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Preliminary Genetic Diversity Study on Different Isolates of *Eimeria tenella* from South India

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Abstract Coccidiosis is an intestinal disease of chickens caused by more than six species of the protozoan parasites of the Genus *Eimeria* and has a major economic impact on the poultry industry throughout the world. Various studies revealed that the diversity of the parasites results in the pathogenicity and in the host pathogen relationship. So, in the present study we analyzed the genetic diversity of *Eimeria tenella* using the small subunit of 18S rRNA from different poultry managements of South India. About 23 variations were observed among the four isolates of *Eimeria tenella* with the similarity ranged from 99.3%–95.6% and 28–39 variations observed when compared the four isolates with the reference sequences obtained from the public domain. More than 90% of the similarity was observed between the study and the reference sequences. A mean distance of 0.03 was observed between the *E. tenella* isolates.
Keywords *Chicken Coccidiosis; E. tenella; 18S rRNA; Genetic Diversity*

1. Introduction

Chicken coccidiosis is a major cause of acute disease in the poultry caused by the protozoan, *Eimeria*. Coccidiosis remained the focus of anxiety in the commercial poultry producers not only due to the losses incurred as a result of mortality in acute infections or lowered production, but also due to the cost input required for effective chemoprophylaxis and immunoprophylaxis (Fayer, 1980).

Seven species of *Eimeria* have been recognized to infect chicken. Three species *E. acervulina, E. maxima* and *E. tenella* are highly recognized as most economically significant species of *Eimeria*. Coccidial infection in chicken normally found to have a minimum of two, or more, of these species at any given time (McDougald et al., 1986; Kucera, 1990; Morris et al., 2007), and it has been shown that individual chickens can be concurrently infected by multiple species of *Eimeria* (Long and Joyner, 1984; Haug et al., 2008; Jenkins et al., 2008). Recently, the correlation between specific species composition and genetic diversity of *Eimeria* species has been addressed in the United States (Scharwz et al., 2009) and Europe (Haug et al., 2008).

Recent reports revealed that the antigenic diversity in the parasites results in the pathogenicity and the host pathogen relationship (Smith et al., 2002; Blake et al., 2006). Hence it is implicit that the identification of the genetic variation in *Eimeria* species as a crucial factor in order to understand the pathogenicity and epidemiology of chicken coccidiosis (Morris and Gasser, 2006). Though there are many reports on the antigenic diversity, parasite genetics and host parasite relationship (Blake et al., 2006; Beck et al., 2009; Smith et al., 2002), there are no studies on the intra specific variation of the *Eimeria* species. Hence, the present investigation was attempted to study the diversity of different isolates of *Eimeria tenella* from South India using the small subunit of 18S rRNA.

2. Materials and Methods

2.1. Sampling

Poultry fecal samples were collected from Commercial Broiler (CB), Commercial Broiler Breeder (CBB), Commercial Layer (CL) and Backyard Poultry systems. The samples were collected in a 50 ml centrifuge tubes containing 1 gm of 1.0 mm glass beads and 4 ml of 2.5% potassium dichromate. Fresh droppings of the chicken from every two to five places was collected depending on the size of the unit until the tube was filled to the 10 ml mark in a 15 ml falcon tube. Each tube was then properly capped, labeled, transported to the laboratory and refrigerated at 4ºC until further process.

2.2. Identification of *Eimeria* Species

The fecal samples collected were processed as described by Eckert et al., (1995) for the identification of *Eimeria* parasite. The enumeration of OPG was done using a 3-chambered McMaster chamber as described by Haug et al., (2008) and the *Eimeria* species were identified using COCCIMORPH, the online diagnostic tool as described in previous studies (Kumar et al., 2014).

2.3. Amplification and Sequencing of 18s rRNA of *Eimeria tenella*

DNA was extracted from the fecal samples was carried out using QIAamp stool DNA isolation kit (Qiagen, Germany). The small subunit of the 18S rRNA of the isolated DNA was amplified and sequenced using published primers (Schwarz et al., 2009) and then sequenced by Sanger sequencing method.

2.4. Phylogenetic Analysis of Small Subunit of 18s rRNA Sequences of *Eimeria tenella*

The sequence alignment was performed using ClustalW program and phylogenetic and molecular evolutionary analyses were conducted using *MEGA* program version 6 (Tamura et al., 2007). A maximum parsimony tree was constructed using the 18S rRNA sequences from Indian isolates with publically available sequence as reference (FJ236372). Pair wise percentage identity was calculated using GeneDoc multiple sequence alignment editor version 2.6.002.

3. Results and Discussion

3.1. Distribution of *Eimeria* species

Based on the curvature characterization, size, symmetry and internal structural characterization for *Eimeria* species using COCCIMORPH identification revealed the presence of *E. acervulina* (79.49%), *E. tenella* (72.88%), *E. mitis* (50.62%), *E. maxima* (35.52%) *and E. necatrix* (10.83%) in the farms screened. *E. brunette* was not recorded in any of the farms screened.

The incidences of the *Eimeria* species were greatly varied between each management. Highest incidence of *Eimeria* infection were observed in the commercial broiler breeder (88.475%), followed by commercial broiler (62.856%). Whereas the incidence observed in commercial layer, backyard

poultry and the colour broiler poultry of managements were 44.69%, 44.44% and 42.81% respectively. Multi species infection was found highly in the commercial broiler breeder, commercial broiler and colour broiler managements with minimum of 4 species in a single farm.

3.2. Phylogenetic and Genetic Diversity Analysis of *Eimeria tenella*

E. tenella isolates of four different poultry managements were amplified and ~1790 bp of small subunit of 18s rRNA of the field *Eimeria* isolated were amplified using specific primers (forward: acctggttgatcctgccag, reverse: cttccgcaggttcacctacgg) and the amplicon (Figure 1) was sequenced and deposited in the NCBI DNA Data Bank with accession nos. JX312812 (CBB), JX093900 (BP), JX093899 (CL) and JX093898 (CB). Overall 48 variations are observed in the study sequences when aligned with 18S rRNA reference sequence (FJ236372). About 29 variations in the *E. tenella* isolate from CBB (JX312812), whereas 34 variations in BP (JX093900), 39 variations in CL (JX093899), and 28 variations in CB (JX093898) were observed. A total of 23 distinct variations were observed in the four *E. tenella* isolates.

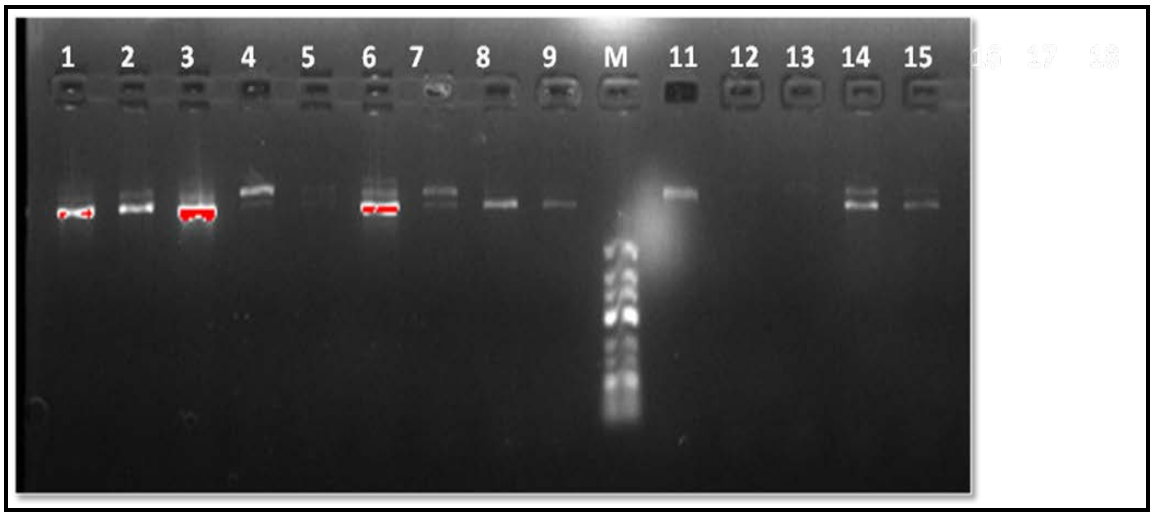

Figure 1: *Agarose Gel Picture Showing Amplification of 18S rRNA Gene of Field Eimeria isolates*

18s rRNA amplicons: ~1790bp (Lane 1,2,3,4,6,7,8,9,11,14,15), Lane M : 100bp-1000bp

Pair wise similarities of the sequences were ranged from 95.3% to 99.3%. The highest sequence homology 99.3% observed between the *E. tenella* isolate from Backyard Poultry and Commercial Broiler (JX093900 andJX093898) and Commercial Layer and Commercial Broiler (JX093899 and JX093898) and the lowest homology 95.6% was observed in the *E. tenella* isolate from Commercial broiler breeder and Commercial Layer (JX312812 - JX093899).

Sequence variation was relatively high between reference sequence and the isolates. The sequence similarity of between reference sequence and JX312812, JX093900, JX093899, and JX093898 were 94.5%, 96%, 94.3% and 96.3% respectively.

Phylogenetic analysis of small subunit of 18S rDNA sequences of four *Eimeria* species isolates showed two different clades (Figure 2). Except Commercial Broiler (JX093898), other three isolates were clustered in monophyletic clades (groups). The clades of JX093898 consisted to be phylogenetically distant from the other isolates. Cluster one contained of JX312812 (CBB), JX093899 (CL), and JX093900 (BP). The second cluster contained of JX093898 and the reference sequence. The overall mean distance of the isolates is 0.03. The tree demonstrates the close relationship of JX312812, JX093899, and JX093900 isolates.

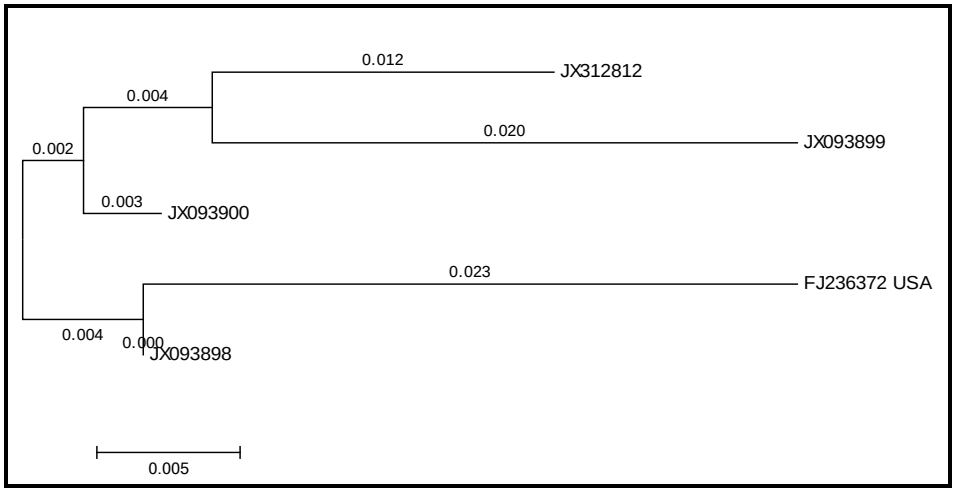

Figure 2: *Phylogetic Analyses of 18S rRNA Sequences of E. tenella isolates*

4. Conclusion

In the present investigation COCCIMORPH is found to be highly effective in the diagnosis of *Eimeria* species. As the measurement of oocysts undergo variations due to changes in metabolism of parasites or birds and even in the value of the shape morphometric indices that overlap, the use of COCCIMORPH can only be used with a sort of limitations to be used as a single tool for diagnosis of *Eimeria* species (Kumar et al., 2014). The present study showed the varying degree of divergence of *Eimeria tenella* isolates from different managements of chicken farms. *E. tenella* isolated from Commercial broilers observed with the highest diversity among the isolates. This study using small subunit of 18S rRNA in screening the genetic diversity of *Eimeria* species is a preliminary study to see the diverse effect of the *Eimeria* among various management system and it is also necessary to study the genetic diversity in the other potential vaccine targets genes and protein coding genes in larger sample size to study the inter and intra specific diversity of *Eimeria* species in different host and different management to understand the evaluation as well as the control of the diseases by the selection of appropriate method.

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