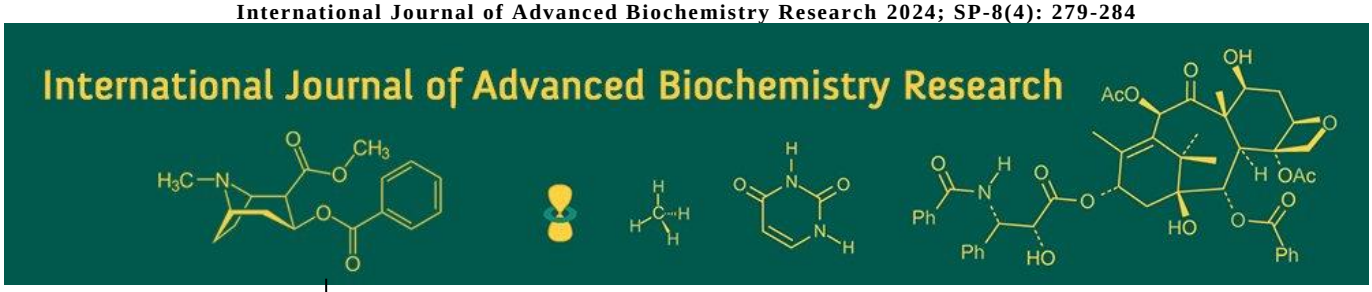

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Canine anemia: Clinical assessment, prevalence and morphological classification

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Abstract
The present study entitled "Canine Anemia: Clinical assessment, Prevalence and Morphological classification" was conducted at Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani. During study, total 146 dogs were screened from dog breeder farms, Veterinary dispensaries, and Veterinary Clinical Complex for detection of anemia. Out of that, 76 cases were considered anemic based on history, clinical signs, and laboratory tests. Suspected cases were examined for morphological classification of anemia.
Clinical evaluation revealed that anemic dogs had rough skin and hair coats, pale visible mucus membranes, lethargy, anorexia, tachycardia and deep breathing as prominent clinical features. Hematology revealed significantly lower values of Hb, PCV, TEC, MCV, and MCHC, whereas, values of TLC, DLC and platelets were found within normal physiological range.
Overall prevalence of anemia was 52.06%. It was found highest in adult dogs that to in males compared to females, in ND breed of dog and during monsoon season. Morphologically, there was marked variation in shape and size of erythrocytes. Morphologically, microcytic hypochromic anemia was recorded as a major type of anemia.

Highlights
1. Dog plays most important role in human lives as a best companion. Due to their fundamental behavioral characters like obedience, loyalty, adoptability, sensitive olfaction, differentiation and investigating behavior, different dog breeds are utilized for various purposes *viz*., sporting, crime detection, explosive searching, etc.
2. Considering the various diseases of dogs, anemia is most common and frequently diagnosed complication caused due to many etiological factors, some of which are life-threatening in veterinary practice.

Keywords: Canine anemia, clinical assessment, prevalence and morphological classification

Introduction
The dog (*Canis familaris*) is the oldest companion animal and is first domesticated species. The dog has fulfilled a variety of roles i.e., as a guard dog, hunting, companion and also guide.
Hytönen, & Lohi, (2016) [11] stated that dog is a large animal model and is physiologically and clinically more similar to human than mouse. Lindblad *et.al.*, (2005) [17] reported that dog genome is less divergent from the human genome than from the mouse genome. Therefore, more of the human genomic sequence can be syntenically aligned to the genome of the dog than that of the mouse. Dogs have approximately the same number of genes as humans, most of them being close Orthologues.
Anemia is defined as a condition in which the body has a decreased number of circulating erythrocytes (RBCs), or decrease in hemoglobin concentration. It can also be defined as reduction in number of the erythrocytes in an animal for that particular age, species, breed, and geographic location (Vegad and Madhu Swamy, 2010) [29]. It can either be due to decreased production of erythrocytes or haemoglobin or increased destruction of red blood cells (RBCs). It is therefore associated with reduction in red cell count or volume of red cell. In a physiological way, anemia is defined as the decreased ability of blood to supply tissues with adequate oxygen for proper metabolic functions (Hoffbrand and Pettit, 1993) [12].

It is characterized by reduction in the hemoglobin (Hb), haematocrit (PCV) or total erythrocytic count (TEC) per unit volume of blood in a normally hydrated animal (Aiello, 1997) [2].

Different researchers *viz*. Moninder (2003) [21], Furlanello *et al*. (2005) [8], Singh (2012) [25], Tandel *et al.* (2016) [28] and Meshram *et al.* (2019) [20] studied anemia in dogs at different places and showed that the prevalence of anemia ranged between 47-75%, which is quite higher and alarming condition.

Wintrobe developed three erythrocytic indices *viz*. Mean corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV) & mean corpuscular hemoglobin concentration (MCHC). The traditional morphological approach for classification of anemia is based on Mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC). This classification is useful and diagnostically sound which classify anemia as normocytic-normochromic, macrocytic-normochromic, macrocytic-hypochromic and microcytic-hypochromic. Based on morphology, there are two more types such as normocytic-hypochromic and microcytic-normochromic. Also, one more addition to type based on aetiology is immunological haemolytic anemia (Chakrabarthi, 2007) [3].

The course and outcome of anemia is mainly dependent upon the degree and severity affecting the blood components and it could be life threatening or at other side it may be asymptomatic and even difficult to detect clinically. Therefore, one should give more attention towards predisposing causes, exciting causes, diagnosis and prevention of anemia rather than treating it.

Considering the importance of RBCs and hemoglobin in transport of oxygen to vital organs and the high prevalence but very little research work on anemia in dogs in India, especially in and around the Parbhani district of Marathwada region, the present study has been conducted.

Materials and Methods

The study was conducted at College of Veterinary and Animal Sciences, Parbhani, Maharashtra. Samples (blood, serum, fecal and urine) for present study were taken from Veterinary Clinical Complex, Veterinary Polyclinics, Veterinary dispensaries and from dog breeder farms in and around Parbhani and screened for anemia.

Clinical assessment of anemia

Anemia was diagnosed on the basis of history, clinical manifestations and laboratory examination. Positive anemic dogs included in this study were selected from, dogs in study area and screened for hematological studies.

The anemic dogs taken for the study were divided into five groups *viz.*, up to 6 months of age (Puppies), 6 months - 1year (Juvenile), 1-2 years (Adolescent), 2-8 years (Adult) and above 8 years of age i.e., Senile, (Harvey, 2021) [10]. For comparison of hematological status of anemic dog with that of healthy dog, a total of 12 apparently healthy dogs from well-maintained dog owners from Parbhani city were taken.

Inclusion criteria for assessment of anemia in dog

Following inclusion criteria was used along with clinical assessment of dogs for incorporation of anemic cases during study.

1. High rectal temperature
2. Pale visible mucus membranes
3. Anorexia
4. Lethargy and weakness
5. Ticks on body
6. Body condition
7. Behavior and activity
8. Skin and hair coat
9. Posture and gait
10. Clinical observations (Dyspnea, tachycardia, bradycardia)
11. Hemogram
12. Urine analysis
13. Fecal Sample examination

(Mahalingaiah *et al.*, 2017) [19] and (Assenmacher *et al.*, 2019) [4].

Collection of blood and serum samples

The blood samples were collected either from cephalic or saphenous vein of dogs suspected for anemia with all aseptic precautions, in vacutainers with anticoagulant and sterile vials containing Ethylene Diamine Tetra Acetate (EDTA) as an anticoagulant for hematological studies. Samples were collected from apparently healthy control animals in a similar pattern as that suspected for anemic dogs.

Classification of data

The data collected was analysed according to age, sex, breed of animals and also, season for prevalence study. For classification of anaemia, 10 clinical parameters were taken such as visible mucus membranes, rectal temperature, heart rate, respiration rate, anorexia, lethargy, body condition, posture and gait, behaviour, skin and hair coat.

Scoring was made between 0-1 grade. Grading for each parameter was made as 0 if it was normal and 1 if it was deviated from normal i.e., abnormal. For example, normally the color of mucus membrane is pink, so it was graded as 0 whereas, other than pink like pale or congested or icteric was graded as 1. This was done for all 10 parameters and score card was made for each case out of 10 clinical parameters noted and after that groups were made according to grading as follows:

1. **Group I (Severe anemia):** The dogs with score 8-10 were categorized into this group.
2. **Group II (Moderate anemia):** The dogs with score 5-7 were categorized into this group.
3. **Group III (Mild anemia):** The dogs with score 1-4 were categorized into this group.
4. **Group IV (Apparently healthy control):** In this group representative number of cases were taken from apparently healthy dog cases. (Kisielewicz *et al.*, 2014) [16].

Morphological classification of anemia

It was done on the basis of erythrocytic indices (MCV and MCHC) and blood smear examination during DLC.

Prevalence of anemia

Overall prevalence was calculated from number of dogs screened and out of which number of positive cases for anemia, which were selected for further studies. Prevalence of Clinical cases was further classified into Age-wise (Puppies, Juvenile group, Adult and Senile group, as

described by Harvey, (2021) [10] and gender-wise (male and female), breed wise and season wise.

Statistical analysis

Data generated was analyzed statistically to find the level of significance by employing Chi-square test for analysis of prevalence of anemia (age, sex, breed and season wise) and Complete Randomized Design (CRD) for clinical assessment, hematological and biochemical studies in both anemic as well as healthy dog with standard statistical method described by Snedecor and Cochran, (1982) [26].

Results and Discussion

Clinical assessment of anemia

Anemia was diagnosed based on the history of illness, clinical manifestations and laboratory investigations. Clinical assessment of anemia was made by recording color of visible mucus membrane, rectal temperature, heart rate, respiration rate, appetite, body condition, behavior, skin & hair coat, posture & gait and lethargy in dogs.

Results revealed pale visible mucus membrane, lethargy or reluctant to move, anorexia, tachycardia, deep breathing, rough and dehydrated skin & hair coat as prominent clinical manifestations observed in anemic cases of dog after thorough clinical assessment.

Prominent clinical signs and symptoms significant of anemia in dogs were reported by several researchers. From the reviewed literature, Joshi (2000) [13], Furlanello *et al*. (2005) [8], Swann *et al*. (2013) [27], Kisielewicz *et al.* (2014) [16], Mahalingaiah *et al.* (2017) [19], Assenmacher *et al.* (2019) [4] recorded similar clinical findings as recorded in present study.

Table 1: Clinical assessment of anemic and apparently healthy dogs

Sr. No.	Parameter Studied	Group- I (Severe anemia)	Group- II (Moderate anemia)	Group- III (Mild anemia)	Group- IV (Healthy)	Significance
1.	Mucus membrane	1.00^{a}±0	0.76^{ab}±0.02	0.56^{bc}±0.03	0.50^{c}±0.03	S
2.	Rectal Temperature	0.83^{a}±0.01	0.05^{b}±0.01	0.11^{b}±0.02	0.06^{b}±0.01	S
3.	Heart Rate	0.93^{a} ±0.01	0.67^{b}±0.02	0.17^{c}±0.02	0.11^{c}±0.02	S
4.	Respiration Rate	0.93^{a}±0.01	0.62^{b}±0.02	0.11^{c}±0.02	0.06^{c}±0.01	S
5.	Anorexia	01.00^{a}±00	0.76^{b}±0.02	0.11^{c}±0.02	0.11^{c}±0.02	S
6.	Lethargy	01.00^{a}±00	0.86^{a}±0.02	0.50^{b}±0.03	0.28^{b}±0.03	S
7.	Body Condition	0.93^{a}±0.01	0.62^{b}±0.02	0.11^{c}±0.02	0.06^{c}±0.01	S
8.	Posture & gait	0.35±0.02	0.29±0.02	0.06±0.01	0.11±0.02	NS
9.	Behavior	0.93^{a}±0.01	0.62^{b}±0.02	00.00^{c}±00	00.00^{c}±00	S
10.	Skin & Hair coat	0.66^{a}±0.02	0.52^{ab}±0.02	0.56^{a}±0.03	0.22^{b}±0.02	S

Where, Different superscripts in a row show significant difference.
S- Significant, NS- Non-significant
(Note: Values of all parameters of anemic dogs are depicted in 0-1 grade)

Prevalence of anemia

Overall prevalence of anemia in dogs was recorded as 52.06% i.e., out of total 146 dogs screened, 76 cases were found anemic.

Different researchers *viz*. Moninder (2003) [21], Furlanello *et al*. (2005) [8], Singh (2012) [25], Tandel *et al.* (2016) [28] and Meshram *et al.* (2019) [20] studied anemia in dogs at different places and showed that the prevalence of anemia was ranged between 47-75%. In present study we recorded 52.06% prevalence of anemia in dogs, which was found analogous to the findings of earlier workers.

Age wise prevalence

It was found highest (32.90%) in adults, followed by Puppies (28.95%), Adolescent (17.11%), Juvenile group (13.16%) and least (7.90%) in Senile group.

Chervier *et al.* (2012) [7] and Tandel *et al.* (2016) [28] observed highest prevalence of anemia in old dogs, whereas, Singh (2012) [25], Brahmbhatt *et al.* (2015) [6], Meshram *et al.* (2019) [20] and Shah *et al.* (2020) [24] recorded higher incidence in young pups. The results found in present study were allied with the study conducted by Chervier *et al.* (2012) [7], Tandel *et al.* (2016) [28] and Meshram *et al*. (2019) [20].

Gender wise prevalence

It was found highest in males (42 cases out of total 76 cases of anemia i.e., 55.26%) than females (34 cases out of total 76 cases of anemia i.e., 44.74%).

The highest prevalence of anemia in males observed during present study has been found akin with the studies conducted by Brahmbhatt *et al.* (2015) [6], Tandel *et al.* (2016) [28], Meshram *et al.* (2019) [20] and Shah *et al.* (2020) [24].

Breed wise prevalence

It was found highest (32.90%) in ND breed of dog, followed by Labrador (23.68%), German shepherd (19.74%), Pomeranian (5.26%), Rottweiler (5.26%), Siberian Husky (2.63%), Doberman (2.63%) and least in Carvan, Crossbreds, Great Dane, Mudhol Hounds, Pug and Lhasa apso (Each were having 1.32% prevalence).

The results obtained during present study regarding breed wise prevalence of anemia in dogs found congrous with the reports of earlier researchers, Singh *et al.* (2012) [25]; Brahmbhatt *et al.* (2015) [6]; Meshram *et al.* (2019) [20] and Shah *et al.* (2020) [24].

Season wise prevalence

It was observed highest during monsoon season (65.79%), followed by summer (22.37%) and then winter (11.84%).

The findings of present study about seasonal prevalence of anemia was found at par with the findings of Ahmad *et al.* (2007) [1] and Gadahi *et al.* (2008) [9].

Table 2: Details of the dog screened during study in and around Parbhani

Dog screened	Anemic	Non anemic	Total
Puppies (upto 6 months of age)	22	8	30
Juvenile (6-12 months)	10	17	27
Adolescent (1-2 years)	13	21	36
Adult (2-8 years)	25	22	47
Senile (above 8 years)	6	2	8
Total	76	70	176

Table 3: Age-wise prevalence of anemia in dog

Total number of Anemic cases 76 (100%)				
Age	% Prevalence	X2	Statistics	
			1%	5%
Puppies	22 (28.95%)	2.61	NS	NS
Juvenile	10 (13.16%)	1.17		
Adolescent	13 (17.11%)	1.25		
Adult	25 (32.9%)	0.01		
Senile	06 (7.9%)	0.81		
Total	76	5.85		

Where, NS- Non-significant

Table 4: Sex-wise prevalence of anemia in dog

Total number of Anemic cases 76 (100%)			
Sex	% Prevalence	X2	Statistics
Male	42 (55.26%)	0.78	NS
Female	34 (44.74%)	0.85	
Total	76	1.63	

Where, NS- Non-significant

Table 5: Season-wise prevalence of anemia in dog

Total number of anemic cases 76 (100%)				
Season	% Prevalence	X2	Statistics	
			1%	5%
Summer	17 (22.37%)	0.12	NS	S
Monsoon	50 (65.79%)	1.68		
Winter	09 (11.84%)	5.06		
Total	76	6.86		

Where, S- Significant, NS- Non-significant

Table 6: Breed-wise prevalence of anemia in dog

Total number of anemic cases 76 (100%)				
Breed	% Prevalence	X2	Statistics	
			1%	5%
Beagle	0	1.04	NS	NS
Carvan	1 (1.32%)	0.001		
Cross	1 (1.32%)	0.001		
Doberman	2 (2.63%)	1.12		
German Shepherd	15 (19.74%)	0.01		
Golden Retriever	0	1.56		
Great Dane	1 (1.32%)	0.001		
Siberian Husky	2 (2.63%)	0.40		
Labrador	18 (23.68%)	0.03		
Mudhol hound	1 (1.32%)	0.001		
Non-Descript	25 (32.90%)	2.09		
Pitbull	0	1.04		
Pomerarian	4 (5.26%)	0.75		
Pug	1 (1.32%)	0.001		
Rottweiler	4 (5.26%)	0.25		
Saint bernard	0	1.04		
Lhasa apso	1 (1.32%)	0.001		
Total	76	9.331		

Where, NS- Non-significant

Morphological classification of anemia

Morphological classification of anemia was done on the basis of blood smear examination during DLC and by using erythrocytic indices *viz*. MCV and MCHC. After examination of 76 blood smears of anemic dogs it was noted that there was anisocytosis in 78.95% cases and poikilocytosis in 94.74%, whereas, 76.34% cases were found with both anisocytosis and poikilocytosis. Other than variation in size and shape there was tendency for raulex formation in 25% cases, reticulocytosis in 13.16% cases, Heinz bodies in 11.84% cases, Howell jolly bodies in 6.58% cases, tendency of agglutination of erythrocytes in 6.58% cases and basophilic stippling in 3.95% anemic cases of dog. In poikilocytosis, there was marked degree of change in shape of erythrocytes. Abnormalities recorded in shape of erythrocyte were acanthocytosis (42.11%), spherocytosis (30.26%), stomatocytosis (25%), target cells (23.68%), ovalocytosis (14.47%), keratocytosis (13.16%), meniscocytosis (2.63%), discocytosis (1.32%), and tear drop cells (1.32%).

Nassiri *et al.* (2005) [22], Zygner *et al.* (2006) [30], Balch & Mackin, (2007) [5] and Shah *et al.* (2009) [23] reported microcytosis, anisocytosis, poikilocytosis, hypochomasia, spherocytosis and agglutination of erythrocytes during their study of anemia in dogs. The findings on morphology of erythrocytes in the present study go well with the observations recorded by all these authors.

Regarding morphological type of anemia, it was concluded that microcytic hypochromic anemia in 44.74% cases was a major type of anemia followed by normocytic normochromic anemia in 19.74% cases, microcytic normochromic anemia in 18.42% cases, macrocytic hypochromic anemia in 15.79% cases, normocytic hypochromic anemia in 6.58% cases, and macrocytic normochromic anemia in 1.32% cases out of 76 anemic dogs screened.

Kim *et al.* (2006) [14] reported microcytic hypochromic anemia as a major type of anemia during their study, which very well go with findings of present study. Several other researchers like King *et al.* (1992) [15], and Lucidi *et al.* (2017) [18] found normocytic normochromic anemia as major type of anemia during their research. This is also a co relating findings between their study and present study.

Conclusion

From the present study it can be concluded as

1. Pale visible mucus membrane, lethargy or reluctant to move, anorexia, tachycardia, deep breathing, rough and dehydrated skin & hair coat are the prominent clinical manifestations observed in anemic cases of dog.
2. Overall prevalence of anemia in dogs was 52.06%. It was found highest in adult dogs that to in males as compared to females and in ND breed of dog during monsoon season.
3. Microcytic hypochromic anemia was a major type of anemia followed by normocytic normochromic anemia then, microcytic normochromic anemia, macrocytic hypochromic anemia, normocytic hypochromic anemia and macrocytic normochromic anemia.

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Conflict of interest

None

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