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Effect of pregnancy and lactation on prevalence and intensity of gastrointestinal nematode infection in cattle

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Abstract
Non-descript (n=96) and crossbred Jersey (n=107) cows from different farms & villages of Khurda, Cuttack and Puri district of Odisha were included in the studies. Sampling from the same animal was done repeatedly for different stage of pregnancy and lactation. A total of 956 faecal samples (461 samples from nondescript cows and 495 from crossbred cows) were collected at different stage of lactation and pregnancy and examined following standard parasitological techniques. Faecal samples found positive for strongyle eggs were subjected to coproculture to identify the various strongyle larvae. Out of 956 faecal samples 332 (34.72%) were found positive for gastrointestinal nematode infection. Out of 114 non-pregnant dry stall fed cows examined, 27 (23.68%) were found positive with a mean EPG (Egg per gram) of 60.08±11.14, whereas out of 108 non-pregnant dry semi ranging cows, 31 (28.70%) samples were found positive with 63.56±14.04 mean EPG. The overall prevalence of gastro-intestinal nematodoses in dry non-pregnant cows was 26.12%. Sixty (50%) out of 120 cows under dry but pregnant category were found positive for gastrointestinal nematodoses. Higher prevalence rate (50.91%) was observed among pregnant free ranging cows than pregnant stall fed cows (49.23%).The intensity of infection in terms of EPG was lowest among both categories (stall fed and semi-ranging) of cows at 5th month and highest during 9th month of pregnancy with mean EPG ranging from 94.21 ±14.76 to 222.22 ± 28.42 in stall fed and semi ranging pregnant cows showing a gradual increase in intensity of infection from 5th month to 9 months of pregnancy. The intensity of infection in dry pregnant cows was significantly higher (p<0.01) than non-pregnant dry cows. Examination of 168 stall fed & 156 Semi ranging lactating cows revealed higher percentage of infection with gastrointestinal nematodes in semi ranging (37.18%) than stall fed cows (35.71%). Egg per gram (EPG) gradually decreased from 1st month of lactation till 7th month. The mean highest EPG in lactating cows (stall fed and semi ranging taken together) was 185.00 ± 27.32 during 1st month of lactation and lowest (43.75 ± 15.19) was recorded in 7th month of lactation. In comparison to non-pregnant dry cows, lactating cows showed significantly higher intensity of gastrointestinal nematode infection (p<0.01). Examination of 148 Stall fed & 142 Semi Ranging lactating Pregnant cows showed that 32.43% stall fed & 33.80% semi ranging cows were infected with gastrointestinal nematodes. Percentage of prevalence was highest in 3rd Trimester (7-9months) in both stall fed and semi ranging cows followed by mid gestation (4-6month) and lowest in 1st Trimester (0-3month). Morphometric and morphological studies revealed three types of nematode eggs which were strongylid, *Strongyloides* spp. and *Trichuris* spp. Coproculture of the faecal samples revealed 3rdstage larvae of three nematode species namely *Haemonchus* spp., *Strongyloides* spp. and *Trichostrongylus* spp.

Keywords: pregnancy, lactation, gastrointestinal nematodes, intensity

Introduction
Endoparasitism in general and gastro-intestinalnematodoses in particular is a major problem in profitable dairy production. Gastrointestinal (GI) nematodes are responsible for inappetence, indigestion, loss of nutrients, fluid and electrolyte which results in malnutrition and, in turn leads to loss of production. In addition, there is also economic loss due to cost of medication, management and labour. The problem due to GI nematode infection, though is worldwide, is more severe in tropics and subtropics due to the hot and humid climate prevailing over these parts which is favourable for development and long viability of the infective 3rd stage larvae in the environment. Stress caused due to hot and humid tropical climate also make the animals more susceptible to GI nematodoses. The stress and susceptibility is further compounded when the animals suffer from physiological stress due to reproduction and production.

There are ample evidences to draw a conclusion that pregnancy and lactation cause immunosuppression. It has been postulated that this suppression in the complex homeostatic immunological equilibrium aids in the survival of the foetus, a uniquely successful natural allograft. However, this immunosuppression is not specific and it appears to extend to a variety of infections. Thus, pregnancy and lactation can have a profound effect on host's immunological responsiveness to a number of bacteria, viruses, protozoa and helminths (Lloyd, 1983) [14].

Influence of pregnancy and lactation has been largely observed and documented in case of ewes and does towards periparturient period resulting in immunosuppression (Chartier *et al*., 1998; Rocha *et al*., 2004; Chaudhry *et al*., 2009; Beasley *et al*., 2010; Notter *et al*., 2017) [4, 20, 5, 3, 15]. The phenomenon is popularly known as periparturient relaxation of immunity. One of the consequences of this relaxation in immunity is increased intensity and fecundity of gastrointestinal nematodes in females. However, information on periparturient relaxation of immunity to parasites with respect to cows is scanty. Despite of availability of many anthelmintic drugs which can be safely used during pregnancy, farmers usually hesitate to deworm their pregnant cows apprehending abortion or teratogenic effects. But this negligence can have serious detrimental effect on postpartum health and production potentiality of cows. This paper presents the influence of pregnancy and lactation on prevalence and intensity of gastrointestinal nematode infection (GIN) in cows in comparison to dry non-pregnant cows.

Materials and Methods

The present investigations were carried out for a period of one year (August 2017 to July 2018) by covering all seasons in a year.

Animals under study

Both non-descript (n = 96) and cross bred cows (n = 107) belonging to different organized farms such as 'ISKON Goshala', 'Instructional Livestock Farm (ILF) of Orissa University of Agriculture & Technology (OUAT), and cows owned by farmers in Khurda, Cuttack and Puri District were included in the present studies. The selected cows were categorized under dry and pregnant, lactating, pregnant and lactating and non-pregnant dry animals. The same individual animal was sampled repeatedly at different stage of pregnancy and lactation. Cows or pregnant heifers, those conceived through Artificial Insemination, were only selected for study on the merit of their recorded breeding and calving history. Animals which were not dewormed within last two months prior to this study were only considered for sampling.

Management and grazing system of animals

In the above-mentioned three districts, viz; Khurda, Cuttackand Purimajority of the privately owned crossbred cows were stall fed with little or no provision of green fodder. Non-descript cows/heifers were reared under semi-intensive system of management. These animals were left for grazing almost every day during day time.

Collection of faecal samples

The faecal samples of individual animal was collected per rectally using disposable glove and kept in individually marked specimen containers/zipped polythene bags. The relevant information regarding region, breed of cattle, age, date of insemination, date of calving, feed, water, managemental practices, lactation yield and deworming history etc. were also recorded before collection of faecal sample. The collected faecal samples were transported to the laboratory in thermo-cool boxes using ice bags and stored in refrigerator (4 °C) for further examination.

Examination of faecal samples

Qualitative examination was conducted to record the gastrointestinal nematodoses in dairy animals on the basis of presence/absence of eggs/larva in the faeces of animals. The faecal samples were qualitatively examined by using standard parasitological techniques of centrifugal sedimentation and centrifugal floatation (Soulsby, 1982) [21].

To obtain accurate information with regard to the intensity of infection, quantitative examination of faecal sample was carried out. Modified McMaster egg counting technique was used to determine the number of eggs per gram (EPG) of faeces (Coles *et al*., 1992) [7].

Three grams of faeces was taken in a mortar and soaked in 42 ml water for few minutes and then emulsified by using mortar and pestle. The emulsion was poured through a tea strainer in to a clean beaker and after stirring, 15 ml of emulsion was centrifuged at 1500 rpm for 5 minutes. The supernatant was gently poured off. The tube was agitated to loosen sediment and floatation solution (Sodium Chloride solution, 1.27specific gravity) was added to make the final volume up to 15 ml. The tube was inverted five to six times and immediately sample/emulsion was withdrawn with a Pasteur's pipette and both thechambers of the McMaster slide were charged by avoiding the trapping of air bubbles in thechambers. The slide was then put on the microscope under low power objective, allowed to stand still for 02 minutes and eggs were counted under low power objectives in two ruled squares containing 0.30 ml of total volume of emulsion. The number of eggs counted in both these squares was multiplied by (dilution factor) to obtain eggs per gram of faeces in the samples.

Copro-culture and identification of larvae

The faecal samples found positive for strongyle eggs were subjected to coprocultureto identify the various strongyle larvae on the basis of gut cells number and morphological details of third stage infective larvae of strongyles as per the identifying morphological features described by Zajac and Conboy (2012) [23].

The faecal samples which were found positive for the ova of Strongyle nematodes were pooled, cultured following the method described by (Zajac and Conboy, 2012; Pal and Sanyal, 2014) [23, 18]. Briefly, about 30-40 grams of faeces were broken up finely, using a large pestle and mortar with spatula. If the lump remained harder a small quantity of water was added to make it desirably soft and if the faeces were too soft in consistency animal charcoal was added to get the required consistency. A large lump of faeces of desirable consistency was taken on a moistened filter paper and spread evenly. The filter paper was then put on a small Petri dish which was kept inverted in another Petri dish of larger diameter containing small quantity of water. Care was taken to avoid contact between water and filter paper containing culture faecal mass. The larger Petri dish was then covered with another Petri dish to minimize evaporative losses and incubated at 25-27 ºC in BOD incubator for 7 days. After

incubation, the culture petri dish was taken out of the incubator. Luke worm water was poured in to the larger petri dish so as to bring water level in contact with the filter paper. The water in larger petri dish contained larvae which migrated from the faecal mass in small petri dish after hatching out from the eggs. Many of the larvae reached the infective third stage which facilitated for the specific identification of nematode. The infective third stage larvae migrating to water in outer Petridish were pipetted out and centrifuged at 1500 rpm for 3 minutes. The supernatant was discarded and the sediment was warmed over the spirit lamp for few seconds to kill and stretch the infective larvae. A drop of sediment was taken on the slide and mixed with a drop of Lugol's iodine. Further the stained larvae were examined under microscope. The micrometry of 3rd stage larvae were performed for total length and extension of tail sheath beyond tip of larvae. The larvae were identified on the basis of key provided by Zajac and Conboy (2012) [23].

Statistical analysis

The difference in the prevalence of gastro intestinal nematode with class, management, stage of pregnancy and stage of lactation were tested by Chi-square test. Analysis of variance was conducted with DMRT to test the significant difference of the mean EPG under various class and management. All the statistical analysis were carried out using frequency procedure and linear model least square analysis procedures using SAS system software, 2011.

Results & Discussion

The intensity and pathogenicity of parasitic infections in general and gastrointestinal nematodoses in particular are very much influenced by factors like breed, age, nutritional status, environmental stress, husbandry practices and physiological state of the animals. Physiological state such as pregnancy, lactation or both put enormous reproductive and productive stress to females due to change in level of some associated hormones. There are reports that due to gastrointestinal parasitism during the peri-partum there is decrease in total milk production in grazing dairy Holstein cows (Perri *et al*., 2011) [19]. In herds, the peripartum cows are considered the main source for the contamination of pastures and subsequent infection of susceptible animals (Barger, 1993) [1]. Studies indicated that during late pregnancy and early lactation there is reduction of the systemic antibody levels (Jeffcoate *et al*., 1990) [11] and reduction of cellular immune response (Huntler *et al*., 2004) [9]. During peripartum there is the "immune relaxation" phenomenon in which the animals are most susceptible, making them vulnerable to parasites (Jansen, 1982) [10]. Periparturient rise in gastrointestinal nematodoses indicated by enhanced EPG (Eggs per gram) have been reported earlier in sheep and goats (Chartier *et al*., 1998; Rocha *et al*., 2004; Chaudhry *et al*., 2009; Beasley *et al*., 2010; Beasley *et al*., 2012; Falzon *et al*., 2013; Chiezey *et al*. 2015; Notter *et al*., 2017) [4, 20, 5, 3, 2, 8, 6, 15].

The present investigation revealed an overall prevalence of gastrointestinal nematodoses in 34.72% of cows. Earlier studies on gastrointestinal nematode infection in cattleconducted by Kashyap *et al*., 1997 [13] in Madhya Pradesh; Pal *et al*., 2001 [17], in Chhatishgarh, Panda *et al*., 2003 [16] in Odisha hadrecorded 34.5%, 32.21% and 34.02% of prevalence respectively, which are in close agreement with present observation.

A total number of 495 faecal samples from stall fed and 461 samples from semi ranging cows were examined during the present investigation which revealed a prevalence rate of 33.74% and 35.79% of gastrointestinal nematode infection respectively (Table 1). No significant variation in the prevalence of gastrointestinal nematode infection among the cows with respect to their managemental condition was observed. This could be attributed to the direct life cycle pattern of all GI nematodes infecting cattle due to which the stall fed as well as semi ranging cows were equally exposed to the infective larvae.

In another investigation carried out on non-pregnant dry cows during the present research the prevalence rate of GI nematode infection in stall fed and semi ranging cows were 23.68% and 28.70% respectively with a respective mean EPG 60.08±11.14 and 63.56±14.04 which were closely similar but, significantly lower than over all prevalence (34.72%). However, no published literature was available to compare the present findings on this category. The non-pregnant dry cows are naturally free from stress which make them comparatively resistant to GI nematode infection.

Higher Prevalence of GI nematodes at rate of 50.91% and 49.23% in semi ranging and stall fed cows respectively belonging to dry pregnant category were observed as compared to dry non pregnant cows. This variation could be attributed to sub optimal immune response of pregnant cows than their non-pregnant counter parts (Aleri *et al*., 2016). However, no data was available with us to compare the present findings.

Intensity of infection in terms of EPG at different stage of pregnancy was lowest among both categories of cows (stall fed and semi-ranging) at 5th month and highest during 9th month of pregnancy with mean EPG ranging from 94.21 ±14.76 to 222.22 ± 28.42 in stall fed and semi ranging pregnant cows showing a gradual increase in intensity of infection from 5th month to 9 months of pregnancy (Table 2). The intensity of infection in dry pregnant cows was significantly higher ($p<0.01$) than non-pregnant dry cows. The gradual increase in intensity of infection in pregnant cows might be due to enhanced level of serum progesterone which has immune suppressant property (Lloyd, 1983) [14]. No published information on similar studies could be available to compare the present observations.

Examination of 168 stall fed & 156 Semi ranging lactating cows revealed higher percentage of infection with gastrointestinal nematodes in semi ranging (37.18%) than stall fed cows (35.71%) which could be attributed to more exposure of semi ranging cows to infective larvae at a period where lactation stress is there. Gradual decline in the intensity of infection expressed in EPG was observed from 1st month of lactation till 7thmonth.The mean highest EPG in lactating cows (stall fed and semi ranging taken together) was 185.00 ± 27.32 during 1st month of lactation and lowest (43.75 ± 15.19) was recorded in 7th month of lactation (Table 4). In comparison to non-pregnant dry cows, lactating cows showed significantly higher intensity of gastrointestinal nematode infection ($p<0.01$). A relative level of prolactin which influence the milk production in dairy cows might be the contributing factor. This is in accordance to the observations reported by Chartier *et al*. (1998) [4] who recorded periparturient rise in fecal egg counts associated with prolactin concentration.

In order to study the prevalence and intensity of gastrointestinal nematode infection in pregnant lactatingcowsfaecal sample of 148 Stall fed & 142 Semi

ranging cows were examined which showed 32.43% stall fed & 33.80% semi ranging cows infected with gastrointestinal nematodes. The higher percentage of prevalence could be attributed to the synergistic effect of both progesterone and prolactin both of which are immuno-suppressant (Lloyd, 1983 and Rocha *et al.*, 2004) [14, 20].

Data obtained on prevalence and intensity of gastrointestinal nematodoses in pregnant cows/heifers were tabulated for different trimester of pregnancy which showed that the percentage of prevalence was highest in 3rd Trimester (7-9months) in both stall fed and semi ranging cows followed by mid gestation (4-6month) and lowest in 1st Trimester (0-3month). Rate of prevalence of gastrointestinal nematode infection in pregnant semi ranging cows in comparison to pregnant stall fed cows were 27.11% vs 26.66%, 35.00% vs 33.87% & 49.37% vs 47.25% during 1st, mid and last trimester of pregnancy respectively. The intensity of infection (mean EPG) in stall fed pregnant cows in 1st, 2nd and 3rd trimester were 58.33±12.86, 103.23±19.13 and 184.62±21.34 respectively. The intensity of infection was varying significantly between 2nd and 3rd trimester of pregnancy among stall fed cows, and the variation in mean EPG of 3rd semester pregnant cows was statistically significant in comparison to stallfed dry-nonpregnant cows (Fig 2). In case of pregnant semi-ranging cows the mean EPG were 63.56±14.04, 119.49±21.79 and 210.13±25.66 in 1st, 2nd and 3rd trimester of pregnancy respectively (Fig 3). The intensity of infection was varying significantly between 2nd and 3rd trimester of pregnancy among semi ranging cows, and the variation in mean EPG of 3rd semester pregnant cows was statistically significant in comparison to semi ranging dry-nonpregnant cows (Table 5 & 6). No published literature and data were available to compare our findings. However, the excess demand of protein for metabolism, lactation, and growth of foetus during mid gestation could be contributing factors.

The nematode eggs detected in the faecal samples of all types of cows (Dry non pregnant, dry pregnant, lactating and pregnant lactating) were subjected to morphometric and morphological studies which revealed three types of nematode eggs which were strongyles, *Strongyloides* sp. and *Trichuris* spp. Coproculture of the faecal samples which were found positive for strongyle eggs revealed 3rd stage larvae of three species of gastrointestinal nematodes namely *Haemonchus* spp., *Strongyloides* spp. and *Trichostrongylus* spp. These findings were inconsonant with earlier reports (Soulsby, 1982; Jithendran and Bhat, 1999; Panda *et al*., 2003; Yadav *et al*., 2008) [21, 12, 16, 22].

Table 1: Prevalence of gastrointestinal nematode infection in cows with respect to the reproductive and productive status

Category of cows	Stall fed cows			Semi ranging cows		
	No. Examined	No. Positive	Percentage of infection	No. Examined	No. Positive	Percentage of infection
Dry Non Pregnant	114	27	23.68	108	31	28.70
Pregnant dry	65	32	49.23	55	28	50.91
Pregnant lactating	148	48	32.43	142	48	33.80
Lactating	168	60	35.71	156	58	37.18
Total	495	167	33.74	461	165	35.79

Table 2: Intensity of infection in cows with respect to the stage of pregnancy

Stage of Pregnancy (in Months)	Mean EPG±SE
1st	94.21±14.76
2nd	87.65±14.84
3rd	80.81±13.49
4th	82.39±14.95
5th	79.45±15.51
6th	130.23±27.25
7th	174.00±29.46
8th	187.72±27.77
9th	222.22±28.42

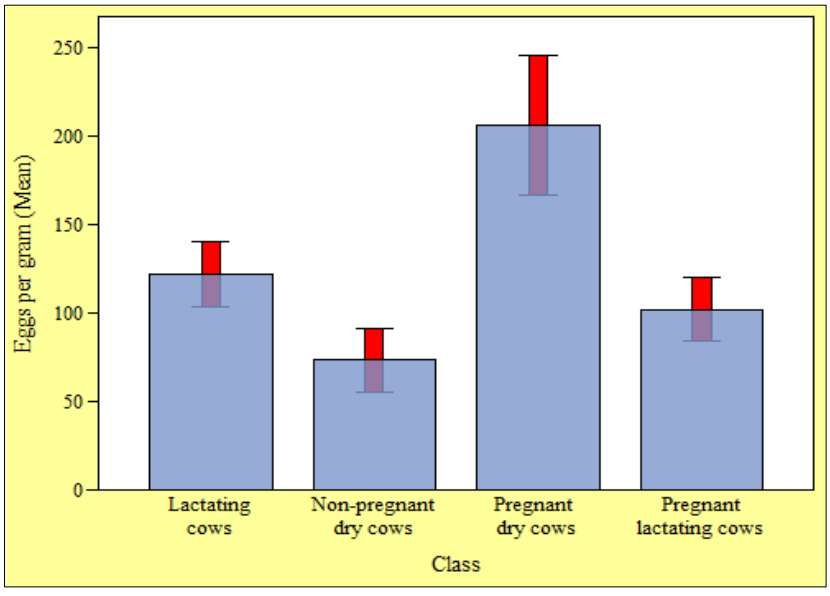

Fig 1: Intensity of GI nematode infection of cows with respect to their productive and reproductive status

Table 3: Intensity of infection of all four class and two managements of animal and their significance

Management	Class	EPG LS Mean ± SE
Stall fed	Dry Non-Pregnant	60.08±15.38^{d}
Sem ranging	Dry Non-Pregnant	86.57±15.80^{dc}
Stall fed	Pregnant dry	197.69±20.37^{ba}
Sem ranging	Pregnant dry	215.45±22.15^{a}
Stall fed	Pregnant lactating	93.58±13.50 ^{dc}
Sem ranging	Pregnant lactating	109.50±13.78^{dc}
Stall fed	Lactating	116.07±12.67^{dc}
Sem ranging	Lactating	126.92±13.15^{bc}

Table 4: Intensity of infection in cows with respect to the length of lactation

Stage of Lactation (in Months)	Mean EPG±SE
1st	185.00±27.32
2nd	157.27±26.06
3rd	129.63±22.80
4th	111.36±23.15
5th	85.56±19.37
6th	70.59±19.25
7th	43.75±15.19

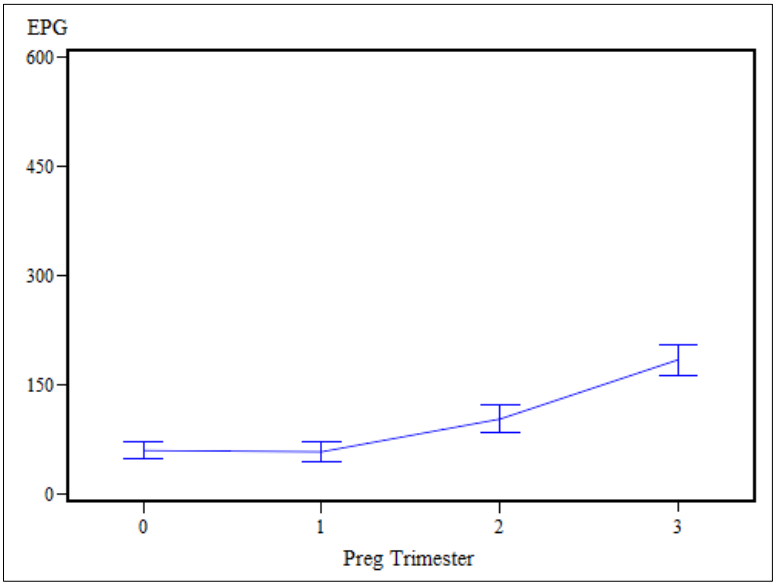

Fig 2: Graph illustrating EPG vs Days of lactation among Dry Non Pregnant and 1st, 2nd and 3rd Trimester of stallfed cows.

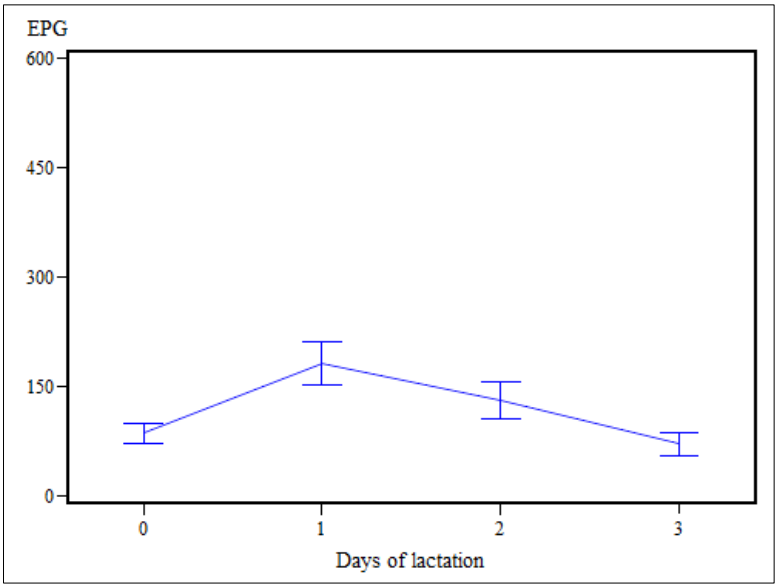

Fig 3: Graph illustrating EPG variation among Dry Non Pregnant and 1-60, 60-120 and above 120 days of lactating semi-ranging cows

Table 5: Significance of variationin EPG among Dry NP cows vs cows in with 1st, 2nd and 3rd Trimester Pregnant cows in SF management

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**No. Examined**	**Trimester**
A	184.62	91	3rd
B	103.23	62	2nd
B			
B	60.09	114	Dry NP
B			
B	58.33	60	1st

Table 6: Significance of variation in EPG among Dry NP cows vs cows in with 1st, 2nd and 3rd Trimester Pregnant cows in SR management

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean EPG**	**No. Examined**	**Trimester**
A	210.13	79	3rd
B	119.49	59	**2nd**
B			
B	86.57	108	Dry NP
B			
B	63.56	59	1st

Prevalence and intensity of GI nematodes with respect to the length of lactation

Conclusion

Intensity of infection with gastro-intestinal nematodes increased with advancement of pregnancy and decreased with advancement in days of lactation. More intensity of GI nematodoses in cows was observed during the advanced stage of pregnancy and early part of lactation in both stall-fed and semi-ranging cows. Cows should be dewormed with safe anthelmintic before last month of pregnancy or parturition and again within one month after parturition/ during 1st month of lactation. Pregnant cows/heifers should not be allowed to graze on community pasture during last trimester of pregnancy and 1st month of lactation in order to reduce the contamination of pasture.

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