Prevalence, Associated Risk Factors and Molecular Characterization of *Eimeria* Species Affecting Backyard Poultry of Jammu Region, North India

Aiman Khursheed
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Anish Yadav (anishyadav25@gmail.com)
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Omer Mohi-U-Din Sofi
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Anand Kushwaha
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Vikas Yadav
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Shafiya I. Rafiqi
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Rajesh Godara
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Rajesh Katoch
SKUAST Jammu: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

Research Article

Keywords: Backyard poultry, Eimeria spp., oocysts, COCCIMORPH, ITS-1 gene

Posted Date: March 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1321738/v1

Abstract

The present study was conducted from January 2018 to December 2019 to know the prevalence of coccidiosis in backyard poultry in Jammu, Samba and Udhampur districts of Union Territory of Jammu and Kashmir, North India. A total of 600 pooled faecal samples collected from backyard poultry were examined for presence of *Eimeria* oocysts. Morphometry and Polymerase Chain Reaction (PCR) based amplification of ITS-1 gene was carried to characterize the *Eimeria* species infecting the backyard poultry of the study area. An overall prevalence of 28.5% *Eimeria* spp. infection among backyard poultry birds was recorded. Among the seasons, highest prevalence was recorded during rainy season (32%) with significantly ($p<0.05$) high oocyst excretion (1.77±0.01) and lowest during summer (19.3%) with low oocyst excretion (0.17±0.006). Young birds upto 3 months of age were found to be more susceptible to infection than older birds, with a significantly ($p<0.05$) high prevalence percentage of 38.02. Morphometry with COCCIMORPH software revealed presence of *Eimeria acervulina, Eimeria maxima, Eimeria necatrix, and Eimeria tenella* species with prevalence rates of 16.5%, 3.6%, 21.3%, and 27.6%, respectively.The amplified fragments of ITS-1 gene presented different sizes of *Eimeria* spp. viz. *E. acervulina* (321bp), *E. tenella* (278 bp), *E. maxima* (145 bp) and *E. necatrix* (383bp). The study concluded that although backyard poultry didn't show clinical form of coccidiosis but may act as source of potential reservoir.

Introduction

Backyard poultry is a manageable and encouraging venture that could help to improve the socioeconomic and nutritional status of rural people, particularly landless or underprivileged households, with a minimal initial investment and significant economic return (Chakrabarti et al., 2014). Backyard poultry accounts for 20% of India's poultry market, which is valued around Rs 800 billion (approximately £8.5 billion), according to the Indian government's National Action Plan for Eggs and Poultry -2022 (NAPEP). Backyard flocks, on the other hand, might be public health and commercial sector problem since they could act as a potential disease reservoir. This is because many of these flocks have inadequate biosecurity and frequent access to the outdoors, allowing them to come into contact with disease-carrying wild birds and other animals like rodents (Whitehead *et al*., 2014; Pohjola et al., 2016). Among the various opportunistic pathogens, parasites play a key role especially among backyard chickens. Coccidiosis is caused by the apicomplexan parasite belonging to the genus *Eimeria*, affect different parts of intestinal tract and causes nutritional malabsorption, inefficient feed intake and clostridial and necrotic enteritis (William, 2005). Although, prevalence of backyard poultry coccidiosis has been reported in Jammu region of Union Territory of Jammu and Kashmir (Sood *et al*., 2009) but keeping in view the advancement made in diagnostic assays, identification and diagnosis must go beyond the traditional methods of identification, which rely on morphological features of sporulated oocysts, sporulation time, and infection site, and are tedious, time-consuming, and limited due to overlapping characteristics among different species (Long and Joyner, 1984). In particular, DNA approaches, specifically PCR approaches, have proven useful for the identification, detection or characterization of *Eimeria* species. The PCR has been utilized to fingerprint avian *Eimeria* species by amplification of ITS-1 gene.

Material And Methods

Study Area and Sample Collection

The study was conductedfrom January 2018 to December 2019 in Jammu, Samba and Udhampur districts of Union Territory of Jammu and Kashmir, North India. The study area is located at 32.73^{O}N and 74.87^{O}E. The region has a subtropical and sub humid climate with four seasons viz. summer (March to June), rainy (July to September), post-rainy (October to November), and winter (December to February). The study area features a humid subtropical climate, with extreme summer reaching 46°C (115°F), and temperatures in the winter months occasionally falling below 4°C (39°F). June is the hottest month with average highs of 40.6°C (105.1°F), while January is the coldest month with average lows reaching 7°C (45°F). Average yearly precipitation is about 42 inches (1,100 mm) with the bulk of the rainfall in the months from June to September, although the winters can also be rather wet.

The study was conducted as a part of societal project entitled “Technological intervention to improve production of dairy and poultry in rainfed areas” funded by Department of Science and Technology (File no. DST No: SEED/TIME/003/2014). As per the mandate of the project, 1500 backyard poultry birds of 40 days of Vanraja breed were procured from government hatcheries, Jammu and distributed among 150 farmers of the study area. Each farmer reared 10 birds, which were kept in the backyard during day time, while in the night hours, the birds were kept in the locally made mud houses having about 4ft. x 4ft. x 3ft. (length x breadth x height) dimensions. As part of routine health check up, visits were made at farmers’ doorstep and 600 pooled faecal samples were collected from different backyard poultry houses and examined for presence of *Eimeria* oocysts. The particulars like location, age and management practices were recorded. The samples were kept at 4°C till further processed.

Examination of Faecal Samples:

Samples were examined by concentration method of faecal examination for the presence of *Eimeria* oocysts. Positive samples were subjected to modified McMaster technique (Soulsby, 1982) for determination of oocysts per gram (OPG) of the faeces. The *Eimeria*n oocysts were allowed to sporulate at 30±2°C in 2 percent W/V potassium dichromate solution. Following sporulation, the oocysts were thoroughly washed thrice in autoclaved distilled water for taking photomicrographs and pelleted for DNA isolation. The *Eimeria* species were identified based on morphometry using COCCIMORPH, the online diagnostic tool as described in previous studies (Kumar *et al*., 2014) and DNA amplification of ITS-1 gene.

DNA extraction and PCR:

As described by Kumar *et al*., 2014, samples with more than 500 OPG of faeces were processed for genomic DNA extraction using the QIAampDNAStool mini kit (Qiagen, Germany). For the identification of *Eimeria* species in poultry, a nested PCR approach employing ITS-1 primers was applied. The genus-specific PCR used primers that amplified the entire ITS-1 sequence with flanking partial 18S rDNA and 5.8S rDNA regions, while species-specific primers targeting the ITS-1 region were used to amplify the individual *Eimeria* species, as described by Lew et al. (2003) with minor modifications. The primers used in the study are listed in Table 1.

Table 1: *Eimeria* genus- and species-specific primers used for PCR assays

Species	Primer name	Primer sequence (5→3)
Eimeria spp	EF1	AAGTTGCGTAAATAGAGCCCTC
	ER1	AGACATCCATTGCTGAAAG
E. tenella	ETF	AATTTAGTCCATCGCAACCCT
	ETR	CGAGCGCTCTGCATACGACA
E. acervulina	EAF	GGCTTGGATGATGTTTGCTG
	EAR	CGAACGCAATAACACACGCT
E. maxima	EMFA1	ct/acaccactcacaatgaggcac
	EMRA1	gtgat/atcgttc/tgg/ag/aagtttgc
E. mitis	EMi5FA	CGGAGCTGGGGTTTTCTTTC
	EMi5RA	CCTGCATATCCACA/GTT/CGAAC/ATAC
E. necatrix	ENF	TAC ATC CCA ATC TTT GAA TCG
	ENR	GGCATACTAGCTTCGAGCAAC
E. praecox	EPFA	AAAA/GCAA/CAGCGATTCAAG
	EPRA	CCAAGCGATTTCATCATT/CGGGGA/G

Statistical analysis

The data obtained on the prevalence of coccidiosis was compared by Chi square test. The mean oocyst per gram of faeces (OPG) was compared by ANOVA using SPSS 16.0 for windows. A p value of <0.05 was considered significant.

Results And Discussion

Out of 600 samples examined, 171 were found positive for *Eimeria* spp. with a prevalence rate of 28.5%.Backyard chickens are raised in a semi-intensive style, in which they spend a significant portion of the day outside and are only housed in the evening. The sick birds in the flock shed oocysts, which are picked up by healthy counterparts. In comparison to litter bedding, it is possible that the ground/soil does not provide the appropriate moisture and temperature conditions for oocyst sporulation. Furthermore, free-ranging tendency of birds aid in the distribution of oocysts across a vast area, reducing the concentrations of infective oocysts in a small region, as seen in broiler house litter. Because there are fewer birds in the free range system of backyard farms, there is a lower stocking density and hence a smaller environmental oocyst load, resulting in less oocyst shedding (Kundu et al., 2020).The present observations are comparable with 20% of coccidiosis infection observed in studies conducted by Sharma *et al.*, (2013) from Jammu, North India, 24.6% Murugan and Durairajan (2020) from Kanchipuram district, South India and 30.12% Das (2021)

from Meghalaya, East India. Similarly, 31.8% prevelance was recorded by Lawal et al., 2016 from Nigeria and 17.9% prevelance was observed by Ketema and Faisal 2019 from Ethiopia.

The infection was observed all round the year and OPG was significantly variable in all seasons. Among the seasons, highest prevalence was recorded during monsoon (32%) with significantly ($p<0.05$) high oocyst excretion (1.77±0.01) and lowest during summer (19.3%) with low oocyst excretion (0.17±0.006) (Table 2). High infection rate in rainy season could be attributed to increase in rainfall which subsequently leads to high humidity and low temperature. Thus, providing optimum environmental conditions for sporulation of oocyst outside the body of bird which inturn results in favourable dispersion and transmission (Sharma et al., 2015). Low infection rate in summer season could be due to increase in temperature above 30°C favouring dryness which results in desiccation of oocysts. The same observations have been recorded from other states in India(Singh et al., 2021).

In this study, the birds upto 3 months and > 6 months of age revealed a significant difference in the rate of infection as compared to other age groups. Young birds upto 3 months of age were found to be more susceptible to infection than older birds, with a significantly ($p<0.05$) high prevalence percentage of 38.02 (Table 2). The explanation for this observation might be linked to the undeveloped immune system of young birds, which makes them more vulnerable to infections. The above findings are consistent with those of Sharma et al., (2015), Prakashbabu et al., (2017) and Das (2021).

Table 2
Risk factors associated with the prevalence of *Eimeria* spp. infection in backyard poultry of Jammu region of North India
a

	Variable	Total Samples	Positive (%)	95%CI	Chi square	df	p value	Odds ratio	95% CI
Age	Upto 3 months	192	73(38.02)	27.6-47.3	19.40	2	(p<0.0001)	constant	-
	3-6 months	244	71(29)	18.7-39.1				0.68	0.45-1.02
	>6 months	164	27(16.4)	14.6-18.4				0.32	0.19-0.54
Season	Winter	150	35(23.3)	16.4-30.2	6.97	3	(p>0.05)	constant	-
	Rainy	150	48(32)	18.0-20.6				1.54	0.92-2.57
	Post rainy	150	41(27.3)	22.1-41.9				1.23	0.73-2.08
	Summer	150	29(19.3)	18.2-36.5				0.78	0.45-1.37

Based on morphometry, four species of *Eimeria* viz. *Eimeria acervulina, Eimeria maxima, Eimeria necatrix, and Eimeria tenella* were found harbouring backyard poultry of Jammu region, North India with prevalence rates of 16.5%, 3.6%, 21.3%, and 27.6%, respectively, (Table 3). *E. tenella* found to be the most predominant species infecting backyard poultry of Jammu. Sharma et al., 2015, Prakashbabu et al., 2017 and Das 2021 also observed that occurrence of *Eimeria* species varies greatly between geographic regions and poultry production systems. Sharma *et al*, 2013 recorded the overall prevalence of 39.58% in backyard poulty of North India and based on morphology identified five *Eimeria* species viz., *E. tenella, E. necatrix, E. maxima, E. acervulina* and *E. mitis.*

Table 3: Morphometric measurements of various *Eimeria* spp. oocysts among Backyard poultry in Jammu region of North India

SPECIES	MEASUREMENTS (Average ± S.E)		%Prevalence of various species from total no. of oocysts measurements taken out of 1000
	Length (µm)	Width (µm)	
Eimeria acervulina	16.99± 0.52	13.19± 0.40	16.5[b]
Eimeria tenella	27.21± 0.58	17.36± 0.19	27.6[a]
Eimeria maxima	34.26± 0.50	20.86± 0.30	3.6[c]
Eimeria necatrix	14.51± 0.32	12.44± 0.31	21.3[a]

Different superscripts indicate significant difference ($p<0.05$) in prevalence among different Eimeria species based on morphometry

The findings of an ITS-1-based nested PCR test were corroborated by Lew et al. 2003. The presence of four *Eimeria* species in backyard poultry faeces samples was confirmed by PCR amplification (Fig. 2 and Fig. 3). The amplified fragments presented different sizes viz. *E. acervulina* (321 bp), *E. tenella* (278 bp), *E. maxima* (145 bp) and *E. necatrix* (383 bp). Similarly, Kaboudi et al., 2016 from Tunisia recorded the overall rate of coccidiosis in the backyard poultry as 31.8% and using PCR tool of ITS-1 gene and revealed the presence of three *Eimeria* species viz. *E. tenella* (61.5%), *E. maxima* (12%), and *E. acervulina* (1.5%). Mixed *Eimeria* species infection was observed with overall prevalence of 26.5%. Though scanty of literature is present regarding molecular characterization of *Eimeria* species in backyard poultry, most common *Eimeria* species

viz. *E. tenella* and *E. acervulina* are found all throughout the world as have been reported in commercial poultry birds (Kumar *et al*., 2014., Siddiki et al., 2014 and Brown Jordan et al., 2018).

Conclusion

In the Jammu region of North India, backyard birds are raised in a semi-intensive manner and the present study reported prevelance of 25.5 percent for *Eimeria* spp. Infection rate was highest during the monsoon season and lowest during the summer. The rate of oocyst shedding was also been found to be closely linked to the rate of infection. Young birds under the age of three months were observed to be more susceptible to infection than older birds. The most common species infecting backyard poultry in the study area was *Eimeria tenella*, and *Eimeria necatrix*.

Declarations

1. **Funding:** The financial support was provided by Department of Science and Technology, New Delhi under grant number DST No: SEED/TIME/003/2014 and Indian Council of Agricultural Research, New Delhi as part of ICAR National Fellow scheme under grant number F.No.Ag.Edn/27/06/NF/2017-HRD. The authors Rajesh Katoch and Anish Yadav has received research support from NABARD and ICAR, respectively. The authors declare that no funds, grant or other support were received during the preparation of this manuscript.

2. **Conflicts of interests:** The authors have no relevant financial or non-financial interests to disclose.

3. **Ethics approval:** Not applicable.

4. **Consent to participate:** Not applicable.

5. **Consent for publication:** Not applicable.

6. **Availability of data and materials:** The data sets generated during the current study are not publicly available but are available from the corresponding author on reasonable request.

7. **Code availability:** Not applicable.

8. **Author contributions:** All authors have read and approved the manuscript. Aiman Khursheed undertook literature search, material preparation, data collection and analysis. Anish Yadav conceptualised and designed the study, aided in manuscript preparation, editing and review and grant arrangement. Omer Mohi-U-Din Sofi, Anand Kushwaha, Vikas Yadav, Shafiya I. Rafiqi, Rajesh Godara assisted in data collection, analysis and manuscript preparation. Rajesh Katoch reviewed the final manuscript and brought part of the grants.

References

1. Brown Jordan, A., Blake, D., Beard, J., Beharry, A., Serrette, L., Soleyn, A. and Oura, C., 2018. Molecular identification of *Eimeria* species in broiler chickens in Trinidad, West Indies. Veterinary Sciences, 5, 12.

2. Chakrabarti, A., Dey, A. and Barari, S.K., 2014. Backyard poultry farming- a source of better livelihood for rural farmers. Krishisewa. http://www.krishisewa.com/articles/livestock/410backyardpoultryfarminghtml/12.04.2019.
3. Das, M., 2021.Diversity of *Eimeria* species in backyard poultry of subtropical hilly region of Meghalaya, India. Journal of Entomology and Zoology Studies, 9, 360–365.
4. Hadipour, M.M., Olyaie, A., Naderi, M., Azad, F. and Nekouie, O., 2011. Prevalence of *Eimeria* species in scavenging native chickens of Shiraz, Iran. African Journal of Microbiology Research, 5, 3296–3299.
5. Hirani, N. D., Hasnani, J. J., Veer, S., Patel, P. V. and Dhami, A. J., 2011. Epidemiological and clinic-pathological studies in Gujarat. Journal of Veterinary Parasitology, 25(1), 42–45.
6. Kaboudi, K., Umar, S. and Munir, M. T., 2016. Prevalence of coccidiosis in free-range chicken in SidiThabet, Tunisia. Scientifica, https://doi.org/10.1155/2016/7075195.
7. Ketema, E. and Fasil, N., 2019. Study on Prevalence and Associated Risk Factors of Poultry Coccidiosis in and Around Alage at vet College, South Western Ethiopia. Journal of Dairy and Veterinary Sciences, 11(1): JDVS.MS.ID.555805.
8. Kumar, S., Garg, R., Banerjee, P.S., Ram, H., Kundu, K., Kumar, S. and Mandal, M., 2015. Genetic diversity within ITS-1 region of *Eimeria* species infecting chickens of north India. Infection Genetics and Evolution, 36, 262–267.
9. Kumar, S., Garg, R., Moftah, 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(1-2), 24–31.
10. Kundu, K., Kumar, S., Banerjee, P.S. and Garg, R., 2020. Quantification of *Eimeria necatrix*, *E. acervulina* and *E. maxima* genomes in commercial chicken farms by quantitative real time PCR. Journal of Parasitic Diseases, 44, 374–380.
11. Lawal, J. R., Jajere, S. M., Ibrahim, U. I., Geidam, Y. A., Gulani, I. A., Musa, G. and Ibekwe, B. U., 2016. Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria. Veterinary World, 9(6), 653.
12. Lew, A.E., Anderson, G.R., Minchin, C.M., Jeston, P.J. and Jorgensen, W.K., 2003. Inter-and intra-strain variation and PCR detection of the internal transcribed spacer 1 (ITS-1) sequences of Australian isolates of *Eimeria* species from chickens. Veterinary Parasitology, 112, 33–50.
13. Long, P.L. and Joyner, L.P.1984.Problems in identification of species of *Eimeria*. Journal of Protozoology, 31, 535–541.
14. Murugan, M. and Durairajan, R., 2020.Prevalence of gastro intestinal parasites in livestock and poultry in southern part of Kanchipuram district. International Journal of Current Microbiology and Applied Sciences, 9.
15. Omer, S.A., Apio, A., Wronski, T. and Mohammad, O.B., 2011. A new coccidian parasite (*Eimeria* farasanii n. sp.) indicates parasite-host specificity in endemic Farasan gazelle. International Journal of Zoological Research, 7, 85–92.
16. Pohjola, L., Nykäsenoja, S., Kivistö, R., Soveri, T., Huovilainen, A., Hänninen, M.L. and Fredriksson-Ahomaa, M., 2016. Zoonotic public health hazards in backyard chickens. Zoonoses Public Health, 63,

420–430.

17. Prakashbabu, B.C., Thenmozhi, V., Limon, G., Kundu, K., Kumar, S., Garg, R., Clark, E.L., Rao, A.S., Raj, D.G., Raman, M. and Banerjee, P.S., 2017. *Eimeria* species occurrence varies between geographic regions and poultry production systems and may influence parasite genetic diversity. Veterinary Parasitology. 233, 62–72.
18. Sharma, S., Iqbal, A., Azmi, S. and Shah, H. A., 2013.Study of poultry coccidiosis in organized and backyard farms of Jammu region. Veterinary World, 6(8), 467.
19. Sharma, S., Iqbal, A., Azmi, S., Mushtaq, I., Wani, Z.A. and Ahmad, S., 2015. Prevalence of poultry coccidiosis in Jammu region of Jammu & Kashmir State. Journal of Parasitic Diseases, 39: 85–89.
20. Shilpa, S., Anish, Y., Vohra, S., Rajesh, K., Ahamad, D. B. and Sanku, B. 2009. Prevalence of coccidiosis in poultry birds in RS Pura region, Jammu. Veterinary Practitioner, 10(1), 69–70.
21. Siddiki, A. Z., Mina, S., Hasan, M. A., Akbar, M. T., Alam, R., Islam, M. A. and Ayesa, B., 2014. Molecular characterization of Eimeria spp. from chicken by Polymerase Chain Reaction based on species-specific SCAR markers. Journal of Agriculture and Veterinary Science, 7(1): 13–17.
22. Singh, M., Kaur, P., Singla, L. D., Kashyap, N. and Bal, M. S., 2021. Assessment of risk factors associated with prevalence of gastrointestinal parasites in poultry of central plain zone of Punjab, India. Veterinary World. 14(4): 972.
23. Soulsby, E.J.L., 1982. Helminth, Arthropods and Protozoa of domesticated animals. 7th Edition. The English Language Book Society, Baillere Tindal, London, pp. 809.
24. Whitehead, M.L. and Roberts, V., 2014. Backyard poultry: Legislation, zoonoses and disease prevention. Journal of Small Animal Practice, 55, 487–496.
25. Williams, R.B., 2005.Intercurrent-coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34,159–180.

Figures

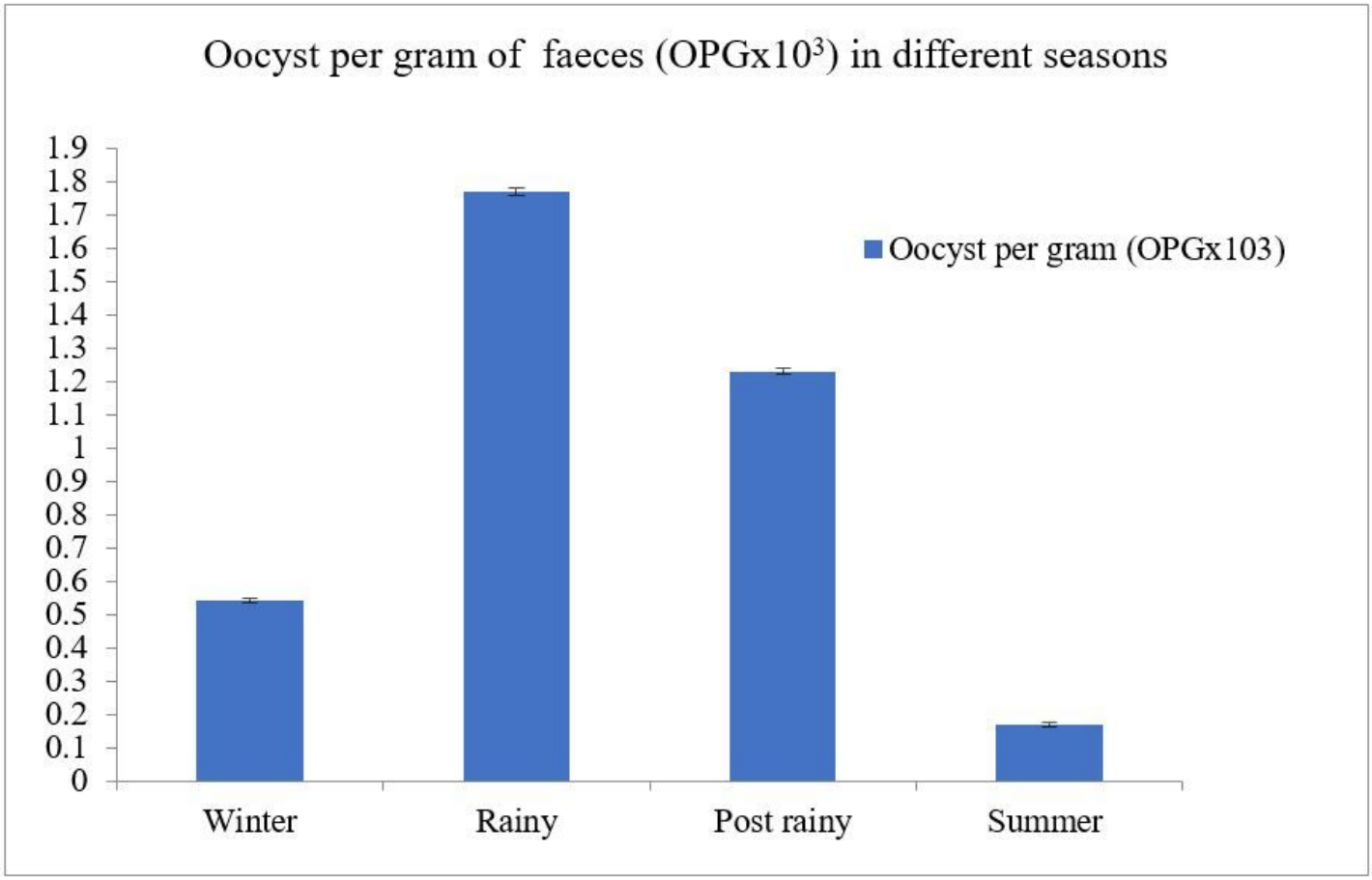

Figure 1

Season wise OPG of *Eimeria* spp. in backyard poultry in Jammu region of North India

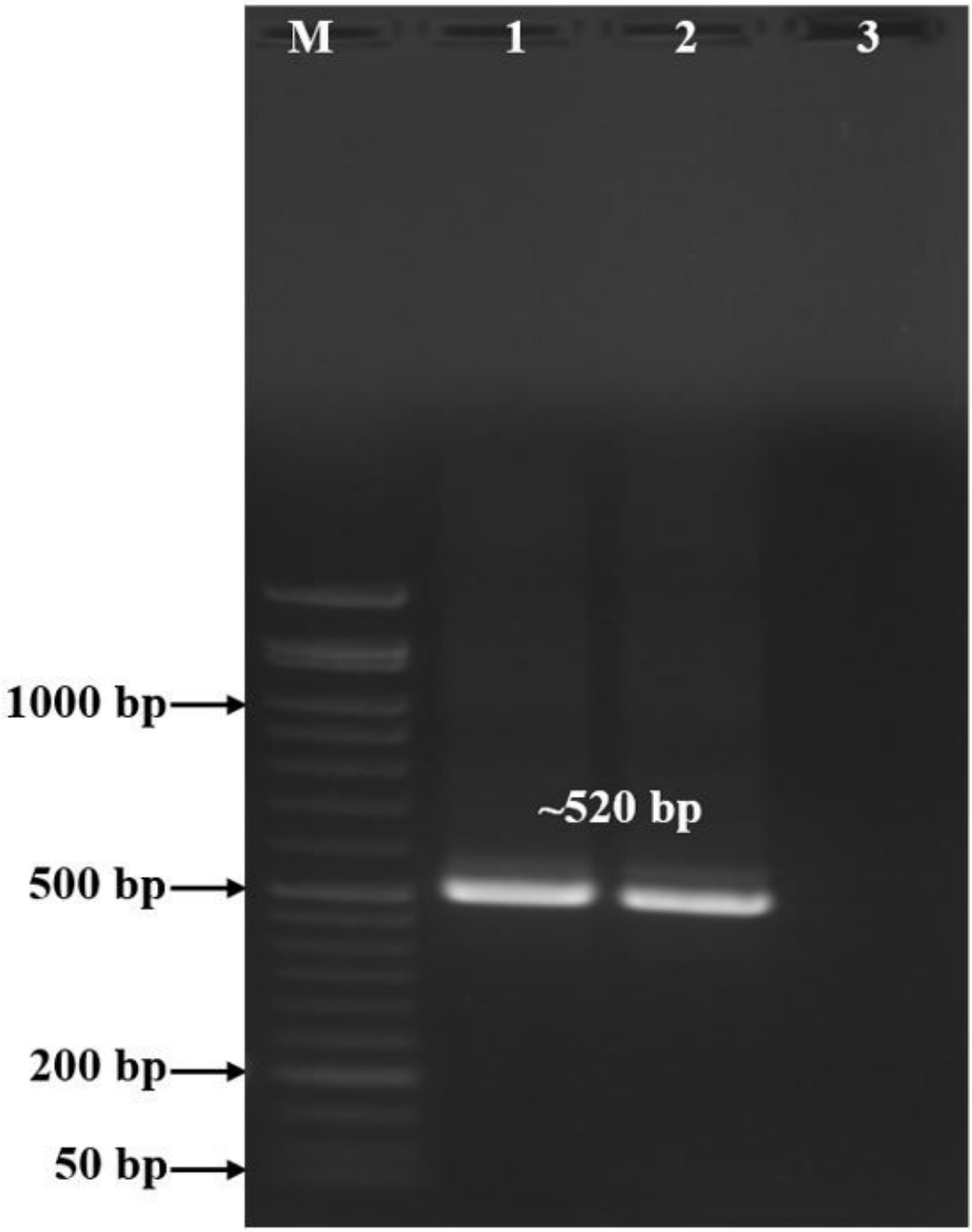

Figure 2

Lane M: 50 bp DNA ladder, Lane 1: Amplified ITS-1 gene of *Eimeria* of chicken (~520 bp), Lane 2: Positive control, Lane 3: No template control

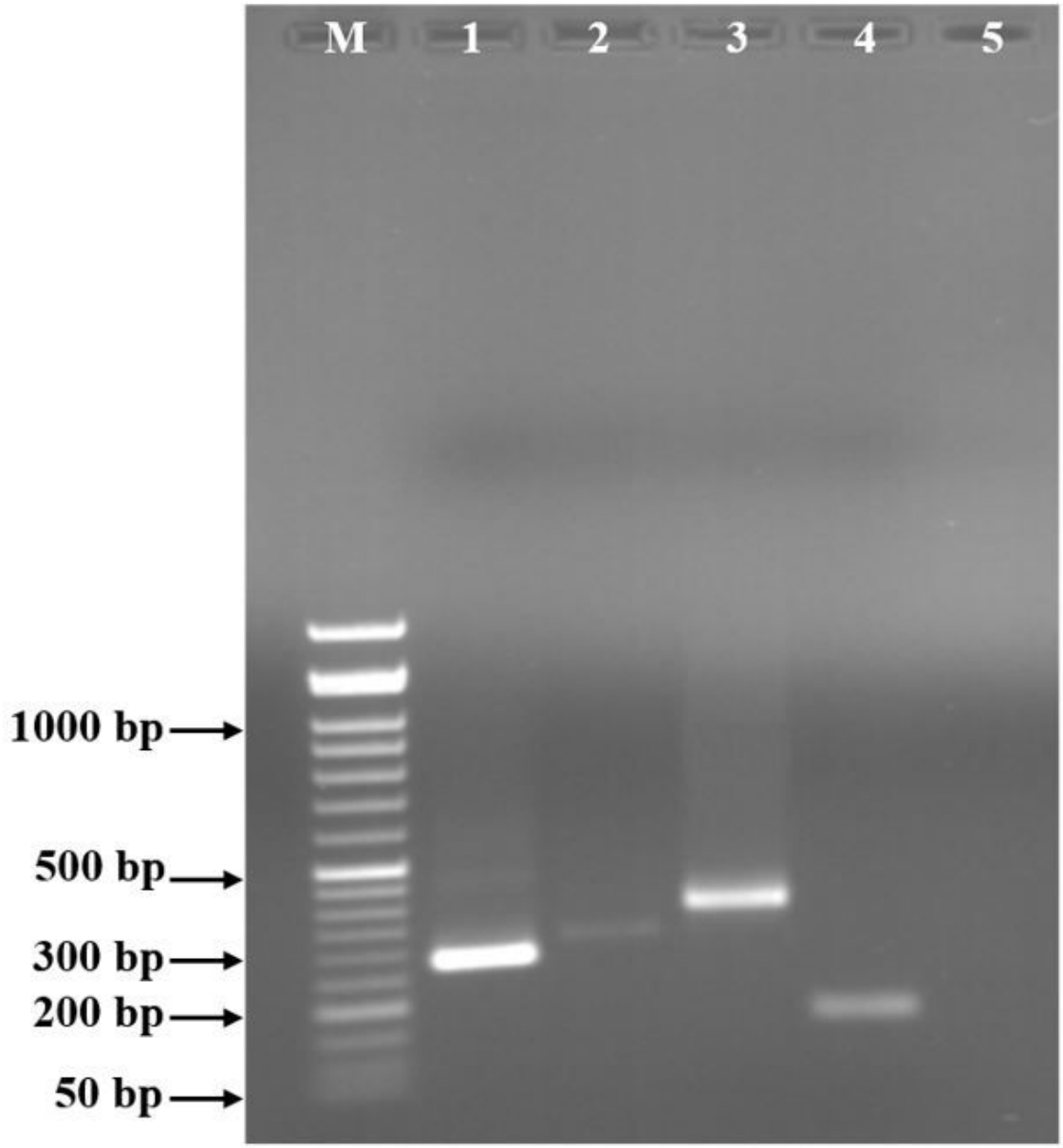

Figure 3

Lane M: 50 bp DNA ladder, Lane 1: Amplified ITS-1 gene of *E. tenella* (~278 bp), *E. acervulina* (~321 bp), *E. necatrix* (~383 bp), *E. maxima* (~145 bp) of chicken, Lane 5: No template control