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Investigation of Physiologic Leukopenia in Belgian Tervuren Dogs

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Background: Physiologic leukopenia in Tervuren dogs was reported in North America with a higher frequency in aged Tervurens. If not recognized, physiologic leukopenia can provoke unnecessary clinical investigations.

Hypothesis: The primary objective was to compare Tervurens and control dogs in Belgium with respect to the numbers of dogs with physiologic leukopenia. The secondary objectives were to compare Tervurens with control dogs and age classes within Tervurens and controls for parameters related to physiologic leukopenia.

Animals: Tervurens ($n = 94$) and control dogs ($n = 48$, maximum of 5 dogs per breed and 5 mixed breed dogs) were entered into the study. Dogs were 1–11 years old and healthy on routine physical examination. Dogs had no history of disease or drug administration in the previous 2 months.

Methods: Hematologic analyses were performed by an automated device within 30 hours of sampling. Blood smears were evaluated for cellular morphologic anomalies.

Results: Only 1 of the 94 Tervuren dogs had physiologic leukopenia (1.06%; 95% confidence interval, 0.05–5.22). Furthermore, the white blood cell (WBC) count in Tervuren dogs (median, $10.00 \times 10^9/L$; range, 5.90–20.80) was not significantly different ($P = .55$) from that of control dogs (median, $9.75 \times 10^9/L$; range, 5.20–20.90). The WBC count decreased significantly ($P < .001$) with age in Tervuren dogs.

Conclusions and Clinical Importance: Physiologic leukopenia is uncommon in the Belgian Tervuren dog. Differences with earlier data published in North America might be due to genetic or environmental differences.

Key words: Breed-specific characteristic; CBC; Physiologic leukopenia; Reference range.

Leukopenia is an absolute decrease in the number of circulating leukocytes below the lower limit of the reference range ($6 \times 10^9/L$).^{1,2} Leukocytes play a key role in host defense mechanisms, and patients with leukopenia have increased risk of infection. Consequently, additional diagnostic tests are indicated in leukopenic patients to identify a cause. In North American studies, otherwise healthy Belgian Tervuren dogs were reported to have decreased leukocyte counts compared to control dogs and a significant number of Tervuren dogs had leukopenia.^{3,4} Physiologic leukopenia resulted from low numbers of neutrophils, lymphocytes, and monocytes and was concluded to be a normal finding in Tervuren dogs. The percentage of leukopenic Tervuren dogs appeared to increase with age, reaching 65% for Tervurens older than 4 years. Additional diagnostic procedures were deemed unnecessary in otherwise healthy leukopenic Belgian Tervuren dogs.⁴

The Tervuren originates from Belgium and remains a popular breed in this country. Therefore, it seemed appropriate to investigate whether similar conclusions with respect to the frequency of physiologic leukopenia, the lower limit of the white blood cell count, and its relationship with age could be drawn for the Belgian

population of Tervuren dogs. If physiologic leukopenia was confirmed, separate reference ranges for this breed should be established to allow clinicians to compare obtained results reliably and avoid unnecessary diagnostic evaluation for physiologic leukopenia.

Materials and Methods

All dogs were recruited through breeders that were affiliated with the Belgian Kennel Club. Dogs had to be between 1 and 11 years of age and underwent thorough physical examination before sampling. The control dogs consisted of mixed breed and other purebred dogs excluding other varieties of the Belgian Shepherd (eg, Groenendael, Lakenois, Malinois), Greyhounds, and Scottish Collies. These breeds were excluded because they are related to the Tervuren or closely related to a breed that is reported to have abnormalities in WBC count.^{3–8} Control dogs belonged to the same owners as the studied Tervuren dogs, or to breeders, relatives, and members of the hospital staff. No more than 5 dogs per breed and 5 mixed breeds were accepted in the control group to eliminate bias because of breed specific characteristics.

At time of sample collection, each owner completed a survey about their dog's general health status, confirming that their pet had been free from disease and had not received any medication for at least 2 months before sampling. Collected information included a copy of the official pedigree of the dog (except for the 5 mixed breeds), the dog's date of birth and its sex. Owners were asked to contact the veterinarian if the dog showed any signs of disease during the week after sampling.

All blood samples were collected by the same 2 veterinarians in the dog's natural environment in order to minimize stress. Blood samples were collected from an unclipped cephalic vein, using a 22-gauge needle and 5-mL syringe. Samples were checked macroscopically for clots after placement in EDTA tubes. Samples were stored at 4°C and shipped at this temperature to the laboratory, where analysis was performed 12 to 30 hours after collection. CBCs were performed with an automated cell counter,^a which was subjected to a quality control check on a daily basis. Basophil numbers were not analyzed, because the low numbers of basophils in individual dogs precludes meaningful comparison.

Results obtained for Tervuren dogs and the controls were used to calculate median and range for each assessed parameter. The age distribution between Tervuren and control dogs was compared by

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Table 1. Hematologic values for Tervuren and control dogs.

Response variable	Reference range	Tervuren		Control dogs	
		Median	Range	Median	Range
Total WBC ^a ($\times 10^9/L$)	6.00–16.00	10.00	5.90–20.80	9.75	5.20–20.90
Neutrophils ($\times 10^9/L$)	3.000–11.500	5.890	3.170–14.850	5.890	2.850–15.870
Lymphocytes ($\times 10^9/L$)	1.000–4.800	2.680	1.280–5.950	2.365	1.010–4.320
Monocytes ($\times 10^9/L$)	0–1.350	0.400	0.160–3.280	0.470	0.120–2.300
Eosinophils ($\times 10^9/L$)	0–1.250	0.865	0.180–3.420	0.795	0.080–3.150
Platelets ($\times 10^9/L$)	164–510	279	63–612	321	68–599

^a Total WBC: total white blood cell count.

the Chi-Square test. The proportion of dogs with physiologic leukopenia in the population was estimated as the percentage of dogs with physiologic leukopenia and a 95% confidence interval was obtained based on the binomial distribution. These proportions were compared between Tervuren and control dogs using Fisher's exact test. Comparison of WBC between Tervuren and control dogs, and among the age classes within the Tervuren and control dog groups was based on the Kruskal-Wallis test at the 5% significance level. To investigate whether a systematic change in WBC (decrease or increase) occurred with age, the Spearman rank correlation was derived and it was tested to determine if it differed significantly from zero. The same analyses were done for other parameters related to physiologic leukopenia.

Results

Ninety-four Tervuren dogs were included in the study. A small number of available dogs (Tervuren and control dogs) were excluded on the basis of their physical examination findings, which disclosed slight ear or dental problems. Age ranged from 1 to 11 years with a median age of 4.4 years for the Tervuren dogs. There were 44 intact males, 2 castrated males, 39 intact females, and 9 spayed females. Body weight varied from 14 to 40 kg, with a median of 24 kg. The control group consisted of 48 dogs, from 16 different breeds: Border Collie (5), German Shepherd (5), Rottweiler (5), Nova Scotia Duck Tolling Retriever (5), Great Dane (4), Munsterlander (4), Australian Shepherd (2), Golden Retriever (2), Newfoundland (2), Saluki (2), Whippet (2), Beauceron (1), Dogo Argentino (1), Giant Poodle (1), Spanish Waterdog (1), Tibetan Terrier (1), and 5 mixed breeds. Age of control dogs ranged from 1 to 11 years, with a median age of 5.3 years. The control group consisted of 17 intact males, and 22 intact and 9 spayed females. Body weight ranged from 12.4 to 64 kg, with a median of 24 kg.

Tervurens and control dogs were subdivided into 7 different age groups (1–2 years, 2–3 years, 3–4 years, 4–5 years, 5–6 years, 6–8 years, and 8–11 years). The number of dogs in each category was, from the youngest to the oldest age group: 15, 9, 9, 20, 5, 20, and 16 for the Tervurens and 2, 8, 7, 5, 9, 5, 11, and 1 dog of unknown age (middle-aged mixed-breed dog) in the control group. The age distribution differed between Tervuren and control dogs ($P = .011$).

Median values of all assessed parameters of both Tervuren and control dogs are shown in Table 1. Only 1

Tervuren (WBC count $5.9 \times 10^9/L$) had leukopenia, resulting in a proportion of 1.06% with 95% confidence interval of 0.03 to 5.79. On the other hand, 2 control dogs (WBC count $5.2 \times 10^9/L$ and $5.9 \times 10^9/L$) were leukopenic (WBC count $<6 \times 10^9/L$) resulting in a proportion of 4.17% with 95% confidence interval of 0.51 to 14.25. There was no significant difference ($P = .264$) between control and Tervuren dogs with respect to the proportion of dogs with physiologic leukopenia.

Leukocytosis (WBC count $>16.0 \times 10^9/L$) was seen in 4 Tervuren and 5 control dogs. The WBC count of Tervuren dogs (median, $10.00 \times 10^9/L$; range, 5.90–20.80) was not significantly different ($P = .55$) from that of the control group (median, $9.75 \times 10^9/L$; range, 5.20–20.90). Neutrophil counts in Tervuren (median, $5.89 \times 10^9/L$; range, 3.17–14.85) did not differ significantly ($P = .978$) from those of the control group (median, $5.89 \times 10^9/L$; range, 2.85–15.87). Lymphocyte count was statistically higher ($P = .003$) in Tervuren dogs (median, $2.68 \times 10^9/L$; range, 1.28–5.95) than in the control group (median, $2.365 \times 10^9/L$; range, 1.01–4.32).

The age categories of the Tervuren dogs (Fig 1) differed significantly from each other in total WBC count ($P < .001$). The Spearman rank correlation coefficient between age and total WBC count was -0.53 and differed significantly from zero ($P < .0001$). The analysis of the individual WBC lines demonstrated significant differences among the age categories in segmented neutrophils ($P = .0297$), lymphocytes ($P = .0016$), eosinophils ($P = .0026$), and monocytes ($P = .0346$). Aging did not result in significant changes in total WBC count for control dogs (Fig 2), with the Spearman rank correlation coefficient between age and total WBC count equal to -0.04 and not significantly different from zero ($P = .77$).

Discussion

Physiologic leukopenia was only observed in 1 of 94 Tervurens and we conclude that it is uncommon in the Belgian population of Tervuren dogs, in contrast with earlier published data, which reported leukopenia in more than 33% of sampled Tervuren dogs.⁴ Stress may be responsible for observed alterations in the WBC count by the effect of corticosteroids and catecholamines on circulating neutrophils and lymphocytes. The effect of corticosteroids would increase the number of

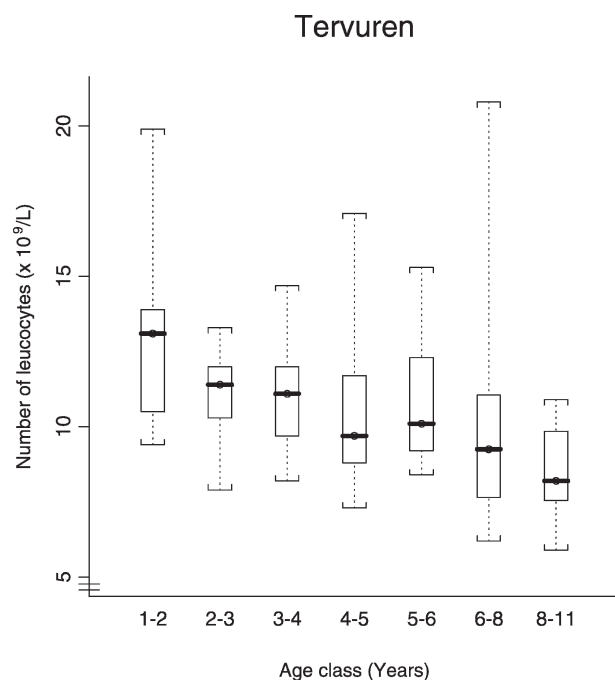


Fig 1. Boxplots of leukocyte counts/L for different age groups in Tervuren dogs. The line in the rectangle corresponds to the median, the upper and lower limit of the rectangle corresponds to the 25th and 75th percentile, and the whiskers correspond to the lowest and highest observed value.

circulating neutrophils and decrease the number of circulating lymphocytes. Catecholamines on the other hand are expected to increase neutrophil and lymphocyte numbers.^{4,9} Attempts were made to minimize the effect of stress, and in contrast to earlier studies sampling conditions were identical among the studied groups. Furthermore, neutrophils tended to be lower and lymphocytes higher in Tervurens compared to control dogs, neither of which would be expected because of catecholamine or glucocorticoid effects.⁹ These factors make any influence of stress very unlikely.

Owners were asked to contact the investigators if the dog displayed any signs of disease during the week after sampling, but no active effort was made by the investigators to question owners about the presence of disease a week after sampling. The investigators however believe that thorough history and physical examinations made the chance of including ill animals unlikely.

The sample size of 94 Tervuren and 48 control dogs provides a power of 80% to detect a difference between Tervuren and control dogs in a one-sided test at the 5% significance level if the population proportion of physiologic leukopenia corresponds to 25% and 6% for Tervuren and control dogs, respectively, which is sufficient for our purposes. No differences could be demonstrated, however, as the proportion of Tervuren dogs with physiologic leukopenia was very low.

The statistically significant decreases in total WBC count and segmented neutrophil and lymphocyte count with increasing age in Tervuren dogs was not observed in the control group. According to the literature, neutrophil and lymphocyte counts decrease in dogs

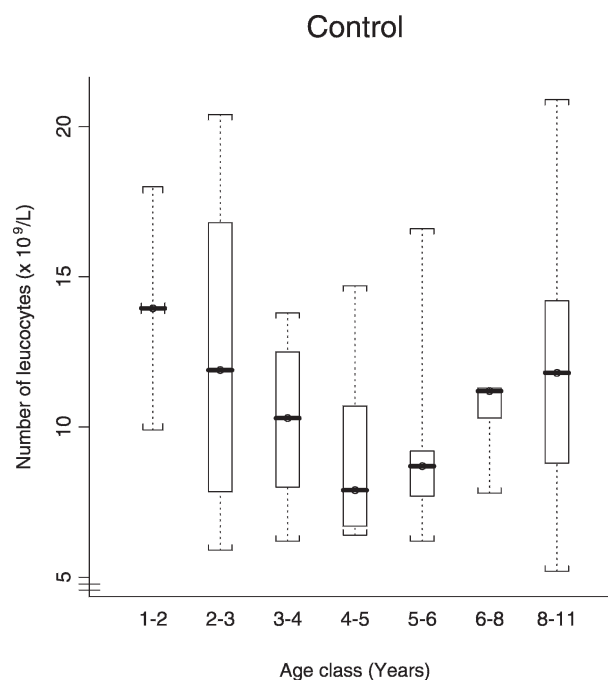


Fig 2. Boxplots of leukocyte counts/L for different age groups in control dogs. The line in the rectangle corresponds to the median, the upper and lower limit of the rectangle corresponds to the 25th and 75th percentile, and the whiskers correspond to the lowest and highest observed value.

from 6 months to 4 years of age, then plateau from 4 to 7 years before increasing again.¹⁰ This pattern was clearly observed in the control group (Fig 2). In contrast, the WBC counts of the Tervuren dogs did not plateau, nor did they increase from 6 years on, but appeared to decline further with aging (Fig 1). These results may reflect the higher proportion of dogs with leukopenia in older Tervurens in earlier published data.⁴ The only leukopenic Tervuren dog in our study was a 9-year-old intact female (a member of the oldest age category).

The reason for the decrease in WBC counts observed with aging in the Tervuren dogs remains unclear, but because environmental factors were similar between Tervuren and control dogs, a genetic trait with delayed penetrance seems likely. The 4 varieties of Belgian Shepherd are classified as 1 breed in Belgium and the Groenendael (which is the black-haired variety of the Tervuren) can be used for breeding to deliver a mixed Groenendael and Tervuren offspring. Special permission however must be given by the Belgian Kennel Club, and this certainly is an exception rather than common practice. Although this procedure highlights the broader genetic base of the Belgian Tervuren population, it is unlikely that genetic mixing with the Groenendael is the sole explanation for the differences in our results from the study by Greenfield et al.⁴

Prospective studies, evaluating the evolution of WBC counts in a selected group of Tervuren dogs with aging might help us better understand this phenomenon.

In human medicine, benign hereditary neutropenia is a well known phenomenon. It is inherited as an autosomal dominant trait, but the exact mechanism

remains unclear.¹¹ The neutropenia probably is caused by a defect in the release of neutrophils from the bone marrow storage pool.¹¹ Striking however is the fact that this phenomenon is restricted to ethnic groups from isolated communities (eg, Yemenite Jews, West Indians).^{11–13} These populations originated from a small number of ancestors, as the population of Tervuren dogs in North America was largely founded by a few imported animals.

Patients with benign hereditary neutropenia are reported to have more dental (gingival recession) and dermatologic problems (inflammation, infection) than normal individuals, which resulted in a change of the name of the condition from physiologic to benign hereditary neutropenia.^{13–15} Gingival inflammation and attachment loss might be associated with neutrophil numbers $<2.000 \times 10^9/L$.¹³ Infections on the other hand appear to be related to very low neutrophil counts ($<0.500 \times 10^9/L$). Coexisting neutrophil function defects have not been detected in these studies.¹⁵

In conclusion, physiologic neutropenia is not common in the Belgian population of Tervuren dogs. However a significant decrease in WBC count with aging was found. A prospective study of a small population of North American Tervuren dogs throughout the aging process and genetic studies in North American Tervuren dogs should be performed to obtain more information regarding this process. On the basis of this study, it appears prudent in Belgium, and probably in all areas besides North America, to further evaluate any Tervuren dog that is presented with leukopenia. Follow-up hematologic examinations or a further investigation and diagnostic evaluation is recommended until additional studies have been performed.

Footnotes

^a ADVIA 120 Hematology System, Bayer, Brussels, Belgium

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