

Original research article

PREVALENCE, RISK FACTORS AND MOLECULAR CONFIRMATION OF *EIMERIA* SPP. IN ONE-HUMPED CAMELS (ALGERIA)

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Abstract

This study had for objective to investigate by molecular tools the intestinal *Eimeria* infection in slaughtered dromedaries (*Camelus dromedarius*) from two abattoirs in Southern Algeria. In all 89 paraffin-embedded intestinal samples were analysed, and the DNA was extracted with a commercial kit. Polymerase chain reaction (PCR) was done after optimising the method, and correlations between the DNA concentrations and PCR results were assessed. Of the 85 DNA extracts tested, 12 (14.1%) were PCR-positive for *Eimeria*. Higher infection rates were observed in Sahraoui camels compared with the Targui breed, and in older animals compared with adults. A strong positive correlation was observed between DNA concentration and the success of PCR amplification. This work provides one of the first molecular confirmations of *Eimeria* infection in Algerian

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dromedaries. Further research is needed to identify *Eimeria* species and construct a phylogenetic tree to support epidemiological control strategies.

Keywords: Algeria, camels, *Eimeria*, molecular characterization, parasites

INTRODUCTION

Often referred to as the “ship of the desert”, the camel is not only used as a means of transport, but is also an important source of meat, milk and wool for desert nomads in Asia and Africa (Toaleb and Raafat, 2024). The world’s camel population continues to grow in most countries, particularly in Africa. It is now estimated to be less than 40 million head (Faye et al., 2023). In Algeria, this species plays an important social and economic role for Saharan families. Its breeding does not require substantial resources or advanced breeding techniques (Boukert et al., 2023). Previous studies claimed that camelids are susceptible to many parasitic diseases (Ahmed et al., 2018; Dubey and Schuster, 2018). For example, Maxamhud et al. (2023) reported that the dromedary camels in Algeria can be infected by many parasitic protozoan, helminth, and arthropod species. Most widely studied gastrointestinal protozoan parasites of camels include *Cryptosporidium spp.*, *Eimeria spp.*, *Giardia duodenalis*, *Balantidium coli* and *Enterocytozoon bieneusi* (Dubey and Schuster, 2018), which all have intracellular life stages that inhabit the intestinal epithelium (Craig et al., 2007). Tafti et al. (2000) reported that the most important and frequent pathological lesions in the gastrointestinal tract of camels were caused by *Eimeria* infections (detected in 63 out of 100 slaughtered camels). *Eimeria* genus is an Apicomplexan group, with plenty of species that can infect various animals (e.g., mammals, birds, cattle, poultry, dogs, cats, sheep and goats), although many remain asymptomatic (Dubey and Schuster, 2018). According to Chartier and Paraud (2012) *Eimeria* are monoxenous. Their life cycle includes an exogenous phase (sporogony) - a free-living phase outside the host and an endogenous parasitic phase inside the host. In the host, an asexual (merogony) and sexual reproduction (gamogony) take place. Unsporulated oocysts are eliminated with faeces, and sporulation occurs in 2 to 7 days, depending on the species and environmental conditions (temperature, oxygen and humidity). *Eimeria* infections are transmitted between animals by the faecal-oral route (Al-Afaleq et al., 2018; Abbas et al., 2019). Eimeriosis, AKA coccidiosis, is an important intestinal parasitic disease in pre-weaned and recently weaned camels (Yakhchalim and Cheraghi, 2007). It is also considered an important parasitic disease of camels (Dubey and Schuster, 2018). Several studies have found three species of *Eimeria* in camels in the Turkestan region: *E. bactriani*, *E. cameli* and *E. dromedarii* (Abbas et al., 2019; Hussein et al., 1987). Research on the prevalence of *Eimeria spp.* in camels reported different infection rates (Metwally et al., 2020). For instance, Djerbouh et al. (2018) reported *E. dromedarii* and *E. cameli*, in 9.6% of the samples in Algeria. In Egypt,

Abbas et al. (2019) found *E. cameli*-like parasites in 31%, *E. rajasthani* in 18%, and *E. dromedarii* in 14% of examined animals. In Iran, the reported range of *Eimeria* infections in dromedary camels was 9.51%–63%. According to Metwally et al. (2020), the different infection rates may reflect risk factors that are summarised by geographical distribution and the influence of varying environmental conditions (humidity, temperature, oxygen levels and soil type) affecting oocyst sporulation, and even age and gender, which play an important role in the infection.

In recent years, research has focused on more sensitive methods to improve the diagnostics of parasitic diseases, such as molecular approaches (Hassan et al., 2016). PCR is a simple, fast, specific, and sensitive assay for the confirmation of protozoa (Verweij et al., 2004). Currently, diagnostic laboratories are increasingly turning to DNA-based technology for the identification of particular *Eimeria* spp. (Ekawasti et al., 2022). It should be noted that PCR diagnosis of *Eimeria* is a more reliable, sensitive and rapid method (Malek and Kuraa, 2018): it is easy to amplify DNA from very small amounts and there is a high degree of variability between closely related species (Ekawasti et al., 2019; Hastutiek et al., 2022). However, few studies have investigated the presence of intestinal parasites in dromedary camels in Algeria, most based on microscopic faecal analysis, as reported by Saidi et al. (2022) and Bouragba et al. (2020). On the other hand, a recent study on the histopathology of intestinal coccidiosis in dromedaries was carried out by Boukert et al. (2024) in Algeria, but molecular diagnostics of coccidiosis in dromedaries has never been done in Algeria. Thus, this study aimed to determine, for the first time, the prevalence of coccidia in the small intestines of dromedaries (*C. dromedarius*) in two regions of the southern Algerian Sahara using molecular diagnostics.

MATERIAL AND METHODS

Study area

The camels in this study were slaughtered in two abattoirs: (1) the Royal Slaughterhouse located in the north-east of the Algerian Sahara, in the province of El-Oued (33° 22' 06" N, 6° 52' 03" E), approximately 512 km from the capital, Algiers, and (2) the municipal slaughterhouse of the province of Tamanrasset (22° 47' 13" N, 5° 31' 38" E), in the far south of Algeria, roughly 1,900 km from Algiers.

Sample collection

The study evaluated the use of the PCR to detect the presence of intestinal *Eimeria* spp. in dromedary camels. The samples analyzed in the present work were derived from the same cohort of animals as reported in our previous publication (Boukert et al., 2024). Specifically, 31 samples were collected from clinically healthy

dromedaries intended for meat consumption, these samples were obtained during post-mortem examinations and included different segments of the intestinal tract, namely the duodenum, jejunum, ileum, and cecum. From these samples, a total of 89 paraffin blocks were prepared for molecular diagnosis. This higher number reflects the fact that multiple tissue segments were collected and processed from each animal. In the present study, all samples from the previous study, regardless of their histopathological status (positive or negative), were subjected to PCR-based molecular analysis.

DNA extraction and purification

The parasite DNA was extracted from paraffin blocks following the protocol described by Pikor et al. (2011). Genomic DNA was isolated using the DNA extraction kit (Molecular Genomique Tools, GM Biolab Co., Ltd). The concentrations and purity of the DNA extracted from the 89 samples were measured using a NanoDrop Micro-Spectrophotometer. The required DNA dilutions were prepared with molecular-grade water. All extraction procedures were done at the Microbiology Research Laboratory, Acibadem University in Istanbul (Turkey). However, PCR was performed under conditions that targeted the partial 18S rRNA and the Internal Transcribed Spacer (ITS) region is commonly used as the target gene. Species-level identification and sequencing were not done in this study. Therefore, the detection is reported at the genus level (*Eimeria spp.*) only, and the lack of sequence confirmation limits the epidemiological and diagnostic specificity of the results.

PCR thermocycler conditions

According to Kawahara et al. (2010), the PCR amplification was used to determine the presence of genomic DNA of *Eimeria spp.* Subsequently, the cycle parameters of the PCR reaction were as follows by the thermocycling procedure as shown in Table 1, using the protocol by Salama et al. (2023). A Bio-Rad thermal cycler was used to do PCR. The reaction protocol consisted of 12.5 µL of 2X PCR Master Mix (#RP02-II-400, GeneMark), 1 µL of 10 µM Forward Primers (Table 2), 1 µL of 10 µM Reverse Primer (ITS-F 5'-GCAAAAGTCGTAACACGGTTTCC-3'); (ITS-R 5'-CTGC AATT CACAATGCGTATCG-3'). These ingredients were provided by Eurofins Genomics. So, 25 ng/µL of DNA sample and dH₂O up to 25 µL (Table 3).

Table 1. Thermocycler protocol

Temperature	Time	Number of cycles
94° C	5 min	X 35
94° C	30 sec	
57° C	40 sec	
72° C	45 sec	
72° C	10 min	

Table 2: Primers Used for PCR Amplification of *Eimeria* spp.

Primer Name	Sequence (5' → 3')
ITS-F	GCAAAAGTCGTAACACGGTTTCC
ITS-R	CTGCAATTCACAATGCGTATCG

Table 3: PCR Reaction Components

Component	Volume / Concentration
2X PCR Master Mix	12.5 µL
Forward Primer	1 µL of 10 µM
Reverse Primer	1 µL of 10 µM
DNA Template	25 ng/µL
dH ₂ O	Up to 25 µL total reaction volume

Standard gel electrophoresis procedures

To detect positivity, 2% agarose gel electrophoresis was run at 120 V for 50 min. Samples were prepared by mixing 5 µL of PCR product with a 1:2 ratio of TIBO Gold: Orange G dyes to observe the DNA. To estimate the size of the amplicons, a 300-1000 bp DNA ladder was used. Finally, the gels were visualised using the ChemiDoc™ MP Imaging System. The first group of samples included a positive sample, one of the samples, which served as a positive control for the remaining samples. On the contrary, negative control without DNA was prepared for each run. However, a non-specific amplification band was observed on the 100 bp line. To address this issue, three different optimization studies were performed.

Optimization experiments

Firstly, gradient PCR was performed between 55 °C and 60 °C for annealing, and although there was still a weak non-specific band, 57 °C was chosen as the most suitable annealing temperature. Secondly, instead of using a commercial master mix, a mix was prepared with different MgCl₂ concentrations in the range of 1 mM and 3.5 mM, but without a significant difference. Finally, as the band was suspected to be due to Taq polymerase contamination by *E. coli*, the Taq polymerase enzyme was treated with DNase. However, the non-specific band did not become weaker. It was therefore concluded that this band did not affect positivity/negativity.

Statistical analysis

Statistical analysis was done with SAS software (SAS, 2005). Descriptive statistics was used to analyze the prevalence of *Eimeria* in dromedaries in the study area and

certain risk factors (sex, age and breed). The geographical area was not taken into consideration due to the inequality in the number of samples. Differences were considered significant at $p < 0.05$ with a 95% confidence interval and the X-Squared statistical test was calculated. In the form of a heat map, a correlation matrix provides a measure of the linear relationship between pairs of numerical variables (amount of DNA, H2O and the positive (1), negative (-1) amplification).

RESULTS

Detection of *Eimeria* spp. by PCR

As suggested by Salama et al. (2023), amplification (positivity) was expected in the 500 bp band. Figure 1 (a, b, c and d) summarises the gel electrophoresis snapshots for positive samples. Eleven samples were identified as positive, namely nos. 14, 22, 27, 30, 39, 58, 64, 65, 66, 80 and 85 (Figure 1).

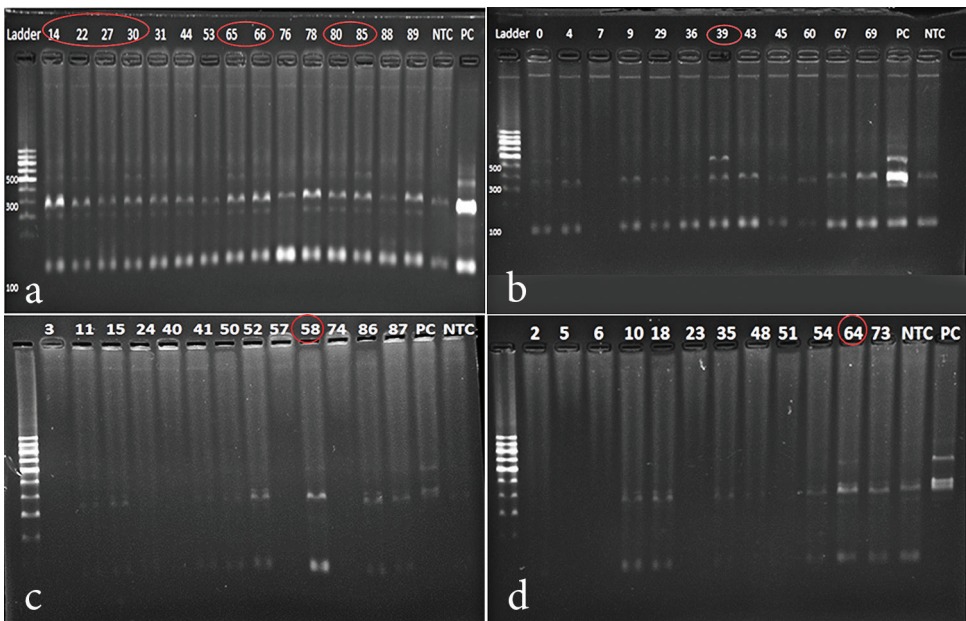


Figure 1. Representative positive PCR amplifications for (a): positive samples: 14, 22, 27, 30, 65, 66, 80, 85 (NTC: Negative Control, PC: Positive Control); (b): 39 was positive; (c): 58 was positive; (d): 64 was positive

Prevalence of Eimeria spp. in camels detected by PCR

Several samples (4 out of 89) showed abnormally high PCR signals and were therefore considered false positives and excluded from the study to prevent their

impact on the statistical analysis. Consequently, 85 DNA samples were retained, out of which 11 tested positive for *Eimeria* spp. (Table 4).

Table 4. Prevalence of *Eimeria* spp. in camels

Technical	No. of DNA samples tested	Positive (+)	Prevalence (%)	IC
PCR	85	11	12.94	5.8 – 20.0

Risk factors for intestinal Eimeria infection in camels

The study identified certain risk factors associated with *Eimeria* spp. infection in dromedaries, including breed, sex and age (Table 5). Statistical analysis showed that there were no significant differences between the three risk factors.

Table 5. Risk factors for Eimeriosis

Variables	Category	EI (%)	NEI (%)	χ^2	<i>p</i> -value
Sex	Female	8.18	47.81	3.87	> 0.05
	Male	4.13	23.18	-	-
Age	< 10 years	4.23	25.76	2.10	> 0.05
	> 10 years	7.66	47.23	-	-
Breed	Sahraoui	9.45	57.53	1.24	> 0.05
	Targui	2.54	15.43	-	-

*EI: *Eimeria* infection; NEI: Non *Eimeria* infection; χ^2 : Chi-square

Correlation matrix for numerical variables used in PCR

A correlation was calculated between the amount of DNA concentration, H₂O and PCR amplification of positive and negative samples. The results are illustrated by a heat map (Figure 2). The results were as follows:

- DNA (25 ng): This variable had a strong negative correlation with DNA (ng/μL) and dH₂O, and a strong positive correlation with PCR amplification (Figure 2).
- dH₂O had a strong negative correlation with ng/μL and DNA (25 ng), and a strong positive correlation with PCR amplification (Figure 2).
- PCR amplification had a weak positive correlation with DNA (ng/μL), DNA (25 ng) and dH₂O, and a perfect positive correlation with itself (autocorrelation). This suggests that amplification increases as DNA concentration and dH₂O increase (Figure 2).

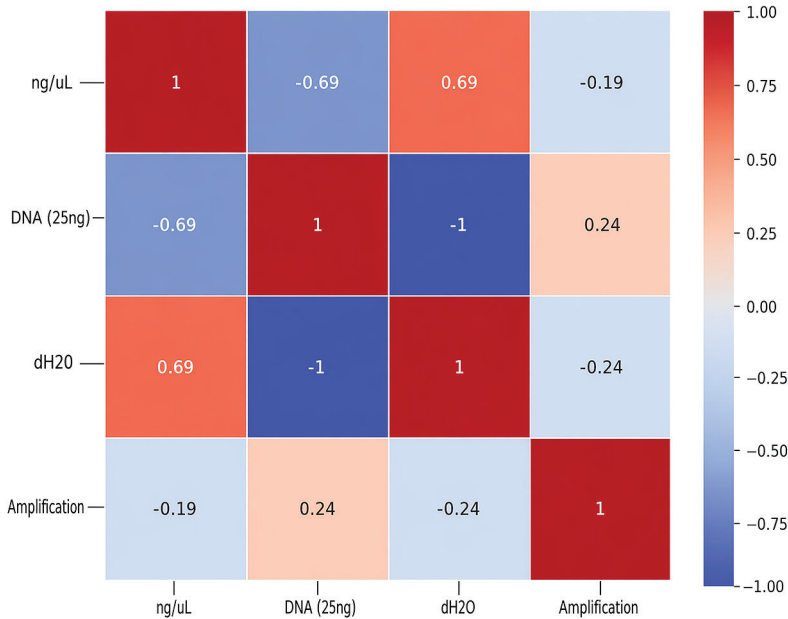


Figure 2. A heat map for three variables

DISCUSSION

Eimeriosis in camelids worldwide has recently been summarised (Dubey and Schuster, 2018). The present study highlighted intestinal infection with *Eimeria spp.* using molecular diagnostics with PCR in camels slaughtered in two abattoirs in southern Algeria. However, there is a paucity of data on intestinal infections in camels, particularly based on molecular methods, which makes it difficult to discuss our results. The studies carried out in Algeria were based exclusively on the detection of intestinal parasites in faecal samples. For example, data reported by Djerbouh et al. (2018) proved *Eimeria dromedarii* (4.8%) and *Eimeria cameli* (9.6%) in five localities in the South of Algeria. According to Bouragba et al. (2020) *Eimeria spp.* (3.21%) were found on 28 farms at the Steppe and Northern Sahara regions and Saidi et al. (2022) detected *Eimeria* species in 20% of camels in the Laghouat region (Southern Algeria). The molecular study, using the PCR assay, confirmed the positive amplification of *Eimeria spp.* DNA in 12.94% (11/85) animals. This result is important due to the high sensitivity and specificity of PCR technique in comparison with other diagnostic methods (Mahmmoud et al., 2010; Qablan et al., 2013). However, this is in sharp contrast to what Salama et al. (2023) found in Egypt, who failed to detect *Eimeria spp.* in dromedaries by PCR, despite the presence of oocysts in faeces. This negative identification of *Eimeria spp.* by PCR is possibly related to fecal PCR inhibition and/or a failure to break oocyst walls

during the DNA extraction (Raj et al. 2013). However, the DNA in our study was extracted directly from the paraffin blocks. Moreover, the investigation method, the time of sampling, and the number of screened animals are considered as important factors (Bouragba et al., 2020). In China (Inner Mongolia), Wei and Wang (1990) detected *Eimeria* spp. in up to 50% Bactrian camels. The study by Tafti et al. (2000) indicated that the most important and frequent pathological lesions in the digestive tract of camels are those resulting from *Eimeria* infections (63% of 100 slaughtered animals). However, the prevalence of *Eimeria* spp. depend on variable factors such as the animal species, age, immune status, stress factors, environment, and management practices (Sazmand et al., 2012). An important fact that contributes to the epidemiology of coccidiosis is the high resistance of oocysts to environmental conditions (Utebaeva et al., 2021). In this study, we considered some risk factors. The seasonal influence was not one of them because we conducted our research in late autumn and winter (November and December 2022). The study by Utebaeva et al. (2021) showed that spring and autumn are the two seasons when *Eimeria* infection is significant. Female dromedaries (8.18%) were more infested by this parasite than the males (4.13%). Therefore, this finding was approved by many studies, such as in histopathological research by Boukert et al. (2024) and by a coprological examination by Metwally et al. (2020). Regarding the age, animals over 10 years old were the most infected by *Eimeria* spp. Our results differ from those published by Sazmand et al. (2012), who found that young males are the most infected with eimerians by coprology examination, and that the Sahraoui breed is more prone to infection compared to the Targui. A statistical analysis was also carried out to assess the association between age, race and the presence of parasites. Therefore, the intestinal damage caused by *Eimeria* spp. leads to both clinical disease and production losses (reduced weight gain, poor feed conversion, lower meat and milk yield). The economic impacts arise from reduced performance, increased management costs, and lower reproductive efficiency, which are significant for producers who depend heavily on camel products (Ahmed et al., 2025). After calculating the X², we found no relationship. The heat map revealed a weak positive correlation between DNA concentration and PCR amplification, indicating that more DNA leads to greater amplification. Conversely, a strong negative correlation was observed between water (dH₂O) and PCR amplification, suggesting that excess water can dilute the reaction and reduce amplification efficiency. Although there was a weak negative correlation between DNA and water, this was not considered a significant factor that influenced the reaction. The heat map confirmed the general understanding of PCR and highlighted the importance of maintaining optimal levels of DNA and water for efficient amplification. According to Kramer and Coen (2001), the efficiency of target DNA amplification in the PCR assay is highly dependent on its initial concentration in a given sample. However, further experiments are needed to establish a direct cause-and-effect relationship and to take into account other factors that may influence the amplification results. It is

important to note that the present study did not include histopathological evaluation or clinical data correlation, despite the availability of paraffin-embedded tissue samples. The absence of microscopic confirmation of *Eimeria* oocysts or associated intestinal lesions limits the ability to link PCR detection to pathological changes or clinical disease. Future investigations should integrate molecular and clinical approaches to provide a more comprehensive understanding of the epidemiology and pathogenicity of *Eimeria spp.* in camels.

It would be plausible to complete this study by sequencing. However, to date, there are no 18S rRNA sequences of *Eimeria cameli* available in GenBank (Narnaware et al., 2024). Consequently, further research is needed to develop new molecular methods, as well as means of prevention and/or treatment of *Eimeria* infection, as indicated by Bussière et al. (2018). Although there are well-established methods for reproducing life cycles in *in vitro* cultures, such as those used for *Toxoplasma gondii* and *Babesia spp.*, they are not yet available for *Eimeria spp.* (Bussière et al., 2018).

CONCLUSION

The results of this study provide, for the first time, a molecular diagnosis of the prevalence of *Eimeria spp.* in the host intestinal epithelium in dromedaries from two slaughterhouses in southern Algeria. In order to reduce this infection, adequate control of coccidiosis must be established, especially in dromedary calves. First, animals should be separated according to age group (avoid mixing young and adult animals), high-quality colostrum should be provided, and stress should be minimised (for example, weaning, transport, dietary changes). However, few studies have been conducted on the treatment of coccidiosis in camels. Some of them have proven the efficacy of aqueous suspension of sulfadimidine administered orally to dromedary calves for 10 days at a dose of 30 mg/kg body weight. Therefore, our previous findings were based solely on molecular investigation by PCR. Thus, a prevalence of *Eimeria spp.* in dromedaries was determined, proving the efficacy of the PCR used. Further molecular studies are needed to determine the exact taxonomic status of various *Eimeria* species found in camels in different areas of southern Algeria. Larger sample sizes are needed to gain a better understanding of the epidemiology of the infection, as eimeriosis can lead to significant economic losses.

Author's contribution

RB and DS designed, wrote the manuscript and reviewed the study. SB, BB and MC analyzed the data. AAB, BŞ, EEIC, KBK, GS and TK contributed to the investigation and methodology. NS reviewed the manuscript. All authors read and approved the final manuscript.

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Ethical statement

Animal experiments in the present study were performed under the approval of the guidelines of the Institutional Committee for the Protection of Animals in Algerian Higher Education and Scientific Research (agreement no. 45/DGLPAG/DVA.SDA.14).

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflict of interest.

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PRELAVENCIJA, FAKTORI RIZIKA I MOLEKULARNA POTVRDA PRISUSTVA VRSTA RODA *EIMERIA* KOD JEDNOGRBIH KAMILA (ALŽIR)

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Kratak sadržaj

Cilj rada bio je primenom molekularnih metoda ustanoviti infekciju creva vrstama *Eimeria* kod kamila (*Camelus dromedarius*) zaklanih u dve klanice na jugu Alžira. Pregledano je ukupno 89 uzoraka creva kalupljenih u parafinu, a DNK je izolovana primenom komercijalnog kita. Lančana reakcija polimerizacije (PCR) je urađena posle metodološke optimizacije, i izračunata korelacija između koncentracije DNK i rezultata PCR. Od 85 izolata DNA koji su testirani, 12 (14,1%) je bilo pozitivno na prisustvo *Eimeria*. Infekcija je bila raširenija kod Sahraoui kamila nego kod kamila rase Targui, kao i kod mlađih životinja u odnosu na odrasle. Ustanovljena je visoka pozitivna korelacija između koncentracije DNK i uspešne amplifikacije u PCR. Ovaj rad je jedan od prvih koji pruža molekularno-biološku potvrdu infekciju vrstama roda *Eimeria* kod kamila u Alžiru. Potrebna su dalja ispitivanja da bi se ustanovile vrste *Eimeria* i konstruisalo filogenetsko stablo kako bi se unapredile strategije epidemiološke kontrole.

Ključne reči: Alžir, kamile, *Eimeria*, molekularna karakterizacija, paraziti