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Research Article

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

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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

yield and affects the fleshly 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 50ºC, 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'GTTTGGTTCAGGTGTTGGTTG'3 and the reverse primer was 3' ATCCAATAACCGCACCAAGAG (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.*

Samples	Length	Width	Shape index
1	19.33	16.65	1.16
2	19.78	18.82	1.05
3	19.74	16.48	1.19
4	19.64	17.85	1.00
5	19.65	15.31	1.28
Average	19.63	17.02	1.13

Table 1: *Measurements (µm) of Eimeria tenella oocysts.*

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 *CO1* was positive allowing the amplification of ~ 809 bp fragment. The banding pattern at ~809 bp obtained with *CO1* 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).

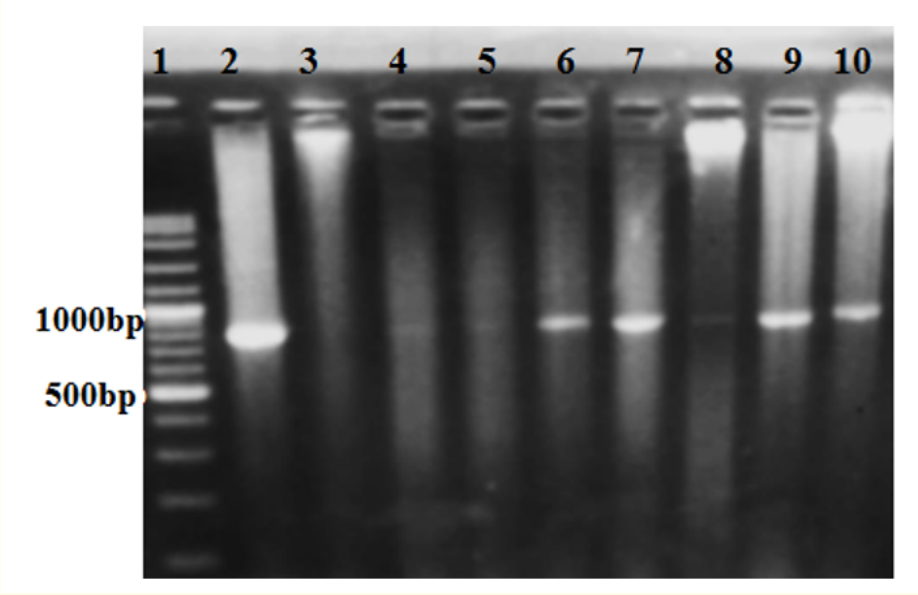
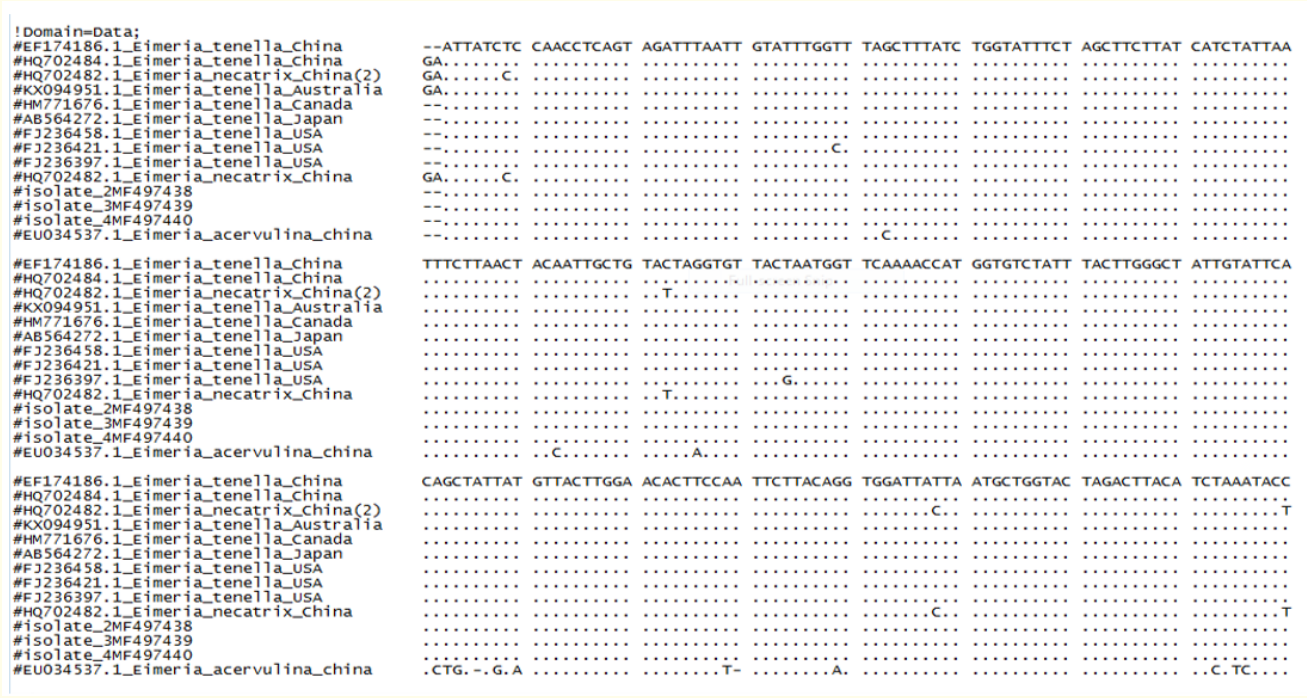


Figure 2: *Agarose gel electrophoresis of amplified part of CO1 gene. Lane 1:100 bp molecular ladder, Lane 2: +ve s control, lane 3: -ve control, lane 4-10 Positive Eimeria species ~809 bp.*

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.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#HQ702484.1_Eimeria_tenella_China GA..... ....
#HQ702482.1_Eimeria_necatrix_China(2) GA.....C. ....
#KX094951.1_Eimeria_tenella_Australia GA..... ....
#HM771676.1_Eimeria_tenella_Canada --..... ....
#AB564272.1_Eimeria_tenella_Japan --..... ....
#FJ236458.1_Eimeria_tenella_USA --..... ....
#FJ236421.1_Eimeria_tenella_USA --..... ....C. ....
#FJ236397.1_Eimeria_tenella_USA --..... ....
#HQ702482.1_Eimeria_necatrix_China GA.....C. ....
#isolate_2MF497438 --..... ....
#isolate_3MF497439 --..... ....
#isolate_4MF497440 --..... ....
#EU034537.1_Eimeria_acervulina_china --..... ....C..... ....
#EF174186.1_Eimeria_tenella_China TTTCTTAACT ACAATTGCTG TACTAGGTGT TACTAATGGT TCAAAACCAT GGTGTCTATT TACTTGGGCT ATTGTATTCA
#HQ702484.1_Eimeria_tenella_China ....
#HQ702482.1_Eimeria_necatrix_China(2) ....T..... ....
#KX094951.1_Eimeria_tenella_Australia ....
#HM771676.1_Eimeria_tenella_Canada ....
#AB564272.1_Eimeria_tenella_Japan ....
#FJ236458.1_Eimeria_tenella_USA ....
#FJ236421.1_Eimeria_tenella_USA ....
#FJ236397.1_Eimeria_tenella_USA ....G..... ....
#HQ702482.1_Eimeria_necatrix_China ....T..... ....
#isolate_2MF497438 ....
#isolate_3MF497439 ....
#isolate_4MF497440 ....
#EU034537.1_Eimeria_acervulina_china ....C..... .....A.... ....
#EF174186.1_Eimeria_tenella_China CAGCTATTAT GTTACTTGGA ACACTTCCAA TTCTTACAGG TGGATTATTA ATGCTGGTAC TAGACTTACA TCTAAATACC
#HQ702484.1_Eimeria_tenella_China ....
#HQ702482.1_Eimeria_necatrix_China(2) ....C.. ....T
#KX094951.1_Eimeria_tenella_Australia ....
#HM771676.1_Eimeria_tenella_Canada ....
#AB564272.1_Eimeria_tenella_Japan ....
#FJ236458.1_Eimeria_tenella_USA ....
#FJ236421.1_Eimeria_tenella_USA ....
#FJ236397.1_Eimeria_tenella_USA ....
#HQ702482.1_Eimeria_necatrix_China ....C.. ....T
#isolate_2MF497438 ....
#isolate_3MF497439 ....
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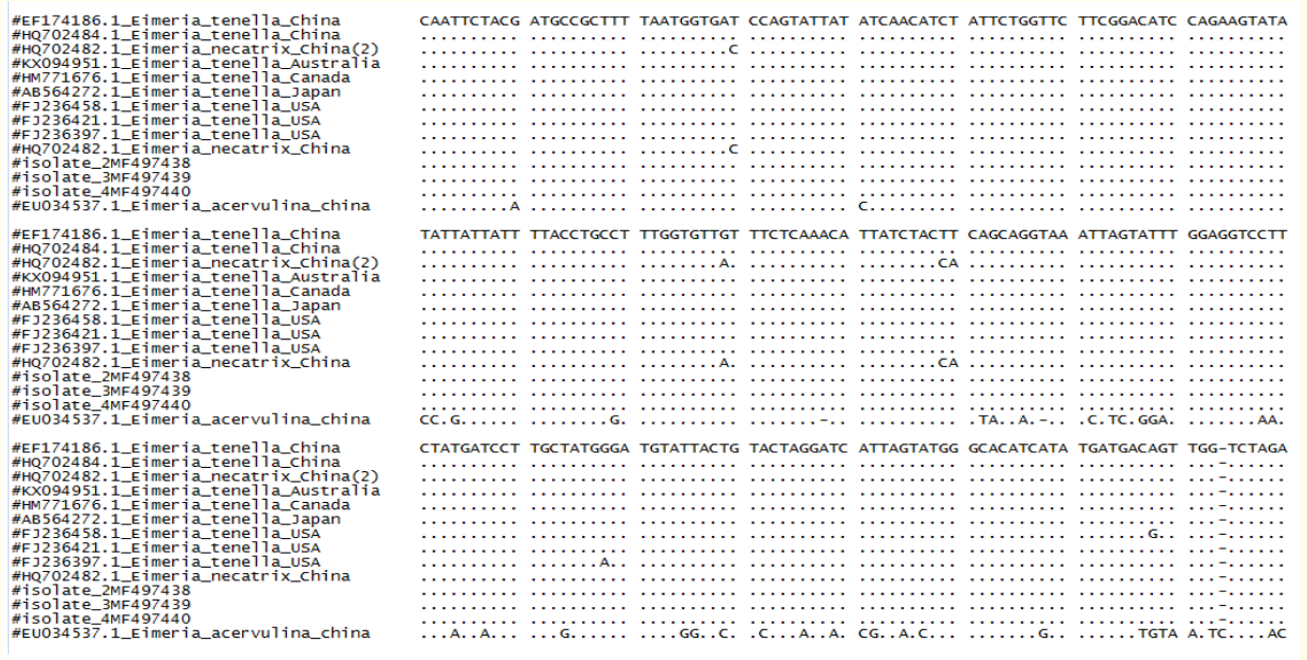
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#HQ702484.1_Eimeria_tenella_China ....
#HQ702482.1_Eimeria_necatrix_China(2) ....C ....
#KX094951.1_Eimeria_tenella_Australia ....
#HM771676.1_Eimeria_tenella_Canada ....
#AB564272.1_Eimeria_tenella_Japan ....
#FJ236458.1_Eimeria_tenella_USA ....
#FJ236421.1_Eimeria_tenella_USA ....
#FJ236397.1_Eimeria_tenella_USA ....
#HQ702482.1_Eimeria_necatrix_China ....C ....
#isolate_2MF497438 ....
#isolate_3MF497439 ....
#isolate_4MF497440 ....
#EU034537.1_Eimeria_acervulina_china .....A .... C..... ....
#EF174186.1_Eimeria_tenella_China TATTATTATT TTACCTGCCT TTGGTGTTGT TTCTCAAACA TTATCTACTT CAGCAGGTAA ATTAGTATTT GGAGGTCCTT
#HQ702484.1_Eimeria_tenella_China ....
#HQ702482.1_Eimeria_necatrix_China(2) ....A. ....CA ....
#KX094951.1_Eimeria_tenella_Australia ....
#HM771676.1_Eimeria_tenella_Canada ....
#AB564272.1_Eimeria_tenella_Japan ....
#FJ236458.1_Eimeria_tenella_USA ....
#FJ236421.1_Eimeria_tenella_USA ....
#FJ236397.1_Eimeria_tenella_USA ....
#HQ702482.1_Eimeria_necatrix_China ....A. ....CA ....
#isolate_2MF497438 ....
#isolate_3MF497439 ....
#isolate_4MF497440 ....
#EU034537.1_Eimeria_acervulina_china CC.G..... .....G. ....-.. ..... .TA..A.-.. .C.TC.GGA. .....AA.
#EF174186.1_Eimeria_tenella_China CTATGATCCT TGCTATGGGA TGTATTACTG TACTAGGATC ATTAGTATGG GCACATCATA TGATGACAGT TGG-TCTAGA
#HQ702484.1_Eimeria_tenella_China ....-.....
#HQ702482.1_Eimeria_necatrix_China(2) ....-.....
#KX094951.1_Eimeria_tenella_Australia ....-.....
#HM771676.1_Eimeria_tenella_Canada ....-.....
#AB564272.1_Eimeria_tenella_Japan ....-.....
#FJ236458.1_Eimeria_tenella_USA ....G.. ...-.....
#FJ236421.1_Eimeria_tenella_USA ....-.....
#FJ236397.1_Eimeria_tenella_USA ....A.. ....-.....
#HQ702482.1_Eimeria_necatrix_China ....-.....
#isolate_2MF497438 ....-.....
#isolate_3MF497439 ....-.....
#isolate_4MF497440 ....-.....
#EU034537.1_Eimeria_acervulina_china ...A..A... ...G..... ....GG..C. .C...A..A. CG..A.C... .....G.. ......TGTA A.TC....AC

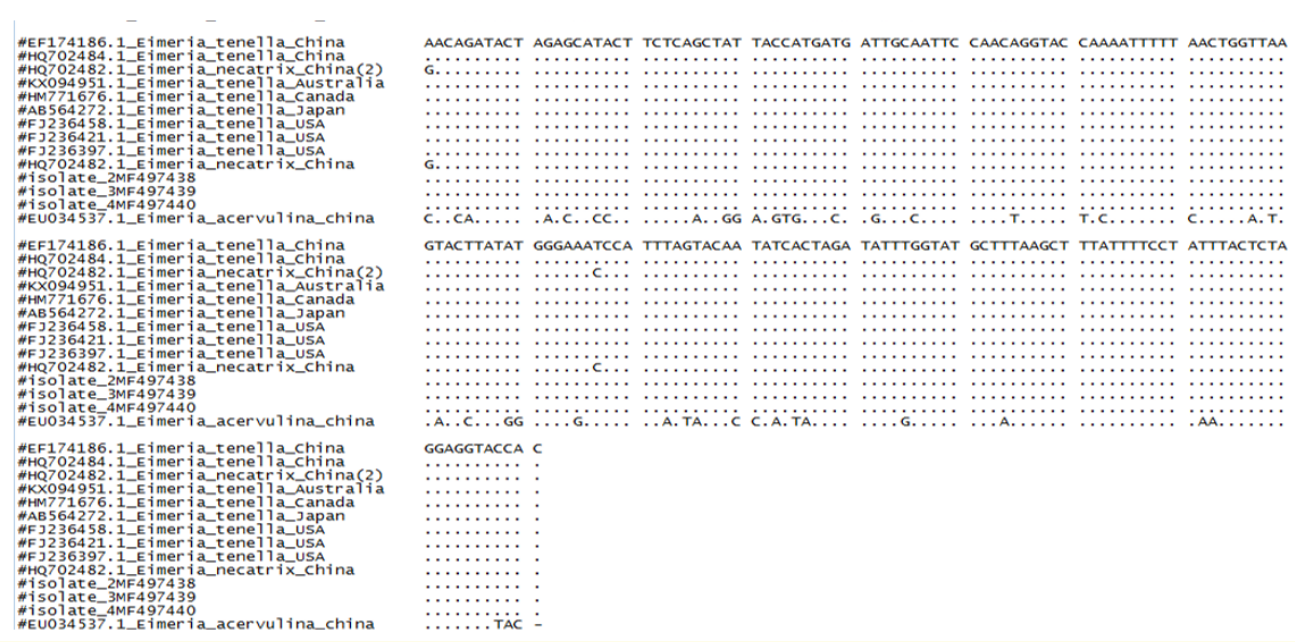

Figure 3: *The alignment results using BioEdit software for the E. tenella, isolated from Khartoum, Sudan), E. tenella, E. necatrix and E. acervulina from GenBank. Notice dots indicate similarity.*

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 Barta., *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.

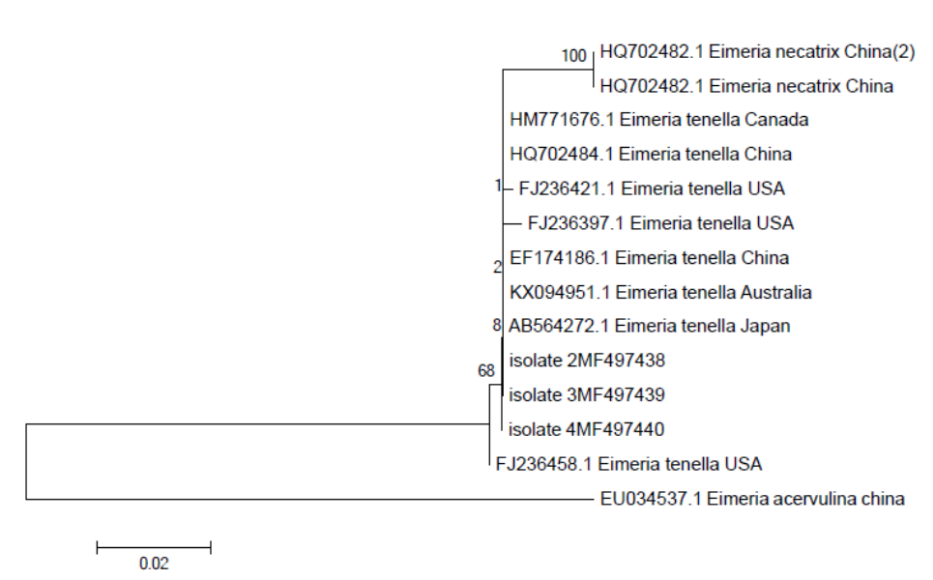

Figure 4: *Phylogenetic tree with Niebuhr Joining (NJ) method and bootstrapping (500) using MEGA7 software.*

Conclusion

The coccidia, *E. tenella* isolated from Khartoum State, Sudan was successfully identified by oocyst measurement technique along with molecular characterization using *CO1* 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

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Bibliography

1. Mcdougald L. "Histomoniasis (Blackhead) and other protozoan diseases of the intestinal tract" (2008).
2. Jatau., *et al*. "Response of 2 breeds of broiler chicks to experimental infection with low dose of *Eimeria tenella* sporulated oocysts". *The Turkish Journal of Veterinary and Animal Sciences* 38 (2014): 398-404.
3. Lillehoj K and Okamura M. "Host Immunity and Vaccine Development to Coccidia and *Salmonella* Infections in Chickens". *Journal of Poultry Science* 40 (2003): 151-193.
4. Lilić S., *et al*. "Coccidiosis in poultry industry". Tehnol Mesa 50 (2009): 90-98.

5. Long P and Millard B. "Studies on site finding and site specificity of *Eimeria* praecox, *Eimeria* maxima and *Eimeria* acervulina in chickens". *Parasitology* 73.3 (1976): 327-336.

6. Chapman H David., *et al*. "A Selective Review of Advances in Coccidiosis Research". *Advances in Parasitology* 83 (2013): 2-357.

7. Essa EA., *et al*. "Isolation and characterization of *Eimeria* species in poultry using conventional methods and coccimorph program in Khartoum state, Sudan". *World Journal of Pharmacy and Pharmaceutical Sciences* 8.2 (2019): 54-67.

8. Long PL and Joyner LP. "Problems in the Identification of Species of *Eimeria*". *The Journal of Protozoology* 31.4 (1984): 535-541.

9. Saif YM., *et al*. "Diseases of Poultry". Ames: Blackwell Academic Publishing Professional (2008): 1095-1105.

10. Saif Y., *et al*. "Diseases of Poultry". 12th Edition, Iowa state press, USA (2003).

11. Schoch CL., *et al*. "Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi". *Proceedings of the National Academy of Sciences* 109 (2012): 6241-6246.

12. Ferri E., *et al*. "Integrated taxonomy: traditional approach and DNA barcoding for the identification of filarioid worms and related parasites (Nematoda)". *Frontiers in Zoology* 6 (2009): 1.

13. Kress W John and Erickson L. "DNA barcodes: Genes, genomics, and bioinformatics". *Proceedings of the National Academy of Sciences of the United States of America* 105.8 (2008): 2761-2762.

14. Teletchea F. "After 7 years and 1000 citations: Comparative assessment of the DNA barcoding and the DNA taxonomy proposals for taxonomists and non- taxonomists". *Mitochondrial DNA* 21.6 (2010): 206-226.

15. Ratnasingham S and Hebert PDN. "BOLD: The Barcode of Life Data System". *Molecular Ecology Resources* 7 (2007): 355-364.

16. Khaier M., *et al*. "Identification and Molecular Characterization of Poultry *Eimeria* (Family: Eimeriidae), Khartoum, Sudan". *Journal of Applied and Industrial Science* 3.5 (2015): 177-181.

17. Alzib AA and Abdelnabi GH. "*Eimeria* Spp. infection in some broiler farms in Khartoum State, Sudan". *Journal of Veterinary Sciences* 3.4 (2017): 1-3.

18. Khaier Mona AM., *et al*. "Isolation, Identification and Molecular Characterization of *Eimeria* spp Infecting Chicken in Khartoum State, Sudan Using ITS1 Gene". *Biochemistry and Molecular Biology* 5.1 (2020): 1-5.

19. Soutter F., *et al*. "Poultry Coccidiosis: Design and Interpretation of Vaccine Studies". *Frontiers in Veterinary Science* 7 (2020): 101.

20. Soulsby E. Helminths, Arthropods and Protozoans of Domesticated Animals. 7th Edition. Bailliere Tindall, London, UK (1982).

21. Speer CA., *et al*. "Structure of the oocyst and sporocyst wall of and excystation of sporocyst of Isosporacanis". *Journal of Parasitology* 59 (1973): 35-40.

22. Schnitzler BE., et al. "Development of a diagnostic PCR assay for the detection and discrimination of four pathogenic. *Eimeria* species of the chicken". *Avian Pathology* 27 (1998): 490-497.

23. William RB. "A survey of *Eimeria* species in commercially reared chickens in France". *Avian Pathology* 25 (1996): 113-130.

24. Conway D and Mckenzie M. "Poultry Coccidiosis Diagnostic and Testing Procedures". 3rd Edition, USA (2007).

25. Miska K., *et al*. "Molecular characterization and phylogenetic analysis of *Eimeria* from turkeys and gamebirds: implications for evolutionary relationships in Galliform birds". *Journal of Parasitology* 96 (2010): 982-986.

26. Alcaide M., *et al*. "Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from arthropod blood meals". *PLoS One* 4 (2009): e7092.

27. Ogedengbe JD., *et al*. "DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata)". *International Journal For Parasitology* 41 (2011): 843-850.

28. Bensch S., *et al*. "MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages". *Molecular Ecology Resources* 9 (2009): 1353-1358.

29. Barta J., *et al*. "Molecular phylogeny of the other tissue coccidia: Lankesterella and Caryospora". *Journal of Parasitology* 87 (2001): 121-127.

30. Ogedengbe ME., *et al*. "Complete mitochondrial genome sequences from five *Eimeria* species (Apicomplexa; Coccidia; Eimeriidae) infecting domestic turkeys". *Parasites Vectors* 7 (2014): 335.

31. Kundu K., *et al*. "Humoral and cytokine response elicited during immunisation with recombinant immune mapped protein-1 (Et-IMP-1) and oocysts of *Eimeria tenella*". *Veterinary Parasitology* 244 (2017): 44-53.

32. El-Sherry., *et al*. "Divergent nuclear 18S rDNA paralogs in a turkey coccidium, *Eimeria meleagrimitis*, complicate molecular systematics and identification". *International Journal for Parasitology* 43.8 (2013): 679-685.

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