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Detection of *Eimeria* Species in Naturally Infected Poultry using Multiplex Polymerase Chain Reaction in Chhattisgarh, India

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ABSTRACT

Background: The present study was undertaken to detect the species of *Eimeria* in naturally infected poultry using multiplex PCR in organized and unorganized farms of four districts namely Bilaspur, Durg, Korba and Rajnandgaon in plain region of Chhattisgarh. **Methods:** Multiplex PCR was carried out using primer pairs of seven species and it revealed that there were only 1-3 species of *Eimeria* per farm and a total of 4 species were only detected in all 4 districts and these were *E. acervulina, E. tenella, E. maxima* and *E. brunetti* with the presence of 50.0%, 43.75%, 37.5% and 12.5% among infected farms, respectively. **Result:** These samples showed the highest incidence of *E. acervulina* and lowest incidence of *E. brunetti*. As per multiplex PCR, mixed infection was present in 37.5% farms and single infection was present in 56.25% farms.

Key words: Coccidiosis, *Eimeria*, Multiplex PCR, Poultry, Species.

INTRODUCTION

Among the important diseases of poultry, coccidiosis produces serious problems and heavy economic losses to the poultry industry across the world (Jadhav *et al*., 2011). The disease is caused by the coccidia parasites of the genus *Eimeria.* There are seven recognized species *viz. E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella* which commonly occur in India (Shivaramaiah *et al*., 2014) and abroad.

The prevalence of poultry coccidiosis has been reported from the different countries including India. The favourable climatic condition of India and other factors like poor management is responsible for high rate of coccidial infection in poultry which has been reported time to time by several workers and almost from every part of India (Sharma *et al*., 2015; Salam and Wani, 2021). The severity of the disease, on the other hand, is determined by the species of *Eimeria* involved. In a subclinical form, however, it may cause immune-suppression in chicken, which is responsible for secondary infection. Thus, disease management and immune function maintenance for maximum performance, growth and production in the poultry industry are critical requirements for profitable farming. Besides, mixed infection *i.e*. more than one species is common (Kalita *et al*., 2021) which will cause different level of pathogenesis depending upon the species present in the birds. Therefore, species identification is required for successful control of the disease which is problematic by using morphological methods. Hence, an alternative diagnostic technique in particular DNA based molecular technique is required to overcome the limitations of traditional methods. Therefore, the objective of this study was to detect the prevalence of *Eimeria* spp. in poultry farms of four important poultry producing districts of Chhattisgarh.

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MATERIALS AND METHODS

Sample collection

The research work was conducted during 2021-22 at the College of Veterinary Science and Animal Husbandry, Dau Shri Vasudev Chandrakar Kamdhenu Viswavidyalaya, Anjora, Durg, Chhattisgarh. The faecal samples were collected randomly from 3 organized farms with 15 samples from each farm and 15 samples from 6 unorganized poultry farms of each district. A total of 240 fecal samples were collected from 12 organized and 24 unorganized farms of 4 districts of plain region of Chhattisgarh. The samples were collected in sterilized polythene zipper bags and brought to the laboratory by placing in ice jars for further investigations (Manjunatha *et al*., 2023). Both the direct and Willis techniques were

used to diagnose and to separate *Eimeria* oocysts in faeces as per the method described by Soulsby (1982). The faeces collected from each group were thoroughly mixed using pestle and mortar. Then, an emulsion of faeces was made using equal amount of water which was sieved using wire mesh to remove the coarser particles. Two milliliters of each emulsion were taken in a 10 ml sterile plastic bottle and the saturated sugar solution was added up to the brim of the bottle and a cover slip was placed on top of each with taking care to exclude air bubbles. The bottles were left upright for 15 minutes. The oocysts adhered on cover slip were then collected by means of rinsing.

Sporulation of oocysts

The Oocysts collected during floatation by Willis method were pooled farm-wise and then washed 3 times in water using centrifuge machine at 2000 rpm for 3 minutes each to remove the sugar. These oocysts were then sporulated by taking in a cleaned Petri dish containing 2.5% potassium dichromate (K_2Cr_2O7) (Munir *et al*., 2018; Murshed *et al*., 2023) solution to avoid fungal growth and left for 5 days at room temperature with frequent aeration. The Sporulation was checked under microscope and the number of sporulated oocysts per milliliter of the solution was recorded by using Mc Master technique before storing at 4°C.

Extraction of genomic DNA from oocysts

The DNA extraction from sporulated oocysts of coccidian parasites was done with DNeasy blood and tissue kit (Qiagen, Germany) as per kit protocol (Kaya *et al*., 2007) with little modification. A 300 µl of solution containing 1 X 10^5oocysts from each sample was centrifuged at 10,000 rpm for 2 minutes to precipitate the oocysts. The supernatant was discarded and the pellet was resuspended in 200 µl nuclease free water after 3 times washing with PBS. Equal volume of glass beads measuring 0.25-0.5 mm in diameter (Sigma-Aldrich, USA) were added and vortex vigorously for 12 minutes to break oocyst and sporocyst walls. Initially, 180 µl of lysis buffer (ATL) was added followed by adding of 20 µl Proteinase K which were mixed by vortexing and incubated at 56°C for 3 hours. Rest part was done as per Manufacturer's protocol.

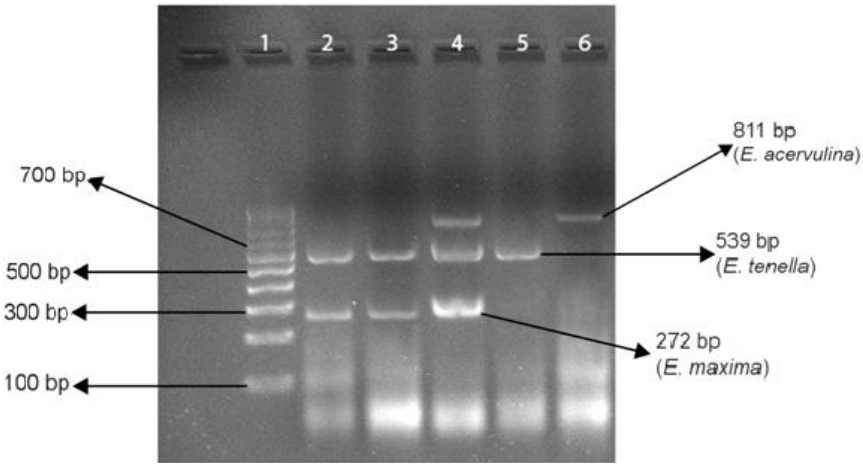
Identification of *Eimeria* spp. by multiplex PCR

The multiplex PCR was used for identification of the *Eimeria* species of poultry using extracted DNA samples as described by Fernandez *et al.* (2003) with slight modification. Initially, the PCR amplification was standardized separately for each species using specific primer pairs to have a common reaction for all seven species. Thermo cycling conditions were set with the denaturation step at 96°C for 5 min followed by 30 cycles at 94°C for 1 min, 64°C for 2 min and 72°C for 1 min with a final extension at 72°C for 7 minutes.

Once the above conditions were standardized, all the primer pairs were put together in a single 35 µl reaction mixture containing 800 µM dNTPs, 5 U Taq Polymerase, 2.4 mM $MgCl_2$, 5.6 µl of 1.6X buffer, 0.9 µM *E. brunetti*, 0.7 µM of *E. acervulina, E. praeco*x and *E. necatrix*, 0.6 µM of *E. tenella, E. maxima* and *E. mitis*, 3.0 µl of genomic DNA and 13.2 µl of nuclease free water for multiplex PCR with the same cycling conditions as described above. The amplification of specific PCR products was checked by gel electrophoresis in 2% agarose gels stained with 0.5 µg/ml ethidium bromide and visualized under gel-Doc UV-transilluminator.

RESULTS AND DISCUSSION

Multiplex PCR was carried out using primer pairs of seven species to know the species present in the poultry of plain region of Chhattisgarh. It revealed that there were only 1-3

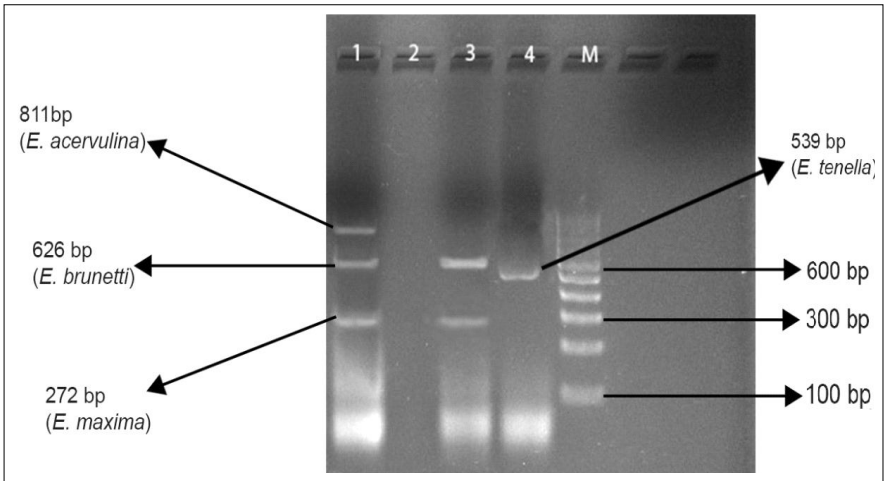


Lane 1-100 bp ladder; Lane 2 and 3 *E. tenella* (539 bp) and *E. maxima* (272 bp); Lane 4- *E. acervulina* (811 bp), *E. tenella* (539 bp) and *E. maxima* (272 bp); Lane 5- *E. tenella* (539 bp) and Lane 6- *E. acervulina* (811 bp).

Fig 1: Agarose gel electrophoresis of *Eimeria* spp. resulting from multiplex PCR.

species of *Eimeria* per farm (Table 1) and a total of 4 species were only detected in all 4 districts and these were *E. tenella, E. acervulina, E. maxima* and *E. brunetti* with the presence of 43.75%, 50.0%, 37.5% and 12.5% among infected farms, respectively (Fig 1 and 2). The incidence of *E. acervulina* was found to be the highest and *E. brunetti* was found to be the lowest in these samples. The *E. necatrix*, *E. mitis* and *E. praecox* were not detected in any farm.

As per multiplex PCR, the mixed infection was present in 37.5% farms and single infection was present in 56.25% farms. This molecular test also indicated that highly pathogenic species *E. tenella* and *E. brunetti* were present in 9 (56.25%) farms of plain region of Chhattisgarh while moderately pathogenic species *E. acervulina* and *E. maxima* were present in 11 (68.75%) farms of which 5 (31.25%) farms were also having highly pathogenic species. The present findings are quite similar with the findings of Lee *et al*. (2012) who observed *E. acervulina* and *E. tenella* as the most prevalent species of *Eimeria* in chicken. Kaboudi *et al*. (2016) also reported high



Lane 1 - *E. acervulina* (811 bp), *E. brunetti* (626 bp) and *E. maxima* (272 bp); Lane 2 -blank; Lane 3- *E. brunetti* (626bp) and *E. maxima* (272 bp); Lane 4- *E. tenella* (539bp) and Lane M- ladder (100 bp).

Fig 2: Agarose gel electrophoresis of *Eimeria* spp. resulting from multiplex PCR.

Table 1: Prevalence of *Eimeria* spp. as detected by multiplex PCR.

District	Name of farms	*Eimeria* spp. identified by Multiplex PCR							Total no. of species recorded
		E.tn	E.nc	E.ac	E.mx	E.mi	E.pr	E.br	
Bilaspur	AS farm, Mangla	-	-	+	-	-		-	1
	Yashashvi farm, Bahtarai	+	-	-	+	-	-	-	2
	Balaji farm, Kota	+	-	-	-		-	-	1
	Unorganized farms	-	-	+	-	-	-		1
Durg	Pappu farm, Chhata	+	-	+	-		-	-	2
	Dewangan farm, Khapari	+	-	+	+	-	-	-	3
	Kevant farm, Kursipar	-	-	-	+		-	-	1
	Unorganized farms	-	-	+	-		-	-	1
Korba	Deshmukh farm, Saida	-		-	+	-	-	+	2
	Sheikh farm, Urga	+	-	-	-		-	-	1
	Vikash farm, Tiwarta	-	-	+	+	-		+	3
	Unorganized farms	+	-	-	-		-	-	1
Rajnandgaon	Sajal farm, Ghorda	-	-	+	-	-	-	-	1
	K. Nishad farm, Khapari	-	-	+	+	-	-	-	2
	Dewangan farm, Somuni	+	-	-	-				1
	Unorganized farm	-	-	-	-	-	-	-	0
Total no. of farms infected (% of infection)		07 (43.75%)	00 (0.0%)	08 (50.0%)	06 (37.5%)	00 (0.0%)	00 (0.0%)	02 (12.5%)	

(E. tn= *E. tenella,* E. nc= *E. necatrix*, E. ac= *E. acervulina,* E. mx= *E. maxima,* E. mi= *E. mitis,* E. pr = *E. praecox,* E. br= *E. brunetti*).

prevalence of *E. tenella* (61.5%) in Sidi Thabet, Tunisia and mixed *Eimeria* species infection with overall prevalence of 26.5%. However, dissimilar result was revealed by Kumar *et al*. (2014). They found 5 species with highest prevalence of *E. necatrix* (43.3%) followed by *E. maxima* (16.7%), *E. tenella* (13.3%), *E. mitis* (3.3%) and *E. praecox* (3.3%) by multiplex PCR. *E. acervulina* and *E. brunetti* were not identified by them**.** The present test could not detect any species of *Eimeria* in the sample of oocysts collected from the unorganized farms of Rajnandgaon district. Although the reason was not clear but it might be due to the presence of a smaller number of oocysts.

CONCLUSION

This multiplex PCR technique demonstrated the presence at least 1 to 3 species of *Eimeria* in one farm. This technique was significant in detection of multiple species of *Eimeria* infecting poultry by polymerase chain reaction and the percentage level presence of multiple species of this parasite in the farms of Chhattisgarh.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Fernandez, S., Pagotto, A.H., Furtado, M.M., Katsuyama, A.M., Madeira, A.M. and Gruber, A. (2003). A multiplex PCR assay for the simultaneous detection and discrimination of the seven *Eimeria* species that infect domestic fowl. Parasitology. 127: 317-325.

Jadhav, B.N., Nikam, S.V., Bhamre, S.N. and Jaid, E.L. (2011). Study of *Eimeria necatrix* in broiler chicken from Aurangabad District of Maharashtra state India. International Multidisciplinary Research Journal. 1(11): 11-12.

Kaboudi, K., Umar, S. and Munir, M.T. (2016). Prevalence of coccidiosis in free range chicken in Sidi Thabet, Tunisia. Scientifica. 1-6. doi: 10.1155/2016/7075195.

Kalita, A., Kakati, P. and Sarmah, P.C. (2021). Isolation and molecular identification of *Eimeria* species circulating in broiler chicken flocks of Assam (India). The Haryana Veterinarian. 60(2): 251-254.

Kaya, G., Dale, C., Maudlin, I. and Morgan, K. (2007). A novel procedure for total nucleic acid extraction from small numbers of *Eimeria* species oocysts. Türkiye Parazitoloji. 31(3): 180-183.

Kumar, S., Garga, R., Moftahb, A., Clark, E.L., Macdonald, S.E., Chaudhry, A.S., Sparagano, O., Banerjee, P.S., Kundu, K., Tomley, F.M. and Blake, D.P. (2014). An optimised protocol for molecular identification of *Eimeria* from chickens. Veterinary Parasitology. 199: 24-31.

Lee, B.H., Kim, W.H., Jeong, J., Yoo, J., Kwon, B.Y., Jung, Y.K., Kwon, J.H., Lillehaug, H.S. and Min, W. (2012). Prevalence and cross-community of *Eimeria* species on Korean chicken farms. J. Vet. Med. Sci. 72: 985-989.

Manjunatha, V., Rout, M., Sreevatsava, V., Kshamaa, L.M., Umashankar, Shankar, B.P. and Byregowda, S.M. (2023). A copro-parasitological surveillance on diverse captive wild avian species. Indian J. Anim. Res. 57(4): 505-510. doi: 10.18805/IJAR.B-4473.

Munir, H.A., Akbar, H., Lateef, M., Rashid, I., Akhtar, R., Nazir, J.J. and Farooqi, S.H. (2018). Anticoccidial activity of different forms of *Aloe vera.* Indian J. Anim. Res. 52(3): 470-473. doi: 10.18805/ijar.B-534.

Murshed, M., Mares, M., Aljawdah, H.M.A. and Al-Quraishy. S. (2023). A comparison in terms of prevalence, morphological and histopathological changes between *Eimeria* spp. that infect local rabbits (*Oryctolagus cuniculus*) in some regions of Saudi Arabia. Indian J. Anim. Res. 57(12): 1662-1669. doi: 10.18805/IJAR.BF-1682.

Salam, S.T. and Wani, S. A. (2021) Prevalence and pathology associated with coccidian parasite *Eimeria tenella* in organised and unorganized poultry of Kashmir Valley, India. Acta Scientific Microbiology. 4(4): 99-104.

Sharma, S., Iqbal, A., Azmi, S., Mushtag, I., Wani, A.Z. and Ahmad, S. (2015). Prevalence of poultry coccidiosis in Jammu region of Jammu and Kashmir State. Journal of Parasitic Diseases. 39(1): 85-89.

Shivaramaiah, C., Barta, J.R., Hernandez-Velasco, X., Tellez, G. and Hargis, B.M. (2014). Coccidiosis: Recent advancements in the immunobiology of *Eimeria* species, preventive measures and the importance of vaccination as a control tool against these apicomplexan parasites. Veterinary Medicine: Research and Report. 5: 23-34.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoan's of Domesticated Animals, 7th Edition. Bailliere Tindall, London. pp-765.