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MOLECULAR DETECTION AND SPECIES IDENTIFICATION OF EIMERIA SPP. IN BROILER FARMS NEAR BY ANAND, GUJARAT, INDIA

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ABSTRACT

Coccidiosis is one of the most economically important protozoan diseases affecting the poultry industry worldwide. The present study was undertaken to determine the prevalence and molecular characterization of Eimeria species infecting commercial broiler farms in Anand district, Gujarat, India. A total of 22 broiler farms comprising 400 birds were surveyed. Faecal and litter samples were collected and examined using flotation and sedimentation techniques. Positive samples were subjected to sporulation using 2.5% potassium dichromate solution and subsequently analyzed by polymerase chain reaction (PCR) for species identification. Microscopic examination revealed that 15 out of 22 farms were positive for coccidiosis, indicating an overall prevalence of 68.19%. Molecular confirmation using genus-specific primers targeting the cytochrome oxidase I (COI) gene amplified an approximately 810 bp fragment confirming the presence of Eimeria species. Species-specific PCR targeting the internal transcribed spacer-1 (ITS-1) region identified six Eimeria species, namely Eimeria acervulina (321 bp), Eimeria brunetti (311 bp), Eimeria mitis (306 bp), Eimeria necatrix (285 bp), Eimeria tenella (278 bp), and Eimeria maxima (145 bp). Eimeria tenella was the predominant species, occurring as a single infection in 40% of positive farms, while mixed infections were observed in the remaining farms. The findings indicate that coccidiosis remains highly prevalent in broiler farms of Central Gujarat and that PCR-based diagnosis provides a sensitive and reliable approach for species identification. Routine molecular surveillance coupled with integrated control measures is recommended to reduce production losses associated with avian coccidiosis.

Keyword: Broiler, Coccidiosis, Eimeria, PCR, ITS-1, Molecular diagnosis, Gujarat

1. INTRODUCTION

The poultry industry has emerged as one of the fastest-growing sectors of animal agriculture and plays a crucial role in ensuring food security through the production of affordable animal protein. Broiler production contributes significantly to the poultry economy owing to its rapid turnover and increasing consumer demand. However, infectious diseases continue to impose major constraints on poultry productivity, among which coccidiosis remains one of the most important parasitic diseases worldwide.

Coccidiosis is caused by protozoan parasites belonging to the genus Eimeria, family Eimeriidae and phylum Apicomplexa. The disease primarily affects the intestinal tract of chickens and results in enteritis, impaired nutrient absorption, poor feed conversion efficiency, reduced body weight gain, increased susceptibility to secondary infections and mortality. Economic losses associated with coccidiosis are attributed not only to mortality but also to decreased growth performance and the cost of preventive and therapeutic interventions.

Seven species of Eimeria are recognized in chickens, namely Eimeria acervulina, Eimeria maxima, Eimeria necatrix, Eimeria tenella, Eimeria brunetti, Eimeria mitis and Eimeria praecox. These species differ in pathogenicity and predilection sites within the intestine. Among them, E. tenella, E. necatrix and E. brunetti are considered highly pathogenic and are frequently associated with severe clinical disease and substantial economic losses.

Conventional diagnosis of avian coccidiosis relies on clinical signs, lesion scoring and microscopic examination of faecal samples. Although these methods remain useful, accurate species identification is often difficult because of similarities in oocyst morphology and the frequent occurrence of mixed infections under field conditions. Molecular diagnostic techniques, particularly polymerase chain reaction (PCR), have emerged as highly sensitive and specific tools for detecting and differentiating Eimeria species.

The poultry industry in Gujarat has experienced rapid expansion over the past decade, leading to increased stocking densities and management challenges that favour the transmission of coccidial infections. Despite the economic importance of coccidiosis, information regarding the prevalence and molecular distribution of Eimeria species in broiler farms of Anand district remains limited.

Therefore, the present investigation was undertaken to determine the prevalence of coccidiosis and identify circulating Eimeria species in commercial broiler farms of Anand district using conventional parasitological techniques and molecular diagnostic methods.

2. MATERIALS AND METHODS

Study rea

The present investigation was conducted in and around Anand district of Gujarat, India. Sample collection was carried out from commercial broiler farms, while laboratory investigations were performed at the Department of Veterinary Parasitology, Department of Veterinary Biotechnology and Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand.

Study population and sample collection

A total of 22 commercial broiler farms comprising approximately 400 broiler birds were included in the study. Fresh faecal samples and litter samples were collected aseptically from different locations within each poultry shed. Details regarding farm management, flock size and age of birds were recorded during sampling.

Microscopic examination

Faecal samples were examined by direct smear, flotation and sedimentation techniques for the detection of Eimeria oocysts. Positive samples were processed for sporulation using 2.5% potassium dichromate solution and incubated at 27°C. Sporulated oocysts were identified on the basis of morphological and morphometric characteristics under a light microscope.

Quantification of oocysts

The intensity of infection was determined by Stoll's dilution technique. Oocyst per gram (OPG) values were calculated and categorized as mild, moderate or severe infection based on oocyst load.

Postmortem examination

Detailed necropsy examination was performed on birds suspected of coccidiosis. Gross lesions in different intestinal segments were recorded and compared with microscopic findings.

Histopathological examination

Representative tissue samples from the duodenum, jejunum, ileum and caecum were collected and fixed in 10% neutral buffered formalin. Samples were processed using standard paraffin embedding procedures. Sections of 5–6 µm thickness were stained with hematoxylin and eosin (H&E) and examined under a light microscope.

DNA extraction

Positive samples containing sporulated oocysts were subjected to genomic DNA extraction. Oocysts were mechanically disrupted using glass beads and DNA was extracted using a commercial stool DNA extraction kit according to the manufacturer's protocol.

Polymerase chain reaction

Genus-specific PCR targeting the mitochondrial cytochrome oxidase I (COI) gene was used for molecular confirmation of Eimeria spp. Species-specific primers targeting the ITS-1 region were employed for identification of Eimeria species.

PCR products were separated by agarose gel electrophoresis and visualized under ultraviolet transillumination. Amplicon sizes were compared with a molecular weight marker for species confirmation.

Statistical analysis

Prevalence was calculated as the percentage of positive farms among the total farms examined. Descriptive statistics were used to summarize the distribution of Eimeria species.

3. RESULTS

Prevalence of coccidiosis

Out of 22 broiler farms examined, 15 farms were found positive for coccidiosis, indicating an overall prevalence of 68.19%.

Table 1. Prevalence of coccidiosis in commercial broiler farms

Particulars	Number
Farms examined	22
Positive farms	15
Negative farms	7
Prevalence (%)	68.19

Species distribution of Eimeria

Species-specific PCR identified six Eimeria species from positive farms.

Table 2. Species-wise distribution of Eimeria infections

Infection type	Positive farms	Percentage (%)
E. tenella alone	6	40.00
Mixed infection (*E. acervulina + E. maxima + E. mitis*)	5	33.34
Mixed infection (*E. necatrix + E. brunetti*)	4	26.27
Total	15	100

Molecular identification

Genus-specific PCR amplified the COI gene producing an expected amplicon of approximately 810 bp.

Species-specific PCR identified six Eimeria species based on characteristic amplicon sizes.

Table 3. PCR amplification profile of Eimeria species

Species	Amplicon size (bp)
E. acervulina	321
E. brunetti	311
E. mitis	306
E. necatrix	285
E. tenella	278
E. maxima	145

Histopathological findings

Microscopic examination revealed characteristic lesions associated with coccidiosis.

Duodenum

Sections showed villous shortening, epithelial degeneration, inflammatory cell infiltration and edema. Numerous developmental stages of Eimeria were observed within enterocytes.

Jejunum

The jejunum exhibited marked disruption of mucosal architecture, inflammatory infiltration and congestion. Villous degeneration was prominent.

Ileum

The ileum showed severe villous distortion, epithelial desquamation and widening of inter-villous spaces. Numerous oocysts were observed in affected tissues.

Caecum

The caecum demonstrated the most severe pathological alterations characterized by hemorrhage, destruction of villi, degeneration of muscular layers and extensive infiltration of inflammatory cells. Multiple developmental stages of Eimeria were present within the mucosa.

3. DISCUSSION

The present study demonstrated a high prevalence of coccidiosis in commercial broiler farms of Anand district. The prevalence observed in this investigation was comparable with earlier reports from various regions of India and abroad, suggesting that coccidiosis remains a major challenge under intensive poultry production systems.

The predominance of E. tenella observed in the present study agrees with previous findings indicating that this species is widely distributed and highly pathogenic. The caecal predilection of E. tenella contributes significantly to production losses due to hemorrhagic enteritis and reduced feed efficiency.

Mixed infections were frequently encountered among positive farms. Such infections are commonly reported under field conditions because multiple Eimeria species can coexist within the same flock. Mixed infections often complicate diagnosis and may exacerbate disease severity through additive pathogenic effects.

The successful amplification of genus-specific and species-specific DNA fragments confirmed the usefulness of PCR as a sensitive diagnostic tool. Molecular techniques offer greater specificity than conventional microscopic methods, particularly when species differentiation is required.

Histopathological examination revealed severe intestinal damage in infected birds. The observed lesions, including villous atrophy, epithelial degeneration and inflammatory infiltration, are indicative of impaired nutrient absorption and reduced productive performance. The most severe lesions were recorded in the caecum, corresponding to infection with E. tenella.

The findings highlight the need for integrated coccidiosis control strategies involving strict biosecurity, litter management, rotational use of anticoccidial drugs and continuous monitoring of Eimeria species circulating in commercial poultry farms.

4. CONCLUSION

The present study revealed a high prevalence (68.19%) of coccidiosis in commercial broiler farms of Anand district, Gujarat. Molecular characterization identified six Eimeria species, namely E. tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti and E. mitis. Eimeria tenella was the predominant species detected.

PCR proved to be a rapid, sensitive and reliable method for species identification and diagnosis of mixed infections. Histopathological examination demonstrated extensive intestinal damage associated with coccidial infection. The implementation of integrated management practices together with routine molecular surveillance is essential for effective control of coccidiosis and reduction of economic losses in broiler production systems.

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