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Risk and managemental factors associated with the prevalence of *Eimeria* (Coccidia) in cattle in Kashmir division

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Abstract
Present study was carried from December 2020 to November 2021 in the division of Kashmir with an aim to determine the effect of season, sex, age and managemental factors for the occurrence of *Eimeria* in cattle. A total of 1198 faecal samples were examined for the presence of *Eimeria* oocysts and 589 (49.16%) were found to be positive. A significant association was found between prevalence of *Eimeria* and season ($\chi 2$ value = 57.386, $p<0.01$). The highest prevalence was recorded in spring (61.00%) followed by autumn (57.74%), summer (41.10%) and winter (35.25%). The monthly highest prevalence was however observed in September (67.34%) followed by October (62.5%) and May (62.26%) and least in January (28.39%). Calves of age less than one year were harboring higher prevalence (60.10%) than other age groups, 1-3 years (52.17%), 3-5 years (43.09%), more than 5 years (34.86%) and the difference was found to be statistically highly significant **(**$\chi 2$ value = 40.977, $p<0.01$). The effect of sex however on the prevalence was found to be relatively non-significant **(**$\chi 2$ value = 7.275). Among the various managemental factors type of floor, feeding pattern herd size was found to have significant impact on prevalence of *Eimeria* in cattle. Highest mean parasitic load was found during autumn season and in animals of age less than one year.

Keywords: *Eimeria*, prevalence, season, age, sex, cattle, Kashmir

1. Introduction
Bovine coccidiosis is a protozoan disease caused by various species of *Eimeria* (Almeida *et al.*, 2011) [2]. It is cosmopolitan in distribution and usually affects cattle under 1 year old, but is occasionally seen in yearlings and even adults, especially if massive infections are acquired (Soulsby, 1986) [19]. More than 20 *Eimeria* species are identified in cattle worldwide (Daugschies *et al.*, 2005) [7]. *Eimeria bovis* and *Eimeria zuernii* are known to be highly pathogenic eimerian species in cattle worldwide, causing morbidity and even mortality associated with diarrhea, mucus and blood stains (Bangoura *et al.*, 2012, Ernst and Benz, 1986) [4, 10]. The prevalence rates and the intensity of *Eimeria* infection vary between sex, ages and from region to region depending on climatic conditions. A limited study regarding prevalence of *Eimeria* in cattle calves has been carried out in the region around Srinagar city by Pandit (2009) [16]. The present study was therefore aimed at to know the holistic view of prevalence rates and parasitic load of *Eimeria* in cattle associated with different risk factors and managemental factors in the division.

2. Materials and Methodology
2.1 Study area & collection of samples
The study was conducted in all the three regions (North, Central and South) of Kashmir division. A total of 1198 fresh faecal samples from both the sexes of animals of different age groups during one calendar year from December 2020 to November 2021 were collected either by per rectal method or freshly defecated samples from local farmers and dairy farms (both private and government). The samples were collected in mini polythene bags and kept at 4 ºC until further examination. The seasons were divided into four-spring (March, April, and May), summer (June, July and Aug ust), autumn (September, October and November) and winter (December, January and February). The age of animals was divided into four age categories *viz*; < 1 year, 1-3 years, 3-5 years and > 5 years.

2.2 Parasitological Examination
Fecal samples were analyzed using floatation technique with saturated Sodium chloride solution as floatation solution for the presence of oocysts as described by Zajac and Conboy (2006) [20] and Shahardar *et al.* (2020) [17]. 25% of all positive samples were selected to calculate opg in each respective category. Opg was calculated using McMasters (1986) counting chamber technique.

2.3 Development of Questioner
A questionnaire was developed for collecting necessary information from farmers regarding the management and associated risk factors. Information regarding the following determinants was collected through questionnaire. Housing condition (ventilated/Non/ventilated), Floor type (cemented with outlet/Non-cemented without outlet), Type of Feeding system (Ground feeding/Manger feeding), Rearing pattern (Mixed/Single Animal type), Flock/herd size (Larger/smaller), Type of watering system (open/tap). Cattle reared along with sheep and goat in the same house were declared as mixed type of rearing while only cattle rearing in a house as single animal type. Herds having more than fifty animals were designated as larger herds where and herds with less than this as smaller.

2.4 Statistical analysis
All the data collected throughout the investigation period were stored on Microsoft (MS) excel spread sheet program and analysis was done by SPSS Version 16 of SPSS software program. The prevalence per cent was calculated by dividing number of positive animals by total number of animas tested and multiplied by 100. The data generated for prevalence study were statistically analyzed using chi square (χ^2) test/Kruskal wallis (H) test as per method of Snedecor and Cochran (1994) [18] to determine whether risk factors had associated with disease significant or not.

3. Results
3.1 Prevalence associated with Season, Age groups and Sex
A total of 589 samples were found to be positive for *Eimeria* oocysts out of 1198 samples depicting a gross total of 49.16% prevalence rate in the animals observed during the study. Spring showed highest prevalence rate (60.96%) followed by autumn (57.74%), summer (41.10%) and winter (35.25%), (Table 1, Fig 1). The monthly highest prevalence was however observed in September (67.34%) followed by October (62.5%), May (62.26%), April (60.39%), March (58.82%) and least in January (28.39%) (Fig 2). A significant association was found between prevalence of *Eimeria* and season ($\chi 2$ Value = 57.386, $p<0.01$). A similar finding was earlier reported by Pandit (2009) in the region who recorded highest rate of prevalence (90.6%) in the months of March and April (spring) and least in winter (58.3%) while studying cattle calves both in organized and un-organized farms in and around Srinagar city. The Division of Kashmir has Dfb type of climate (humid continental/submediterrean) according to Koppen system of classification with warm summers and more or less moist throughout the year with no characteristic dry season (Bagnouls, 1959) [3]. However the highest amount of rainfall is received during March and April months (mausam.imd.gov.in/srinagar/mcdata/city) due to western disturbances which contribute about 60-65% of total precipitation in Kashmir division (Dimri *et al.* 2015, Mukhtar *et al.* 2022) [9, 15]. The temperature slowly starts accelerating by mid-March. The moisture content and an increase in temperature from March onwards provide favourable conditions for the sporulation of oocysts. By March certain animals also begin to feed in open on sprouting green grass which increases the chances of ingesting the oocysts. The prevalence rate and intensity of infection may therefore increase by April and May after series of sporulation cycles. There is decrease in precipitation from mid-May to mid-July after which amount of precipitation again increases and continues till mid-September though the amount of precipitation is lower than received during March and April (mausam.imd.gov.in/srinagar/mcdata/city) [14]. The mean maximum temperature (29.7 ^{o}C) also reaches its peak in the month of July/August which starts receding after September and reaches its lowest in the month of December and January (mausam.imd.gov.in/srinagar/mcdata/city) [14]. The months of August and September therefore also provide suitable conditions for the maximum sporulation and spread of oocysts. During July, August and September months some animals are also flocked in large numbers in open grazing grounds (high alpine pastures) that increases the chances of transmission of oocysts from one animal to other. The decrease in temperature from November to February may restrict the sporulation of oocysts contributing to lower cases of *Eimeria* during winter. The trend of reporting higher cases of *Eimeria* with an increase in temperature and moisture conditions is supported by various other researchers. Das *et al.* (2015) [6] has recorded highest *Eimeria* infection during post-monsoon (16.29%) followed by monsoon (15%), winter (9.44%) and pre-monsoon (7.49%) season in the dairy cattle of Assam.

The prevalence of infection has also found an age pattern being 60.10% in animals of age < 1 year, 52.17% in 1-3 years, 43.09% in 3-5 years and 34.86% in animals of age > 5 years and the relationship was found to be statistically significant ($\chi 2$ Value = 40.977, $p<0.01$), (Table 1, Fig 3). Das *et al.* (2015) [6]. has also reported an age pattern in the prevalence rate of *Eimeria* infection in cattle being highest in heifer (45.4%), followed by calves (33.2%) and adult (21.4%). Immature immunity can be determining factor for the greater *Eimeria* infection in young animals than old ones. Chibunda *et al.* (1997) [5] and Faber *et al.* (2002) [11] also pointed to the presence of an immature immune system in younger calves resulting in their higher susceptibility to (Eimeriosis) coccidiosis.

In the study, the infection rate was 44.53% in males and 52.41% in females (Table 1, Fig 4). The difference however was statistically non-significant ($\chi 2$ Value = 7.275). Similar to this finding, Dawid *et al.* (2012) [8] did not find a significant association with sex. The absence of a significant correlation between infection and animal sex might suggest that both male and female animals have an almost equal likelihood of being infected with coccidia. Yet, females harbour more coccidia than males this could be attributed to the greater physiological stress experienced by female animals in relation to pregnancies and giving birth (Dawid *et al.* 2012) [8].

Table 1: Prevalence of *Eimeria* in cattle in different Seasons, Age groups and sexes

Risk Factor	variable	Prevalence (%) of males	Prevalence (%) of females	Total Prevalence (%)	χ2Value
Season	Spring	51.58 (65/126)	67.39 (124/310)	60.96 (189/310)	57.386**
	Summer	36.36 (48/132)	44.63 (79/309)	41.10 (127/309)	
	Autumn	54.96 (72/131)	60.23 (103/302)	57.74 (175/302)	
	Winter	33.33 (35/105)	36.62 (63/278)	35.25 (98/278)	
Age group	< 1 year	56.49 (100/177)	63.58 (117/184)	60.11 (217/361)	40.977**
	1-3 years	48.63 (71/146)	55.11 (97/176)	52.17 (168/322)	
	3-5 years	34.82 (39/112)	48.10 (89/185)	43.09 (128/297)	
	> 5 years	16.94 (10/59)	41.50 (66/159)	34.86 (76/218)	
Sex	Males			44.53 (220/494)	7.275 NS
	Females			52.41 (369/704)	
Grand total of samples in each risk factor category				49.16 (589/1198)	

Figures in the parenthesis show total no. of samples positive to total no. of samples observed
** Significant (p<0.01); NS- Non-significant

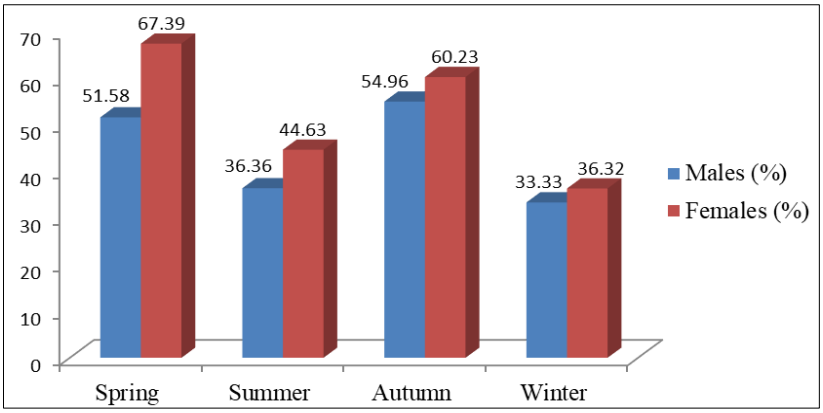

Fig 1: Seasonal prevalence of *Eimeria* in cattle of both the sexes

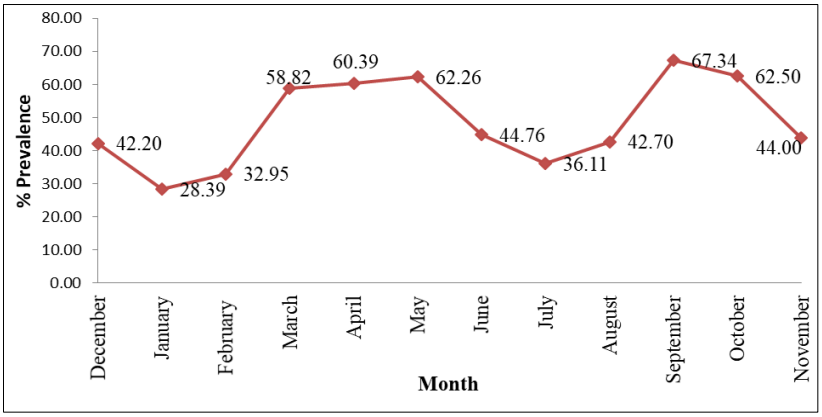

Fig 2: Monthly prevalence of *Eimeria* in cattle from December 2020 to November 2021

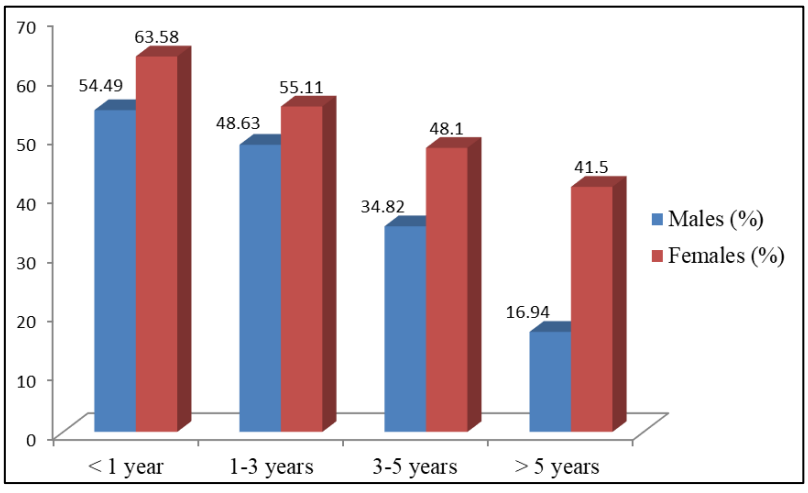

Fig 3: Age-wise prevalence of *Eimeria* in cattle

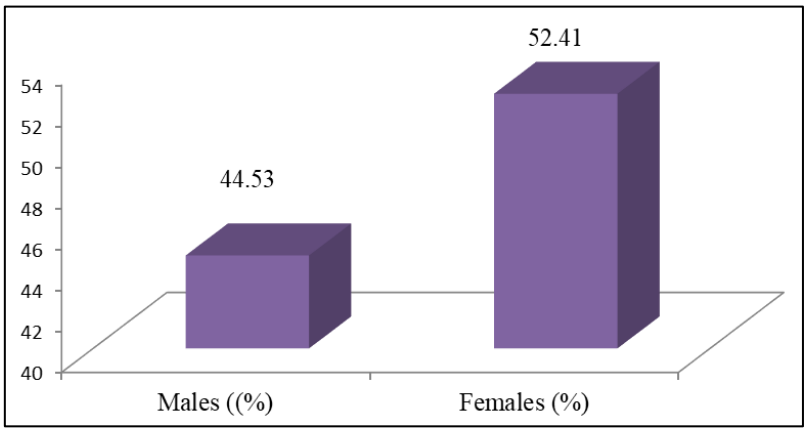

Fig 4: Sex-wise prevalence of *Eimeria* in cattle

3.2 Prevalence associated with Managemental Factors

Among the managemental factors, floor type, feeding pattern and herd size had a significant impact on the prevalence rate of *Eimeria* than other managemental factors (Table 2). Animals reared in ventilated houses were harboring lesser infection (46.84%) than in non-ventilated houses (50.69%). The difference however was statistically non-significant ($\chi 2$ Value = 1.686). A significant association was found between the type of floor and prevalence percentage of *Eimeria* ($\chi 2$ Value = 16.803, $p<0.05$). Houses with cemented floor and with a urine outlet had lower prevalence (42.23%) than with non-cemented floor and without urine outlet (54.16%). Similar type of pattern of incidence was reported by Khan *et al.* (2011) [12] who observed more prevalence in non-cemented floor ($p<0.05$) as compared to cemented floor in small ruminants. The disparity could emanate from the fact that in the non-cemented floor type urine accumulates in the floor and increases the temperature; thereby providing warm and wet environment favourable for the sporulation of the Oocysts, moreover higher prevalence of coccidiosis in non-cemented (mud/mud-brick) type of floor may be associated with more chances of coccidian oocysts to survive in cracks and crevices of mud/ mud-brick type of floor which may be difficult for effective cleanliness of houses (Lawrence, 2011) [13] Feeding pattern also strongly influenced the prevalence rate of *Eimeria*. Animals feeding on ground recorded higher prevalence (53.00%) than animals with manger/trough feeding (42.36%) and the difference was statistically significant ($\chi 2$ Value = 12.179, $p<0.05$). Findings of ground fed animals being at higher risk to *Eimeria* infection than manger fed cattle are in agreement with the findings of Abebe *et al.* (2008) [1] who described a significant association between different feeding systems of calves and the risk of infection with *Eimeria* species. In ground feeding animals the chances of ingesting oocysts are more as compared to manger or trough feeding and thereby increasing the chances of infection. Higher prevalence was recorded in mixed type of rearing (50.55%) than single animal type of rearing (48.03%), the difference however was statistically non-significant ($\chi 2$ Value = 0.66). In mixed type of rearing (cattle reared with goat and sheep) it can be assumed that greater number of animals in small space increase the temperature and humidity thus providing favourable conditions for sporulation of oocysts. Prevalence of coccidiosis was strongly predisposed by the herd size ($\chi 2$ Value = 8.883, $p<0.05$). Higher prevalence was observed in larger herds (56.09%) as compared to smaller ones (46.55). Crowding increases the temperature and humidity thus may favour the sporulation of oocysts and increase the chances of transmission of oocysts since animals are in close contact with each other. Insignificant association ($\chi 2$ Value = 0.87) was found between watering system and *Eimeria* infection being higher in open/stream (50.77%) than tap water system (47.94%). Stream water is the main source of water supply in Kashmir division. Animals that assemble at streams for drinking water may also defecate there and thus polluting the streams with dung carrying oocysts. In many areas of the region, stream water is either directly supplied through taps or after unscientific filtering thus making tap water also a carrier of oocysts.

Table 2: Prevalence of *Eimeria* in cattle under different Managemental conditions

Determinant	Variable	No. of samples observed	No. of samples positive	Prevalence (%)	$\chi 2$Value
Housing condition	ventilated	476	223	46.84	1.686 ^{NS}
	Non- ventilated	722	366	50.69	
Floor type	Cemented with outlet	502	212	42.23	16.803**
	Non cemented without outlet	696	377	54.15	
Feeding pattern	Ground	766	406	53.00	12.179**
	Manger	432	183	42.36	
Rearing pattern	Mixed	536	271	50.55	0.66 ^{NS}
	Single Animal Type	662	318	48.03	
Herd/flock size	Large	328	184	56.09	8.883*
	Small	870	405	46.55	
Watering source	Open/stream	516	262	50.77	0.87 ^{NS}
	Tap	682	327	47.94	

** Significant ($p<0.01$); * Significant ($p<0.05$); NS- Non-significant

3.3 Parasitic Load (OPG+SE)

The season and age also had a great impact on the parasitic load (opg count) of animals as well. Highest mean parasitic load/opg (Table 3, Table 4) was shed during autumn (3590.90±236.12) followed by spring (2812.5±231.78), winter (1375±521.7) and summer (1312.5±408.52). The difference was found to be statistically significant (H-value = 9.99, p<0.05). This could be attributed to favourable climatic conditions in these seasons (as above) which may provide opportunity to complete multiple life cycles in a short span of time thus increasing the intensity of infection. Animals of age group less than one year harbored more mean opg values than animals of higher age group (< 1 year = 4009.09±231.74, 1-3 years = 2357.14±231.43,3-5 years =906±279.53, > 5 years = 736.84±701.78) and the difference was found to be statistically significant (H-value = 10.48, p<0.05). Similarly females relatively had more mean opg values (2538.04±123.67) than males (2327.27±212.43) but the difference was statistically non-significant. The disparity could be attributed to lower immunity in lower age groups and physiological stress in females.

Table 3: Frequency of Mean opg values (Parasitic load)

Risk factor	variables	Opg values				H- value (Kruskal wallis- value)
		< 1000	1000-5000	5000-10000	>10,000	
Season	Spring	30	8	5	5	9.99*
	Summer	24	6	2	0	
	Autumn	21	11	6	6	
	Winter	20	4	1	0	
Age-group	< 1year	26	11	9	9	10.48*
	1-3 years	23	13	4	2	
	3-5 years	28	3	1	0	
	>5 years	18	2	0	0	
Sex	Males	31	10	5	4	0.067^{NS} (χ2Value)
	Females	62	19	9	7	

* Significant (p<0.05); NS- Non-significant

Table 4: Mean opg values + standard error of *Eimeria* oocysts in different Seasons, Age groups and Sex

Mean Opg values + SE of *Eimeria* in cattle in different seasons					Over all mean
Season	Spring	Summer	Autumn	Winter	
Mean	2812.5±231.78	1312.5±408.52	3590.90±236.12	1375±521.7	2272.72±349.53
Mean Opg values + SE of *Eimeria* in cattle in different Age groups					Over all mean
Age group	< 1year	1-3 years	3-5 years	5 years	
Mean	4009.09±231.74	2357.14±231.43	906±279.53	736.84±701.78	2002.26±359.12
Mean Opg values + SE of *Eimeria* in cattle in different Sexes					Over all mean
Sex	Males		Females		
Mean	2327.27±212.43		2538.04±123.67		2432.65±168.05

4. Conclusion

The present study reveals that season, age and some managemental factors have a significant impact on the prevalence and intensity of *Eimeria* infection in the region.

5. Acknowledgements

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6. Declaration of competing interest

The authors have no conflict of interest

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