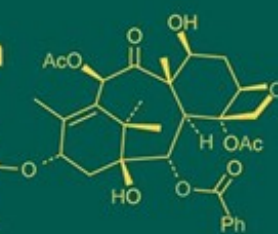
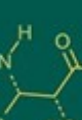
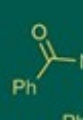


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Haematological alterations in dogs with canine distemper virus-associated respiratory tract infection in Guwahati, Assam

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ABSTRACT

Canine distemper is a multisystemic viral disease of dogs in which the blood picture changes with the stage of infection and with secondary complications. This study evaluated the haematological alterations in dogs with respiratory tract infection associated with canine distemper virus (CDV) under field conditions in Guwahati, Assam, India. Twelve dogs formed two groups of six: dogs with respiratory signs that were positive for CDV antigen in a rapid immunochromatographic test of nasal and conjunctival swabs (CDV group) and apparently healthy dogs (control group). Haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), thrombocyte count, total leukocyte count (TLC), and differential leukocyte percentages were measured with an automated haematology analyser. Groups were compared by the independent samples *t*-test, with $P < 0.05$ considered significant. Dogs in the CDV group had significantly ($P < 0.01$) lower Hb (42.3%), TEC (32.3%), PCV (25.5%), and thrombocyte count (40.3%) than the control group, and all four values were below the canine reference limits. The TLC was significantly ($P < 0.01$) higher (46.2%), with higher percentages of granulocytes and monocytes and a lower percentage of lymphocytes. The absolute granulocyte ($14.45 \times 10^3/\mu\text{L}$) and monocyte ($1.68 \times 10^3/\mu\text{L}$) counts estimated from the group means exceeded the upper reference limits, whereas the estimated lymphocyte count ($1.77 \times 10^3/\mu\text{L}$) remained within the reference interval. The results indicate anaemia, thrombocytopenia, and granulocytic leukocytosis with monocytosis in CDV-infected dogs and suggest that the fall in lymphocytes was relative and not absolute.

Keywords: Canine distemper virus, Haematology, Anaemia, Thrombocytopenia, Leukocytosis, Rapid antigen test

Introduction

Dogs (*Canis lupus familiaris*) live in close association with humans as companion, guard, and working animals, and infectious diseases remain a major threat to their health. Canine distemper is among the most important viral diseases of dogs worldwide. It is caused by canine distemper virus (CDV), an enveloped, single-stranded RNA virus of the genus *Morbivirus* in the family *Paramyxoviridae* (Martella *et al.*, 2008) [1]. The virus infects a wide range of domestic and wild carnivores, which makes its control difficult even where vaccination is practiced (Beineke *et al.*, 2015) [2]. Affected dogs develop a multisystemic disease with respiratory, gastrointestinal, cutaneous, and neurological signs, and young, unvaccinated dogs suffer the most (Greene *et al.*, 2006; Buragohain *et al.*, 2018) [4, 3].

Canine distemper virus is strongly lymphotropic. It replicates in lymphoid tissues and destroys lymphocytes, particularly CD4⁺ T cells, which results in lymphopenia and marked immunosuppression (Iwatsuki *et al.*, 1995; Greene *et al.*, 2006) [5, 4]. This immunosuppression leaves affected dogs open to secondary bacterial infections. The virus also persists in the bone marrow, where it causes erythroid hypoplasia and shortens the life span of erythrocytes, leading to anaemia (Ezeibe *et al.*, 2008) [6]. Thrombocytopenia is a further recognised effect of CDV infection (Axthelm *et al.*, 1987) [7].

Haematological examination is a simple, rapid, and inexpensive investigation available in most veterinary clinics, and it helps to judge the severity of disease and the presence of complications. The haemogram of distemper, however, is not uniform. Almeida *et al.* (2009) [8] recorded a normal total leukocyte count with lymphopenia in 55.6% of affected dogs. Barbosa *et al.* (2011) [9] observed leukopenia in 16% and leukocytosis in 12% of 25 cases, whereas leukocytosis was more frequent in other reports, at 33.3% (Sousa *et al.*, 2015) [10] and 62% (Willi *et al.*, 2015) [11]. In experimentally infected dogs, leukocyte and lymphocyte counts fell during the early phase and rose later (Ezeibe *et al.*, 2008) [6]. The blood picture, therefore, depends on the stage of the disease and on its complications.

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In North East India, Behera *et al.* (2014) [12] reported anaemia, thrombocytopenia, and neutrophilic leukocytosis with lymphopenia in ten dogs with distemper, and Buragohain *et al.* (2018) [3] documented the occurrence of CDV infection in dogs in Guwahati. Immunochromatographic kits that detect CDV antigen allow a rapid antemortem diagnosis at the clinic (An *et al.*, 2008) [13], so that blood changes can be studied in dogs in which the antigen has been detected at the time of sampling. A leukocyte differential expressed only as percentages can hide whether a change is relative or absolute. However, studies describing the erythrocyte values, the thrombocyte count, and both the relative and the absolute leukocyte counts in dogs with CDV infection from this region are still limited.

The aim of the present work was to describe the haematological alterations in dogs with CDV-associated respiratory tract infection, diagnosed with a rapid antigen test kit, in comparison with healthy dogs.

Materials and methods

Study location, selection, and grouping of animals

The work was carried out at the Veterinary Clinical Complex (VCC), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India. Dogs presented to the VCC with a history of pyrexia, oculo-nasal discharge, cough, dehydration,

weakness, or anorexia were subjected to detailed clinical and physical examination, and the history of illness was recorded from the owner. The study included 12 dogs in two groups of six dogs each. Dogs in the CDV group were clinical cases with respiratory signs that tested positive for CDV antigen with the rapid test kit, whereas apparently healthy dogs served as the control group.

Collection of clinical samples

Nasal and conjunctival swabs were collected aseptically from each suspected dog using sterile swabs (Fig. 1a, b).

Detection of CDV antigen by rapid test kit

The presence of CDV antigen in the nasal and conjunctival swabs was tested with a rapid CDV antigen test kit (Bionote Inc., Republic of Korea), following the manufacturer's protocol.

The kit is a lateral-flow chromatographic immunoassay in which antibodies against CDV act as the capture and the detector antibodies. Each swab was mixed in the assay diluent supplied with the kit, four drops of the mixture were placed in the sample well of the test device, and the result was read between 5 and 10 min later. A test was recorded as positive when both the control (C) and the test (T) lines appeared in the result window, and as negative when only the control line appeared (Fig. 1c). A test in which the control line did not appear was treated as invalid, and the sample was retested with a new device.

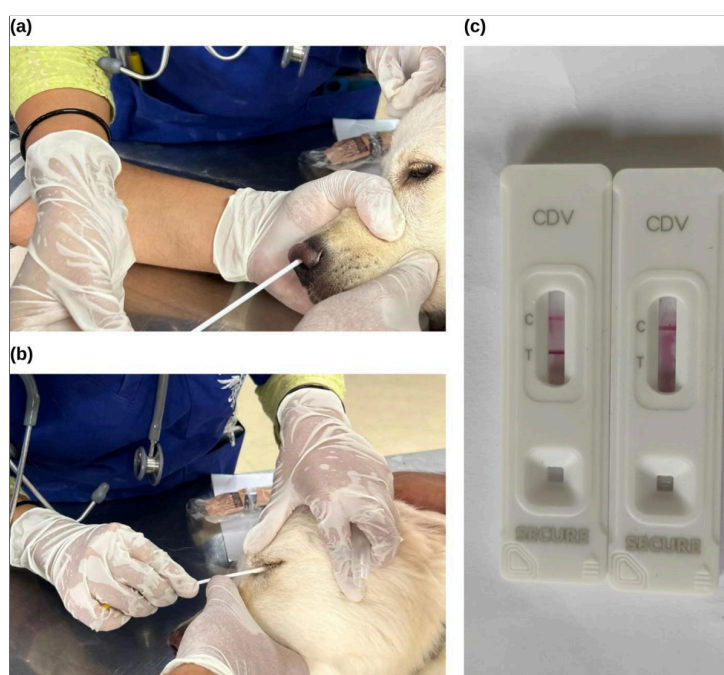
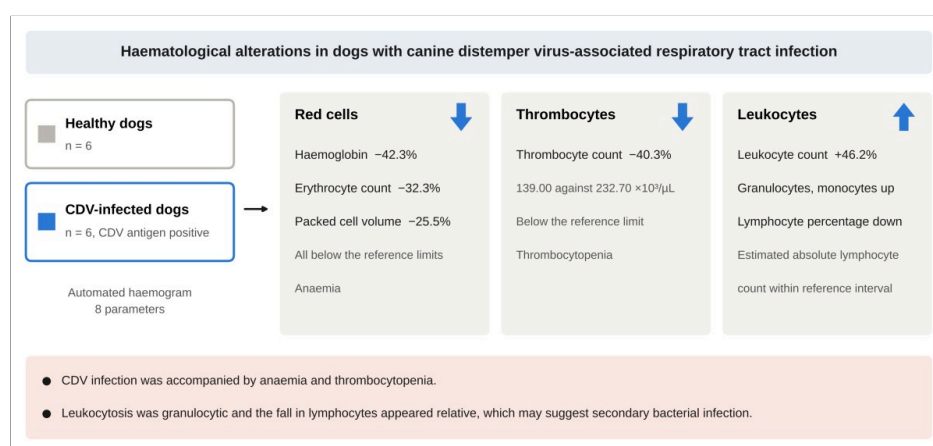


Figure 1. Collection of samples and detection of canine distemper virus (CDV) antigen. (a) Collection of a nasal swab. (b) Collection of a conjunctival swab. (c) Rapid CDV antigen test devices showing a positive result (left; control and test lines) and a negative result (right; control line only)

Collection of blood and haematological analysis

Approximately 2.0 mL of blood was collected aseptically from the cephalic or saphenous vein of each dog using a sterile disposable syringe and transferred immediately into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA). The samples were analysed using a fully automated haematology analyser (MEK-6550, Nihon Kohden, Tokyo, Japan).

Haemoglobin (Hb, g/dL), total erythrocyte count (TEC, $\times 10^6/\mu\text{L}$), packed cell volume (PCV, %), thrombocyte count ($\times 10^3/\mu\text{L}$), and total leukocyte count (TLC, $\times 10^3/\mu\text{L}$) were recorded. The analyser gives a three-part leukocyte differential, from which the percentages of granulocytes, lymphocytes, and monocytes were recorded.

Estimation of absolute leukocyte counts

The absolute count of each leukocyte type was estimated for each group from the mean TLC and the mean differential percentage:

Absolute count ($\times 10^3/\mu\text{L}$) = TLC ($\times 10^3/\mu\text{L}$) $\times$ differential percentage / 100

These estimates were not compared statistically. All haematological values were interpreted against the canine reference intervals given by Fielder (2024) [14]. Because the analyser does not separate the types of granulocytes, the interval for segmented neutrophils was used for granulocytes.

Statistical analysis

Statistical tests were run in SPSS 20.0 for Windows (IBM Corp., Armonk, NY, USA). The independent-samples *t*-test was used to compare the CDV and control groups. Differences were taken as significant at $P < 0.05$, and values are given as mean $\pm$ standard error. The difference between the groups was also expressed as a percentage of the control mean. Figures were drawn with Matplotlib version 3.11.2 in Python (<https://matplotlib.org/>).

Results

Erythrocyte values and thrombocyte count

The erythrocyte values and thrombocyte count of the two groups are shown in Table 1 and Fig. 2. Dogs in the control group had an Hb of 14.90 g/dL, a TEC of $6.20 \times 10^6/\mu\text{L}$, and a PCV of 42.80%. Dogs in the CDV group had a significantly ($P < 0.01$) lower Hb (8.60 g/dL), TEC ($4.20 \times 10^6/\mu\text{L}$), and PCV (31.90%), which corresponded to falls of 6.30 g/dL (42.3%), $2.00 \times 10^6/\mu\text{L}$ (32.3%), and 10.90 percentage points (25.5%), respectively, compared to the control group. All three values in the CDV group were below the lower limits of the ca-

nine reference intervals (11.9 g/dL, $4.95 \times 10^6/\mu\text{L}$, and 35%, respectively).

The thrombocyte count of the CDV group ($139.00 \times 10^3/\mu\text{L}$) was 40.3% lower ($P < 0.01$) than that of the control group ($232.70 \times 10^3/\mu\text{L}$) and lay below the lower reference limit of $211 \times 10^3/\mu\text{L}$.

Table 1. Haemoglobin, total erythrocyte count, packed cell volume, and thrombocyte count (mean $\pm$ SEM) of healthy dogs and dogs naturally infected with canine distemper virus (CDV)

Parameters	Control group	CDV group
Haemoglobin (g/dL)	14.90 ^a $\pm$ 0.14	8.60 ^b $\pm$ 0.13
Total erythrocyte count ($\times 10^6/\mu\text{L}$)	6.20 ^a $\pm$ 0.11	4.20 ^b $\pm$ 0.15
Packed cell volume (%)	42.80 ^a $\pm$ 0.27	31.90 ^b $\pm$ 0.26
Thrombocyte count ($\times 10^3/\mu\text{L}$)	232.70 ^a $\pm$ 3.02	139.00 ^b $\pm$ 2.49

$n = 6$ per group. Means with different superscripts (a, b) in a row differ significantly ($P < 0.01$)

Total and differential leukocyte counts

The TLC and differential leukocyte percentages are presented in Table 2 and Fig. 2. The TLC of the CDV group ($17.90 \times 10^3/\mu\text{L}$) was significantly ($P < 0.01$) higher than that of the control group ($12.24 \times 10^3/\mu\text{L}$), an increase of $5.66 \times 10^3/\mu\text{L}$ or 46.2%, and it exceeded the upper reference limit of $14.1 \times 10^3/\mu\text{L}$. The percentage of granulocytes increased from 76.10% to 80.70% and that of monocytes from 7.70% to 9.40% ($P < 0.01$), whereas the percentage of lymphocytes decreased from 16.20% to 9.90% ($P < 0.01$). The difference of each parameter from the control mean is presented in Fig. 3a.

Table 2. Total leukocyte count and differential leukocyte percentages (mean $\pm$ SEM) of healthy dogs and dogs naturally infected with canine distemper virus (CDV)

Parameters	Control group	CDV group
Total leukocyte count ($\times 10^3/\mu\text{L}$)	12.24 ^b $\pm$ 0.22	17.90 ^a $\pm$ 0.23
Granulocytes (%)	76.10 ^b $\pm$ 0.18	80.70 ^a $\pm$ 0.31
Lymphocytes (%)	16.20 ^a $\pm$ 0.13	9.90 ^b $\pm$ 0.12
Monocytes (%)	7.70 ^b $\pm$ 0.17	9.40 ^a $\pm$ 0.16

$n = 6$ per group. Means with different superscripts (a, b) in a row differ significantly ($P < 0.01$)

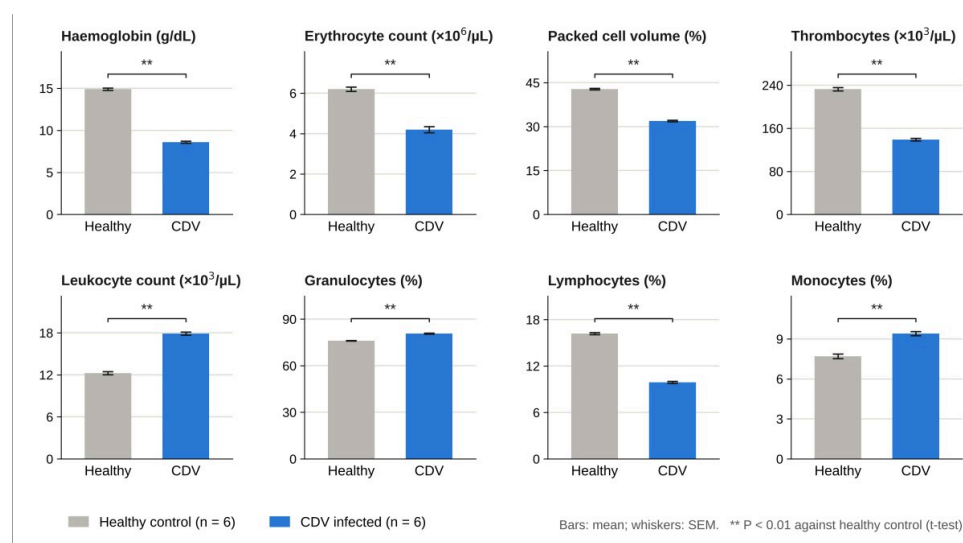


Figure 2. Haematological parameters of healthy dogs and dogs naturally infected with canine distemper virus (CDV). Bars show the mean and whiskers the SEM ($n = 6$ per group). ** $P < 0.01$ against the control group

The estimated absolute leukocyte counts are given in Table 3 and Fig. 3b. The granulocyte count rose from $9.31 \times 10^3/\mu\text{L}$ in the control group to $14.45 \times 10^3/\mu\text{L}$ in the CDV group and exceeded the upper

reference limit of $12.0 \times 10^3/\mu\text{L}$. The monocyte count rose from 0.94 to $1.68 \times 10^3/\mu\text{L}$, above the upper reference limit of $1.4 \times 10^3/\mu\text{L}$. In contrast, the lymphocyte count changed little, from 1.98 to $1.77 \times 10^3/\mu\text{L}$, and remained within the reference interval.

Leukocyte type	Control group	CDV group	Reference interval
Granulocytes	9.31	14.45	2.9–12.0
Lymphocytes	1.98	1.77	0.4–2.9
Monocytes	0.94	1.68	0.1–1.4

(2024) [14]; the interval for segmented neutrophils is given for granulocytes

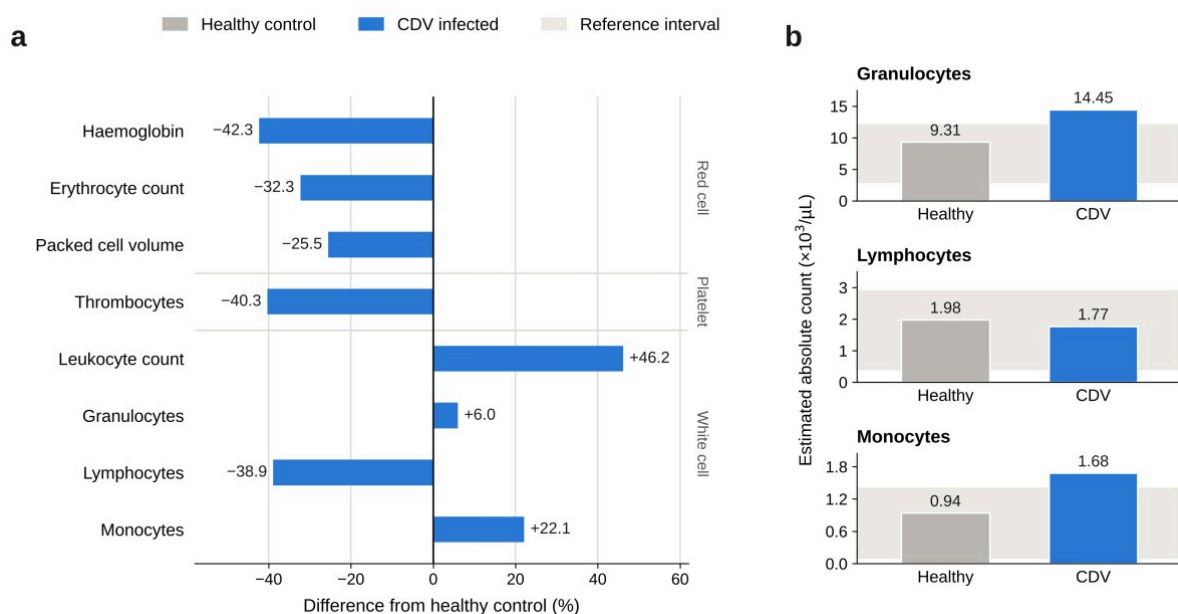


Figure 3. (a) Difference between CDV-infected and healthy dogs, expressed as a percentage of the control mean. (b) Estimated absolute granulocyte, lymphocyte, and monocyte counts. The shaded band is the canine reference interval

Canine distemper continues to occur in dogs in and around Guwahati, particularly in young and unvaccinated animals (Buragohain *et al.*, 2018) [3]. Because the virus attacks lymphoid tissue, bone marrow, and epithelial surfaces, changes in the blood are expected in most affected dogs (Greene *et al.*, 2006) [4]. Haematology is not specific for distemper, but it is widely available, and it helps the clinician to assess the severity of the disease and to recognise complications. In the present study, all eight haematological parameters of dogs positive for CDV antigen differed from those of healthy dogs.

In our study, Hb, TEC, and PCV were markedly reduced in the CDV group, and all three fell below the canine reference limits (Fielder, 2024) [14], which indicates anaemia. This agrees with earlier reports, in which anaemia was recorded in 64% (Barbosa *et al.*, 2011) [9], 69% (Willi *et al.*, 2015) [11], 72.2% (Sousa *et al.*, 2015) [10], and 88.9% (Almeida *et al.*, 2009) [8] of dogs with distemper. The mean Hb of the present dogs (8.60 g/dL) was lower than the values reported by Jesus *et al.* (2021) [15] (10.87 g/dL) and by Saeed *et al.* (2021) [16] (10.51 g/dL), whereas the TEC and PCV were close to theirs ($4.47 \times 10^6/\mu\text{L}$ and 33.29%, and $4.95 \times 10^6/\mu\text{L}$ and 31.42%, respectively). The morphological type of the anaemia was not assessed in the present study. In earlier reports it has varied, from normocytic normochromic (Almeida *et al.*, 2009) [8] and normocytic hypochromic (Behera *et al.*, 2014) [12] to microcytic hypochromic (Saeed *et al.*, 2021) [16].

Several mechanisms may explain the anaemia of distemper. The virus persists in the bone marrow, causes erythroid hypoplasia, and shortens the life span of erythrocytes (Ezeibe *et al.*, 2008) [6]. Almeida *et al.* (2009) [8] found apoptotic cells in the peripheral blood and bone marrow of affected dogs and suggested that apoptosis is involved in the haematological changes. In addition, inflammatory cytokines reduce the availability of iron, the survival of erythrocytes, and the regenerative capacity of the marrow (Kendall, 2024) [17]. Blood loss from intestinal parasites or poor nutrition could add to this, but neither was assessed in the present dogs.

The thrombocyte count of the CDV group was 40.3% lower than that of the control group and lay below the reference limit (Fielder, 2024) [14]. These results match earlier studies, in which thrombocytopenia was recorded in 33.3% of dogs with distemper (Sousa *et al.*, 2015) [10], in affected dogs from North East India (Behera *et al.*, 2014) [12], and in a severely affected pup (Headley *et al.*, 2009) [18]. Thrombocytopenia induced by CDV has also been described in experimentally infected dogs (Axthelm *et al.*, 1987) [7].

The present study showed a significant increase in TLC, with higher percentages of granulocytes and monocytes and a lower percentage of lymphocytes. Leukocytosis in distemper was also reported by [Behera *et al.* \(2014\)](#) [12], [Sousa *et al.* \(2015\)](#) [10], and [Willi *et al.* \(2015\)](#) [11]. When the differential was converted into absolute counts, the estimated granulocyte and monocyte counts exceeded the reference limits, whereas the estimated lymphocyte count stayed within the reference interval. The lymphocyte percentage of the CDV group (9.90%)

also lay within the reference interval of 8–21% (Fielder, 2024) [14]. The fall in the lymphocyte percentage therefore appears to be mainly relative, a result of the rise in granulocytes. This differs from the absolute lymphopenia usually described in distemper, which was present in 55.6% of the dogs studied by Almeida *et al.* (2009) [8], with a mean count of 1,054 cells/ μ L, and in 76% of those studied by Barbosa *et al.* (2011) [9].

The stage of the disease is the likely reason for this difference. In experimental infection, leukocyte and lymphocyte counts fall first and rise later (Ezeibe *et al.*, 2008) [6]. Naturally infected dogs usually reach the clinic after the early viraemic phase, when the immunosuppression caused by the virus may already have allowed secondary bacterial infection. A granulocytic leukocytosis with monocytosis and relatively few lymphocytes is the pattern of an inflammatory or stress leukogram (Kritsepi-Konstantinou *et al.*, 2016) [19]. In a previous study at the same clinic, dogs with bacterial respiratory tract infection had TLC values of 19.26 to 22.96 $\times 10^3/\mu$ L, compared with 10.17 $\times 10^3/\mu$ L in healthy dogs, together with neutrophilia (Sarkar *et al.*, 2024) [20]. The leukogram of the present cases is thus compatible with distemper complicated by bacterial infection, although this was not confirmed by culture.

Conclusion

This study showed that dogs with CDV-associated respiratory tract infection in Guwahati had anaemia and thrombocytopenia, together with a leukocytosis made up of granulocytes and monocytes. The lower lymphocyte percentage appeared to be a relative change, as the estimated absolute lymphocyte count remained within the reference interval. While haematology cannot confirm distemper, it helps to recognise anaemia, a low thrombocyte count, and possible bacterial complications that need attention during treatment.

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