The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(1): 175-179, 2025 www.thebioscan.com

THE PREVALENCE OF EIMERIA AND CRYPTOSPORIDIUM IN CATTLE IN AL-DIWANIYAH AND AL-NAJAF PROVINCES

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DOI: https://doi.org/10.63001/tbs.2025.v20.i01.pp175-179

KEYWORDS
intestinal protozoa, Eimeria, Cryptosporidium, cattle.

Received on:

12-11-2024

Accepted on:

10-12-2024

Published on:

24-01-2025

ABSTRACT

This study is to isolate and diagnose some intestinal protozoa, namely *Eimeria* and *Cryptosporidium*, that cause diarrhea in cattle. To this end, the study investigates the frequency of infection in different districts and localities in Al-Diwaniyah and Al-Najaf Provinces. Also, the study examines whether age and sex are effective factors in the prevalence of that disease in animals. The results of the laboratory and microscopical examination of feces revealed that while 41 (out of 160) animals were infected with *Eimeria* spp., that is 25.62 %, these animals tested negative for *Cryptosporidium* spp. Additionally, the infection was higher in both the young animals (47.36 %) and in cows (26.82 %). The highest rates of infection with *Eimeria* spp. have been detected in Al-Diwaniyah province (33.33 %) and the lowest rates have been detected in the zoological field of the Faculty of Veterinary Medicine, University of Kufa (14.28 %). In terms of age, statistically significant differences ($p< 0.05$) in infection frequencies have been found. Non-significant differences in infection frequencies have been found between sex on the one hand and the geographical region on the other hand ($p>0.05$).

Conclusions: The infections with coccidia were found in all the studied regions. As for age, they were common in young and older animals but most frequent in young animals. Also, both male and female animals were vulnerable to infection with coccidia.

INTRODUCTION

Cattle herds predominantly host the unicellular parasites Eimeria and Cryptosporidium, which can cause health problems, especially in young animals (Lassen et al., 2009). Eimeriosis is linked to the presence of bloody diarrhea and occasionally watery diarrhea (Engidaw et al., 2015). Coccidiosis is a prevalent global illness that affects cattle in general, with a specific impact on calves. Coccidiosis is primarily a gastrointestinal disease that affects young calves, typically those under six months of age. It is particularly severe in animals who have been weaned at an early stage. The infection rate in this particular age cohort of animals ranges from 0% to 100% during the period from birth to two months of age (Bangoura and Daugschies, 2019). Bovine coccidiosis results in significant economic losses in the livestock industry. Hence, extensive research has been conducted to examine the dissemination, life cycle, and disease-causing potential of Eimeria species worldwide (Ashfaq et al., 2023). Eimeria is a type of coccidian protozoa that belongs to the phylum Apicomplexa and the family Eimeriidae. It infects ruminants, poultry, horses, and rabbits. These parasites have a global distribution and primarily impact dairy cows that are younger than one year old (Bruhn et al., 2011). Eimeriosis is a significant contributor to financial losses in the global breeding of ruminant animals. Subclinical coccidiosis in cattle results in significant economic losses, amounting to millions of dollars yearly in most countries (Rashid et al., 2019). Europe, including the UK and Wales, has had a significant prevalence of coccidia in cattle, with high infection rates being a typical occurrence. For instance, the presence of coccidiosis, specifically E. zuernii and E. bovis, was detected in 63.2% of the facilities that were examined (Stewart et al., 2008). In Switzerland, the presence of E. bovis was detected in 36% of calves under 30 months old, while E. zuernii was observed in 19% of cases (Bangoura et al., 2012). Research undertaken in many countries, such as France, Poland, Austria, and Belgium, has documented instances of Eimeria spp., which have been recognized as prevalent internal parasites (Pilarczyk et al., 2002). This study aims to examine the prevalence of Eimeria and cryptosporidium spp. infections in cattle in various regions of Al-Najaf and Al-Diwaniyah Provinces. It will analyze the age and sex of the animals to determine their susceptibility to infection.

MATERIALS AND METHODS:

Materials: Solutions

1) Formalin 10% used for preservation of samples, prepared by adding 100 ml of formalin 40% into 1 lit. of water.
2) Normal saline, prepared by solving 8.50 gr. of Nacl in 1 lit. of distal water.
3) Sheather's solution, prepared by heating 355 ml of water, adding 454 gr. of granulated sugar (table sugar), and mixing them with 6.70 gr. of crystalline phenol (Hendrix and Robinson, 2006).
4) potassium dichromate solution, prepared by solving 25 gr. of potassium dichromate powder in 1 lit. of distal water.
5) phosphate buffer saline (PH=7.2)
6) Acidic alcohol, prepare by mixing 2 ml of concentrate hydrochloric acid with 98 ml of ethanol 95 % (Coles, 1986).
7) Methylene blue solution.

Stains

Carbol fuchsin stain, alcohol basic fuchsin.

Methods

Field study

The current study was carried out through weekly visits to various areas of Al-Najaf province, including the Al-Najaf slaughterhouse and the zoological field of the Faculty of Veterinary Medicine at the University of Kufa, as well as the Al-Diwaniyah province. The data pertaining to the animals were documented in specifically designed forms that included information such as the number of samples, geographical region, owner's name, sex, and age. The permanent dentition formula, as described by Reece (2005), was employed to ascertain the age of the animal. This formula is represented as 2 (I 0/4, C 0/0, P 3/3, M 3/3), resulting in a total of 32 teeth. The research was carried out between early January and mid-March, 2013. The study encompassed a total of 160 cattle, comprising animals of various ages and both genders.

Drawing on Reece's (2005) approach, the animals were classified into two age groups: the first group consisted of 38 animals under the age of two, while the second group consisted of 122 animals over the age of two. Moreover, the study employed a random selection process to choose 160 animals, with 78 samples obtained from males and 82 samples obtained from females.

Collection of Fecal Samples

160 fecal samples were collected randomly from the cattle. Five grams of fecal samples were collected directly from the rectum or immediately after defecation. Some fecal samples were kept in plastic containers while others were stored in containers containing 10 % formalin. All the containers were marked with the animal data.

Methods of fecal examination:

Direct smear method:

The direct smear method is a simple microscopical examination of fecal parasites. The method involves placing a small amount of feces directly on the microscope slide, followed by the addition of several drops of saline to the same amount of feces. The solution and feces are mixed together until they get homogenous. The solution is smeared over the slide to create a thin film. The slip is covered. The slide under the microscope is examined by 40 X and 100 X (10).

Flotation method:

A beaker was filled with 4-5 grams of feces and a small quantity of distilled water was added for mixing. The liquid was filtered through two layers of gauze and subsequently collected in a new 15-ml test tube. The combination underwent centrifugation at 1000 rpm for 3 minutes, after which the supernatant was removed. The sheather's solution was introduced to the sediment and carefully centrifuged for 5 minutes. The combination was re-centrifuged at 1000 rpm for 5 minutes. Subsequently, the opening of the test tube was sealed with a slip. The tube was left for 5 to 10 minutes. Subsequently, the cover slip was taken off. The specimen was positioned on a microscopic slide and scrutinized with a 10X and 40X magnification (Bowman and Lynn, 2009).

Modified Zeihl-Neelsen's Stain (Acid fast stain):

A small amount of feces was spread thinly. Subsequently, the item was allowed to dry, and then treated with a 99% menthol solution for 3-5 minutes. Then, the specimen was treated with carbol fuchsine for 3 minutes. It was cleansed by a feeble water current and permitted to air dry. The combination was discolored by adding an acidic alcohol, followed by water. The specimen was stained with methylene blue for 2 minutes, followed by a thorough water rinse. Finally, it was allowed to air dry and microscopically examined under magnifications of 40X and 100X (Bessat, 2019).

Sporulation:

The oocysts are sporulated in a solution containing 2.5 % potassium dichromate, and incubated them for one week at room temperature with constant aeration. *Eimeria* spp. were identified on the basis of the morphological characteristics of the oocysts and sporocysts (Hendrix and Robinson, 2006).

The Statistical Analysis

A Chi-square **(X^2)** was used to analyze the results of the present study at a (0.05) probability level (Al-Rawi, 2000).

RESULTS

Among the 160 fecal samples analyzed, only 41 samples (25.62%) were found to contain oocysts of Eimeria spp. However, laboratory testing for diagnosing Cryptosporidium spp. yielded negative results (Fig. 1).

The Effect of Age and Sex on Infection with *Eimeria* spp.

The findings of this study indicate that the infection rates were significantly higher in cattle under the age of two (47.36%) compared to animals over the age of two (18.85%). Female cattle (cows) had a higher prevalence of Eimeria spp. (26.82%) compared to male cattle (bulls) (24.35%). Similarly, the statistical analysis revealed that there were significant differences in infection rates among animals of different ages ($p<0.05$), as indicated in Table (1). However, the differences in infection rates based on the sex of the animals were not found to be significant ($p>0.05$).

Table (1) The age-sex divergence in the frequency of infections with *Eimeria* spp. in cattle:

Animal's age		Number of the examined cattle	Number of the infected cattle	Percentage of infection%
Less than 2 years		38	18	47.36
More than 2 years		122	23	18.85
Total		160	41	25.62
Chi-Square x2 (*p* value)		12.362 (0.000) *		
The sex	**Male**	78	19	24.35

	Female	82	22	26.82
Total		160	41	25.62
Chi-Square x2 (*p* value)		0.128 (0.721) NS		

* $P \leq 0.05$, NS =Non-significant P > 0.05

Geographic distribution of the infections with *Eimeria* spp.

The current investigation unveiled that the Al-Diwaniyah region exhibits the greatest rates of infection with Eimeria spp., with a prevalence of 33.33%. This is followed by the Al-Najaf slaughterhouse, which has a lower but still significant infection rate of 20.89%. The zoological field exhibited the lowest infection rates, which stood at 14.28%. Furthermore, the statistical analysis of the results indicated that there were no significant differences in infection rates across different geographical locations (p> 0.05), as shown in Table (2).

Table (2) The frequency of infections with *Eimeria* spp. in the studied areas:

The studied region	**Number of the examined cattle**	**Number of the infected cattle**	**Percentage of infection%**
AL- Najaf Slaughterhouse	67	14	20.89
Al-Diwaniyah province	72	24	33.33
The zoological field of vet. Med. College university of Kufa	21	3	14.28
Total	160	41	25.62
Chi-Square x2 (*p* value)	4.447 (0.108) NS		

* NS =Non-significant (P > 0.05)

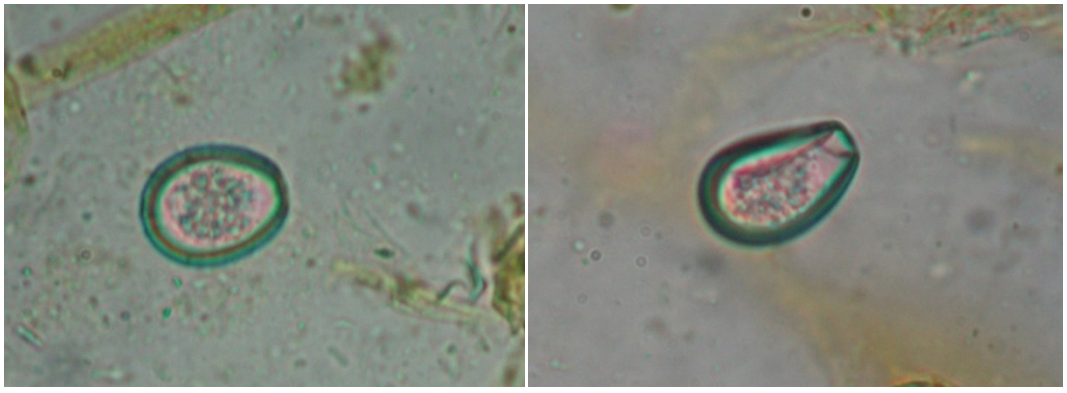

Figure 1.: Some species of *Eimeria* 40 x.

DISCUSSION

The study examining the presence of Eimeria and Cryptosporidium spp. infections in cattle of various ages did not identify any characteristic instances of clinical coccidiosis, although some cattle had diarrhea. The overall prevalence of Eimeria spp. was 25.62%. This finding is consistent with the studies conducted by (Al-Bakry, 2009, and Kim et al., 2018), which revealed infection rates of 25.71% and 25.9%, respectively. The present study reports a greater prevalence of infections compared to previous studies conducted by (GÜL et al., 2008; Heidari et al., 2014; Das et al., 2015; and Hussin, 2016), who reported infection rates of 22.53%, 8.25%, 11.97%, and 9.5%, respectively. However, this finding indicates a lower prevalence of infections compared to the studies conducted by (Almeida et al., 2011; Dong et al., 2012; Bangoura et al., 2012; Rehman et al., 2011, and Koutny et al., 2012, Lopez-Osorio et al., 2020, and Tomczuk et al., 2015). Their findings reported infection rates of 33.33%, 95.4%, 52.8%, 47.09%, 47.1%, 83.67%, and 75.5%, respectively. Similarly, the study conducted by (Klockiewicz et al., 2007) revealed that Eimeria spp. was present in 93% of the farms examined. The findings of the present study indicate that the highest prevalence of Eimeria spp. infections was observed in Al-Diwaniyah and the Al-Najaf slaughterhouse, with rates of 33.33% and 20.89%, respectively. Conversely, the lowest infection rates were recorded in the zoological field, at 14.28%. Therefore, there were substantial variations in both the number and percentage of infections across different geographical regions ($p<0.05$). These findings indicate that geo-environmental factors such as climate, geographical location, and seasonal variations have a substantial impact on the spread of diseases. In addition, pastures polluted with oocysts create favorable conditions for sporulation and transmission, facilitating the development of diseases. Furthermore, this study revealed that the prevalence of infections was greater in cattle under the age of two (47.36%), whereas it was lower in cattle above the age of two (18.85%). The statistical study revealed a strong relationship ($p< 0.05$) between age and the spread of Eimeria infections in animals. This shows that the age of the animal plays a significant role in the transmission of Eimeria. Calves are susceptible to infections due to factors such as weaning, grazing on grasslands contaminated with Eimeria, and their immature immune system. This observation, which is related to age, is consistent with the studies conducted by (Lassen et al., 2009; Koutny et al., 2012; Dong et al., 2012; Bangoura et al., 2012; Heidari et al., 2014; and Lopez-Osorio et al., 2020). However, it contradicts the findings of (Tomczuk et al., 2015). Similarly, female cattle, specifically cows, exhibited a higher prevalence of infections with Eimeria spp. (26.82%) compared to male cattle, specifically bulls (24.35%). This disparity can be attributed to the stress experienced by cows during breeding, pregnancy, and dairy production. The results showed that there was no statistically significant difference in the prevalence of infections between male and female animals ($p>0.05$), suggesting that the sex of the animals did not influence the occurrence of illnesses. This finding aligns with the findings presented by (Ali et al., 2005; Heidari et al., 2014, and Chandra Deb et al., 2022). However, it diverges from the outcomes described by (Ekawasti et al., 2021). In a similar vein, the research conducted by (Koutny et al., 2012) suggested that the various methods and procedures employed in the management, housing, watering, and feeding of cattle have a significant influence on the occurrence of Eimeria in cattle.

CONCLUSION

Coccidia infections were detected in all of the examined regions. In terms of age, the infections were prevalent among both juvenile and geriatric animals, but were most frequent among the younger individuals. Furthermore, both male and female animals were susceptible to infection with coccidia. Due to the rarity of diarrheal samples, there was a negative correlation between diarrhea and *Eimeria spp*. infection. Furthermore, the

subclinical impacts indicate that coccidia are likely responsible for health issues in the animals' lifetimes, significantly impacting their health and productivity. Hence, future research should prioritize a more in-depth exploration of the pathogenic consequences of *Eimeria*. Furthermore, there is a need to enhance knowledge and understanding of coccidian infections in cattle among individuals responsible for the well-being of animals, including owners, farmers, and veterinarians.

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