First Report on Barcoding of *Eimeria tenella* (Family Eimeriidae) from Khartoum State, Sudan

Mona AM Khaier1*, Mai A Masri2, Mortada M Elhassan3, Abuugasim I Abdelhalim2 and Sumaia MA Abukashawa2

1Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
2Department of Zoology, Faculty of Science, University of Khartoum, Khartoum, Sudan
3Department of Epidemiology and Infectious Diseases, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan

*Corresponding Author*: Mona AM Khaier, Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan.

Received: June 22, 2020; Published: July 08, 2020

Abstract

**Background:** Chicken caecal coccidiosis is an invasion and destruction of caecal mucosa by the protozoa *Eimeria tenella*. The infection is characterized by extreme caecal damage resulting in diarrhea, morbidity and mortality with consequent serious economic losses.

**Aim:** The aim of this study was to characterize the *Eimeria tenella* existing in Khartoum State, using oocyst measurements and molecular barcoding approach.

**Materials and Methods:** Oocysts of *E. tenella* were collected from 10 positive samples. The supernatant containing the oocysts (after being sporulated) was used for length measurements using light microscope equipped with calibrated ocular micrometer. DNA was extracted using modified Cetyl-Trimethyl Ammonium Bromide (CTAB) method. The CO1 positive PCR products were purified and sequenced by Sanger sequencing method. The edited sequences were aligned with ClustalW using BioEdit software. A bootstrap Niebuhr-Joining tree (500 replicates) was created using MEGA7 software.

**Results:** The average length of the oocyst was 19.63 μm and the width was 17.02 μm. The shape index was 1.13 μm. The positive CO1 isolates showed 100% identity with *E. tenella* from GenBank with zero P-distance value.

**Conclusion:** *E. tenella* was successfully identified by CO1 barcoding primer, and the sequences showed identity to the global isolates forming sister clades.

**Keywords:** CO1; Barcoding; Eimeria tenella; Khartoum; Sudan

Introduction

Caecal coccidiosis in poultry was instigated by an Apicomplexan *Eimeria tenella* (Family: Eimeriidae). The severe disease is characterized mostly by bleeding, high morbidity and mortality, lost weight gain, emaciation and loss of skin pigmentation. Important economic losses were due to mortality, losses due to decreased body weight gain and losses due to increased feed conversion ratio [1]. Decreased performance was also observed in subclinical cases [2]. Moreover, the infection facilitates enteric bacterial diseases as a result of destruction of the caecal lining mucosa [3]. Not only does coccidiosis cause mortality and impaired growth, but it also reduces meat and protein

Citation: Mona AM Khaier, et al. "First Report on Barcoding of *Eimeria tenella* (Family Eimeriidae) from Khartoum State, Sudan". *EC Veterinary Science* 5.8 (2020): 01-10.
yield and affects the fleshy characteristics of meat [4]. In addition, E. tenella was more common and exerts a greater impact on poultry production [5].

Conventionally, the identification of Eimeria tenella was based on morphological features of the sporulated oocyst, sporulation time and the location/scoring of pathological lesions in the intestine [6,7]. However, the aforementioned procedures have serious limitations due to their subjective nature and overlapping characteristics among different species [8]. Diagnosis of E. tenella is dependent upon finding caecal lesions with prominent blood and often firm bloody cores and accompanying clusters of large schizonts and oocysts [9,10]. Barcoding is a beginning of a short DNA sequence(s) that enables species identification or recognition in a particular domain of life [11]. DNA barcoding is a reliable, consistent, and autonomous tool for species discrimination in routine identification of parasites [12]. A DNA barcode gene region must satisfy three criteria: (i) contain significant species-level genetic variability and divergence, (ii) have conserved flanking sites for developing universal PCR primers for wide taxonomic application, and (iii) have a short sequence length so as to facilitate current capabilities of DNA extraction and amplification. All these criteria were found in CO1 region [13]. Mitochondrial COI has been the most widely used genetic target in animal barcoding and has being useful in species allocation on a large scale [14]. More than one million COI sequences were deposited in the Barcode of Life Data System (BOLD) [15]. These developments have given rise to the International Barcode of Life Project (iBOL.org) which is calling for a barcode-based identification system for animals, including parasites.

In Sudan, the identification of E. tenella was mainly by oocyst measurements, sporulation time, lesion region [16,17]. E. tenella is the most dominant species in Sudan [17,18]. Improving knowledge on identifying the genetic structure of coccidia is of great importance for the development of effective control programs by designing a suitable drug and/or vaccine [19].

**Aim of the Study**

The aim of this study was to identify E. tenella isolated from broiler farms in Khartoum State, Sudan.

**Material and Methods**

**Sample collection**

A total of 10 positive caecum samples were collected. The caeca were opened by a longitudinal incision then and the contents were collected and examined by light microscope [20].

**Purification and sporulation of oocysts**

The oocysts were collected from the caecal contents and purified using saturated sodium chloride flotation technique. The oocysts were then aspirated using disposable pasture pipette and counted using cell McMaster counting slide. The mixture was diluted with distilled water (4:1) to remove sodium chloride and the sediment obtained was subjected to sporulation [21]. The purified sporulated oocysts were stored in 2.5% potassium dichromate for further nucleic acid extraction.

**Parasitological examination**

The supernatant containing sporulated oocysts was examined under light microscope at x400 magnification. Five random oocysts were selected for measuring their length and width using calibrated ocular lens. The oocyst shape index was then worked out. Oocysts were identified as Eimeria tenella using the key described by Soulsby [20].

**DNA extraction and quantification**

Fifty cycles of freezing and thawing (using liquid nitrogen and a water path at 50°C, respectively) were carried out for complete rupturing of the oocysts walls. Ten microliter of the …. was taken and examined under the microscope to ensure complete destruction of the oocyst wall. The Cetyl-Trimethyl Ammonium Bromide (CTAB) buffer (2% weight/volume CTAB, 1.4M NaCl, 0.2% [beta]-mercapto-ethanol, 20 mM EDTA, 100 mM TRI) protocol of Murray and Thomson (1980) was used to extract the DNA.
DNA concentration and purity were measured using a Nanodrop Spectrophotometer (ND1000 Spectrophotometer; Nanodrop Technologies, Inc.).

Polymerase chain reaction (PCR) for *E. tenella*

PCR was done using iNtRON Biotechnology (Maxime PCR premix Kit (i-Taq)). Sequences were amplified from extracted DNA as described by Schnitzler, et al [22]. A pair of primer specific for *Eimeria species* on the mitochondrial region of *CO1* gene was used: the forward primer was 5’ GTTTGGTTTACGTTGGTTG 3’ and the reverse primer was 3’ ATCCAATAACGGAACCAAGG (allowed the amplification of ~ 809 bp fragment). A denaturing step at 94°C for 2 minutes was coupled to 40 cycles of denaturation (94°C for 20s), annealing (57°C for 30s) and extension (72°C for 1 minutes), and a final extension step at 72°C for 7 minutes. PCR products were run in 3% agarose gel and the gel was observed under ultraviolet light to visualize the bands.

Amplified *E. tenella* CO1 gene sequencing

Five PCR products were sent for commercial sequencing; all samples were purified prior to sequencing (BGI, Begin Co., Ltd., China). The multiple sequence alignment (BLAST) of the isolates with other similar *Eimeria* sp. nucleotides sequences at GenBank nucleotide database (NCBI) was carried out to find the homology and evolutionary relation between the sequences through the BioEdit version 7.0.9.0 software.

Molecular and bioinformatics analysis

Aligned sequences were analyzed by Neighbour-joining tree to infer evolutionary relationships among the *Eimeria tenella* species using MEGA7 software. A comparison between current isolates and other *E. species* from database was made using BioEdit and MEGA-7 software.

Results and Discussion

Understanding of the genetic structure of *Eimeria* population is critical for addressing important biological and control issues such as vaccination, spreading of anti-coccidial resistance alleles and developing for control strategies. Poultry caecal coccidiosis is generally characterized by parasite replication in the caecum.

Measurements obtained for *Eimeria tenella* oocysts recovered from the examined material are given in table 1. The width and shape index of *Eimeria tenella* oocyst. Unsporulated and sporulated oocysts isolated from infected birds, magnified x400 are shown in figure 1.

![Figure 1: Unsporulated and sporulated oocysts isolated from infected birds.](image-url)
Table 1: Measurements (μm) of Eimeria tenella oocysts.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Length</th>
<th>Width</th>
<th>Shape index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.33</td>
<td>16.65</td>
<td>1.16</td>
</tr>
<tr>
<td>2</td>
<td>19.78</td>
<td>18.82</td>
<td>1.05</td>
</tr>
<tr>
<td>3</td>
<td>19.74</td>
<td>16.48</td>
<td>1.19</td>
</tr>
<tr>
<td>4</td>
<td>19.64</td>
<td>17.85</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>19.65</td>
<td>15.31</td>
<td>1.28</td>
</tr>
<tr>
<td>Average</td>
<td>19.63</td>
<td>17.02</td>
<td>1.13</td>
</tr>
</tbody>
</table>

In the present study, the mean values of Eimeria tenella oocyst measurements were 19.63 μm length, 17.02 μm width and 1.13 μm shape index. These findings are in agreement with William [23], Conway and McKenzie [24] for the same species. In this study, the amplified COI was positive allowing the amplification of ~ 809 bp fragment. The banding pattern at ~809 bp obtained with COI primer amplified with DNA from caecum samples is in agreement with Miska., et al [25]. PCR products of expected band size were obtained (Figure 2).

Figure 2: Agarose gel electrophoresis of amplified part of CO1 gene. Lane 1:100 bp molecular ladder, Lane 2: +ve s control, lane 3: -ve control, lane 4-10 Positive Eimeria species ~809 bp.

Four isolates were successfully sequenced and submitted in the GenBank. They were given the following accession numbers, MF497437, MF497438, MF497439 and MF497440 respectively. These four E. tenella isolates when aligned with those retrieved from GenBank (EF174186.1, HQ702484.1, KX094951, HM771676.1, AB564272.1, FJ236458.1, FJ236421.1 and FJ236397.1) were found to be genetically similar with 100% identity using BioEdit software.

The sequences from this study were also aligned by ClustalW using BioEdit software with E. necatrix and E. acervulina sequences retrieved from GenBank in addition to E. tenella to infer the differences and similarities. The alignment was shown in figure 3.

Citation: Mona AM Khaier, et al. "First Report on Barcoding of Eimeria tenella (Family Eimeriidae) from Khartoum State, Sudan". EC Veterinary Science 5.8 (2020): 01-10.
First Report on Barcoding of Eimeria tenella (Family Eimeriidae) from Khartoum State, Sudan

<table>
<thead>
<tr>
<th>Domain</th>
<th>Taxon</th>
<th>Accession</th>
<th>Nucleotide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eimeria tenella</td>
<td>China</td>
<td>#1741461_Eimeria.tenella.China</td>
<td>CAATTCCTG ATGCGCTTTT TAATGGTATG CCAAGATTAT ATCCACATCT ATTTGCTGCT TCCGGACAT CAGAGATA</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>China</td>
<td>#5072486_Eimeria.tenella.China</td>
<td>CAGCTTATTAT GTATTCTGCA AACTTCCAA TCTTTCAAG TGGATTAT TATGCTTCAC TCTAAATC</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>China</td>
<td>#5072482_Eimeria.necatrix.China</td>
<td>TTTTCTACTT ACAATTCTG TACTAGTTG TACTAATGG TCAAAACCAT GGTGCCTTT TACTTGCGG TATTGTACAA</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>China</td>
<td>#5072481_Eimeria.necatrix.China</td>
<td>CTCTTTATC TCGTATGCA CAGAATCTA TAGTACGTC TATTGTAGA</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>China</td>
<td>#5072480_Eimeria.necatrix.China</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>China</td>
<td>#5072479_Eimeria.necatrix.China</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>Japan</td>
<td>#1741468_Eimeria.tenella.Japan</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>Japan</td>
<td>#1741467_Eimeria.tenella.Japan</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>Japan</td>
<td>#1741466_Eimeria.tenella.Japan</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>Japan</td>
<td>#1741465_Eimeria.tenella.Japan</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>USA</td>
<td>#1741462_Eimeria.tenella.USA</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>USA</td>
<td>#1741461_Eimeria.tenella.USA</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>USA</td>
<td>#1741460_Eimeria.tenella.USA</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
<tr>
<td>Eimeria tenella</td>
<td>USA</td>
<td>#1741459_Eimeria.tenella.USA</td>
<td>CCGATCTG CAGGTAGAT CAGGTAGAT CAGGTAGAT CAGGTAGAT</td>
</tr>
</tbody>
</table>

The utility of mitochondrial COI sequences for species identification and phylogeny reconstruction has been demonstrated for several parasite groups [26-28]. A multiple sequence alignment comparison between 3 of the current isolates and other *Eimeria sp.* including *Eimeria tenella*, *Eimeria necatrix* and *Eimeria acervulina* sequences from database using multiple sequence alignment by ClustalW in MEGA7 software showed that the isolates under study are highly similar to the reference sequence from Canada, China, USA, Australia and Japan (Figure 3). The Newick format was transferred to MEGA7 software to construct phylogenetic tree for the aligned sequences. Phylogenetic analysis of the sequences revealed *E. tenella* and *E. necatrix* together formed a well-supported monophyletic clade. Clade one contained *E. necatrix*, clade two contained most of the *E. tenella* from GenBank and the isolates from this study. Clade three contains a USA *E. tenella*. *E. acervulina* as an outgroup in the fourth clade. Analyses showed that *E. tenella* and *E. necatrix* together formed a well-supported monophyletic clade. Results were shown in figure 4.

The fact that *Eimeria necatrix* is closely related to *E. tenella* the isolates, this is in agreement with most of the previous reports Bar-ta., et al. [29], Ogedengbe., et al. [30] and Kundu., et al [31]. Cytochrome c oxidase subunit I (COI) partial sequences are more reliable species-level molecular markers than 18S rDNA [32]. This was the primary identification by barcoding for *E. tenella* in Sudan, managing to identify local strains, better understanding of the parasite biology and designing for a suitable drugs and vaccines. Combining target-based approaches with parasite *in vitro* and *in vivo* testing and medicinal chemistry generates a comprehensive view on the genotype-to-phenotype-to-compound correlation, which could allow for the design of novel drug candidates. The mitochondrial genome or barcoding sequences are highly suited for molecular diagnostics and phylogenetic of coccidia and, potentially, genetic markers for molecular epidemiology. More research is needed to cover the other different species of *Eimeria* to complete the diagnosis and epidemiology of the parasite, due to the economic importance of coccidiosis in poultry industry, rapid diagnosis of the disease and early treatment should be carried out.

Citation: Mona AM Khaier., et al. “First Report on Barcoding of *Eimeria tenella* (Family Eimeriidae) from Khartoum State, Sudan”. *EC Veterinary Science* 5.8 (2020): 01-10.
Conclusion

The coccidia, *E. tenella* isolated from Khartoum State, Sudan was successfully identified by oocyst measurement technique along with molecular characterization using COI primer barcoding method. The sequences of *E. tenella* showed identity to the global isolates forming sister clades.

Data Availability Statement

All sequence data are available at NCBI database under the accession numbers MF497437, MF497438, MF497439 and MF497440.

Acknowledgments

University of Bahri, College of Veterinary Medicine was acknowledged for funding the research. Thanks are also due to the Center of Excellence, Genetics and Molecular Biology Laboratory, Faculty of Science, Department of Zoology, University of Khartoum, Sudan for providing laboratory facilities.

Bibliography

1. McDougald L. “Histomoniasis (Blackhead) and other protozoan diseases of the intestinal tract” (2008).
First Report on Barcoding of *Eimeria tenella* (Family Eimeriidae) from Khartoum State, Sudan


**Citation:** Mona AM Khaier, et al. “First Report on Barcoding of *Eimeria tenella* (Family Eimeriidae) from Khartoum State, Sudan”. *EC Veterinary Science* 5.8 (2020): 01-10.


Citation: Mona AM Khaier, et al. “First Report on Barcoding of Eimeria tenella (Family Eimeriidae) from Khartoum State, Sudan”. EC Veterinary Science 5.8 (2020): 01-10.