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Prevalence of anaemia in sheep with special reference to parasitic and micromineral deficiency anaemia

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Abstract
The present study was carried out in the department of Veterinary Pathology, College of Veterinary and Animal Sciences (COVAS), Parbhani. Total 320 cases of sheep admitted in TVCC, COVAS, Parbhani and sheep farms in and around Parbhani were screened. Of which 78 cases were found positive for anaemia. Noticeable clinical observations in cases of anaemia were pale mucous membranes, reduced feed and water intake, tachycardia, hypopnea, dehydration, emaciation, rough body coat, reluctance to move, increased respiratory rate and rectal temperature. Overall, prevalence of anaemia was found to be 24.73%. Age-wise prevalence of anaemia was found highest in above 1-year age group sheep. Gender-wise prevalence of anaemia was found highest in females as compared to males. Etiological classification of anaemia was made on the basis of assessment of parasitic infections associated with anemic cases (EPG, fecal sample examination, sedimentation); nutritional deficiency anaemia (assessed on the basis of serum micromineral studies).

Keywords: Anaemia, prevalence, sheep, parasitic and micromineral deficiencies anaemia

Introduction
Sheep farming is one of the profitable sectors for alleviating poverty all over India as well as world. But the low production and mortality due to a wide range of diseases and nutritional deficiency may cause the economic losses to Sheep rearing. Sheep is known as a museum of parasites. These are the crucial impediments for profitable sheep farming in so many parts of the world including India.

Broadly Anaemia can be classified on the basis of 1) Bone marrow response 2) Size of RBC and Haemoglobin concentration and 3) On aetiology. In anaemia, haematological and biochemical alterations provide important indices for its diagnosis (Brar, 2014) [5].

On etiological classification anaemia categorized into Blood loss anaemia, hemolytic anaemia, Nutritional deficiency anemias and Bone marrow depression anemias (Brar, 2014) [5].

Serum microminerals study showed marked decrease in values of serum microminerals like copper (46.05- 60.23 ug/dl) and iron (45.58- 94.21 ug/dl) as stated by Jayalaxmi *et al.*, (2020).

Haemonchosis is a common problem in Sheep and lambs throughout the year. In *Haemonchosis* losses 0.05 ml of per day causing serious ill effects like pallor of mucous membrane, especially in affected sheep conjunctivae, progressive weakness, submandibular oedema (Paul *et.al.*, 2020) [19].

Materials and methods
Clinical assessment of anaemia
The history of sheep was recorded with the duration of illness, examination of visible mucosa, eyes, extremities, skin and anus, physical condition, general appearance, gait, posture and other behavioural changes. Other clinical manifestations and general clinical examinations including temperature, pulse and respiration was recorded in the affected and apparently healthy sheep. Clinical data obtained was analysed for clinical assessment of anaemia.

Overall prevalence was calculated from number of Sheep screened out of which number of positive cases for anaemia selected for further studies. Prevalence of Clinical cases was further classified into Age-wise (Up to1 year group, above 1-year group) and gender-wise (male and female).

The data collected was analysed according to age, sex of animals for prevalence study and evaluation of parameters selected for study for classification of anaemia.

Age wise prevalence of anaemia

Data generated was categorized in to following age groups:

a) Sheep up to 1-year age group
b) Sheep above 1-year age group

Gender wise prevalence of anaemia

Data generated was categorized into the following genders:

a) Male
b) Female

Parasitic infections associated with anaemia

Faecal samples were collected directly from rectum of Sheep showing anaemic conditions in a properly labelled sterile plastic vial. The collected faecal samples were examined by parasitological techniques as per the methods described by Soulsby (1982) [24].

Nutritional deficiency anaemia

Serum samples collected from apparently healthy animals and anaemic sheep, were processed for biochemical and micro-mineral estimations to assess the nutritional deficiency anaemia.

Serum micro-mineral estimation

Estimation of Copper, Cobalt, Iron and Zinc from Serum were processed by Atomic Absorption spectroscopy (Perkin Elmer 200). For this serum samples were digested by the procedure used by Hilliard and Smith (1979) [13].

Procedure of digestion of serum

One ml serum sample mixed in 1 ml of Nitric acid was added to a 100 ml volumetric flask and stirred until it wet the added samples. Then 1 ml of Perchloric Acid were carefully added in Flask and slightly stirred after the addition. It was heated on a hot plate and a strong effervescence was produced. When the brown fumes were less dense, the solution was allowed to cool. A slightly yellow dissolution and a small white solid quantity in suspension still remained. The solution was filtered with Whatman's filter paper, washed with 0.5 ml of Perchloric Acid and diluted up to 10 ml with triple distilled water (Hilliard and Smith, 1979) [13].

Statistical analysis

Data generated was analysed statistically to find the level of significance by employing suitable, standard statistical method described by (Snedecor and Cochran, 1982) [25]. Chi-square test was employed for analysis of prevalence of anaemia (age and sex). Completely Randomized Design (CRD) for serum micromineral studies in both anaemic as well as healthy Sheep was applied.

Result and discussion

Anaemia due to lower than normal in RBC's per cumm, the amount of hemoglobin and the volume of packed cells per 100 ml of blood, is probably one of the most common condition seen in Indian Sheep. Physiologically, it is characterized by insufficient circulating hemoglobin and clinically reduced exercise tolerance and pale mucus membranes.

Anaemia can be assessed on the basis of clinical observations and laboratory investigations. Table 1 (A), 1 (B) and 1 (C) represents the criteria used for clinical assessment of 78 anemic cases.

Clinical assessment of anaemia was made on percentage basis viz. in case of body condition it was thin/emaciated/dehydrated in 70 cases (89.74%) subsequently by 8 healthy cases (10.25%). As per as behavior is concerned it was dull and depressed in 72 cases (92.30%), whereas in 6 cases (7.69) were active. In case of gait, 33 cases (42.30) were showed move slow gait and normal gait was observed in 45 cases (57.69). 73 cases (93.59%) were found in standing posture followed by recumbent posture in 5 cases (6.41%). For skin and hair coat, 70 cases (89.75%) were with rough skin and hair coat, 05 cases (6.41%) were with smooth skin and hair coat followed by shiny skin and hair coat in 03 cases (3.84%).

For clinical examination such as respiratory rate, 40 cases (51.28) were found with normal respiratory rate followed by polypnea in 30 cases (38.46%) and dyspnea in 08 cases (10.25%). For clinical examination such as heart rate, 40 cases (51.28%) were with normal heart rate followed by tachycardia in 32 cases (41.02%) and bradycardia in 06 cases (7.69%). For rectal temperature as assessment, there were increased rectal temperature in 48 cases (61.53%) subsequently normal rectal temperature in 25 cases (32.05%) and decreased rectal temperature in 05 cases (6.41%).

Visible mucus membranes were pale in 63 cases (80.76%), whereas in 15 cases (19.23%) there were white mucus membranes. For water intake as a criterion, it was reduced in 65 cases (83.33%) subsequently normal water intake was in 13 cases (16.66%). For appetite as a clinical assessment criterion, it was reduced 75 cases (96.15%) followed by completely reduced in 03 cases (3.06%).

Popular clinical signs and symptoms of anemic sheep were reported by several researchers. From the reviewed literature Darzi *et al*., (2011) [7], Saminathan *et al.*, (2015) [21], Ganguly *et al.*, (2016) [11], Jayalakshmi *et al*., (2017) [14], Ebrahim, Z. K., (2018) [8], Reddy *et al*., (2018) [20], Gangane *et al*., (2019) [10] and Namratha *et al*., (2019) [18] recorded similar signs and symptoms such as inappetence to anorexia, emaciation, pale mucus membrane, decreased growth, depression, rough body coat, diarrhoea, fever and dehydration in cases of Sheep anaemia. The clinical studies done in 78 cases of anaemic Sheep in present study can be well endorsed by the findings recorded by earlier works.

Overall prevalence of anaemia

Table 2 shows the details of clinical cases of sheep admitted to TVCC polyclinic, College of Veterinary and Animal Sciences, Parbhani and sheep farms in and around Parbhani during period of study (February, 2020 to January, 2021). Total 320 sheep were screened, out of screened sheep 78 cases (24.73%) were found anemic.

Age-wise prevalence of anaemia

Age-wise prevalence of anaemia is depicted in table 3.

On applying chi-square statistical analysis to know age-wise prevalence of anaemia, it was appeared significant. Afterwards, the percentage of prevalence of anaemia when calculated, it was found highest in the age group of above 1-year age group sheep.

Darzi *et al*., (2011) [7], Amin and Wani, (2012) [1] and Haq *et al*., (2017) [12] conducted study on anaemic sheep which were shown highest prevalence of anaemia in adult animals than that of younger animals. In the present study, it was observed that in age wise prevalence of anaemic cases, age group above year age group sheep showed highest prevalence, the results observed in present study were found at par with the study

conducted by Darzi *et al*., (2011) [7], Amin and Wani, (2012) [1] and Haq *et al*., (2017) [12].

Gender-wise prevalence of anaemia

Gender-wise prevalence of anaemia is shown in table 4.

By doing statistical chi-square analysis to know gender-wise prevalence of anaemia, it was found nonsignificant. Later, percentage of prevalence of anaemia when calculated, it was observed that prevalence of anaemia in females were found highest (56 cases, 71.79%) as compared with the prevalence of anaemia in males (22 cases, 28.20%). Highest incidence observed in anaemic female group in the present study were found akin with the study conducted by Tehrani *et al*., (2012) [27], Tak *et al*., (2013) [26] and Sivajothi and Reddy, (2018) [20].

Parasitic infections associated with anaemia

Table 5 shows details of parasitic infections associated with anemic cases of sheep.

By using modified Stoll's dilution method EPG was calculated in all positive anemic cases and different parasitic eggs were identified by the morphological examination of fecal samples.

Parasitic infections identified by Microscopic examination of fecal sediment

Out of 78 anemic cases of sheep 70 cases were found positive for parasitic infections (89.74%). It was observed that parasitism was the major cause of anaemia in sheep.

In this present study, mixed parasitic infection was found highest i.e., 46 cases (65.71%) followed by individual infections of *stongyles spp*. i.e., 22 cases (31.42%) followed by *Trichuris spp*. in 01 case (1.42%) and *strongyloides spp*. in 01 case (1.42%).

In mixed type of infections, *Strongyles spp. and Trichuris spp.* mixed infection found highest i.e., 14 cases (20%) followed by *Strongyles spp.* and *Strongyloides spp.* mixed infection (11 cases, 15.71%). Whereas, 08 cases (11.42%) were found positive for *Strongyles spp., Trichuris spp., Eimeria spp.* and *Strongyloides spp.* type of mixed infection and mixed infection of *Strongyles spp., Trichuris spp. and Strongyloides spp.* showed positivity in 06 cases (8.57%).

The findings of present study were found akin with earlier studies conducted by Bhat *et al*., (2012) [3], Lone *et al*., (2012) [17], Tehrani *et al*., (2012) [27], Kumar *et al*., (2015) [16], Kaur *et al*., (2018) [15], Reddy *et al*., (2018) [20], Satish *et al*., (2018) [22], Sivajothi and Reddy, (2018) [20] and Gangane *et al*., (2019) [10].

EPG

From the findings of present study, it can be concluded that there was high grade of infection in 30 cases (42.85%) having EPG>1000 and low grade of infection in 40 cases (57.14%) having EPG<1000.

Serum Micro-mineral study

Table 6 depicts serum micromineral values in healthy control group sheep and anemic group sheep.

Randomly serum samples were collected from healthy control group sheep (12) and anemic group sheep (18) and which were analyzed for iron (Fe), copper (Cu), cobalt (Co) and zinc (Zn) by AAS (Perkin Elmer 200) at Department of Soil Sciences, VNMKV, Parbhani to correlate the micro-mineral status with anaemia.

Serum iron (Fe, ug/dl)

0-1 year age and male group

It was observed that serum iron values of anemic group sheep (76.35 ± 17.43) were significantly lower than that of healthy control group sheep (158.46[a] ± 5.34).

0-1 year age and female group

Serum iron values of anemic group sheep (78.77 ± 10.83) were markedly lower than the healthy control group sheep (146.46[a] ± 3.07).

Above 1 year age and male group

Mean serum iron values of anemic group sheep (60.28 ± 3.85) were significantly lower than the healthy control group sheep (155.86 ± 13.30).

Above 1 year age and female group

Mean ± SE serum iron values of anemic group sheep (71.55 ± 5.50) were markedly lower than the healthy control group sheep (150.23 ± 8.28).

Overall, there were decrease in serum iron values in the anemic group sheep when compared with that of apparently healthy control groups.

Decrease in serum iron values in anemic cases in present study were found in accordance with the study conducted by Awad *et al*., (2016) [22], Ebrahim, (2018) [8] and Jayalaxmi *et al.*, (2020) in anaemic sheep.

For the synthesis of haem in haemoglobin iron is essential and the deficiency of iron leads to the iron deficiency anaemia because of less synthesis of haem affect the haemoglobin levels (Bhattacharyya (2015) [6].

Serum copper (Cu, ug/dl)

0-1 year age and male group

Serum copper values of anemic group sheep (55.60 ± 7.62) were significantly lower than the healthy control group sheep (73.43 ± 1.93).

0-1 year age and female group

Serum copper values (Mean ± SE) of anemic group sheep (53.23 ± 6.08) were markedly lower than the healthy control group sheep (73.00 ± 3.66).

Above 1 year age and male group

Mean ± SE values serum copper of anemic group sheep (51.99 ± 2.87) were significantly lower than the healthy control group sheep (88.76 ± 2.98).

Above 1 year age and female group

Serum copper values of anemic group sheep (47.70 ± 3.40) were markedly lower than the healthy control group sheep (84.53 ± 5.93).

Overall serum copper values were found decreased in present study of anaemic group sheep.

In present study on micromineral estimation by AAS, serum copper values were observed lower in the anaemic group sheep. The results obtained during study goes well with the study done by Galbat *et al*., (2015) [9] and Jayalaxmi *et al.*, (2020).

Copper is essential component for synthesis of vitamin B12 in the ruminants. Also, copper is required for the erythropoiesis i.e copper and manganese together are necessary for the incorporation of iron in haemoglobin (Bhattacharyya (2015) [6].

Serum cobalt (Co, ug/dl)

0-1 year age and male group

Serum cobalt values of anemic group sheep (8.97 ± 1.13) were markedly lower than the healthy control group sheep (15.60 ± 0.98).

0-1 year age and female group

Serum cobalt values of anemic group sheep (7.86 ± 0.41) were significantly lower than the healthy control group sheep (17.76 ± 2.58).

Above 1 year age and male group

Serum cobalt values of anemic group sheep (8.42 ± 1.02) were markedly lower than the healthy control group sheep (11.40 ± 1.99).

Above 1 year age and female group

Mean ± SE values serum cobalt of anemic group sheep (8.03 ± 0.89) were significantly lower than the healthy control group sheep (14.00 ± 0.95).

Overall, there were decrease in the serum cobalt values in the anaemic group sheep as compared to the healthy control group sheep.

The serum copper values were observed low in anaemic group sheep as compared with the healthy control group. In present study, decrease in serum cobalt values found akin with the study conducted by Tuncer *et al.*, (2020) in anaemic sheep.

Serum Zinc (Zn, ug/dl)

0-1 year age and male group

Serum zinc values of anemic group sheep (77.82± 4.55) were found lower than the healthy control group sheep (89.00 ± 4.26).

0-1 year age and female group

Serum zinc values of anemic group sheep (81.06 ± 1.93) were markedly lower than the healthy control group sheep (91.33 ± 6.36).

Above 1 year age and male group

Serum zinc values of anemic group sheep (69.82 ± 4.69) were significantly lower than the healthy control group sheep (89.40 ± 2.23).

Above 1 year age and female group

Serum zinc values of anemic group sheep (71.82 ± 5.81) were markedly lower than the healthy control group sheep (87.86 ± 1.09).

Overall, there were decrease in serum zinc values of anemic group sheep in present study.

Serum zinc values were significantly decrease in the anemic group sheep of present study. Decrease in serum zinc values observed in present study are found similar with the study of Awad *et al*., (2016) [22] and Tuncer *et al.*, (2020).

Conclusions

1. Overall prevalence of anaemia was 24.73% in sheep. Age-wise prevalence of anaemia was found highest in age group of above 1-year age of sheep, whereas, prevalence of anaemia was found highest in females as compared to that of males.
2. Microcytic normochromic anaemia and normocytic normochromic anaemia was a prominent feature in sheep anaemia.
3. There was high incidence of parasitic anaemia (89.74%) and it was characterized by high grade mixed parasitic infections in 46 cases (65.71%), followed by individual infections of *Stingless spp*. i.e., 22 cases (31.42%) followed by *Trichuris spp*. in 01 case (1.42%) and *Strongyloides spp*. in 01 case (1.42%).
4. Serum micromineral studies revealed marked decrease in levels of iron, copper, cobalt and zinc in anemic sheep.

Table 1(A): Represent the inclusion criteria applied for the clinical assessment of anaemia in Sheep.

Inclusion criteria applied for clinical assessment of anemic cases					
Visible mucous membrane		Water intake		Appetite	
White (%)	Pale (%)	Normal (%)	Reduced (%)	Reduced (%)	Completely Reduced (%)
15 (19.23)	63 (80.76)	13 (16.66)	65 (83.33)	75 (96.15)	3 (3.06)

Table 1(B): Represent the inclusion criteria applied for the clinical assessment of anaemia in Sheep.

Inclusion criteria applied for clinical assessment of anemic cases										
Body condition		Behavior		Gait		Posture		Skin and hair coat		
Healthy (%)	Thin/Emaciated/ Dehydrated (%)	Active (%)	Dull and Depressed (%)	Normal (%)	Move slowly (%)	Standing (%)	Recumbent (%)	Smooth (%)	Rough (%)	Shiny (%)
8 (10.25)	70 (89.74)	6 (7.69)	72 (92.30)	45 (57.69)	33 (42.30)	73 (93.59)	5 (6.41)	5 (6.41)	70 (89.75)	03 (3.84)

Table 1(C): Represent the inclusion criteria applied for the clinical assessment of anaemia in Sheep.

Inclusion criteria applied for clinical assessment of anemic cases								
Respiratory Rate			Heart rate			Rectal Temp.		
Normal (%)	Dyspnea (%)	Polypnea (%)	Normal (%)	Tachycardia (%)	Bradycardia (%)	Normal (%)	Increase (%)	Decrease (%)
40 (51.28)	08 (10.25)	30 (38.46)	40 (51.28)	32 (41.02)	06 (7.69)	25 (32.05)	48 (61.53)	05 (6.41)

Table 2: Overall prevalence and details of Sheep screened during study at TVCC, COVAS, Parbhani and Sheep farms in and around Parbhani.

	Sheep screened	Total	Prevalence%
Anaemic cases	Females upto 1 yr age group	9	
	Females above 1 yr age group	47	
	Males upto 1 yr age group	10	
	Males above 1 yr age group	12	
	Total	78	24.37
Non-Anemic cases (excluding those selected for healthy control group)	Females upto 1 yr age group	60	
	Females above 1 yr age group	104	
	Males upto 1 yr age group	46	
	Males above 1 yr age group	32	
	Total	242	75.62
	Grand total	320	100

Table 3: Shows Age-wise prevalence of anaemia in Sheep

Total number of anaemic cases 78 (100%)					
Age	**% Prevalence**	**X^2**	**Statistics**	**Chi square table**	
				1%	**5%**
Upto 1 year age group	19 (24.35%)	4.31	HS	6.63	3.84
Above 1 year age group	59 (75.64%)	2.76			
Total	78	7.07			

Table 4: Shows Gender-wise prevalence of anaemia in Sheep

Total number of anaemic cases 78 (100%)			
Sex	% Prevalence	X^2	**Statistics**
Male	22(28.20%)	0.23	NS
Female	56 (71.79%)	0.10	
Total	78	0.33	

Table 5: Indicates different parasitic infection in anaemia Sheep of different age and sex

EPG	No. of cases	Parasite identified	Positive cases	Total cases examined	Percentage (%)
EPG> 1000 (High level of infection)	30	a) Individual infection			
		Strongyles spp.	22	70	31.42
EPG<1000 (Low level of infection)	40	*Trichuris spp.*	01	70	01.42
		strongyloides spp.	01	70	01.42
		Total	24		
		b) Mixed infections	46		
		Strongyles spp.+ Trichuris spp.+Eimeria spp. + Strongyloides spp.	08	70	11.42
		Strongyles spp.+ Trichuris spp.+Eimeria spp.	01	70	01.42
		Strongyles spp.+ Trichuris spp.+ Strongyloides spp.	06	70	08.57
		Strongyles spp.+ Trichuris spp.	14	70	20.00
		Strongyles spp.+Eimeria spp.	01	70	01.42
		Strongyles spp.+Eimeria spp. + Strongyloides spp.	05	70	07.14
		Strongyles spp.+ Strongyloides spp.	11	70	15.71
Total	70		70		

Table 6: Depicts serum micro-mineral parameters of Sheep

Group of animals		Values of serum micromineral parameters			
I. Anaemic groups		**Iron (ug/dl)**	**Copper (ug/dl)**	**Cobalt (ug/dl)**	**Zinc (ug/dl)**
0-1 year age group	Male (n=10)	$76.35^{b} \pm 17.43$	$55.60^{c} \pm 7.62$	$8.97^{c} \pm 1.13$	$77.82^{abc} \pm 4.55$
	Female (n=9)	$78.77^{b} \pm 10.83$	$53.23^{c} \pm 6.08$	$7.86^{c} \pm 0.41$	$81.06^{abc} \pm 1.93$
Above 1 year age group	Male (n=12)	$60.28^{b} \pm 3.85$	$51.99^{c} \pm 2.87$	$8.42^{c} \pm 1.02$	$69.82^{c} \pm 4.69$
	Female (n=47)	$71.55^{b} \pm 5.50$	$47.70^{c} \pm 3.40$	$8.03^{c} \pm 0.89$	$71.82^{bc} \pm 5.81$
II. Control groups					
0-1 year age group	Male (n=6)	$158.46^{a} \pm 5.34$	$73.43^{ab} \pm 1.93$	$15.60^{ab} \pm 0.98$	$89.00^{ab} \pm 4.26$
	Female (n=6)	$146.46^{a} \pm 3.07$	$73.00^{b} \pm 3.66$	$17.76^{a} \pm 2.58$	$91.33^{a} \pm 6.36$
Above 1 year age group	Male (n=6)	$155.86^{a} \pm 13.30$	$88.76^{a} \pm 2.98$	$11.40^{bc} \pm 1.99$	$89.40^{a} \pm 2.23$
	Female (n=6)	$150.23^{a} \pm 8.28$	$84.53^{ab} \pm 5.93$	$14.00^{ab} \pm 0.95$	$87.86^{a} \pm 1.09$
Statistics		HS	HS	HS	S
CD values	1%	42.49	21.22	5.74	-
	5%	31.26	15.61	4.22	17.46

Mean with similar superscripts in column do not differ significantly
n= Number of sheep tested

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