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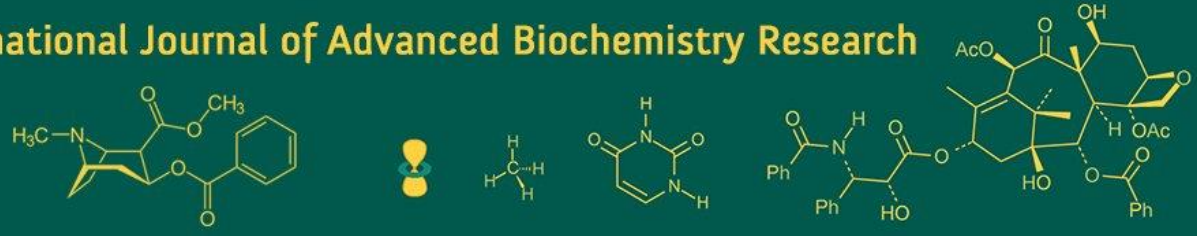

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Cytohistopathological evaluation and immunophenotyping of canine lymphoma

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Abstract
The aim of this study is to determine the chemo-profile of aqueous extract of *Nauclea latifolia* using GC-MS. The GC-MS analysis of the plant extract was performed by using GC-MS QP-2010 (Shimadzu) and the mass spectra of the compounds found in the extract were matched with the data in the library of the National Institute of Standards and Technology (NIST) The result revealed the presence of eighteen compounds. The most prevailing compounds were 9-Octadecenoic acid (Z)-methyl ester, Oleic acid, and Benzamide 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl] having a percentage area of 16.93%, 14.01%, and 9.18% respectively. The least abundant compound was 5-Methyl-2-phenylindolizine (0.66%). Some of these phytocompounds' bioactivities include anti-inflammatory, antibacterial, and antimalarial activities. As a result, the leaf extract has the potential to be a source of novel treatments. The results of this investigation thus validate the plant's traditional usage for medicine.

Keywords: Canine lymphoma, cytology, histopathology, immunophenotyping, CD79a, diffuse large B-cell lymphoma

Introduction
In veterinary oncology, the incidence of cancer in dogs is considerably high, with spontaneous tumors such as malignant melanoma, osteosarcoma, and lymphoma serving as valuable comparative models for human oncology (Gordon *et al.*, 2009, and Paoloni *et al.*, 2009) [16, 28]. Among these, canine lymphoma is one of the most common hematopoietic malignancies, closely mirroring human non-Hodgkin lymphoma (NHL) in terms of morphology, immunophenotype, biological behavior, and therapeutic response (Marconato *et al.*, 2011) [22]. Diagnosis of canine lymphoma is based on clinical signs, particularly generalized painless lymphadenopathy, and involves WHO staging, hematological analysis, imaging, cytology, histopathology, and immunophenotyping for confirmation and subtyping (Zandvliet, 2016 and McLinden *et al.*, 2024) [43, 25].
As per the World Health Organization (WHO) classification, canine lymphoma is categorized based on morphology, immunophenotype, and clinical characteristics, encompassing 25 distinct subtypes (Valli *et al.*, 2011; Shevchik and Samoyluk, 2020) [38, 32]. Approximately 70 Percent of cases are of B-cell origin, and most frequently diagnosed as Diffuse Large B-cell Lymphoma (DLBCL), which closely parallels its aggressive human counterpart (Ponce *et al.*, 2010 and Będkowska *et al.*, 2025) [30, 6].
Canine lymphoma is one of the most frequently diagnosed malignancies in dogs, accounting for approximately 7-24 Percent of all tumors and 80-90 Percent of hematopoietic neoplasms, with reported incidence rates ranging from 13-114 cases per 100 000 dogs across different regions (Liptak, 2007; Chandrasekar, 2009; and Meuten, 2020) [20, 9, 26]. It predominantly affects middle-aged to older dogs, with peak incidence between 5 and 10 years of age and a mean age at diagnosis of around 8y, though certain breeds such as Bullmastiffs may develop the disease earlier (Edwards *et al.*, 2003; Villamil *et al.*, 2009; and Matise-Van Houtana and Geine-Romanova, 2022) [13, 41, 23]. Certain breeds show a markedly higher predisposition to canine lymphoma, with Boxers, Golden Retrievers, Rottweilers, Bullmastiffs, Labrador Retrievers, German Shepherds, and Cocker Spaniels being most commonly affected, and distinct breed-related associations observed for B-cell and T-cell

subtypes, supporting a strong genetic influence on disease susceptibility (Modiano *et al.*, 2005 and Zandvliet, 2016) [27, 43]. Most studies report a slight male predominance in the occurrence of canine lymphoma, though some findings indicate a relatively balanced sex distribution, suggesting minimal overall sex-related influence on disease incidence (Matise-Van Houtana and Geine-Romanova, 2022 and Van der Heiden *et al.*, 2025) [23, 39].

Cytological evaluation is a fundamental tool for diagnosing canine lymphoma, especially high-grade variants, with fine-needle aspiration (FNA) of enlarged lymph nodes being a rapid, minimally invasive, and cost-effective technique (Teske, 1994 and Aresu *et al.*, 2015) [35, 2]. High-grade lymphomas, such as DLBCL, are characterized by a monomorphic population of large lymphoblasts with high nuclear-to-cytoplasmic (N:C) ratios, conspicuous nucleoli, basophilic cytoplasm, and frequent mitoses, with >50% lymphoblasts considered diagnostic and >80-90 Percent typical for aggressive forms (Zwingenberger *et al.*, 2012; Batista *et al.*, 2022) [44, 5]. Low-grade lymphomas, including small lymphocytic, centrocytic, and T-zone lymphoma, exhibit smaller, less proliferative lymphoid cells with distinct nuclear and cytoplasmic features (Thamm *et al.*, 2007 and Valli, 2008) [36, 37]. Certain cytological variants, such as Mott cell differentiation in B-cell lymphoma, null-cell lymphoma, and Burkitt-like lymphoma, provide additional diagnostic clues (Bain, 2009; Kim *et al.*, 2022; and Sirivisoot *et al.*, 2024) [4, 18, 33]. Cytology also helps differentiate lymphoma from reactive hyperplasia, which shows a heterogeneous lymphoid population (Pinheiro *et al.*, 2014) [29].

Histopathological evaluation is essential for the accurate diagnosis and classification of canine lymphoma, particularly in low-grade or cytologically ambiguous cases, as it assesses tumor architecture, nuclear features, and mitotic index to guide prognosis and therapy (Valli *et al.*, 2011 and Będkowska *et al.*, 2025) [38, 6]. According to the WHO classification, canine lymphoma comprises over 25 subtypes based on B-and T-/NK-cell lineage, though approximately 80% of cases are represented by five major subtypes: DLBCL, Marginal Zone B-cell lymphoma, Peripheral T-cell lymphoma not otherwise specified, nodal T-zone lymphoma, and T-lymphoblastic lymphoma (Valli *et al.*, 2011 and Zandvliet, 2016) [38, 43]. In the present study, another subtype, Burkitt's lymphoma, was also diagnosed, highlighting the diversity of canine lymphoma subtypes.

Diffuse Large B-cell Lymphoma (DLBCL) shows diffuse effacement of lymph node architecture with large neoplastic cells 2-2.5× the size of erythrocytes, rounded to cleaved nuclei, coarse or fine chromatin, prominent nucleoli, basophilic cytoplasm, and numerous mitoses; subtypes include centroblastic, immunoblastic, and anaplastic forms, with macronucleolated medium-sized cells (MMCs) sometimes observed (Valli *et al.*, 2011; Ponce *et al.*, 2010 and Chen *et al.*, 2019) [38, 30, 10].

Marginal Zone B-Cell Lymphoma (MZL) is characterized by a nodular growth pattern with faded germinal centers, paler corona of neoplastic B cells, marginated chromatin, single central nucleoli, low mitotic activity, and gradual inward progression sparing medullary cords and perinodal fat in early stages (Meuten, 2020) [26].

Burkitt's Lymphoma in dogs is characterized by sheets of medium-sized lymphocytes with high mitotic activity, a "starry sky" pattern due to tingible body macrophages, basophilic cytoplasm, nuclei up to 1.5× RBC diameter with prominent nucleoli (3-5 per nucleus), and focal capsular invasion; necrosis is typically absent (Valli *et al.*, 2011 and Meuten, 2020) [38, 26].

Immunophenotyping classifies canine lymphomas based on lymphocyte lineage with prognostic significance (Frantz *et al.*, 2013 and Zandvliet, 2016) [15, 43]. B-cell lymphomas are the most common (≈60-75%), generally have a better prognosis, and express markers such as CD20, CD21, CD79a, and PAX5. T-cell lymphomas account for 30-40% of cases, are often more aggressive, and express CD3, CD4, and CD8 (Appelbaum *et al.*, 1984 and Bienzle and Vernau, 2011) [1, 8]. Null cell lymphomas are rare (<5%) and lack detectable B-or T-cell markers (Ponce *et al.*, 2010 and Valli *et al.*, 2011) [30, 38].

Materials and Methods

In this seven-month study, 40 suspected cases of spontaneously occurring canine lymphoma were collected from the Veterinary College Hospital, Bengaluru, and various government veterinary hospitals in and around Bengaluru. These samples were submitted to the Department of Veterinary Pathology for cytological evaluation, along with detailed records of the animals' clinical signs, history, breed, age, and sex.

Fine Needle Aspiration Cytology (FNAC)

FNAC was performed by stabilizing the enlarged lymph node and aspirating cellular material with a 22-G needle from multiple directions. The aspirates were smeared onto clean, dry slides and stained using Giemsa or Field's stain. For Giemsa staining, air-dried smears were methanol-fixed for one minute, stained with Giemsa diluted in phosphate buffer (pH 7.2) for 40 minutes, rinsed, air-dried, and mounted with DPX (Coles, 1986) [11]. For Field's staining, air-dried smears were methanol-fixed, sequentially stained with Field's B (red, 5-6 sec) and Field's A (blue, 10-30 sec), rinsed, air-dried, and mounted with DPX (Mbassi *et al.*, 2022) [24].

Histopathology

Following surgical excision, tissue samples were fixed in 10% neutral buffered formalin, paraffin-embedded, and sectioned at 4 µm using a rotary microtome. Sections were stained with Hematoxylin and Eosin (H&E) (Luna, 1968) [21] and examined microscopically for classification of canine lymphoma according to the WHO guidelines (Valli *et al.*, 2011) [38].

Immunophenotyping

Immunochemicals

Primary antibodies for CD79a (mouse monoclonal IgG1κ, Clone: HM47) and CD3 (mouse monoclonal IgG1, Clone: CA17.2A12) detection were obtained from Thermo Fisher Scientific®. A ready-to-use HRP-conjugated goat anti-mouse IgG secondary antibody (Thermo Fisher Scientific®) was used for all targets. Visualization was performed using a DAB Substrate Kit (10X DAB solution and stable peroxide substrate buffer, Thermo Scientific®).

Protocol

Immunohistochemical detection of B-and T-cell lymphomas was performed on paraffin-embedded canine lymph node sections using mouse monoclonal antibodies against CD79a

and CD3. Following deparaffinization, endogenous peroxidase blocking, and heat-induced epitope retrieval in 10 mM citrate buffer (pH 6.0), tissue sections were incubated overnight at 4 °C with primary antibodies, followed by peroxidase-labeled secondary antibody at 37 °C for 1 hour. Visualization was achieved with DAB substrate, and sections were counterstained with Harris hematoxylin, dehydrated, cleared, and mounted in DPX. Canine lymph node sections served as positive controls (Ramos-Vara, 2005 and Valli *et al.*, 2011) [31, 38].

Results

Age

The occurrence of canine lymphoma was observed in dogs aged between 2 and 13y, with a mean age of 6.27y. The highest occurrence (32%) was noted in the 4-6y age group, followed by 20 Percent in the 2-4y age group. Both the 6-8 and 8-10y groups accounted for 16 Percent each, while 12 Percent of cases were recorded in dogs aged 12-14y and 4 Percent in the 10-12y age group (Table 1).

Breed

Breed-wise analysis of canine lymphoma revealed the highest occurrence in non-descript breeds (32%), followed by Rottweiler, Labrador Retriever, and Beagle (16% each), then Shih Tzu (12%), and American Bully and Pakistani Mastiff, each accounting for 4 Percent (Table 2).

Sex

In the present study, analysis of sex-wise distribution revealed that the majority of canine lymphoma cases occurred in male dogs, accounting for 68 Percent (n = 17) of the total cases. In contrast, female dogs constituted 32 Percent (n = 8) (Table 3).

Gross Pathology

Gross examination of the affected lymph nodes revealed marked enlargement, measuring between 3 and 9 cm at their greatest diameter. The nodes were firm in consistency, with cut surfaces predominantly creamy white to pinkish in color. In some cases, focal to diffuse hemorrhages were noted on the cut surfaces of the neoplastic lymph nodes (Fig. 1 and 2).

Cytology

A total of 40 canine lymph node FNAC samples were collected from the Veterinary Clinical Complex, Bengaluru, and nearby clinics. Among these, 35 samples were cytologically diagnosed as lymphoma and five as reactive hyperplasia.

Cytological examination of lymphoma cases revealed a predominantly homogeneous population of lymphoid cells, mainly intermediate to large-sized lymphocytes with occasional lymphoblasts. The neoplastic cells were arranged in monolayers or loose, poorly cohesive sheets. Morphologically, the cells were round to oval with a high N:C ratio. The cytoplasm varied from scant to abundant with variable basophilia. Marked cellular atypia was observed, characterized by moderate to marked anisocytosis and anisokaryosis. The nuclei displayed vesicular chromatin with prominent, centrally placed single (Fig. 3) or multiple bizarre-shaped nucleoli (Fig. 4), and in intermediate-sized lymphocytes, the nuclei were equal to or larger than the diameter of two erythrocytes (Fig. 5). In Burkitt's lymphoma and DLBCL cases, tingible body macrophages imparted a characteristic starry-sky appearance (Fig. 6). Mitotic figures ranged from moderate to high in frequency, and abundant lymphoglandular bodies were also evident (Fig. 7).

In reactive hyperplasia, cytological smears showed a diffuse distribution of lymphoid cells predominantly arranged in monolayers. The cell population was heterogeneous, consisting mainly of small lymphocytes with scattered intermediate and occasional large lymphocytes. There was no evidence of anisocytosis or anisokaryosis, indicating cellular uniformity. The small lymphocytes had round to oval nuclei, equal to or smaller than the size of two erythrocytes, with vesicular chromatin and a distinct single nucleolus. The cytoplasm was well-defined with basophilic staining, and plasma cells along with lymphoglandular bodies were also observed.

Histopathology

Out of the 35 canine lymphoma cases, histopathological evaluation was performed on 25 tissue samples. Among these, 17 cases (68%) were diagnosed as diffuse large B-cell lymphoma (DLBCL), while four cases each (16% each) were identified as Burkitt's lymphoma and marginal zone B-cell lymphoma (Table 4).

Diffuse Large B-Cell Lymphoma (DLBCL)

Microscopically, the lesional tissue was composed of solid nests of lymphoid cells arranged in sheets, exhibiting a characteristic "starry-sky" appearance. The normal architecture of the lymph node was completely effaced, with total loss of germinal centers (Fig. 8). The neoplastic lymphocytes formed a monomorphic population and demonstrated capsular invasion extending into the peri-nodal areas. These cells were predominantly large lymphocytes, uniform in size and shape, with an increased N:C ratio. Nuclear pleomorphism was evident, characterized by the presence of distinct, large central nucleoli (Fig. 9) or multiple bizarre nucleoli impinging upon the nuclear membrane (Fig. 10).

Burkitt's Lymphoma

The lesional tissue exhibited solid nests of lymphoid cells arranged in sheets, displaying a characteristic "starry-sky" appearance due to the presence of tingible body macrophages (Fig. 11). The entire nodal architecture was completely effaced, with total loss of germinal centers. The neoplastic lymphocytes were uniform in size and shape, predominantly intermediate-sized, and showed an increased N:C ratio. Nuclear pleomorphism was evident, characterized by distinct, large central nucleoli or multiple small nucleoli (2-4), with no evidence of nucleolar impingement on the nuclear membrane. The nuclei were round to oval, with occasional elongation or indentation, and the chromatin was coarsely clumped (Fig. 12). The cells formed a monomorphic population of lymphocytes, and mitotic figures were frequent, ranging from 8 to 19 per high-power field (Fig. 13).

Marginal Zone B-Cell Lymphoma

The lesional tissue exhibited irregularly sized germinal centers with a fading germinal histoarchitecture (Fig. 14). The nuclei of the lymphoid cells appeared vesicular, with marginally placed chromatin and thickened nuclear

membranes. The neoplastic cells displayed very prominent single central nucleoli, and the cytoplasm was moderate to abundant with irregular yet distinct borders (Fig. 15). Mitotic figures were frequently observed. In the marginal areas, the neoplastic cells showed pale staining and appeared to coalesce or bridge with adjacent germinal marginal regions. Focal invasion of peri-nodal adipose and connective tissue by neoplastic cells was also evident.

Immunophenotyping

In the present study, immunophenotyping was done by immunohistochemistry on canine lymph node tissue samples to differentiate into B-cell and T-cell types using CD79a and CD3 markers, respectively.

In the control canine lymph node tissue, CD79a produced characteristic cytoplasmic and membranous staining, appearing brown to dark brown, confirming its specificity for B-lymphocytes (Fig. 16). Lymph node sections from canine lymphoma cases that exhibited a similar cytoplasmic and membranous staining pattern were classified as B-cell lymphomas. For T-cell identification, CD3 staining in control lymph node tissue showed distinct membranous and cytoplasmic positivity of T-lymphocytes, confirming marker reactivity (Fig. 17).

Based on the immunohistochemical findings, all 25 examined cases were identified as B-cell lymphomas, exhibiting strong cytoplasmic and membranous immunopositivity for CD79a and absence of CD3 expression (Fig. 18). The representative lymphoma sections showed no CD3 immunoreactivity, confirming the lack of T-cell lineage involvement (Fig. 19).

Table 1: Age-wise occurrence of canine lymphoma

Age Group (y)	Occurrence	Percent Occurrence
2 to 4	5	20
4 to 6	8	32
6 to 8	4	16
8 to 10	4	16
10 to 12	1	4
12 to 14	3	12
Total	25	100

Table 2: Breed-wise susceptibility of canine lymphoma

Breed	Occurrence	Percent Occurrence
Non-descript	8	32
Rottweiler	4	16
Labrador Retriever	4	16
Beagle	4	16
Shih Tzu	3	12
American Bully	1	4
Pakistani Mastiff	1	4
Total	25	100

Table 3: Sex wise occurrence of canine lymphoma

Sex	No. of dogs	Percent Occurrence
Male	17	68
Female	8	32
Total	25	100

Table 4: Histopathological classification of canine lymphoma (n = 25)

Classification	No. of dogs	Percent Occurrence
DLBCL	17	68
Burkitt's lymphoma	4	16
Marginal B-cell lymphoma	4	16
Total	25	100

Fig 1: Gross picture of enlarged right prescapular lymph node

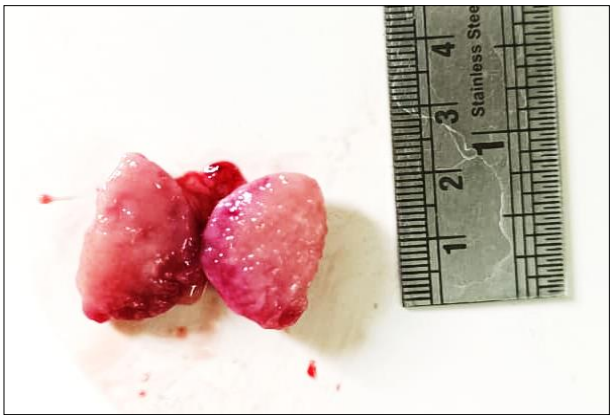

Fig 2: Gross view of the lymph node cut surface exhibiting a creamy white to pinkish coloration

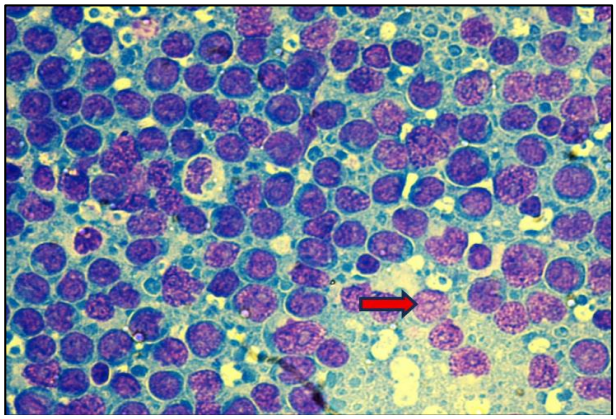

Fig 3: Cytological picture of lymphocytes showing a centrally placed single large nucleolus (red arrow). Fields stain X 400

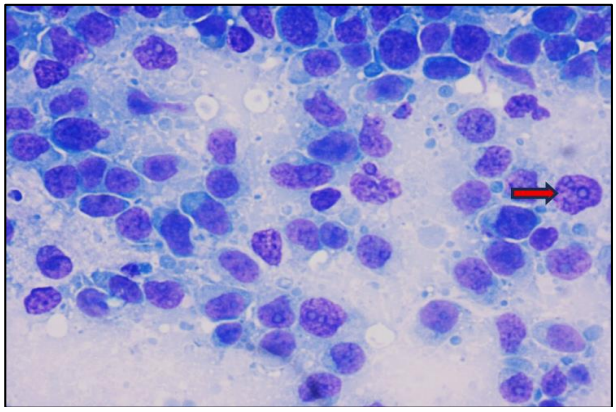

Fig 4: Cytological picture of lymphocytes showing multiple bizarre-shaped nucleoli (red arrow). Fields stain X 400

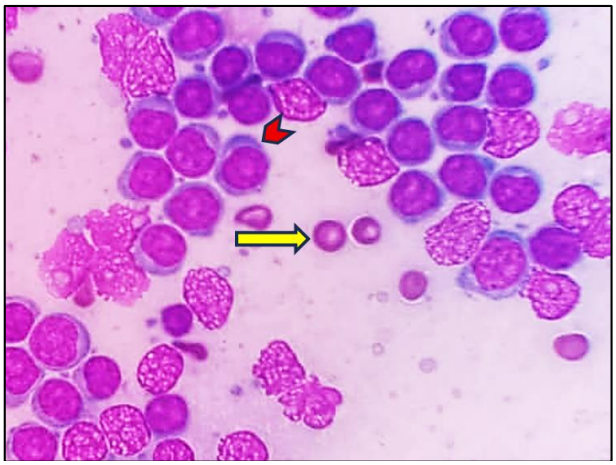

Fig 5: Cytological picture of a lymph node showing the majority of intermediate-sized lymphocyte population (red arrow head) measuring size ≥2 RBCs (yellow arrow) in size. Fields stain X 400

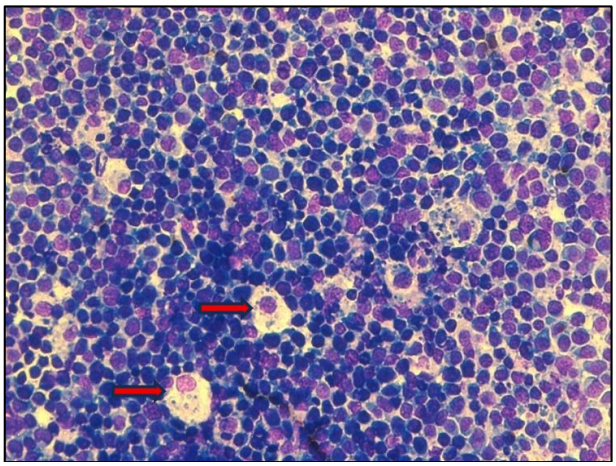

Fig 6: Cytological picture of a lymph node showing tingible body macrophages, giving a starry sky appearance (red arrows). Fields stain X 100

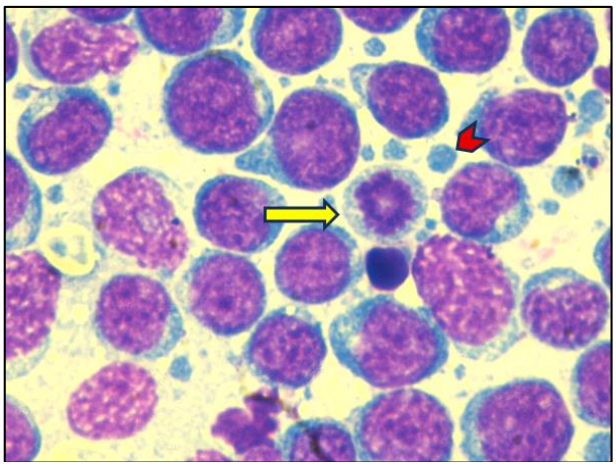

Fig 7: Cytological picture of a lymph node showing lymphoglandular body (red arrow head) and mitotic figure (yellow arrow) in size. Fields stain X 1000

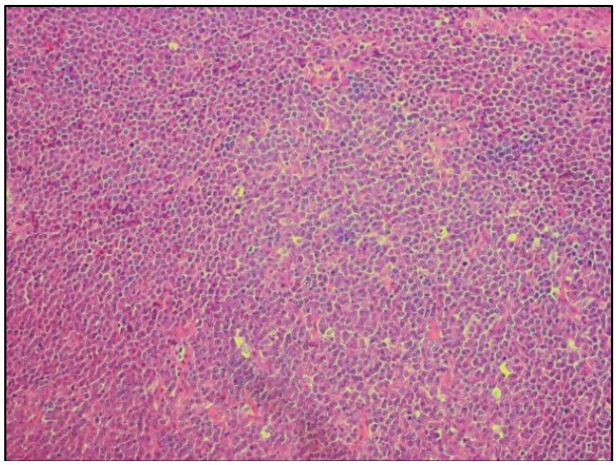

Fig 8: Histological section of neoplastic lymph node showing effacement of architecture, i.e., loss of germinal centres. H & E X 40

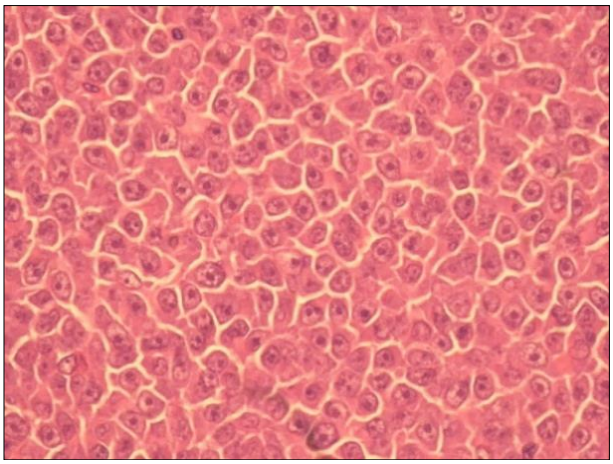

Fig 9: Histological section of a neoplastic lymph node showing large lymphocytes with a centrally placed single large nucleolus. H & E X 400

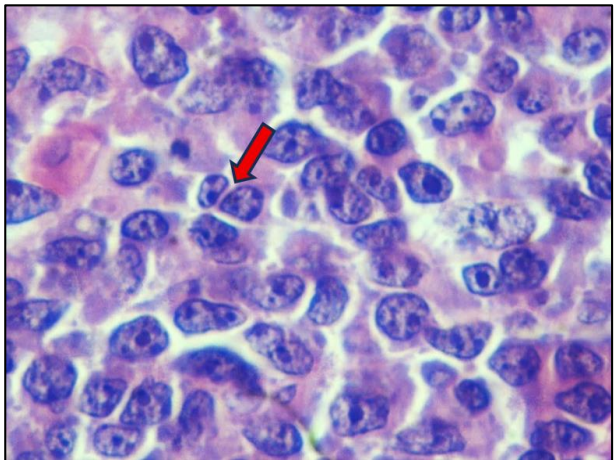

Fig 10: Histological section of a neoplastic lymph node showing lymphocytes with multiple bizarre-shaped nucleoli impinging on the nuclear membrane (red arrow). H & E X 1000

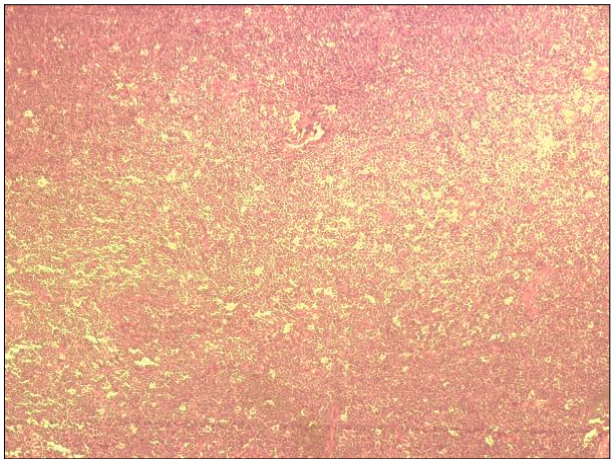

Fig 11: Histological section of neoplastic lymph node showing a solid nest of lymphocytes arranged in solid sheets with a “starry sky” appearance, due to the presence of tangible body macrophages. H & E X 40

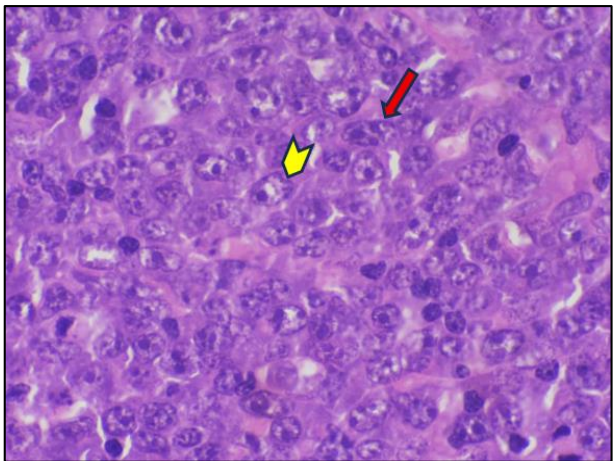

Fig 12: Histological section of a neoplastic lymph node showing the majority of round to oval nuclei and a few cells showing elongated (red arrow) and indented nuclei (yellow arrowhead). H & E X 400

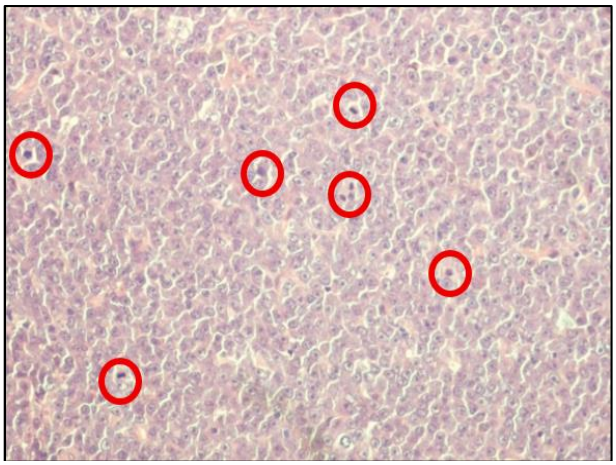

Fig 13: Histological section of a neoplastic lymph node showing mitotic figures (red circles). H & E X 200

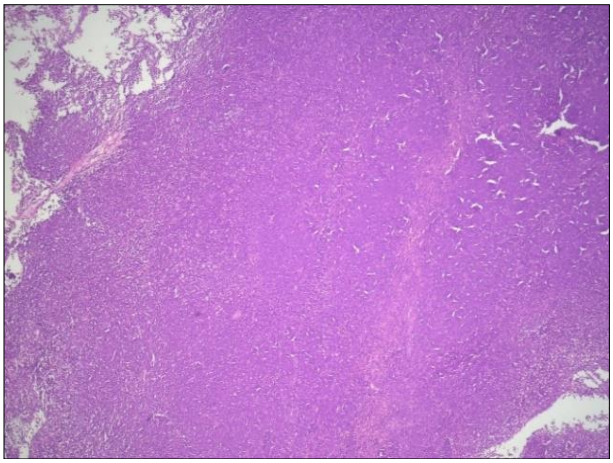

Fig 14: Histological section of a neoplastic lymph node showing fading germinal centre architecture. H & E X 40

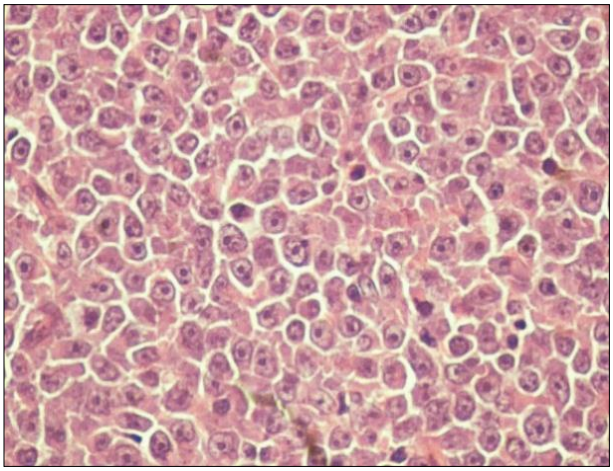

Fig 15: Histological section of a neoplastic lymph node showing lymphocytes with central large nucleoli and thickened nuclear membrane. H & E X 400

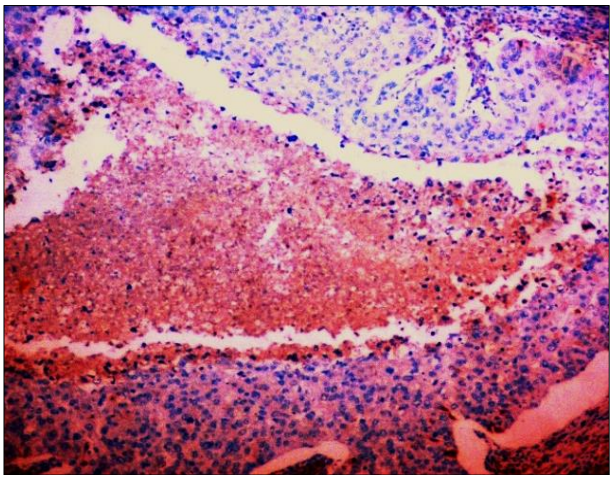

Fig 16: Immunohistochemical section of a canine lymph node used as a positive control, showing cytoplasmic and membranous positivity for CD79a. DAB brown, IHC X100

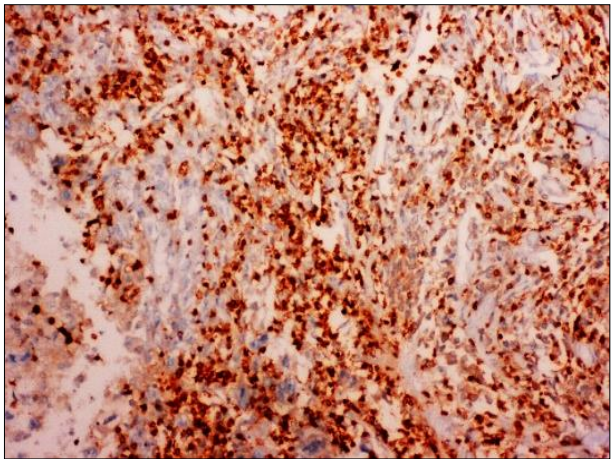

Fig 17: Immunohistochemical section of a canine lymph node used as a positive control, showing cytoplasmic and membranous positivity for CD3. DAB brown, IHC X200

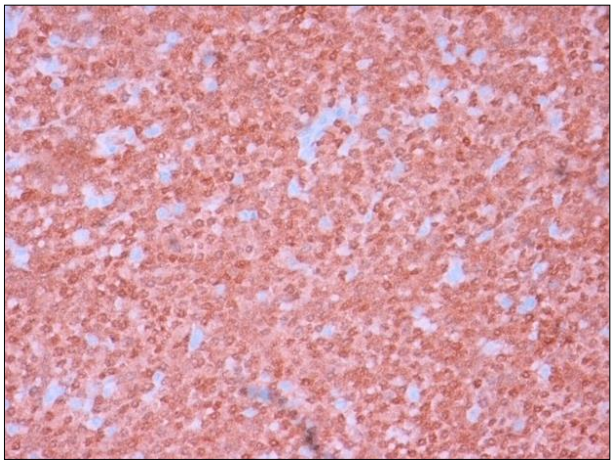

Fig 18: Immunohistochemical section of a lymph node tissue of a canine lymphoma case showing cytoplasmic and membranous positivity for CD79a-Positive. DAB brown, IHC X 200

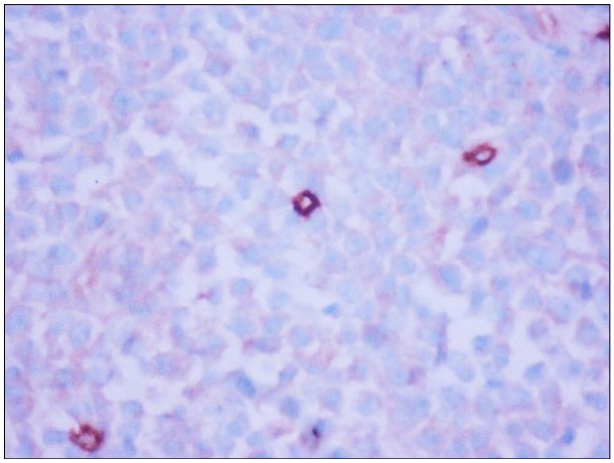

Fig 19: Immunohistochemical section of a lymph node tissue of a canine lymphoma case showing cytoplasmic and membranous positivity for CD3-positive. DAB brown, IHC X 400

Discussion

The present study was undertaken to determine the occurrence of canine lymphoma with respect to age, breed, and sex, and to evaluate the cytological and histopathological characteristics, and immunophenotyping.

The mean age of occurrence of canine lymphoma was 6.27y, with peak occurrence observed in the 4-6-y age group. These findings align with previous reports indicating that lymphoma predominantly affects middle-aged dogs. Certain breeds, such as Bullmastiffs, may develop lymphoma at a younger age (Edwards *et al.*, 2003; Villamil *et al.*, 2009; and Matise-Van Houtana and Geine-Romanova, 2022) [13, 41, 23]. Despite the peak in middle-aged dogs, lymphoma can occur across a wide age range, indicating that age alone does not independently determine the risk of tumor development, highlighting the importance of considering both age and breed in clinical assessment and management.

Breed-wise analysis showed the highest occurrence of canine lymphoma in non-descript dogs (32%) and the lowest in American Bully and Pakistani Mastiff (4%). These findings partially align with previous studies, which indicate that medium to large breeds are generally more susceptible, whereas smaller breeds have a lower risk (Modiano *et al.*, 2005 and Zandvliet, 2016) [27, 43]. The predominance of ND dogs may reflect their larger population, greater preference as pets, and higher presentation at veterinary hospitals. These findings underscore the importance of considering breed and population characteristics in clinical assessment and management.

Sex-wise analysis in the present study revealed a male predominance in canine lymphoma. These findings are generally consistent with previous reports indicating a slight male predisposition (Matise-Van Houtana *et al.*, 2022) [23], although some studies reported a balanced sex distribution (Van der Heiden *et al.*, 2025) [39] or higher occurrence in females in certain breeds such as Boxers and Golden Retrievers (Jankowska *et al.*, 2019 and Eraghi *et al.*, 2025) [17, 14]. Despite differences in incidence between sexes, most studies, including a review by Bennett *et al.* (2023) [7], indicate that sex alone is not a consistent prognostic factor. The observed male predominance in this study may be attributable to the larger male population, suggesting that sex plays a limited and variable role in the development of canine lymphoma.

The findings of the present study are in agreement with previous reports on the utility of cytology for diagnosing and differentiating canine lymphoma (Landgren *et al.*, 2004; Vezzali *et al.*, 2010; Zwingenberger *et al.*, 2012; Pinheiro *et al.*, 2014; Xie *et al.*, 2015; Aresu *et al.*, 2015; Aresu, 2016; Suwa and Shimoda, 2018; Chen *et al.*, 2019; and Batista *et al.*, 2022) [19, 40, 44, 29, 42, 2, 3, 34, 10, 5]

The histopathological findings of the present study are in agreement with previous reports describing DLBCL as the most common and clinically significant subtype in dogs, exhibiting high-grade features, aggressive behavior, and characteristic histomorphology (Valli *et al.*, 2011; Chen *et al.*, 2019; and Meuten, 2020) [38, 10, 26]. The observed histopathological patterns also align with the WHO classification of canine lymphomas, supporting accurate differentiation and prognostication (Valli *et al.*, 2011 and Meuten, 2020) [38, 26]. Overall, the findings support the importance of detailed histopathological assessment for precise classification, prognosis, and therapeutic planning in canine lymphoma.

Immunophenotyping in the present study revealed that all 25 canine lymphoma cases were of B-cell origin based on immunohistochemistry. These findings align with previous reports indicating that B-cell lymphomas are the predominant subtype in dogs, generally accounting for 60-75% of cases and associated with a more favorable

prognosis compared to T-cell lymphomas (Zandvliet, 2016; Bennett *et al.*, 2023; and Eraghi *et al.*, 2025) [43, 7, 14]. The absence of T-cell and null-cell lymphomas in this study contrasts with literature reporting T-cell lymphomas in 10-40% of cases (Dobson *et al.*, 2002 and Eraghi *et al.*, 2025) [12, 14], likely reflecting the randomized sample selection and the short seven-month study duration rather than true population prevalence.

Conclusion

The present study highlights that canine lymphoma predominantly affects middle-aged dogs, with non-descript breeds showing the highest occurrence and a slight male predisposition. Cytological evaluation proves to be a rapid and reliable diagnostic tool for differentiating lymphoma from reactive lymphoid conditions and for identifying high-grade lymphoma. Histopathological assessment confirms DLBCL as the most common and clinically significant subtype, exhibiting characteristic high-grade features and supporting the WHO-based classification for accurate prognostication. Immunophenotyping demonstrates that all cases in this cohort were of B-cell origin, reinforcing the predominance of B-cell lymphomas in dogs and their relatively favorable prognosis. Overall, the study underscores the importance of integrated cytohistopathological evaluation and immunophenotyping for precise diagnosis, subtyping, and informed clinical management of canine lymphoma.

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