $P<0.01$ ) and OPG number ( $X^2=31.24$ ,  $df=2$ ,  $P<0.001$ ) among different age categories (Table 6). The overall prevalence and OPG decreased significantly ( $r_s=-0.391$ ,  $P<0.001$ ), OPG load ( $r_s=-1.08$ ,  $P<0.001$ ) with increasing host age.

The infection prevalence of *Eimeria* species in cattle was significantly higher (37.01%; 94/254) in wet season than that in dry season (26.07%; 73/280) (Table 7;  $X^2=7.39$ ,  $df=1$ ,  $P<0.01$ ). OPG numbers were significantly higher (224.24±29.23) when compared to that in dry season ( $P<0.001$ ). The maximum numbers of oocysts in this study varied from 2000 in *E. bovis* to 300 in *E. subspherica*.

Multiple-species infections (simultaneous infections with multiple parasite species in one individual host) of more than one *Eimeria* species were commonly present in the two age categories and declined with increasing host age. Multiple-species infections were detected in 89.22% (149/167) of positive cattle carried two to five species, and 77.84% (130/167) of positive cattle had two to three species. Infections with 4 species were less common (7.78%; 13/167) (Table 8). Some *Eimeria* species consistently co-occurred. Three positive pair-wise associations were found. These were between (i) *E. bovis* and *E. zuernii* ( $r_s=-0.94$ ,  $P<0.001$ ); (ii) *E. bovis* and *E. ellipsoidalis* ( $r_s=-0.29$ ,  $P<0.001$ ) and (iii) *E. zuernii* and *E. ellipsoidalis* ( $r_s=-0.28$ ,  $P<0.001$ ). No negative associations were found.

## Discussion

Subclinical coccidiosis is a serious economic problem in 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 [2,3]. In Al-Baha no attention has yet been paid

**Table 4:** Prevalence (%) of *Eimeria* species in cattle. Abundance (mean ± SE) and intensity (mean ± SE) of *Eimeria* species in cattle. Prevalence and parasite abundance (mean ± SE) of *Eimeria* oocysts in cattle in different localities of Al-Baha area.

Locality	Examined number	Infected number	Prevalence (%)	Significance among location	Mean no. of oocysts per gram of faeces (range)
Al-Baha	286	93	32.51	$X^2=0.44$ , $df=1$ , $P=0.51$	245.82±37.51 (0-1600)
Baljurashi	248	74	29.84		220.56±25.58 (0-1450)

**Table 5:** Prevalence and abundance (mean ± SE) of *Eimeria* oocysts per host sex of cattle.

Host sex	Examined number	Infected number	Prevalence (%)	Significance between host sex prevalence	Mean no. of oocysts per gram of faeces (range)
Male	294	85	28.91	$X^2=5.14$ , $df=1$ , $P<0.01$	126.94±20.63 (0-1400)
Female	240	82	34.16		112.53±18.73 (0-1600)
Overall	534	167	31.27		210.22 ± 19.59 (0-1600)

**Table 6:** Prevalence and parasite load (mean ± SE) of *Eimeria* oocysts in different age categories of cattle.

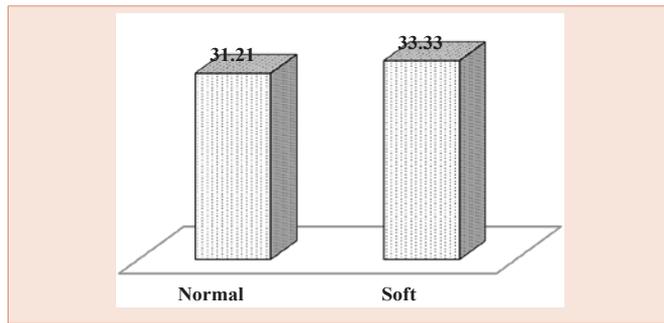
Age categories	Examined number	Infected number	Prevalence (%)	Significance among age categories prevalence	Mean no. of oocysts per gram of faeces (range)
6-12 months old	414	153	36.96	$X^2=27.63$ , $df=1$ , $P<0.001$	343.45±43.54 (0-1600)
≥12 months old	120	14	11.66		159.25±20.72 (0-500)
Overall	534	167	31.27		210.22 ± 18.52 (0-1600)

**Table 7:** Prevalence and parasite load of *Eimeria* oocysts in different seasons.

Season	Examined number	Infected number	Prevalence (%)	Significance among age categories prevalence	Mean no. of oocysts per gram of faeces (range)
Dry season	280	73	26.07	$X^2=7.39$ , $df=1$ , $P=0.007$	165.67±23.45 (0-600)
wet season	254	94	37.01		224.24±29.23 (0-1600)

**Table 8:** Percentage of zero, single and multiple-species infections of *Eimeria* species in cattle.

The number of <i>Eimeria</i> species	0	1	2	3	4	5
Infected cattle	0	18	48	82	13	6
Percentage relative to infected cattle (%)	0	10.77	28.74	49.10	7.78	3.59
Percentage relative to examined cattle (%)	68.73%	3.37%	9.99%	15.35%	2.43%	1.12%



**Figure 1:** Prevalence of *Eimeria* infection according to fecal consistency.

to *Eimeria* infection because of the lack of detailed information on the species composition and infection prevalence of *Eimeria* spp. which is important to implement effective control programs. In the present study, 31.27% of cattle were infected with *Eimeria* spp. The susceptibility of hosts to eimerian infection 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 [26]. The overall prevalence of *Eimeria* spp. (31.9%) is lower than previous findings reported in Saudi Arabia by Kasim & Al-Shawa [15], (34.1%), in the coastal plain area of Georgia (USA) by Ernst et al. [27], (82.28%), in sub-humid tropical climate by Rodriguez-Vivas et al. [28], (87.8%) and in Addis Ababa and Debre Zeit by Abebe et al. [18] (68.1%). This variation is most likely attributed to the differences in agro-ecology, and husbandry practices of the study animals in different countries [18].

Earlier, study already described the occurrence of seven *Eimeria* species in cattle in Saudi Arabia [15]. In the present study, the species composition of *Eimeria* consisted of eight species namely, *E. bovis*, *E. zuernii*, *E. ellipsoidalis*, *E. canadensis*, *E. alabamensis*, *E. auburnensis*, *E. cylindrica* and *E. subspherica*. The recorded species is similar to those recorded by Kasim and Al-Shawa (1984) except *E. wyomingensis* which were not observed in the present work. *E. alabamensis* and *E. canadensis* are the first finding in Saudi Arabia. Similarly, other authors reported the same finding in Saudi Arabia [15], Austria [7], Pakistan [29], and Iran [9].

The two pathogenic species of *Eimeria* spp. were identified in this study; *E. bovis* (25.84%) and *E. zuernii* (20.78%), while six non-pathogenic species were detected: *E. ellipsoidalis* (13.11%), *E. canadensis* (10.86%), *E. alabamensis* (8.05%), *E. auburnensis* (7.78%), *E. cylindrica* (5.43%) and *E. subspherica* (2.06%). This is in agreement with data reported by other researchers Ernst et al. 1984, Almeida et al. 2011, Heidari et al. [9]. 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 [30-32].

The fecal consistency in this study was normal to soft; there was no statistically significant relationship between increase in OPG and fecal consistency (Figure 1,  $P = 0.503$ ). In studies from Iran and Brazil, all fecal samples were normal to soft and animals were asymptomatic despite infection with pathogenic species [9]. In contrast, Bangoura et al. [4], demonstrated a positive correlation between oocyst excretion

and fecal consistency in cattle herds infected by *E. bovis* and *E. zuernii*. They also reported that the infection dose has a marked influence on the extent of clinical symptoms. The OPG levels were generally low on average. In many epidemiological studies about bovine coccidiosis clinical cases have been reported rarely or not at all [33,34]. It can be assumed that coccidiosis mostly occurs in a subclinical form. No statistically significant difference was found in prevalence and OPG load between localities in Al-Baha area. The highest prevalence (32.51%) and OPG ( $245.82 \pm 37.51$ ) were observed in Al-Baha locality. Kasim and Al-Shawa [15], found variation in *Eimeria* infection of sheep per study sites and correlate it to differences in rainfall and humidity. We attribute the differences in infection per location is probably due to difference in ecological parameters (such as rainfall, humidity and temperature).

The overall infection prevalence of *Eimeria* spp. of cattle in female (34.16%) was higher than that in male (28.91%). Similar findings were found by Priti et al, Rehman et al. [29], Pyziel et al. [35] and Heidari et al. [9], in India, Pakistan, Poland and Iran, respectively. Dawid et al. [36] did not find a significant association in relation to 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 cattle harbor more coccidia than male ones; this could be attributed to the greater physiological stress experienced by female animals in relation to pregnancies and giving birth [36].

The maximum numbers of oocysts in this study varied from 2000 in *E. bovis* to 300 in *E. subspherica*. The maximum OPG levels observed in other countries were 5000 in Iran [9], 67 000 in Ethiopia [18], 109 000 in Canada [30], 30 600 in Kenya [37], and 52 000 in Turkey [38]. OPG values over 5000 may indicate a clinical case [38].

Age is one of the risk factors in coccidiosis and risk of infections is greater in young animals [9,18,39]. All species of *Eimeria* were more prevalent and intense in younger cattle compared to older ones. There was a about 3-fold increase in overall *Eimeria* spp. infection in  $\leq 1$  year animals (36.96%, Table 6) compared to  $>1$  year old animals (11.66%). This general young bias in *Eimeria* infection is well documented in cattle throughout the world [9,18,36,39-41]. Acquired immunity has been shown to cause a decrease in infection of various *Eimeria* species with host age [40,42]. The higher infection rate that found in young cattle may be attributed to lower resistance or less immunity to *Eimeria* species in young animals compared to the older animals. Previous exposure might have contributed to the development of a certain level of immunity in older animals as compared to the younger ones that did not experience previous exposure [36]. Faber et al. [16], also pointed to the presence of an immature immune system in younger calves resulting in their higher susceptibility to coccidiosis. In contrast, older animals can develop immunity in response to previous exposure, and hence be more resistant to subsequent reinfections.

The prevalence and oocyst counts of *Eimeria* species in wet season (37.01%; 94/254) were significantly higher than that in dry season (26.07%; 73/280). Similarly, Rehman et al. [29], Koutny et al. [7] and Ibrahim and Afsa [39], found higher *Eimeria* oocyst counts during wet season. El-Bahy et al. [43], reported that *Eimeria* spp are

abundant during April to June and their incubation period is about 1-2 week, so new infection could be occur in the same period as end of March till end of June. On the contrast, other researchers observed that there was no significant difference in OPG during seasons [44].

In this study demonstrated that mixed infections with two to five *Eimeria* species were more commonly observed. This finding is consistent with the findings of other researchers [7,18,39]. Three positive pair-wise associations were found between *Eimeria* species. This finding is consistent with the finding of Craig et al. [12], explained the positive pair-wise correlations between *Eimeria* species for one of two reasons. The first one is the overdispersion of parasites in natural populations, that is most individuals harbour few parasites and a few harbour many. They relate the aggregation may be due to variation in the exposure or susceptibility to infection within the host population. The positive pair-wise interactions that found in this study may be result from synergistic effect of concurrent parasite infection. This study was confirmed that the pathogenic coccidian, *E. bovis* and *E. zuernii* are the highest prevalent species in Al-Baha cattle populations. Further epidemiological investigations are required to investigate the different agro-ecological risk factor on the occurrence of bovine coccidiosis.

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