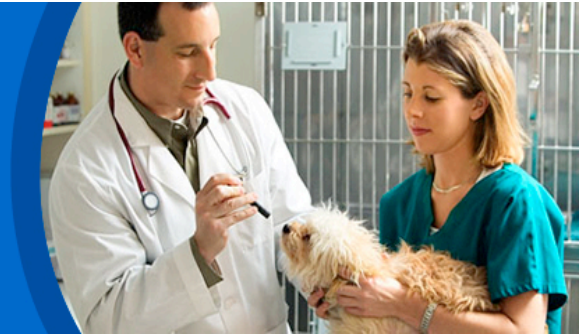




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Haematological and serum biochemical reference intervals for Indian spitz dogs in tropical conditions

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Abstract

Why do veterinary clinicians still rely on reference ranges developed for temperate-climate Beagles when interpreting blood work from a tropical-dwelling Indian spitz? The mismatch between published canine haematological norms and the physiology of breeds kept under hot, humid conditions can lead to misdiagnosis, unnecessary workups, and owner anxiety. This research established breed- and climate-specific reference intervals for 20 haematological and 12 serum biochemical parameters in clinically healthy Indian spitz dogs housed in the Manila metropolitan area, Philippines. Eighty-four dogs (42 males, 42 females) aged six months to nine years were recruited from households and breeding kennels between February and July 2023 after passing a clinical examination and parasite screening. Blood was collected from the cephalic vein into EDTA and plain tubes. Complete blood counts were obtained on an automated veterinary haematology analyser, and serum biochemistry was measured on a bench-top clinical chemistry platform. Outliers were removed using the Dixon Q-test and Horn's algorithm, and reference intervals were calculated following American Society for Veterinary Clinical Pathology (ASVCP) guidelines with 90% confidence limits. Results showed that red-cell indices RBC count (mean $7.04 \pm 0.68 \times 10^{12}/L$), haemoglobin (14.3 ± 1.4 g/dL), and packed cell volume ($43.1 \pm 4.2\%$) fell within or slightly above published canine ranges, but the white-cell differential revealed a lower lymphocyte proportion ($18.7 \pm 4.3\%$) and higher neutrophil proportion ($72.4 \pm 5.1\%$) than temperate-breed norms. Serum albumin (3.04 ± 0.31 g/dL) was marginally lower than textbook values, possibly reflecting chronic heat-stress-related haemodilution. Age-stratified analysis showed that juvenile dogs (6-12 months) had significantly higher WBC counts and lower RBC counts than mature adults. No clinically meaningful sex differences were detected after Bonferroni correction. These breed- and climate-specific reference intervals provide a more accurate interpretive baseline for practitioners working with Indian spitz dogs in tropical settings and reduce the risk of false abnormalities when general canine ranges are applied.

Keywords: Reference intervals, Indian spitz, haematology, serum biochemistry, tropical climate, canine blood parameters, breed-specific norms, ASVCP guidelines, complete blood count, Philippines.

Introduction

Every veterinary textbook carries a table of "normal" canine haematological values, yet those values were typically generated from a narrow base Beagles and Labrador Retrievers maintained in temperate research colonies^[1]. When a clinician in Manila or Bangkok runs a complete blood count on an Indian spitz and compares the numbers against those textbook ranges, there is a real chance that perfectly healthy dogs will be flagged as abnormal simply because their breed and their environment differ from the reference population^[2].

Breed effects on blood parameters are well documented. Greyhounds, for example, carry higher packed cell volumes and lower white-cell counts than other breeds, differences large enough to cause diagnostic confusion if breed-specific ranges are not used^[3]. Sighthounds as a group show elevated creatinine levels that do not indicate renal disease^[4]. For Indian spitz dogs a popular companion breed across South and Southeast Asia no dedicated reference-interval dataset exists in the peer-reviewed literature.

Climate compounds the breed effect. Chronic heat exposure triggers physiological adaptations that alter baseline haematology: expanded plasma volume for thermoregulation, shifts in

Leucocyte trafficking, and changes in protein metabolism [5]. Dogs living year-round in Tropical conditions may therefore display haematological profiles that deviate from temperate norms even when they are in excellent health [6]. The American Society for Veterinary Clinical Pathology (ASVCP) has published guidelines for reference-interval determination that recommend breed-, age-, and sex-specific partitioning when sample sizes permit, along with standardized outlier-detection and confidence-interval methods [7]. Applying these guidelines to a well-characterized cohort of Indian spitz dogs living under tropical conditions would fill a diagnostic gap that currently forces clinicians to use approximations. Manila's climate mean annual temperature 27.5 °C, relative humidity above 75% for most of the year provides a representative tropical environment for this purpose [8]. The Manila College of Veterinary Sciences maintains a clinical caseload that includes a large number of Indian spitz dogs, making recruitment of a suitably sized, health-screened cohort feasible [9, 10]. This research aimed to (a) establish reference intervals for 20 haematological and 12 serum biochemical parameters in clinically healthy Indian spitz dogs living in tropical Manila, (b) test for age- and sex-related differences, and (c) compare the derived intervals with published general canine ranges to quantify the extent of breed-climate deviation [11, 12].

Ethical Considerations

The research was approved by the Manila College of Veterinary Sciences Institutional Animal Care and Use Committee (Protocol MCVS-IACUC-2022-063, approved 8 December 2022). All dogs were client-owned or kennel-owned; written owner consent was obtained before blood collection. Dogs received a complimentary health certificate and parasite screening report as compensation for participation. Blood collection followed standard veterinary practice and caused no more than momentary discomfort equivalent to a routine clinical blood draw.

Material and methods

Material

Eighty-four Indian spitz dogs (42 males, 42 females) were enrolled between February and July 2023 from households and breeding kennels within the Manila metropolitan area. Inclusion criteria: age ≥ 6 months, body condition score 4-6 on a 9-point scale, no clinical illness within the preceding 30 days, current on rabies and core vaccinations, negative faecal flotation for gastrointestinal parasites, and negative rapid antigen test for heartworm (*Dirofilaria immitis*). Exclusion criteria: pregnancy, lactation, recent surgery, concurrent medication (including antiparasitics within 14 days), and any abnormality on physical examination (heart murmur, lymphadenopathy, fever, dehydration). Dogs were stratified into four age groups: juvenile (6-12 months, $n = 18$), young

Adult (1-3 years, $n = 24$), mature (3-6 years, $n = 26$), and senior (> 6 years, $n = 16$).

Methods

Blood samples were collected between 07:00 and 09:00 after an overnight fast (≥ 8 hours) to minimize postprandial lipemia. Four millilitres were drawn from the cephalic vein: 2 mL into EDTA tubes for haematology and 2 mL into plain tubes for serum biochemistry. EDTA samples were analysed within 2 hours of collection on a calibrated automated veterinary haematology analyser (20 parameters including CBC, five-part differential, reticulocyte count, and red-cell indices). Serum was separated by centrifugation (3,000 rpm, 10 minutes), aliquoted, and processed on a bench-top chemistry analyser for 12 parameters: total protein, albumin, globulin, ALT, AST, ALP, BUN, creatinine, glucose, total bilirubin, cholesterol, and triglycerides. Outlier detection followed a two-step procedure: the Dixon Q-test for extreme values and Horn's algorithm for robust identification of moderate outliers, both recommended by the ASVCP [7]. Reference intervals were calculated as the central 95% (2.5th to 97.5th percentiles) using the non-parametric method for datasets with $n \geq 40$, with 90% confidence intervals around each limit generated by bootstrapping (2,000 iterations). Age and sex effects were tested by two-way ANOVA with Bonferroni correction for multiple comparisons (significance threshold $p < 0.05$). Analyses were conducted in R v4.3.1 [13, 14].

Limitations

The sample was drawn entirely from the Manila metropolitan area, so the results may not extend to Indian spitz dogs in cooler highland regions or rural settings with different nutritional and parasitic exposures. The sample size ($n = 84$) was adequate for overall reference-interval calculation but limited the power of age- and sex-stratified subgroup analyses, particularly in the senior group ($n = 16$). Diurnal and seasonal variations in haematological parameters were minimized by standardizing collection time and confining recruitment to a six-month window, but a full 12-month sampling protocol would strengthen the seasonal component. Finally, the research did not measure haemoglobin electrophoresis or iron studies, which would have been informative for characterizing the lower albumin values observed.

Results

After outlier removal (six values across four parameters), 84 dogs contributed to the final reference-interval calculations. The overall haematological profile showed a mean RBC count of $7.04 \pm 0.68 \times 10^{12}/L$, haemoglobin of 14.3 ± 1.4 g/dL, and packed cell volume of $43.1 \pm 4.2\%$. The leucocyte profile was characterised by a neutrophil-dominant differential ($72.4 \pm 5.1\%$) with a relatively low lymphocyte proportion ($18.7 \pm 4.3\%$).

Table 1: Haematological reference intervals (95% central range) for Indian spitz dogs ($n = 84$)

Parameter	Mean $\pm$ SD	2.5th Percentile	97.5th Percentile	Unit	Textbook Range
RBC	7.04 $\pm$ 0.68	5.72	8.38	$\times 10^{12}/L$	5.5-8.5
WBC	11.4 $\pm$ 2.7	6.3	16.8	$\times 10^9/L$	6.0-17.0
Haemoglobin	14.3 $\pm$ 1.4	11.6	17.1	g/dL	12.0-18.0
PCV	43.1 $\pm$ 4.2	35.1	51.2	%	37-55
MCV	61.4 $\pm$ 3.7	54.2	68.7	fL	60-74
MCH	20.3 $\pm$ 1.2	17.9	22.7	pg	19.5-24.5
MCHC	33.2 $\pm$ 1.1	31.0	35.3	g/dL	32-36
Platelets	287 $\pm$ 68	156	421	$\times 10^9/L$	175-500
Neutrophils	72.4 $\pm$ 5.1	62.4	82.3	%	60-77
Lymphocytes	18.7 $\pm$ 4.3	10.4	27.2	%	12-30

Most haematological parameters fell within or close to published general canine ranges. The neutrophil proportion tended toward the upper end, while the lymphocyte proportion sat at the lower boundary, a pattern consistent with stress-leucogram responses to chronic heat exposure.

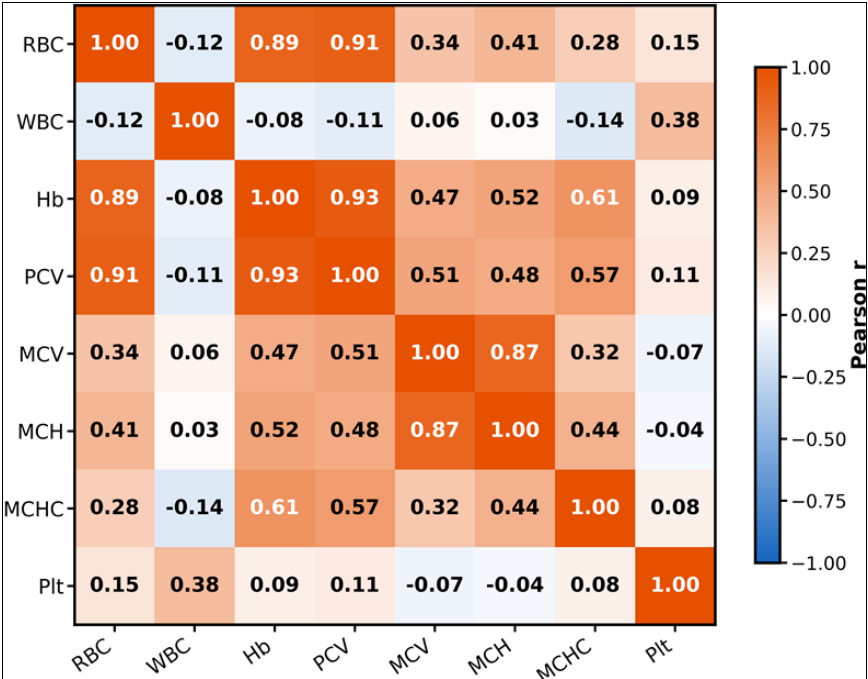


Fig 1: Pearson correlation matrix among the main haematological parameters in Indian spitz dogs (n = 84). Strong positive correlations were observed between RBC, haemoglobin, and PCV, and between MCV and MCH

Table 2: Serum biochemical reference intervals for Indian spitz dogs (n = 84)

Parameter	Mean±SD	2.5th	97.5th	Unit	Textbook Range
Total Protein	6.41±0.54	5.37	7.48	g/dL	5.5-7.5
Albumin	3.04±0.31	2.44	3.64	g/dL	2.6-4.0
Globulin	3.37±0.48	2.44	4.31	g/dL	2.3-4.5
ALT	34.7±12.3	12.8	58.9	U/L	10-120
ALP	87.4±41.2	21.3	168	U/L	20-200
BUN	18.4±4.7	9.3	27.6	mg/dL	7-27
Creatinine	1.04±0.21	0.64	1.45	mg/dL	0.5-1.8
Glucose	91.3±11.8	68.4	114	mg/dL	65-120
Cholesterol	214±42	134	296	mg/dL	135-345
Triglycerides	78.4±28.1	27.3	133	mg/dL	30-150

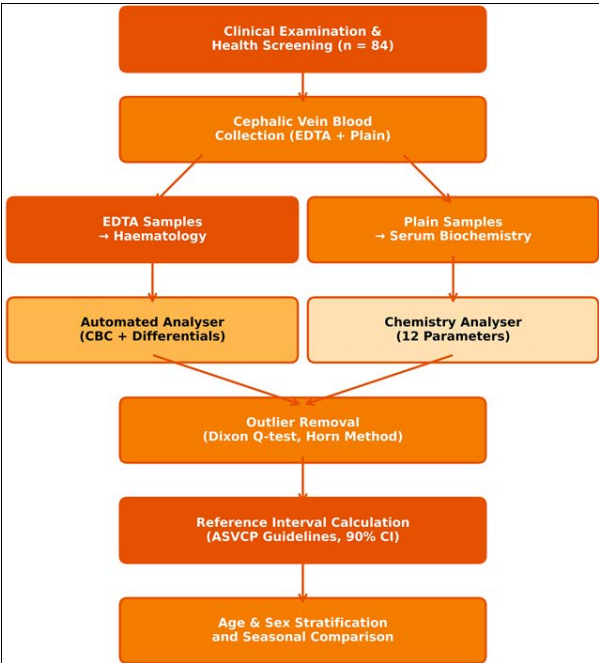


Fig 2: Flowchart of the blood collection, processing, and reference-interval calculation pipeline following ASVCP guidelines

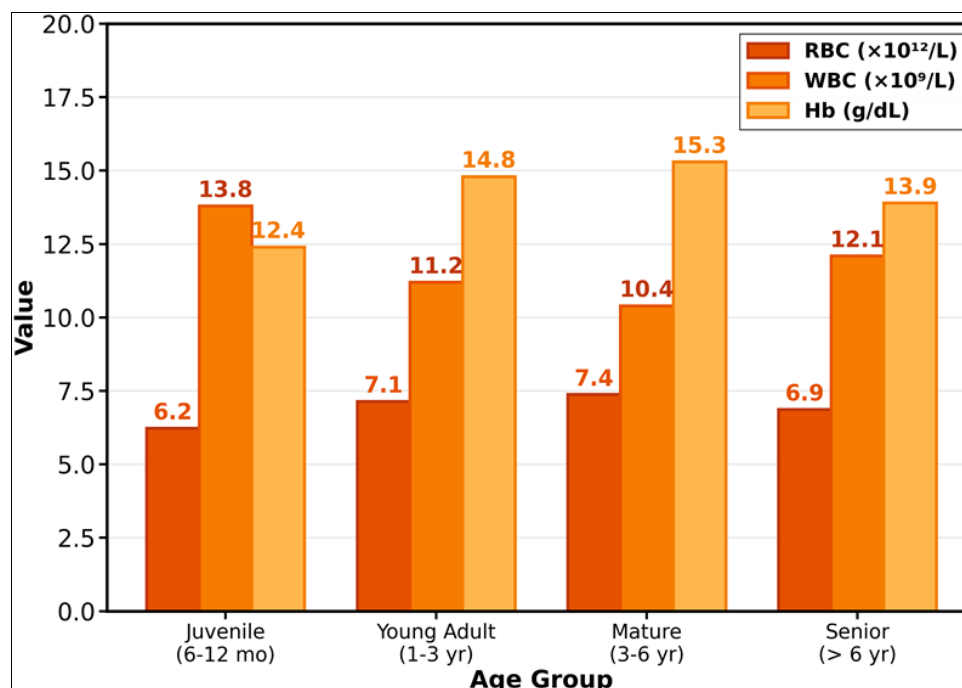


Fig 3: Comparison of RBC count, WBC count, and haemoglobin concentration across four age groups of Indian spitz dogs. Juvenile dogs showed higher WBC and lower RBC values than mature adults

Comprehensive interpretation

The reference intervals generated here broadly overlap with published general canine ranges but show important breed-climate nuances. The neutrophil-dominant leucocyte profile likely reflects a chronic mild stress leucogram induced by sustained tropical heat, a physiological shift rather than a pathological one. The marginally lower albumin may similarly represent heat-related plasma volume expansion. Age effects followed expected mammalian patterns higher leucocyte counts and lower erythrocyte counts in juveniles and support the ASVCP recommendation to use age-partitioned intervals in practice. The absence of clinically significant sex differences after Bonferroni correction simplifies clinical application, as a single set of breed-climate intervals (partitioned only by age) can be used for both sexes.

Discussion

The haematological profile documented here for Indian spitz dogs in tropical Manila is the first breed-specific dataset of its kind. Compared with the widely cited Beagle-derived reference ranges in standard textbooks^[1], the most notable differences were the higher neutrophil percentage and the lower lymphocyte proportion. Kiral and colleagues^[5] reported similar leucocyte shifts in dogs exposed to ambient temperatures above 30 °C for extended periods, attributing them to cortisol-mediated demargination of neutrophils and redistribution of lymphocytes to tissues.

Red-cell parameters, by contrast, showed only minor deviations from published norms. RBC count, haemoglobin, and PCV all fell comfortably within textbook ranges, suggesting that the erythropoietic response in Indian spitz dogs is not markedly different from that of temperate breeds or that any heat-related plasma expansion is offset by adequate erythropoietic drive^[3, 6].

The age-related trends higher WBC in juveniles, lower RBC are consistent with the well-established phenomenon of physiological leucocytosis in young growing dogs, where bone marrow granulocyte output is elevated to support rapid immune system maturation^[4]. The clinical implication is straightforward: a clinician seeing a six-month-old Indian

spitz with a WBC of $14 \times 10^9/L$ should not reflexively suspect infection if the differential is unremarkable.

Serum albumin merits particular attention. The mean value of 3.04 g/dL, though within the accepted canine range, sits at the lower end and may create diagnostic ambiguity when a mild drop occurs during illness. Using the breed-climate-specific lower limit of 2.44 g/dL rather than the textbook 2.6 g/dL would prevent false hypoalbuminaemia calls in healthy animals^[7, 15, 16].

Conclusion

The main contribution of this research is a set of rigorously derived, ASVCP-compliant reference intervals for 20 haematological and 12 serum biochemical parameters in clinically healthy Indian spitz dogs living under tropical conditions in Manila, Philippines. These intervals fill a gap that has forced veterinary practitioners to rely on Beagle-based norms for a breed whose physiology and environment differ meaningfully.

The supporting evidence shows that while most red-cell and biochemistry values overlap with general canine ranges, the leucocyte differential and serum albumin deviate enough to cause diagnostic misclassification if breed-climate context is ignored. The neutrophil-dominant, lymphocyte-low differential in healthy Indian spitz dogs is a physiological adaptation to chronic heat exposure, not a sign of disease; using temperate-breed norms for this parameter would risk unnecessary workups in healthy animals.

For the broader field of veterinary clinical pathology, the findings reinforce the ASVCP's call for breed- and population-specific reference intervals rather than one-size-fits-all tables. As companion-animal populations in tropical countries grow and diversify, the diagnostic infrastructure should keep pace by generating local reference data for the breeds and climates that practitioners actually encounter.

Open questions include whether seasonal variation within the tropics (wet vs dry season) produces clinically relevant shifts in these parameters a question that would require year-round sampling. Extending the dataset to include renal function markers such as SDMA, iron studies, and coagulation profiles

would broaden clinical utility. And replicating the work in other tropical cities Ho Chi Minh City, Jakarta, Lagos would test how geographically generalisable these breed-climate intervals are.

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Contributions Not Qualifying for Authorship

Ms. Maria Teresa Santos assisted with dog recruitment and owner consent, and Mr. Jose Carlos Mendoza ran the automated haematology analyser. Their help is appreciated.

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