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Stephanie S Pradhan
CSKHPAU, DGCN COVAS, Palampur, Himachal Pradesh, India

Stephanie S Pradhan
CSKHPAU, DGCN COVAS, Palampur, Himachal Pradesh, India

Correspondence
Stephanie S Pradhan
CSKHPAU, DGCN COVAS, Palampur, Himachal Pradesh, India

Importance of haematological profile in bovine calves with respiratory diseases

Stephanie S Pradhan and Vipan Kumar Gupta

Abstract
The present study has been conducted to determine the haematological parameters of calves suffering from respiratory diseases for a proper diagnosis and prognosis of a disease in addition to the clinical examinations and other diagnostic procedures. Blood was collected via jugular vein from extremely sick and apparently healthy animals from Palampur, Himachal Pradesh. Blood smears were prepared instantly on glass slides for assessing the blood picture. Total leucocyte count (TLC) and Differential leucocyte count (DLC) were carried out according to the standard protocols. The TLC between the healthy and sick calves did not differ significantly. However, in DLC the sick animals showed significant neutrophilia associated with a fall in lymphocytes. In more severe cases there was a hike in the number of band cells and metamyelocytes. Neutrophils are the first cells that respond to bacterial infection so an elevation in neutrophil count could be suggestive of some ongoing bacterial infection.

Keywords: Bovine calves, total leucocyte count, differential leucocyte count, Himachal Pradesh

1. Introduction
Livestock is the major source of livelihood in India where the majority of the population still lives in rural areas where 80% are small and marginal farmers [1]. Calves are important assets of the poor dairy farmers as they play a major role in uplifting their socioeconomic condition. As a result, successful rearing of the young calves exclusively determines the profitability of the dairy farms and the farmers. Calf mortality of 20% may reduce the net profit by 38% [2]. The total number of cattle in India as per 2012 censuses is 190.90 million contributing around 37.28% of the total livestock population. According to the 18th livestock census (2007) the total bovine population in Himachal Pradesh (HP) was 3.03 million, which is approximately 1% of India's bovine population. Among the three age groups, the mortality rate was highest in calves 21.53%, followed by young stocks 9.35% and adults 4.73% [3].
Calf mortality and morbidity have been mostly attributed to respiratory affections and digestive disorders [4, 5]. Diseases of the respiratory system are some of the leading causes of morbidity and mortality in preweaned dairy calves [6] and weaned heifers [7]. This leads to economic loss not only due to loss of the present value of the calf but also due to loss of genetic potential for herd improvement [8]. Respiratory problems have been known to increase by 34% in the last 20 years with 21% neonatal mortality [6].
Pneumonia is one of the major respiratory infections, which take a heavy toll on the life of the calves during their first few months of life. It appears to be the most predominant cause of mortality in 1-6 months old calves [9, 10]. Pneumonia decreases the body weight gain by 0.8 kg per day during the first 3 months of their lives [11]. Calf pneumonia is a multifactorial disease, involving the interplay of infectious agents such as viruses, bacteria, mycoplasma and parasites, managerial errors, stresses and host susceptibility. Despite the availability and use of many antimicrobial drugs, calf morbidity and mortality still remains an important cause of economic losses on dairy farms worldwide.
The present study has been conducted to determine the haematological parameters of the calves suffering from respiratory diseases for a proper diagnosis and prognosis of a disease in addition to the clinical examinations and other diagnostic procedures carried out by the examiner.

2. Material and Methods
2.1 Experimental design
The blood samples from sick and apparently healthy animals for the present study were collected from the University farm of CSKHPAU and surrounding districts of Palampur,

Himachal Pradesh. Haematology (TLC and DLC) was carried out on the blood samples from animals exhibiting severe disease condition. Till date no report on haematological profile of calves with respiratory diseases has been reported from Himachal Pradesh. Hence, this study was undertaken to determine the type of ongoing infection.

2.2 Collection of blood
2-3 ml blood was collected in EDTA vials via jugular vein [12] puncture using 18 gauge needles for analyzing haematological parameters (TLC and DLC) from six healthy and 14 sick calves exhibiting severe clinical condition. Blood smears were prepared instantly on clean grease free slides in duplicate for assessing the blood picture.

2.3 Total leucocyte count estimation (TLC)
Thoma diluting pipette or white blood cell (WBC) diluting pipette was taken and blood drawn upto 0.5mark. End of the pipette was properly wiped. The column of the blood was reduced if it exceeded the 0.5 mark. The leukocyte diluting fluid was then taken upto the 11 mark and shaken well. The first two to three drops were discarded before filling the haemocytometer or the counting chamber. The end of the pipette was placed at the junction of the counting chamber and the cover slip. Diluted blood flows under the coverslip due to the capillary action. The pipette was removed as soon as the chamber was completely filled. The cells were then allowed to settle down for sixty seconds and their number counted under low power in the four primary squares. All the cells in the four primary squares were added and multiplied by 50 to obtain the number of WBC in one cubic millimeter [12].

2.4 Differential Leucocyte count (DLC)
The slides with thin blood smears were taken and stained with Giemsa. Prior to staining, the smears were fixed with methanol for 3 minutes and were allowed to air dry. Working solution was prepared by mixing 6 ml of neutral distilled water or buffered distilled water and 1 ml of Giemsa stain. The smears were flooded by the stain and left for 30-45 minutes. Buffered distilled water was used for washing off the stain. The slide was then drained, dried and examined under oil immersion (100X) for distribution and enumeration of cells. At least 100 leucocytes were counted and the different cells were expressed as a percentage [12, 13].

3. Results
Total leucocyte count (TLC) and differential leucocyte count (DLC) were carried out on the blood samples collected from animals exhibiting severe disease condition. The results of the present study are:

3.1 Haematology
Blood samples collected from 14 clinically sick and 6 apparently healthy animals were subjected to total leucocyte count (TLC) and differential leucocyte count (DLC). Sick animals showed significant neutrophilia. In more severe cases there was a hike in the number of band cells (immature neutrophils) and metamyelocytes (cells undergoing granulopoiesis) (Fig-1, 2, 3 & 4). Out of 20, 10 and one animal showed degenerative shift to the left and moderate regenerative shift to the left respectively. Unpaired T test has been implemented with the following results (Table 1).

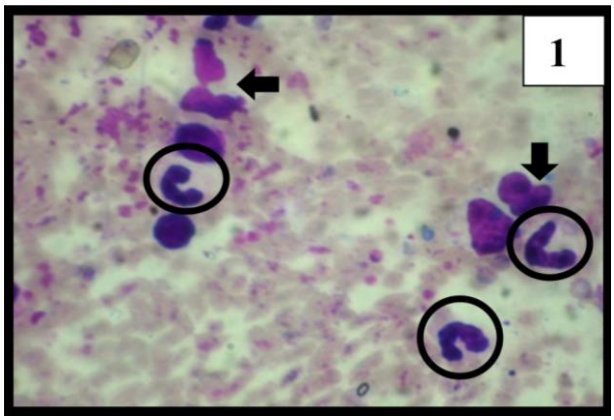

Fig 1: Band cells (circle) and metamyelocytes (arrow) (330X).

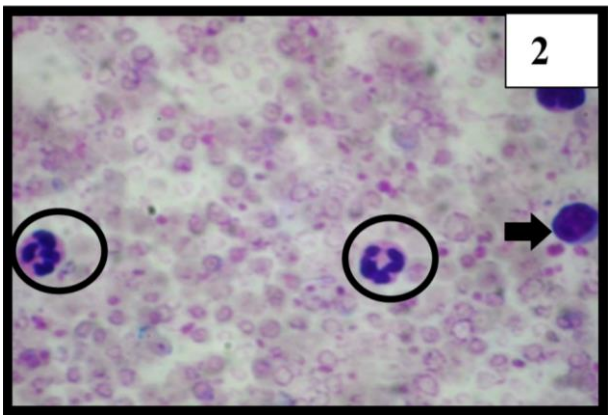

Fig 2: Mature neutrophils (circle) and lymphocyte (arrow) (330X).

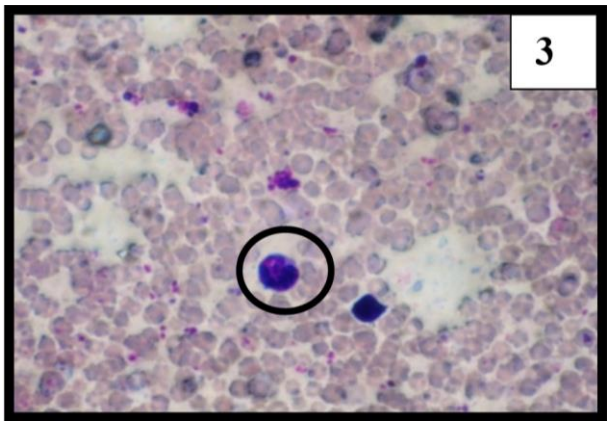

Fig 3: Monocyte (circle) (330X).

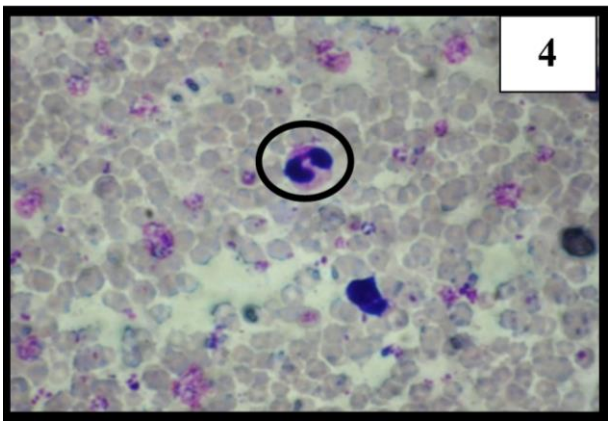

Fig 4: Eosinophil (circle) (330X).

Blood smear examination: differential leucocyte count (DLC) (Giemsa stain)

Table 1: Total and differential leucocyte count of healthy and animals manifesting respiratory symptoms

Parameter	Apparently healthy	Calves manifesting respiratory signs with nasal discharge	p(<0.05)	S/NS
TLC (10^3/cumm)	5.35±0.45	4.86±0.56	0.5925	NS
Lymphocyte (%)	75.17±1.62	44.14±3.68	0.0001	S (0.0001)
Neutrophil (%)	16.67±1.17	31.86±4.53	0.0456	S (0.0456)
Band cell (%)	3±0.58 (Range:1-5)	13.57±4.53 (Range:3-48)	0.0574	NS
Metamyelocyte (%)	2.5±0.85(Range:3-5)	7.86±2.58(Range:3-35)	0.2006	NS
Eosinophils (%)	0.50±0.22	0.43±0.23	0.8531	NS
Monocyte (%)	2.17±0.70	2.14±0.25	0.9684	NS

p=level of significance; S= significant; NS=non- significant; Results are presented as mean values ± standard error**;** p<0.05 was considered as significant.

The statistical significance was tested using unpaired t test. The TLC between the healthy and sick calves did not differ significantly. Lymphocyte count was significantly lower (P<0.0001) in sick calves. This was related to the rise in neutrophil count. Neutrophil count was significantly higher (P<0.0456) in sick animals. Band cell (highest-48) and metamyelocyte (metamyelocyte-35) count were higher in sick animals but the observed difference was not statistically significant.

4. Discussion

In the present study the TLC between the six healthy and 14 sick calves did not differ significantly. Kovacic and colleagues analyzed 23 three-month old calves with mild clinical signs of bronchopneumonia and 15 age-matched healthy calves. It was reported that the peripheral blood leukocyte count of bronchopneumonic and healthy calves showed no difference [14]. This report is consistent with our present finding.

A significant increase in TLC count is directly related to the rise in the total number and percent of neutrophils in the calves suffering from enzootic bronchopneumonia in comparison to healthy calves [15]. In this present study, 14 sick calves showed significant neutrophilia associated with a decrease in lymphocytes with similar reports from other authors [16, 17]. However, in the present study rise of immature neutrophils was associated with an increase in TLC count in one case. This can be described as a moderate regenerative shift to the left as there is an increase in the immature cells (band and metamyelocytes) with increase in the total leucocyte count. This happens due to moderately severe to severe infection or severe stress. Other 10 cases showed increase in neutrophil count (mature and immature) associated with low or normal TLC count. A low, normal or slightly elevated total leucocyte count associated with an increase in immature cells of the granulocytic series is suggestive of degenerative shift to the left. This indicates that the animals are either in initial stages of inflammatory process or they have been suffering from some grave disease condition. However, in cattle marked leucopenia with an increase in immature granulocytes is common in the initial stages [12]. Compared with other species, cattle have a small bone marrow reserve for granulocytes. This results initially in a neutropenic rather than a neutrophilic reaction in an early inflammatory process. Neutrophilia and a left shift might be observed only after the speed of granulopoiesis is increased [18, 19].

Previous workers stated that an elevation in total leucocytic count in calves could be linked with bacterial pneumonia [20, 21]. Furthermore, the elevated leucocytic count was recorded in many infectious diseases [20]. Peripheral blood leukocyte count is considered to be the easiest method to identify infections in humans and animals [14]. The mechanisms underlying leukocytosis include an increased release from the bone marrow, decreased emigration into the tissues, and a shift of cells from the marginal into the circulatory pool [22]. The most common causes for neutrophilia are chronic inflammation and stress. Infection of the respiratory tract has been considered to be one of the most common causes of chronic inflammation [18].

5. Conclusion

In the present study haematology (TLC and DLC) was carried out on the blood samples collected from sick animals and the healthy ones that acted as controls. TLC between the healthy and sick calves did not differ significantly. However, DLC showed significant neutrophilia in sick animals associated with lymphocytopenia. This could be suggestive of some ongoing bacterial infection as the neutrophils are the first cells that respond to bacterial infection.

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