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Changes in Haematological and Biochemical Parameters with Ageing in Dobermann Pinscher Dogs - A Pilot Study

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ABSTRACT: Ageing is a natural, non-pathological process initiated at birth and concluding with death, involving morphological and functional changes in all organs. Recent research has identified various cellular and molecular parameters associated with ageing in mammals, including altered intercellular communication, genomic instability, mitochondrial dysfunction, and epigenetic changes. As the population of elderly dogs increases globally, these animals frequently exhibit multi-organ impairments requiring continuous veterinary attention. This study aimed to assess the impact of ageing on the physiological characteristics of clinically healthy Dobermann pinscher dogs by analyzing haematological and biochemical parameters across different age groups. Twenty-four clinically healthy Dobermann Pinscher dogs were divided into four age groups and evaluated through clinical examinations, complete blood counts, and serum chemistry analyses. Results indicated that, while most parameters remained within normal ranges, geriatric dogs showed significant differences in haemoglobin, HCT, TEC, TLC, and MCV levels compared to younger dogs. Geriatric dogs also exhibited significant increases in total protein, globulin BUN, ALT, and ALP, along with reductions in blood glucose, serum calcium, and chloride levels. These findings emphasize the need for regular laboratory evaluations to distinguish between typical age-related changes and signs of underlying disease, thereby improving the quality of life and well-being of ageing dogs.

Keywords: Ageing dogs, biochemical, Dobermann pinscher, geriatric, haematology.

INTRODUCTION

Ageing is a natural, non-pathological process that begins at birth and concludes with death. This process induces morphological and functional changes in all the organs (Bellows *et al.,* 2015). Recently, numerous cellular and molecular parameters like altered intercellular communication, genomic instability, mitochondrial dysfunction, and epigenetic alterations were found to be associated with ageing in mammals. Older dogs make up a significant proportion of the pet dog population around the world. Ageing dogs frequently show multi-organ impairments, necessitating continuous veterinary care (Metzger & Rebar 2012).

Research studies indicates that haematological anomalies are prevalent in otherwise healthy older dogs (Willems *et al.,* 2016) and emphasizes the importance of routine laboratory tests to safeguard their welfare (Bellows *et al.,* 2015).

In recent years, numerous studies have focused on understanding the biochemical basis of ageing. As the elderly population expands, understanding the ageing process is crucial to thwart age-related sicknesses as intricate biological processes are closely connected with longevity across various animal species. Sharing their daily lives with humans, dogs are exposed to almost identical social and environmental factors. Subsequently, they are susceptible to similar age-related ailments, such as a type of dementia resembling Alzheimer's, a condition not naturally observed in mice. As pet dogs age, they experience an increase in ageing-related illnesses, which poses a significant financial burden. Therefore, advancing research on the biological effects of aging in dogs is important for enhancing their healthy and productive longevity.

As cherished family members, older dogs typically receive excellent veterinary care. When examining these senior dogs, veterinarians should be able to distinguish between typical age-related changes (*i.e.,* healthy ageing) and signs of underlying disease or abnormalities.

While aging-related biological analyses are well-documented in human medicine, similar data in veterinary medicine are limited, making the connection between age and clinical changes in dogs a subject of ongoing debate. Haematological and serum biochemistry profiles have been found to be more realistic and specific than routine physical examinations to investigate physiological changes in geriatric dogs. However, comprehensive data on the haematology and serum biochemistry of apparently healthy older and geriatric Dobermann dogs are poorly documented. This study evaluated the effects of ageing on the physiological characteristics of clinically healthy Dobermann Pinscher dogs by analyzing and comparing hematological and biochemical parameters across four different age groups

MATERIAL AND METHODS

This study examined 24 clinically healthy Dobermann Pinscher dogs (22 males and two females) brought for routine general health check-ups at the Referral Veterinary Polyclinic (RVC), ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh. The dogs were divided into four groups based on their age: Group I- 8 to <10 years of age, Group II- 10 to <12 years of age, Group III - > or equal to 12 years of age and Control group with 1-7 Years of age. As the ageing process differs with breeds, the groups of young, old and geriatric dogs were defined by available data on ageing in large dog breeds (Egenvall *et al*., 2005; Fernandes *et al.,* 2013). All dogs were clinically assessed by taking history, routine preliminary data, complete blood count& serum chemistry values. All examinations were performed with manual animal restraint, without sedation or anaesthesia. The exclusion criteria were situations of illness or treatment with drug administration.

A blood sample of 5 ml was collected from each dog through a cephalic vein puncture. Each sample was divided into two parts: a sterile tube with an anticoagulant (EDTA K3) and a sterile tube with a coagulation activator (dry tube). All the samples were collected between 8 and 11 a.m. and immediately sent to the clinical laboratory of the RVC for evaluation. Parameters such as mean haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total erythrocyte count (TEC), total leukocyte count (TLC), and differential leukocyte count (DLC) were estimated using a veterinary-specific automatic blood cell counter (model Urit 3000 VET Plus, Via Casa Sicignano, 42 – Sant'Antonio Abate (Na) – ITALY). The biochemical parameters and mineral profiles, namely total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, blood urea nitrogen (BUN), blood glucose, serum creatinine, serum calcium, sodium, potassium, chloride, uric acid, Phosphorous, alanine transaminase (ALT), AST and alkaline phosphatase (ALP) were estimated using appropriate biochemical test kits procured from Erba-Mannheim; Trans Asia Biomedicals, Himachal Pradesh.

Statistical analysis

The student's t-test for independent samples and Tukey's post-hoc test was used to compare differences between groups (intergroup analysis), and the significance level was established at 5% ($p < 0.05$). The statistical analyses were done using Graph Pad Prism 8.0 software.

RESULTS AND DISCUSSION

A. Analysis of haematological parameters of different age groups of Dobermann

The analysis results of blood and serum samples collected from 24 Dobermann Pinscher dogs of four different age groups were presented in Table 1 and 2. Across all groups studied, majority of the haematological parameters remained within normal limits, with the exceptions of MCV and MCH in groups 2 and 3. Significant differences ($p < 0.05$) were observed between the control group and group 3 in terms of haemoglobin levels, HCT, TEC, TLC, and MCV. Group 2 also showed significant differences ($p < 0.05$) from the control group in haemoglobin, HCT, and MCV. Specifically, MCV levels were significantly elevated ($p < 0.05$) in both groups 2 and 3 compared to the control group. Group 3 animals exhibited a notable reduction ($p < 0.05$) in haemoglobin concentration, HCT, and total erythrocyte count compared to the control animals. Similarly, group 2 animals showed a significant decrease ($p < 0.05$) in haemoglobin and total erythrocyte count. Both groups 2 and 3 displayed a significant increase ($p < 0.05$) in MCV relative to the control group.

B. Analysis of parameters related to serum biochemistry of different age groups of Dobermann

The findings from the serum biochemistry analysis across four age groups of Dobermann are detailed in Table 2. While most parameters fell within normal ranges, exceptions were noted for blood glucose, AST, ALT, globulin, uric acid, BUN, chloride, and ionized calcium. Group 3 demonstrated significant ($p < 0.05$) increases in total protein, albumin, globulin, BUN, ALT, and ALP compared to the control group. Additionally, this group showed significant ($p < 0.05$) reductions in blood glucose, serum calcium, and chloride levels. In Group 2, there were significant ($p < 0.05$) increases in total protein, BUN, and chloride levels, along with a significant reduction in blood calcium levels, when compared to the control group.

Dogs are generally considered elderly when they have reached 75% of their breed's typical life expectancy. In dogs, senility varies according to the breed and size of the animal. Large and giant dogs age earlier than smaller dog breeds and are considered geriatric from 9 years onwards (Bellows *et al*., 2015; Wade *et al.,* 2023). In this study, the apparently healthy dogs were divided into four groups and considered geriatric if they

were over 12 years old (Bruno *et al*., 2022). Although the dogs in group 2 and 3 of the present study were aged, they did not exhibit any clinical manifestations commonly observed in sick geriatric dogs.

In the present study, though there were significant variations in some of the haematological and biochemical parameters between control, geriatric and elderly dogs, the statistical means for the parameters remained within the reference means for the species, except for MCV, BUN, globulin and total protein. Many diseases affect one or more protein fractions. An increase in total protein and globulin is often noted in older dogs, which can be interpreted based on clinical signs or other laboratory parameters.

The Geriatric and elderly groups (Group 3 and 2) of dogs in the present study revealed significantly reduced haemoglobin concentration, haematocrit percentage and total erythrocytic and lymphocytic count compared to young control dogs. Moreover, there was a significant increase in MCV in geriatric dogs. Though non-significant, platelet counts decreased, and monocyte counts increased in geriatric dogs. These findings are in line with reports from previous studies (Pati *et al.,* 2015; Radakovich *et al.,* 2017; Bruno *et al.,* 2022), and these changes can be attributed to physiological, immunological and biological changes in the body due to ageing, and/or decreased production of erythrocytes from bone marrow, and splenomegaly (Sanjeeta *et al.,* 2013).

The mean urea value was found in another study to be higher in geriatric dogs than in elderly dogs and can be probably due to protein diet, intestinal haemorrhage, use of certain medicines, dehydration or old age (Finco and Duncan 1976; Radakovich *et al*., 2017). However, there were also reports of reduced BUN in geriatric dogs (Lee *et al.*, 2020; Bruno *et al*., 2022). In the present study, the mean blood glucose level in geriatric dogs was significantly lower than that in control dogs, and in geriatric dogs, the mean blood glucose level was slightly lower than the reference value. However, the blood sugar concentration was well above 60 mg/dl, which corresponds to a hypoglycaemic state. The reduced blood-glucose level could also be attributed to the age-related reduction of hepatic glycogen stores (Lowseth *et al.,* 1990; Lee *et al.,* 2020). It was also observed that the plasma phosphorus levels were in the normal range in all groups but tended to be higher in the geriatric dogs, which agreed with previous reports (Aguilera-Tejero *et al.,* 1998).

Table 1: Serum biochemical parameters and mineral profile in different age groups of Dobermann pinscher dogs (Mean ± SE).

Parameters	Control	Group-1	Group 2	Group 3
Total protein (g/dl)	5.61±0.25^{a}	5.42±0.14^{a}	6.74±0.27^{ac}	8.78±0.68^{d}
Albumin (g/dl)	2.50±0.15^{a}	2.53±0.17^{ab}	3.10±0.20^{ac}	3.52±0.27^{c}
Globulin (g/dl)	3.12±0.16^{a}	2.90±0.08^{ab}	3.64±0.31^{ac}	4.76±0.63^{c}
A:G ratio	0.81±0.05^{a}	0.88±0.08^{a}	0.90±0.10^{a}	0.68±0.08^{a}
AST (U/L)	48.47±2.83^{a}	47.27±3.17^{a}	48.82±7.17^{a}	59.93±12.07^{a}
ALT (U/L)	47.53±9.34^{a}	47.36±7.99^{a}	64.05±9.24^{a}	86.19±13.0^{b}
ALP (U/L)	81.67±7.92^{a}	76.85±11.19^{b}	113.92±8.84^{a}	115.22±2.43^{c}
CKMB (U/L)	3.4±0.42^{a}	3.67±0.51^{a}	4.27±0.41^{a}	4.75±0.39^{a}
Glucose (mg/dl)	87.87±3.81^{a}	79.32±3.29^{a}	74.05±2.27^{a}	65.48±4.48^{b}
Direct bilirubin (mg/dl)	0.02±0.00^{a}	0.02±0.01^{a}	0.08±0.03^{a}	0.11±0.03^{a}
Total bilirubin (mg/dl)	0.12±0.03^{a}	0.01±0.02^{a}	0.14±0.05^{a}	0.24±0.05^{a}
Indirect bilirubin (mg/dl)	0.01±0.03^{a}	0.08±0.03^{a}	0.06±0.02^{a}	0.13±0.03^{a}
Creatinine (mg/dl)	0.89±0.11^{a}	1.03±0.10^{a}	1.37±0.10^{a}	1.37±0.14^{a}
BUN (mg/dl)	15.70±2.15^{a}	15.70±2.15^{ab}	33.99±5.43^{c}	40.24±2.13^{c}
Sodium (mEq/L)	138.50±4.85^{a}	140.15±1.51^{a}	141.48±2.67^{a}	141.68±1.83^{a}
Potassium (mEq/L)	4.33±0.16^{a}	4.75±0.14^{a}	4.73±0.17^{a}	4.50±0.15^{a}
Chloride (mEq/L)	109.80±2.56^{a}	108.17±2.8^{a}	111.85±1.56^{a}	102.40±3.91^{c}
Calcium (mg/dl)	10.67±0.27^{a}	10.67±0.27^{a}	8.12±0.26^{b}	6.95±0.31^{c}
I Calcium(mg/dl)	1.30±0.04^{a}	1.19±0.05^{a}	1.17±0.02^{a}	1.14±0.06^{a}
Uric acid(mg/dl)	0.20±0.05^{a}	0.17±0.04^{a}	0.33±0.13^{a}	0.43±0.14^{a}
Phosphorous(mg/dl)	3.46±0.24^{a}	3.67±0.08^{a}	4.08±0.24^{a}	4.85±0.20^{a}

Values within a row, having different superscripts, differ significantly (P<0.05) with each other

Table 2: Serum haematological parameters and mineral profile in different age groups of Dobermann pinscher dogs (Mean ± S.E.).

Parameters	Control	Group-1	Group 2	Group 3
Haemoglobin (gm/dl)	16.73±0.54^{a}	15.65±0.36^{ab}	14.30±0.25^{b}	14.25±0.20^{bc}
HCT (%)	45.83±1.39^{a}	42±0.47^{a}	41..98±0.29^{a}	40.8±0.481^{b}
TEC (million cells /µL)	6.48±0.29^{a}	6.06±0.12^{a}	5.29±0.15^{b}	5.11±0.09^{b}
TLC (Thousand cells/µL)	10.72±1.0^{a}	13.73±0.42^{a}	10.98±1.09^{a}	6.88±0.31b
Platelet count (10^{9}/L)	4.1±0.37^{a}	2.93±0.33^{a}	3.03±0.4^{a}	3.79±0.14^{a}
Lymphocyte (%)	24.0±3.32^{a}	18.17±1.92^{a}	17.67±0.95^{a}	16.83±1.30^{a}
Neutrophil (%)	73.50±1.82^{a}	73.33±2.09^{a}	73.50±1.41^{a}	67.67±4.17^{a}
Eosinophil (%)	2.0±0.37^{a}	0.83±0.4^{a}	1.33±0.56^{a}	1.33±0.6^{a}
Monocyte (%)	6.0±0.82^{a}	8.0±0.77^{a}	7.50±0.81^{a}	7.0±1.15^{a}
Basophils (%)	0±0	0±0	0±0	0±0
MCV (femtoliters)	71.14±2.37^{a}	69.45±1.04^{a}	79.73±2.32^{b}	80.01±2.06^{bc}
MCH picograms (pg.) per cell	26.06±1.34^{a}	25.94±1.06^{a}	27.18±1.03^{a}	27.90±0.24^{a}
MCHC (percentage)	36.75±1.96^{a}	37.29±1.00^{a}	34.08±0.73^{a}	34.97±0.85^{a}

Values within a row, having different superscripts, differ significantly (P<0.05) with each other

CONCLUSIONS

The study found that older and geriatric dogs exhibited elevated levels of total protein, globulin, ALT, BUN, and MCV, along with decreased haemoglobin levels, HCT, and total erythrocytic and leukocytic counts. Serum chloride values and blood glucose concentrations were also lower in geriatric dogs. While the observed mean values of these haematological and biochemical parameters generally fell within the reference ranges across all age groups; exceptions were noted for BUN, ALT, and MCV. Although all the animals examined appeared healthy in the present study, more haematological and biochemical changes were observed in the geriatric age group. This highlights the importance of laboratory evaluations for prognosis and early diagnosis, ultimately enhancing dogs' quality of life and well-being.

FUTURE SCOPE

In recent decades, the life expectancy of dogs has increased significantly,leading to an increase in the aged population. Recent studies suggest domestic dogs are a valuable model for translational gerontology, as the leading causes of morbidity and mortality are the same in humans and dogs.This research concept is in line with the One Health concept.

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Conflict of Interest. None.

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