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Dr. Raghavendra SV
Department of Veterinary Pathology, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Dr. Anand Kumar A
Associate Dean, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Dr. Amaravathi P
SLDL, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Dr. Rayulu VC
Department of Veterinary Parasitology, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Dr. Anusha V
Veterinary Assistant Surgeon, Komarole, Prakasam district, Andhra Pradesh, India

Correspondence
Dr. Raghavendra SV
Department of Veterinary Pathology, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Faecal examination and haemato biochemical changes of ailing lambs in Rayalaseema region of Andhra Pradesh

Dr. Raghavendra SV, Dr. Anand Kumar A, Dr. Amaravathi P, Dr. Chengalva Rayulu V and Dr. Anusha V

Abstract
Out of 100 faecal samples collected from ailing lambs, 68 samples were positive for different parasitic ova and oocysts. It was maximum in the age group of 4-6 months. Various parasitic ova identified were eggs of Strongyle, *Moniezia* sp, *Schistosoma* sp, *Haemonchus* sp, *Paramphistomum* sp, *Trichuris* sp and oocysts of *Coccidia* sp. Among 102 blood samples collected from ailing lambs decreased PCV, Hb, TEC, MCHC, neutrophils, monocytes, eosinophils and increased TLC, MCV, MCH, lymphocytes were observed when compared with apparently healthy group (n=10). Out of 50 serum samples from ailing lambs screened, increased TP, albumin, globulin, A/G ratio, creatinine and decreased calcium, ALT were observed when compared with apparently healthy group (n=10).

Keywords: Ailing lambs, faecal, ova, blood, serum

Introduction
In four districts of Rayalaseema region, lamb population was more in Anantapur district (1.240 million) followed by Kurnool (0.457 million), Kadapa (0.457 million) and Chittoor (0.424 million) (19th Livestock Census, India 2012) [1]. Sheep are widely distributed and are of great importance as a major source of income and for uplifting socioeconomic conditions of small and the landless farmers of rural India (Acharya, 1982) [2]. The important cause of lamb mortality includes pneumonia, diarrhoea and pneumo enteritis (Mahamoud *et al*., 1999 and Binns *et al.*, 2002) [17, 7]. In the present study the mortality observed might be due to parasitic infestation in combination with haemato biochemical changes.

Material and Methods
Fresh faeces were collected from healthy control and the clinical cases. The faecal samples were processed by sedimentation and floatation techniques. Parasitic objects including eggs, cysts and oocysts were identified based on their morphological features according to Soulsby (1982) [31]. Blood samples of 10 ml were collected, out of which 5 ml transferred into sterile EDTA coated vials. The collected unclotted blood samples were examined on the same day for estimation of haemoglobin (Hb), Packed cell volume (PCV), Total erythrocytes count (TEC), Total leukocytes count (TLC), Differential leukocyte count (DLC) and erythrocyte indices (Jain, 1993).
The serum was separated from the additional blood of about 5 ml collected earlier and gently transferred into small tubes and centrifuged to get clean supernatant serum. That was collected into sterile screw capped vials and stored at -20^{0}C for biochemical analysis. Biochemical parameters in serum were determined using the Eppendorf spectrophotometer. The collected serum samples were subjected to estimation of total protein, albumin, calcium, creatinine, AST and ALT by using kits. Total protein, albumin, AST and ALT kits were obtained through Autospan (arkary care Pvt. Ltd.), Surat, India. Calcium LS and creatinine kits were obtained through Ensure biotech Pvt. Ltd. Hyderabad, India and ERBA diagnostics GmbH, Trans asia bio-medicals Ltd, Solan district, Himachal Pradesh respectively.

Results and Discussion
Diarrhoea was the foremost symptom in parasitic infestation and confirmed by presence of different parasitic ova and in the present study the ova that were identified in faecal sample include eggs of *Strongyles*, *Moniezia* sp, *Schistosoma* sp, *Haemonchus* sp, *Paramphistomum* sp, *Trichuris* sp and oocysts of *Coccidia*.

The parasitic infestation in lambs might be due to over stocking of animals, grazing of young and adults together with poorly drained land provide an idyllic condition for transmission of the parasites to build up clinical infection of the host. The prevalence of parasitic infections in this region might be due to lower immunity of lambs as a result of under nourishment. All the lambs in the area under investigation largely depended on grazing in water lagged lands. Reid and Armour (1976) [29] discussed as helminths in sheep will cause mortality at any age. Diarrhoea, weight loss and pale visual mucus membrane (anaemia) were the chief signs in parasitic infestations and that resulted death in Moniezia Sis (Muslin *et al.*, 1988) [21] and protozoal infections (Karl Skirnisson and Hakon Hansson, 2006) [15] and Palanivel *et al.* (2011) [25] in Coccidiosis and also in Haemonchosis (Misra and Ruprah, 1972; Bali and Fotedar, 1974; Jensen, 1974 and Movsesijan *et al.*, 1975) [20, 6, 14, 19]. Sravan Kumar (2011) [32] reported *Strongyle*, *Strongyloides* sp and *Moniezia* sp led to mortality might be due to obstruction or damaging of mucosal portion of gastrointestinal tract that led to decreased absorption. The maximum parasitic ova in the present study was observed at the age group 4-6 months and findings were in correlation with the results of Jensen (1974) [14] but Kumar *et al.* (2015) [33] reported in 6-12 months age might be due to over eating of green pasture by rapidly growing lambs leading to more access to parasitic infestation. In females parasitic ova were observed more than males in the present study. (Figs. 1-7), (Tables: 1-2).

Table 1: Age wise prevalence of parasitic infections by faecal sample in ailing lambs: n=100

S. No	Faecal sample positive for	<1 Month		1-3 Month		4-6 Month		7-9 Month		Total
		F	M	F	M	F	M	F	M	
1	*Strongyle*	-	-	6	1	8	3	1	-	19
2	*Moniezia* sp.	-	-	1	-	1	-	1	-	03
3	*Schistosoma* sp.	-	-	-	1	1	-	-	-	02
4	*Coccidia* oocysts	1	4	7	2	5	3	-	1	23
5	*Haemonchus* sp.	-	-	-	-	-	2	-	-	02
6	*Paramphistome* sp.	-	-	3	1	4	7	2	-	17
7	*Trichuris* sp.	-	-	1	-	1	-	-	-	02
	Total	1 (1.47%)	4 (5.88%)	18 (26.47%)	5 (7.35%)	20 (29.41%)	15 (22.06%)	4 (5.88%)	1 (1.47%)	68

Table 2: Sex wise prevalence of parasitic infections by faecal sample in ailing lambs: n=100

Sample No	Faecal sample positive for	Females	Males	Total
1	*Strongyle*	15	04	19 (27.94%)
2	*Moniezia* sp.	03	-	03 (4.41%)
3	*Schistosoma* sp.	01	01	02 (2.94%)
4	*Coccidia* oocysts	13	10	23 (33.82%)
5	*Haemonchus* sp.	-	02	02 (2.94%)
6	*Paramphistome* sp.	09	08	17 (25%)
7	*Trichuris* sp.	01	01	02 (2.94%)
	Total	42 (61.76%)	26 (38.24%)	68

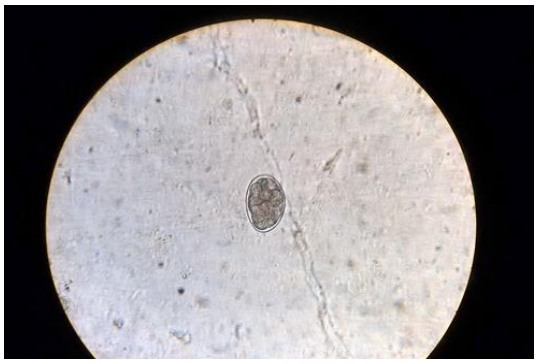

Fig 1: Presence of Strongyle egg in the faecal sample

Fig 3: Presence of Trichuris sp egg in the faecal sample

Fig 2: Presence of Moniezia sp egg in the faecal sample

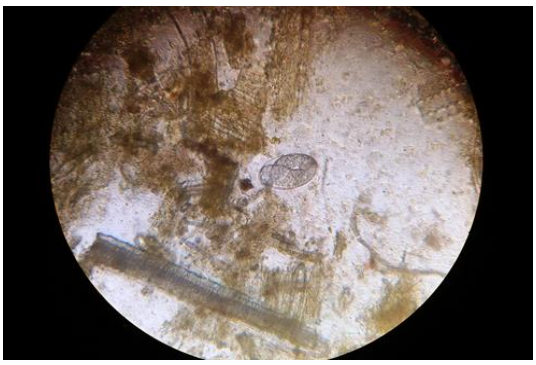

Fig 4: Presence of Strongyloid sp egg in the faecal sample

Fig 5: Presence of Coccidia sp oocysts in the faecal sample

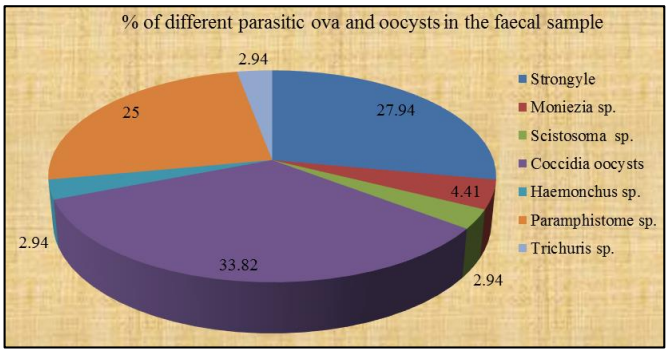

Fig 6: Pie chart showing the parasitic ova and oocysts in faecal sample

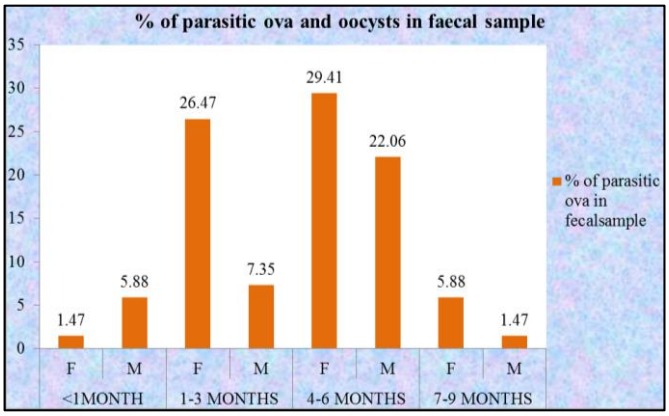

Fig 7: Age wise prevalence of parasitic infections by faecal sample examination in ailing lambs

Haematological studies showed different clinical signs viz; decreased PCV, Hb and TEC. The decreased PCV, Hb and TEC, that were in agreement with the observations of Misra and Ruprah (1972) [20], Ogunsusu (1978) [22], Dhanalakshmi *et al.* (2002) [8], Purohit *et al.* (2003) [27], Zaki *et al.* (2003) [34] and Sravan Kumar *et al.* (2015) [33]. The decrease PCV, Hb and TEC might be due to presence of parasites *Coccidia* sp, *Strongyle*, *Paramphistomum* sp and followed by *Haemonchus* sp which were recognized as active blood suckers in stomach, intestines and some of them lead to blood loss from GIT. Haemoglobin is the iron-containing oxygen-transporting protein in the red blood cells of Vertebrates. The reduction of Haemoglobin in the red blood cells decreases blood oxygen carrying capacity leading to symptoms of anemia and may result death in later stages. The increased TLC was correlated with the results of Misra *et al.* (1996), Radostits *et al*. (2007) [28], Eldin *et al*. (2013) [9], Hassan *et al.* (2013) [12] and Sravan Kumar *et al.* (2015) [33]. Leukocytosis might have occurred due to normal reaction of body defence mechanism against infection. MCV and MCH values were increased whereas MCHC value was decreased. The results of decrease in RBCs count and Hb, PCV and MCHC values were similar with the results of Saleh and Allam (2014) [30]. Increased MCV and MCH results were in agreement with Ganguly *et al*. (2016) [10]. Increased lymphocytes and decreased neutrophils, monocytes and eosinophils were observed in the present study. The lymphocytosis might be due to defence mechanism, reactive lymphocytosis in chronic inflammatory conditions or due to lymphosarcoma. In males increased neutrophils, eosinophils, MCV and decreased TLC, lymphocytes than in females was observed. The values of all hematological parameters except neutrophils, eosinophils, MCH, MCHC were lowest in <1 month age group animals when compared with other age group lambs. The increased neutrophils and eosinophils in <1 month age group animals might be due to bacterial and parasitic infections due to lowered immunity and improper management like poor cholesterol feeding, sanitation that led to lowered immunity and infections respectively. The values of all hematological parameters in Anantapur, Chittoor, Kadapa and Kurnool districts were almost similar but increased neutrophils and decreased lymphocytes were observed in Kurnool district when compared with other districts (Figs. 8-11), (Tables: 3-6).

Table 3: Hematological changes in ailing lambs (age wise) in Rayalaseema region; Mean±SE

Parameters	<1 Month	1-3 Month	4-6 Month	7-9 Month	10-12 Month
PCV (%)	24±3.56	30.88±1.86	30.02±0.99	30±1.84	29.32±1.58
Hb (g%)	8.9±1.81	9.25±0.35	9.40±0.27	8.98±0.49	9.27±0.53
TLC (10^3/µl)	9.33±1.35	10.42±4.74	10.38±2.11	9.43±7.35	9.89±3.39
TEC (million/µl)	6.05±0.62	6.79±0.31	6.22±0.18	6.93±0.36	6.61±0.39
N (%)	41.25±6.94	37.68±3.17	34.17±1.67	34.25±4.68	39.86±4.48
L (%)	57.25±6.52	60.63±3.06	64.91±1.64	63.75±4.66	58.67±4.59
M (%)	0±0	0.32±0.13	0.07±0.05	0.08±0.08	0.14±0.08
E (%)	1.5±0.96	0.84±0.34	0.57±0.16	1.08±0.34	1.33±0.95
MCV (fl)	39.49±3.46	45.32±1.47	49.49±1.56	44.08±2.8	46.93±1.98
MCH (pico gram)	14.34±1.97	13.84±0.52	15.61±0.56	13.15±0.79	15.76±0.78
MCHC (%)	36.02±2.58	30.23±1.02	31.47±0.70	30.74±1.11	32.2±1.01

Table 4: Hematological changes in ailing lambs (sex wise) in Rayalaseema region; Mean ±SE

Parameters	Male	Female
PCV (%)	30.15±1.10	29.53±0.90
Hb (g%)	9.22±0.31	9.31±0.25
TLC (10^3/µl)	9.75±3.21	10.4±1.98
TEC (million/µl)	6.43±0.18	6.5±0.19
N (%)	41.48±2.47	32.65±1.63
L (%)	57.33±2.52	65.93±1.59
M (%)	0.07±0.05	0.17±0.05
E (%)	1.12±0.48	0.7±0.17
MCV (fl)	48.35±1.58	46.48±1.2
MCH (pico gram)	14.96±0.47	14.91±0.48
MCHC (%)	30.97±0.68	31.89±0.61

Table 5: Hematological changes in ailing lambs (district wise) in Rayalaseema region; Mean ±SE

Parameters	Anantapur	Chittoore	Kadapa	Kurnool
PCV (%)	29.64±1.86	29.83±1.08	29.92±1.25	29.4±1.72
Hb (g%)	8.87±0.48	9.51±0.3	9.18±0.33	8.74±0.5
TLC (10^3/µl)	10.91±4.41	10.15±2.73	9.68±2.95	10.24±0.44
TEC (million/µl)	6.56±0.43	6.23±0.17	6.9±0.28	6.65±0.49
N (%)	36.79±4.15	34.06±1.93	34.21±1.77	49.5±5.89
L (%)	62.5±4.14	64.29±1.97	64.5±1.7	49.83±5.9
M (%)	0.14±0.14	0.13±0.05	0.04±0.04	0.25±0.18
E (%)	0.57±0.37	1.12±0.41	0.75±0.18	0.42±0.23
MCV (fl)	47.26±2.43	48.69±1.36	44.75±2.03	44.48±2.22
MCH (pico gram)	14.36±0.76	15.77±0.47	13.83±0.76	13.54±0.96
MCHC (%)	30.68±0.96	32.31±0.68	31.18±0.89	29.46±1.39

Table 6: Hematological changes in ailing lambs (n=102) in Rayalaseema region; Mean ±SE

Parameters	Mean ±SE of ailing lamb	Healthy group
PCV (%)	29±0.7	32.9±0.87
Hb (g%)	9.28±0.19	10.55±0.28
TLC (10^3/µl)	10.14±1.77	8.44±0.79
TEC (million/µl)	6.47±0.13	9.43±0.28
N (%)	36.28±1.45	44.1±3.77
L (%)	62.39±1.45	52.7±3.94
M (%)	0.13±0.04	1±0.21
E (%)	0.87±0.22	2.6±0.3
MCV (fl)	47.17±0.95	35.08±1.05
MCH (pico gram)	14.93±0.34	11.24±0.34
MCHC (%)	31.51±0.46	32.07±0.27

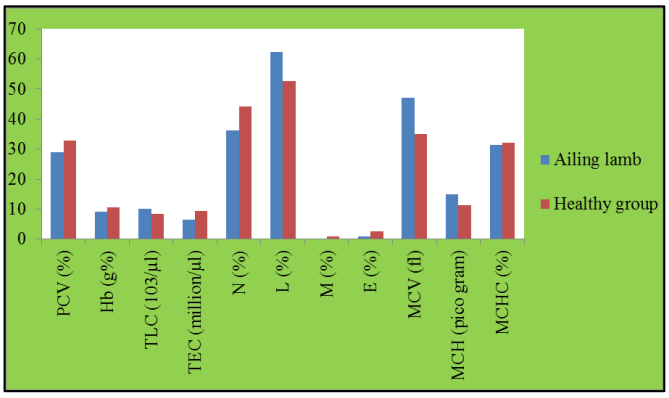

Fig 8: Hematological changes in ailing lambs (n=102) in Rayalaseema region (mean).

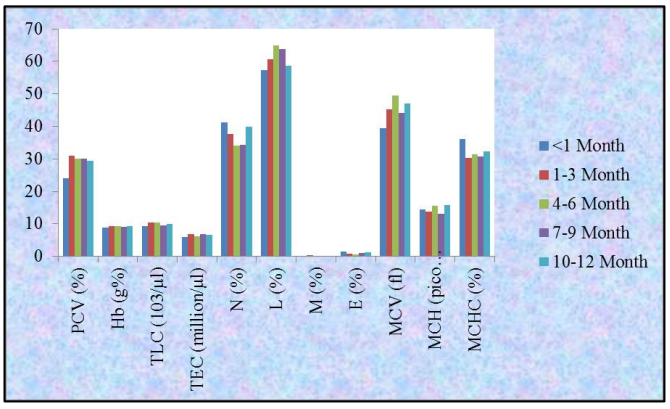

Fig 9: Hematological changes in ailing lambs (age wise) in Rayalaseema region (mean).

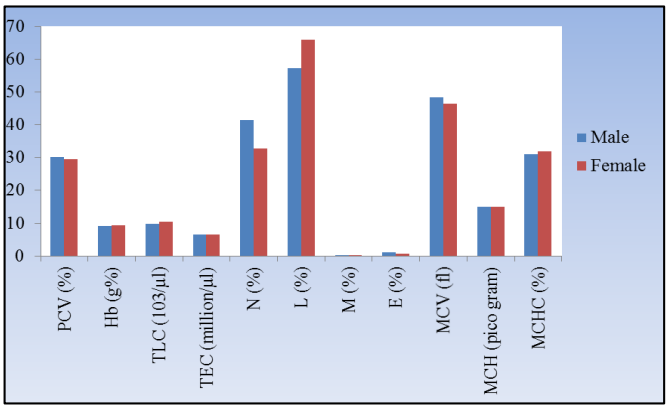

Fig 10: Hematological changes in ailing lambs (sex wise) in Rayalaseema region (mean).

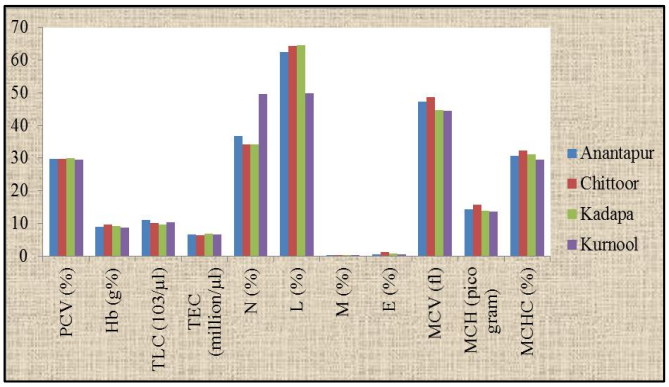

Fig 11: Hematological changes in ailing lambs (district wise) in Rayalaseema region (mean).

The serum analysis of ailing lambs showed increased TP, albumin, globulin, A/G ratio, creatinine, AST whereas the calcium, ALT values were slightly decreased when compared with apparently healthy group. Increased A/G ratio, AST was also reported by Maiti *et al.* (1999) [18], Prasanthi *et al.* (1999) [26], Dhanalakshmi *et al.* (2002) [8], Purohit *et al.* (2003) [27], Zaki *et al.* (2003) [34], Pandit *et al.* (2009) [24], Al-Hadithy *et al.* (2013) [4], Saleh and Allam (2014) [30], Sravan Kumar *et al.* (2015) [33] and Ganguly *et al.* (2016) [10]. Since these enzymes have their function and greatest concentration within the cell, increase in enzymatic activities reflects cellular abnormalities. Increased TP, albumin, globulin values were consonance with Khan *et al.* (2006) [16], Ganguly *et al.* (2016) [10]. An increased creatinine value was concurrence with the results of Nasir *et al.* (2013) [23], Saleh and Allam (2014) [30] and it might be due to renal damage. The results of lowered calcium levels were in agreement with the results of Anil *et al.* (2011) and Saleh and Allam (2014) [30]. Increased TP, albumin, globulin and ALT were noted in males whereas increased A/G ratio, calcium, creatinine and AST were observed in females. The results of increased TP, albumin, globulin in males and increased AST in females were in agreement with the results of Al-Fartosi *et al.* (2010) [3]. Increased ALT in male lambs was also reported by Al-Hadithy *et al.* (2013) [4]. The lowest levels of TP and albumin at 1-3 months age group, globulin, calcium, creatinine and AST levels at 7-9 months age group, A/G ratio at 10-12 months age group and lowest ALT was at <1 month age group were noted. Maximum levels of AST in 1-3 months, ALT in 10-12 months and creatinine in 4-6 months age groups were observed. Reports on age wise serum biochemistry are not available (Figs. 12-15), (Tables: 7-10).

Table 7: Serum biochemical changes in ailing lambs (district wise) of Rayalaseema region: Mean +SE

Parameters	Anantapur	Chittoor	Kadapa	Kurnool
TP (g/dl)	5.94±0.74	8.2±0.52	8.5±0.52	6.85±1.27
Albumin (g/dl)	2.63±0.41	3.21±0.15	3.17±0.47	2.54±0.47
Globulin (g/dl)	3.31±0.47	4.98±0.41	5.33±0.55	4.32±0.98
A/G	0.95±0.23	0.71±0.05	0.71±0.19	0.63±0.12
Calcium (mg/dl)	9.4±0.65	9.49±0.5	9.67±1.01	8.9±1.12
Creatinine (mg/dl)	1.57±0.13	1.87±0.08	2.07±0.12	1.5±0.14
AST (IU/L)	64.24±11.32	78.97±10.5	77.4±9.84	76.02±23.02
ALT (IU/L)	20.23±3.94	18.4±1.65	28.29±16	21.92±9.63

Table 8: Serum biochemical changes in ailing lambs (age wise) of Rayalaseema region: Mean ±SE

Parameters	<1 Month	1-3 Month	4-6 Month	7-9 Month	10-12 Month
TP (g/dl)	9.21±0.15	6.83±0.81	7.91±0.59	5.61±1.17	8.87±0.36
ALBUMIN (g/dl)	3.64±0.1	2.65±0.37	3.09±0.21	2.76±0.81	3.28±0.11
GLOBULIN (g/dl)	5.57±0.05	4.18±0.52	4.82±0.49	2.85±0.6	5.59±0.42
A/G	0.65±0.01	0.64±0.08	0.79±0.1	1.01±0.29	0.62±0.06
CALCIUM (mg/dl)	9.95±0.11	8.91±0.76	9.54±0.55	8.55±1.0	10.14±0.86
CREATININE (mg/dl)	1.87±0.13	1.72±0.13	1.94±0.1	1.47±0.09	1.79±0.11
AST (IU/L)	87.52±11.49	95.47±28.11	71.87±5.63	40.66±11.71	80.09±7.17
A LT (IU/L)	11.49±2.65	19.27±3.58	16.91±1.93	25.46±8.64	31.12±14.03

Table 9: Serum biochemical changes in ailing lambs (sex wise) of Rayalaseema region: Mean ±SE**.**

Parameters	Male	Female
TP (g/dl)	8.43±0.66	7.27±0.41
Albumin (g/dl)	3.18±0.26	2.94±0.17
Globulin (g/dl)	5.24±0.52	4.33±0.32
A/G	0.69±0.1	0.78±0.08
Calcium (mg/dl)	9.16±0.56	9.62±0.46
Creatinine (mg/dl)	1.78±0.1	1.84±0.08
AST (IU/L)	64.02±6.39	82.92±9.7
ALT (IU/L)	28.19±7.65	16.37±1.58

Table 10: Serum biochemical changes in ailing lambs (n=50) of Rayalaseema region: Mean ±SE

Parameters	Ailing Lambs	Apparently healthy group
TP (g/dl)	7.71±0.36	7.2±0.17
ALBUMIN (g/dl)	3.03±0.15	2.7±0.06
GLOBULIN (g/dl)	4.68±0.28	4.4±0.17
A/G	0.74±0.06	0.63±0.03
CALCIUM (mg/dl)	9.45±0.35	9.66±0.18
CREATININE (mg/dl)	1.81±0.06	1.45±0.11
AST (IU/L)	75.74±6.57	70.4±2.73
ALT (IU/L)	20.86±3.13	21.4±3.47

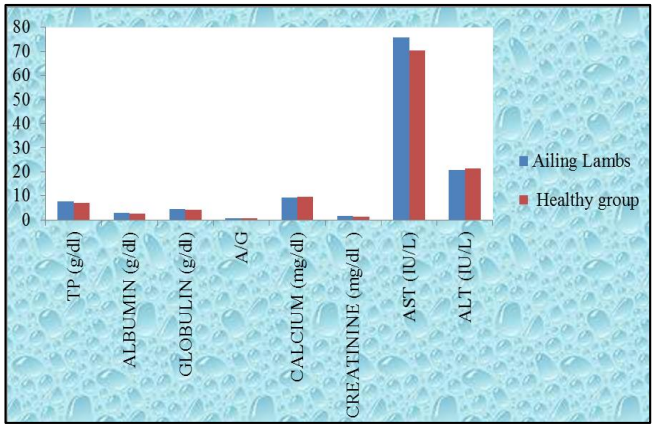

Fig 12: Serum biochemical changes in ailing lambs (n=50) of Rayalaseema region (mean).

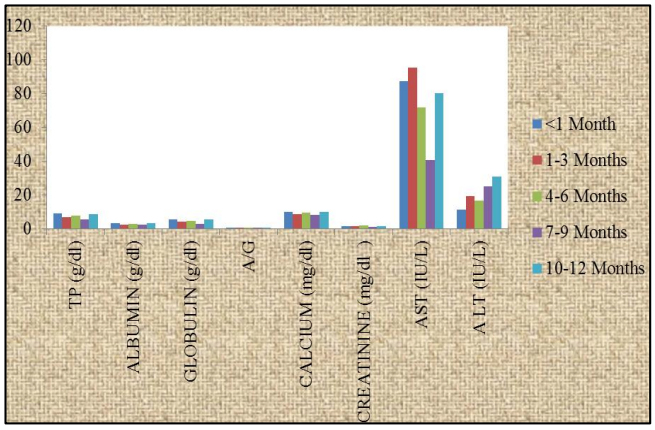

Fig 13: Serum biochemical changes in ailing lambs (age wise) of Rayalaseema region (mean).

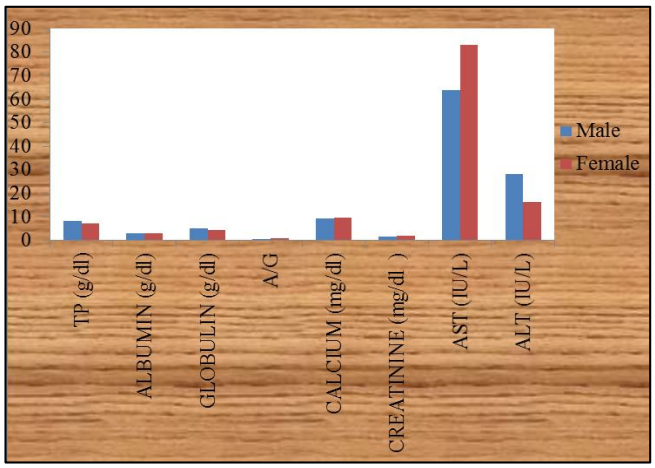

Fig 14: Serum biochemical changes in ailing lambs (sex wise) of Rayalaseema region (mean).

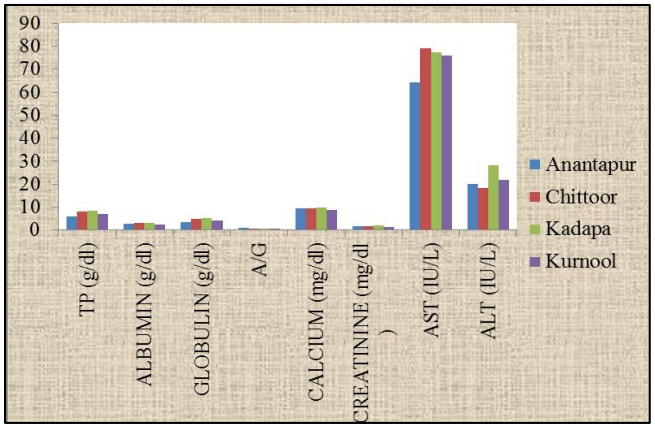

Fig 15: Serum biochemical changes in ailing lambs (district wise) of Rayalaseema region (mean).

Summary and Conclusion

Identified parasitic ova and oocysts were *Strongyle* 19 (27.94%), *Moniezia* sp 3 (4.41%), *Schistosoma* sp 2 (2.94%), *Haemonchus* sp 2 (2.94%), *Paramphistomum* sp 17 (25%), *Trichuris* sp 2 (2.94%) and *Coccidia* sp 23 (33.82%) in the faecal samples. The blood picture revealed decreased PCV, Hb, TEC, MCHC, neutrophils, monocytes, eosinophils and increased TLC, MCV, MCH, lymphocytes when compared with apparently healthy group (n=10). Males showed increased PCV, neutrophils, eosinophils, MCV and in females increased TLC, lymphocytes, monocytes, MCHC whereas almost similar Mean ±SE values of Hb, TEC were noted in males and females. In serum samples increased TP, albumin, globulin, A/G ratio, creatinine and decreased calcium, ALT was observed when compared with apparently healthy group (n=10). Increased TP, albumin, globulin, AST were reported in males and increased A/G ratio, calcium, ALT in females. Similar values of creatinine in both sexes were noted.

Infectious origin might be due to improper manage mental practices including prophylactic measures, treatment schedule and sanitation due to inadequate knowledge of the farmers. Educating the farmers, about simple improvements in the flock management both ewes and lambs before, during and after lambing will greatly reduce the death rate in lambs, which in turn improves the economy of farmer. Effective monitoring of animals included provision of proper housing facilities based on seasonal variation, respecting the fundamentals of hygiene, good quality fodder, colostrum feeding, deciding on a health plan with a Veterinarian and adopting prophylactic measures like deworming, vaccination against specific infectious diseases would help to overcome lamb mortality in sheep flocks.

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