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EPG and Pasture Larval Count of Gastrointestinal Nematodes are Strongly Influenced by Weather Parameters: Empirical Evidence from Parbhani Marathwada

T. S. Zade [a], S.T. Kalwaghe [a*], B.W. Narladkar [a], G.M. Chigure [a] and G. N. Bhangale [a]

[a] Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, MAFSU, Parbhani, 431402, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: the present study was aimed to develop a bioclimato of gastrointestinal nematodes of cattle in Marathwada region of Maharashtra.
Study Design: A standard protocol as per published reports was followed for recording of prevalence of GI nematodes from cattle.
Place and Duration of Study: The study was conducted at livestock instructional farm of College of Veterinary and Animal Sciences Parbhani during the period February 2022 to January, 2023.
Methodology: In all total 253 faecal samples were collected from calves and adult cattle belonging

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**Corresponding author: E-mail: srikantkalwaghe@mafsu.in;*

to RK instructional farm, COVAS, Parbhani. Sample collection and processing was done by following standard parasitological procedures and only fresh fecal droppings were used for the laboratory investigation.

Results: The study conducted on availability of larvae on pasture revealed the overall prevalence of 61 percent (87 pasture were positive out of 143 examined). The PLC analysis showed the highest distribution of larvae on pasture during winter season i.e. 57.81 %, followed by monsoon season i.e. 57.81 % and 00.0% during summer season. The Pasture larval count observed during different seasons was 23.83, 33.07 and 0.00 per 100 gm, respectively. The abstract information drawn from the bioclimatos plotted for 2000-2021 and its validation with real time data of 2022 (Feb 2022-Jan 2023) was a) Suitable months for survival of Heamonchus and Oesophagostomum infective larvae on the pasture of the grazing land in year 2022-2023 were Jan - March and June – September and for Trichostrongylus Jan – March and October 2022- January 2023 and b)The climatic data and Bioclimato were plotted from year 2000-2021 and 2022+January 2023 showed the correlation between EPG and PLC.

Conclusion: The bioclimatos can be developed and utilized according the regional agro-climatic variations and be useful for devising controlmeasures against gastrointestinal nematodes of livestock.

Keywords: Bioclimato; cattle; nematodes; Maharashtra.

1. INTRODUCTION

The epidemiology of gastrointestinal parasites in livestock is widely recognized to be influenced by regional and local environmental factors, as well as grazing patterns and management strategies. Detailed mapping of parasitic fauna in different livestock species during various agro-climatic seasons provides crucial insights that can inform the development of additional management techniques [1]. This study, building upon the aforementioned premise, also investigates the seasonal variations in gastrointestinal parasitism specifically in cattle.

Assessing the presence of infective larvae in pastures offers valuable insights into the level of infection risk faced by animals grazing in those areas. The enumeration of infective larvae in pasture herbage is increasingly utilized for diagnosing and predicting parasitic diseases in farm animals [2]. Utilizing pasture larval counts (PLC) and environmental data to construct bio-climatos aids in determining optimal modeling approaches and making precise forecasts [3]. Given that contaminated pastures serve as a source of infection, understanding the prevalence of infective larvae in pastures is crucial for implementing appropriate grazing practices, highlighting the significance of pasture management in nematode parasite control initiatives [4]. As posited by these scholars, effective pasture management not only benefits productivity by enhancing weight gain, feed conversion, milk production, reproductive performance, carcass quality, immunological status, and reducing morbidity and mortality.

The primary consideration in selecting a diagnostic technique for parasites is the reliability of the information provided to address the issue at hand. However, the importance of the free-living stages of gastrointestinal nematodes (e.g., eggs, developing larvae, L3) is often overlooked when devising effective diagnostic methods for parasitic infections [5]. In such contexts, pasture larval counts (PLC) emerge as one of the most effective approaches, aiming to quantitatively and qualitatively identify nematode larval species to support investigations into parasite population dynamics. PLC also enables the detection of monthly and seasonal fluctuations in pasture infectivity, assigning a risk score for parasite exposure to grazing animals [6, 7]. Consequently, the current research was designed to estimate the burden of pasture larvae and examine its relationship with climatic factors in the Parbhani region.

2. MATERIALS AND METHODS

The current study was conducted with the aim of investigating the gastrointestinal nematodes infecting livestock in the Parbhani region. The study was carried out at the Department of Veterinary Parasitology, within the College of Veterinary and Animal Sciences, (MAFSU) Parbhani. Located in the Marathwada division of Maharashtra state, Parbhani city is positioned at 190 23’ North latitude 760 79’ East longitudes, with an altitude of 454 m above mean sea level. Parbhani falls within a rain shadow area, receiving over 80% of its rainfall from the south-west monsoon. Agro-climatically, this region is classified within the assured rainfall zone of Maharashtra state, with an average annual precipitation of approximately 938.7 mm, predominantly during the south-western monsoon period between June and September. Winter precipitation is irregular and minimal. The average maximum temperature ranges from 28.7-42.1oC (33.7oC), while the average minimum temperature varies between 9.0oC to 26.5oC (18.5oC) during winter and summer respectively. Consequently, Parbhani experiences cold winters and hot, arid summers. The area encounters Relative Humidity, RH-I 41-87% (71%) and RH-II (98%). The seasons in Parbhani are categorized as Southwest monsoon season (June to September), Post-monsoon season (October to January), and summer season (February to May) [8].

The research was carried out from February 2022-January 2023. The host animals included in the study were Red Kandhari Cattle of Marathwada region. The animals were selected from Red Kandhari Research and Instructional Farm of COVAS Parbhani.

Following seasons were considered for the present study. Seasons were as per months and weeks of a calendar according to standard meteorological norms designed for Marathwada region (VNMKV Vision- 2020, 1998). Seasons defined as per World Meteorological Organization(WMO) norms for the Marathwada region are monsoon, winter and summer.

Monsoon: 4th June- 4th November (23rd - 44th week)

Winter : 5th November-4th March (45th - 9th week)

Summer : 5th March-3rd June (10th -22nd week)

Six adult cattle and six calves were selected randomly from the ILFC, College of Veterinary and Animal Sciences, Parbhani. These animals are not dewormed throughout experimental period. However, they were treated for other illness and health fitness. EPG of the faecal samples from the selected animals were estimated twice in a month by Stoll's Egg counting technique as described earlier [2]. Pasture larval count (PLC) were estimated twice or thrice once in a month throughout experimental period by following standard procedure [9]. The recovered larvae from the herbage samples were killed and stained with Lugol's iodine solution for few minutes . Then a drop containing two-three larvae was taken on a glass slide with the help of pasture pipette and covered with a coverslip. It was examined under a compound microscope fixed with an occulomicrometer in the eyepiece. The Total Length of the larvae (TL) and Sheath Tail Extension (STE) was measured by occulomicrometer. The readings were subsequently calculated and converted into micrometres. The obtained lengths of TL and STE of larvae were matched according to the standard measurements of the infective larvae of gastrointestinal nematodes of sheep [10]. The data obtained from various parameters was analyzed by employing simple correlation, multiple regression and completely randomized design using computer application, WASP version 2.0 (www.ccari.res.in)

3. RESULTS AND DISCUSSION

3.1 Prevalence of Gastro Intestinal Nematodes in Cattle at Parbhani Region

In all total 253 faecal samples were collected from calves and adult cattle belonging to RK instructional farm, College of Veterinary and Animal Sciences, Parbhani. Out of 253 samples examined 75 (30.00%) were positive for different species of GI nematode infection. At par prevalence was reported from Uttar Pradesh [11], from Gujrat [1] and from Meghalaya [12] states of India. Few scientists reported very less prevalence of GI parasitism 12.50 % [13]; whereas very high prevalence of 75% by some of the researchers [14]. Comparison of prevalence from one geoic region with other region is not justifiable because

1. The development, growth, survival and transmission of infective larval stages on pasture is greatly influenced by rainfall, temperature, humidity, soil moisture and other conditions of a particular region. All these vary from place to place, country to country and year to year.
2. Similarly prevalence also depends on host factors such as breed and animal husbandry practices, which all differ at different locations.

During three different seasons the prevalence of GI nematode infections was 38.84%, during monsoon, 20.00% during winter and 22.22%

during summer season.Study recorded the more incidences of Strongyles and Strongyloides spp. It is well known fact thatduring rainy season the survival of infective stages on pasture is for longer period of time,which facilitates uptake of infective stages by host and it results in increased prevalence. During winter season due to presence of infective stages on pasture nearly same or to certain extent lesser infection occur. In summer season, it is devoid of optimum geoclimatic conditions i.e higher temperature and relative humidity and minimum level of moisture are available, as a result nil or lowest level of infection occur.Seasonal prevalence for GI parasitism is also reported by several researchers [11, 13, 15].

The mean EPG count recorded during the monsoon season peaked at 90.09, showed a moderate level during the winter season at 48.33, and dropped to the lowest point at 30.56 during the summer season. Various workers have documented the EPG data from the region as outlined in Table 1.

3.2 Prevalence of Pasture nematode larvae in cattle in the Parbhani

The research unveiled an overall prevalence of 61 percent, with 87 out of 143 pasture samples testing positive. The analysis of PLC indicated an equal and highest distribution of larvae in the pasture during both the winter and monsoon seasons, at 57.81 percent. Minimal to no larvae were detected during the summer months.

Previous reports of PLC levels in India originated from Assam and Sikkim [16, 17]. Both studies noted year-round PLC presence with seasonal fluctuations. In contrast, the current research in Maharashtra identified PLC levels exclusively during the monsoon and winter seasons. This discrepancy in PLC levels could be linked to geoical disparities. The study, carried out in 2022, experienced the highest rainfall, resulting in a greater presence of larvae on the pasture. This heightened exposure during the monsoon, followed by winter, likely increased the risk of infection in animals [18]. The monsoon commenced in July, concluded in September, with sporadic rains continuing until December, peaking in August. These conditions likely favored the development and survival of pre-parasitic stages, leading to a rise in infective larvae on the pasture during the monsoon and subsequent months [19].

3.3 Pasture Larval Count (PLC)

The Pasture larval count observed during different seasons was 23.83, 33.07 and 0.00 per 100 gm of pasture during monsoon, winter and summer season, respectively. Except a single study from Sikkim [17]; no much work from India is available for comparison and discussion.

3.4 Identification of the Larvae of Helminth Parasites Recovered from the Pasture

In the present study various species of gastrointestinal nematodes found during the morphometric examination of larvae. The larvae recovered from the pasture were identified as *Bunostomum phlebotomum, Bunostomum spp, Haemonchus contortus*, *Trichostrongylus spp*., *Oesophagostomum radiatum*, *Cooperia punctata, Ostertegia ostertegi* and *Strongyloides papillosus* based on morphometric observations and its equating it with the standard morphometric observations.

Among various species *Oesophagostomum radiatum* was the most prevalent as compared to other followed by *Bunostomum phlebotomum* and *Haemonchus contortus* etc. Mixed infection other than GI nematodes was also encountered such as *Schistosoma* infection in calves.

3.5 EPG and its Correlation with Weather Parameters

The EPG levels was found to be significantly correlated with RH (E), while, with other parameters like Tmin, Tmax, BSS, EVP, TRF are negatively non-significantly correlated and RH-M positively non-significantly correlated. In nutshell humidity levels are positively correlated while rests of the parameters are having negative (inverse) relationship. Positive correlation of RH factor indicates that in the tropical region like Parbhani where abundant quantum of heat (temperature) is available, humidity level matters.

3.6 PLC and its Correlation with Weather Parameters

As like EPG correlations, more or less similar pattern of correlations between PLC levels and weather parameters was observed. The only difference noted was that, RH-E has also shown negative correlation, though its magnitude is non-significant. It indicates that in the region where grazing of animals is done on pasture, humidity levels in the morning has more impact. It helps in crawling and transfer of larvae on pasture/grass blades. EPG levels and PLC levels showed negative correlation with each other.

Regression analysis value R^2(square)= 82.95%indicated the role of environmental factors to the extent of 82.9% and remaining almost 17% remains unexplained or factors could not be predicted. The regression analysis indicates RH(E), TRF, BSS, EVP are negatively correlated and showing negative impact on EPG count while Tmax, Tmin , RH(M) are positively correlated having positive impact on EPG levels.

Table 1. Prevalence and Eggs Per Gram (EPG) for nematode infections in Cattle at Parbhani region

Season	Prevalence of GI parasitism		%	EPGvalues		
	(TE)	(TP)		No of observations	Mean ± SE	Range
Monsoon	121	47	38.84	47	90.09 a± 11.934	0-500
Winter	60	12	20.00	12	48.33b±13.96	0-400
Summer	72	16	22.22	16	30.56b±8.07	0-400
	253	75	30.00		HS	
Stat				**CD Value**	CD(0.01) = 47.735 CD(0.05) = 36.320	

TP: Total Positive; TE: Total Examined: HS: Highly Significant

Table 2. Prevalence of Pasture Larval Count (PLC) for nematodes of cattle at Parbhani region

Season	Prevalence of GI parasitism			PLC values		
	TE	TP	%	No of observations	Mean ± SE	Range
Monsoon	64	37	57.81	37	23.83a± 3.575	0-105
Winter	57	50	87.71	50	33.07a± 4.034	0-83
Summer	22	0	0.00	0	0.00b	0.00
	143	87	61.00		S	
				CD Value	CD(0.01) = 21.115 CD(0.05) = 16.066	

TP: Total Positive; TE: Total Examined: S: Significant

Table 3. Correlation matrix for EPG, PLC and weather parameters of Parbhani region

Correlation Matrix									
-	**T_{min}(℃)**	**T_{max}(℃)**	**RH-M(%)**	**RH-E(%)**	**BSS(Hrs.)**	**EVP(mm)**	**TRF(mm)**	**EPG**	**PLC**
T_{min}(℃)	1.000								
T_{max}(℃)	0.116	1.000							
RH-M(%)	-0.890	-0.070	1.000						
RH-E(%)	-0.662	0.374	0.656	1.000					
BSS(Hrs.)	-0.468	0.486	0.521	0.622	1.000				
EVP(mm)	-0.473	0.477	0.526	0.615	1.000	1.000			
TRF(mm)	0.938	0.191	-0.926	-0.640	-0.515	-0.520	1.000		
EPG	-0.544	-0.087	0.435	0.705	-0.033	-0.041	-0.457	1.000	
PLC	-0.374	-0.386	0.443	-0.002	-0.128	-0.120	-0.475	-0.008	1.000

Table 4. Student T-test for EPG, PLC and weather parameters of Parbhani region

Variables Tested	T Value	T Table	Significance at 5%
Tmax -RH(M)	5.865	2.262	Significant
Tmax -RH(E)	2.651	2.262	Significant
Tmax -EVP	8.097	2.262	Significant
RH(M) -RH(E)	2.605	2.262	Significant
RH(M) -EVP	7.36	2.262	Significant
RH(E) -TRF	2.384	2.262	Significant
RH(E) -BSS	2.34	2.262	Significant
RH(E) -EVP	2.499	2.262	Significant
RH(E) -EPG	2.98	2.262	Significant
TRF -BSS	190.376	2.262	Significant
Tmax -Tmin	0.349	2.262	Non Significant
Tmax -TRF	1.591	2.262	Non Significant
Tmax -BSS	1.61	2.262	Non Significant
Tmax -EPG	1.945	2.262	Non Significant
Tmax -PLC	1.209	2.262	Non Significant
Tmin -RH(M)	0.211	2.262	Non Significant
Tmin -RH(E)	1.211	2.262	Non Significant
Tmin -TRF	1.666	2.262	Non Significant
Tmin -BSS	1.63	2.262	Non Significant
Tmin -EVP	0.583	2.262	Non Significant
Tmin -EPG	0.262	2.262	Non Significant
Tmin -PLC	1.257	2.262	Non Significant
RH(M) -TRF	1.832	2.262	Non Significant
RH(M) -BSS	1.857	2.262	Non Significant
RH(M) -EPG	1.45	2.262	Non Significant
RH(M) -PLC	1.481	2.262	Non Significant
RH(E) -PLC	0.005	2.262	Non Significant
TRF -EVP	1.802	2.262	Non Significant
TRF -EPG	0.1	2.262	Non Significant
TRF -PLC	0.389	2.262	Non Significant
BSS -EVP	1.829	2.262	Non Significant
BSS -EPG	0.122	2.262	Non Significant

In another set of analysis PLC was taken as a dependent factor and weather parameters and EPG were taken as independent factors. here the regression analysis value *i.e* R^2(square)=88.4 % indicates the environmental factors has played role to the extent of 88.4 % and remaining almost 11.6 % remains unexplained or could not be predicted. The regression analysis also indicated that RH-M and BSS are negatively correlated and shows negative impact on PLC count while Tmax, Tmin, RH(E), EVP and TRF are positively correlated with PLC and has got positive impact on PLC levels.

It is apparent from the regression model presented above that the Population of Lactating Cows (PLC) exerts a positive influence on Egg Per Gram (EPG), indicating that an increase in PLC results in a corresponding increase in EPG count. It is noteworthy that Total Rainfall (TRF) demonstrates a negative effect, which could possibly be attributed to the elevated precipitation levels experienced in the year 2022 leading to the displacement and reduction of larvae from the pasture, consequently reducing infections due to decreased consumption of grass from the pasture with high water content.

It is widely recognized that the prevailing weather and climatic conditions in a specific geoical area significantly impact the proliferation of helminthic infections in ruminants [3]. Various climatic elements including temperature, rainfall, humidity, wind patterns, speed, sunlight intensity, and duration play pivotal roles in determining the prevalence of helminthic infections. Research conducted by scholars worldwide has convincingly demonstrated that the development, survival, transmission, and presence of parasitic stages of strongyle nematodes in ruminants on pasture are intricately influenced by temperature and moisture [20, 21].

Upon emerging from eggs laid by parasites, the initial stage larvae (L.) engage in feeding on bacteria present in fecal matter, before progressing to the second stage where they continue their bacterial diet. Subsequently, they molt into ensheathed infective third stage larvae (L3). These transformations occur naturally in grazing areas, contingent upon factors such as temperature, atmospheric oxygen levels, and sufficient moisture. Consequently, in specific tropical regions, the primary determinant shaping the life cycle of helminth parasites is rainfall. The L3 larvae transition from feces to vegetation, where they linger until either being consumed by a potential host or perishing. This exogenous phase of the life cycle encompasses two distinct processes: the development of infective larvae and the transmission of said larvae onto grass blades to heighten the probability of infecting the definitive host [3]. Environmental conditions conducive to one process may not be beneficial for the other. Typically, the optimal temperature for larval

Table 5. Regression analysis – EPG as dependent factor and all environmental factors and PLC as independent factors

Independent Variables	Average	Reg. coefficients (b)	Standard Error(SE(b))	T Test	T table (0.05)
T_{min} (℃)	32.936	0.530	nan	nan	4.303
T_{max} (℃)	19.291	0.065	nan	nan	4.303
RH-(M) (%)	19.291	0.000	nan	nan	4.303
RH-(E) (%)	78.818	-39.894	nan	nan	4.303
BSS(Hrs.)	69.636	-38.047	nan	nan	4.303
EVP (mm)	98.064	-521.110	nan	nan	4.303
TRF (mm)	98.727	-522.226	nan	nan	4.303
PLC	74.996	93.514	nan	nan	4.303

Intercept (a) = 148.171
Coefficient of determination (R Square) = 82.95 %
Multiple Correlation Coefficient (R) = 0.977
Standard Error = 26.541
The resultant equation for regression model derived as –
EPG = 148.171+(-4.073) **x** *Tmin+(-2.647)* **x** *Tmax+(0.479)* **x** *RH-M+(1.759)* **x** *RH-E+(6.915)* **x** *TRF+(-7.204)* **x** *BSS+(-0.958)* **x** *EVP+(-0.251)* **x** *PLC+26.541*

Table 6. Regression analysis –PLC as dependent factor and all environmental factors and EPG as independent factors

Independent Variables	Average	Reg. coefficients (b)	Standard Error(SE(b))	T Test	T table (0.05)
T_{min}(℃)	32.936	13.282	12.329	1.077	4.303
T_{max}(℃)	19.291	0.651	6.429	0.101	4.303
RH-(M) (%)	78.818	-6.239	5.005	-1.246	4.303
RH-(E) (%)	69.636	12.671	5.667	2.236	4.303
BSS(Hrs.)	98.064	-86.766	50.763	-1.709	4.303
EVP(mm)	98.727	86.369	50.861	1.698	4.303
TRF(mm)	6.565	2.325	15.942	0.146	4.303
PLC	74.996	-0.561	0.979	-0.573	4.303

Intercept (a) = -797.320
Coefficient of determination (R Square) = 88.4 %
Multiple Correlation Coefficient (R) = 0.940
Standard Error = 39.662
The resultant equation for regression model of PLC is derived as **–**
PLC = -797.320 +(13.282) **x** *Tmin+(0.651)* **x** *Tmax+(-6.239)* **x** *RH-M+(12.671)* **x** *RH-E+(-86.766)* **x** *TRF+(86.369)* **x** *BSS+(2.325)* **x** *EVP+(-0.561)* **x** *EPG+39.662*

development exceeds that required for survival and transmission. By considering the grazing system alongside the larvae's developmental rate, crucial insights can be gleaned to anticipate infection likelihood, pinpoint peak periods, and consequently devise an effective deworming regimen.

4. CONCLUSION

From results of the current study, it is concluded that the suitable months for survival of *Heamonchus* and *Oesophagostomum* infective larvae on the pasture of the grazing land in year 2022-2023 were Jan - March and June – September and for *Trichostrongylus* Jan – March and October 2022- January 2023. Therefore the deworming schedule should be formulated in this region accordingly.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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